

EFFECT OF POLYETHYLENE GLYCOL (PEG) ON GERMINATION AND SEEDLING PARAMETERS OF MAIZE VARIETIES

ABSTRACT

In an experimental framework employing a Factorial Randomized Complete Block Design (FRBCD), a study was conducted in July 2022 at the Crop Science Institute, NARC, Islamabad. The objective was to assess the impact of different Polyethylene Glycol (PEG) concentrations on two maize cultivars Sargodha 202 and OPV-3 under drought stress conditions. With triplicate replications, each experimental unit consisted of a pot filled with 4.5 kg of dry sand, where 10 seeds were planted. The 45-day study incorporated four treatment groups: T0 (Control), T1 (10% PEG), T2 (20% PEG), and T3 (30% PEG).

Data analysis revealed that increasing PEG concentrations led to a decline in key parameters such as germination rate, root elongation, and shoot development, while concurrently escalating seedling mortality in both cultivars. Statistical evaluations, carried out through the Least Significant Difference (LSD) method at a 5% significance level, highlighted notable differences between the treatment outcomes. Sargodha 202 exhibited superior performance relative to OPV-3, recording a germination rate of 91.66% as opposed to OPV-3's 83.33%. Furthermore, Sargodha 202 demonstrated enhanced root (13.08 cm) and shoot (50.08 cm) lengths in comparison to OPV-3, which showed 10.58 cm for root and 45.83 cm for shoot length. Sargodha 202 also displayed elevated levels of both root and shoot moisture content and a higher crop growth rate compared to OPV-3. Collectively, these results underscore the greater resilience and adaptability of Sargodha 202 under PEG-induced drought conditions.

Keywords: Fodder and forage, Germination parameter, Maize, Pots experiment, Polyethylene glycol (PEG), Seedling.

1. INTRODUCTION

Maize or corn (*Zea Mays* L.) is an important annual cereal crop of the sector belonging to family Poaceae. Maize is a crucial staple crop worldwide and serves multiple purposes such as food production, animal feed, and manufacturing. The maize plant has particular needs when it comes to water and climate, which are crucial for its growth and development. Specifically, the plant requires a temperature between 15 and 20°C in order to germinate successfully [1]. According to the FAOSTAT (2021) [2] report, maize is among the most significant crops globally. This annual plant has a single stalk and leaves that are arranged alternately, and it can reach up to 3 meters in height. The inflorescences, namely the tassel and the ear, produce unisexual flowers, with the former being male and the latter female. The grains of maize are arranged in rows on the cob and are covered with a husk, as described in the (FAO, 2017) [3] report. Maize, also referred to as corn, is a widely cultivated crop in Pakistan that is mainly grown

as a fodder crop. According to the Pakistan Agricultural Research Council (PARC), maize ranks as the third-largest crop in terms of both area and production output after rice and wheat (Pakistan Agricultural Research Council, 2020) [4]. Maize holds the third position among cereals cultivated in Pakistan, following wheat and rice. The two major maize-growing provinces in the country are Khyber Pakhtunkhwa and Punjab. This crop serves as a primary food source for people in Khyber Pakhtunkhwa, particularly in mountainous areas where poverty is widespread, owing to its affordability. Additionally, it is widely recognized as a valuable fodder crop, especially for producing silage. Maize is often referred to as the "king of crops" for silage purposes [5]. Besides Pakistan, other nations such as the United States, China, and Brazil heavily depend on maize as a crop due to its versatility in multiple industries, including food, feed, and biofuels [2].

Maize has an energy density of 365 Kcal/100 g and is made up of around 72% starch, 10% protein, and 4% fat. While maize has a lesser protein content than wheat and rice, it is higher in fiber, B vitamins, and other minerals. But it is deficient in several essential vitamins, such as vitamins B12 and C, and is typically not a rich source of calcium, folate, and iron [6]. Additionally, several foods or elements of the diet, such as tea (oxalates), coffee (polyphenols), eggs (phosvitin), and milk (calcium), might impede the absorption of iron from nonheme sources in maize [6,7]. Maize flour and cornmeal have been enhanced in nations where anemia and iron deficiency are public health problems. As a way to increase micronutrient consumption and avoid iron insufficiency, maize flour, and cornmeal are fortified with iron and other vitamins and minerals in nations where anemia and iron deficiency are public health issues [8]. Due to its high starch and low fiber contents, which produce a concentrated source of energy for animal production, maize is a great choice for livestock feed. Compared to other grains, maize exhibits a superior conversion ratio to meat, milk, and eggs. The extent of maize utilization in livestock feed remains unknown, however, substantial usage for poultry feed is believed to occur in tropical regions. Yellow maize is the favored variant for livestock feed and can be administered as whole grains, dry or wet, cracked or ground, or steamed. Typically, maize is supplemented with proteins and vitamins. It is anticipated that maize incorporation in formulated feed will experience growth in forthcoming times [9].

Drought is a pervasive problem worldwide that affects a substantial amount of agricultural land with varying degrees of severity. A deficiency of water, high temperatures, and low humidity in the atmosphere can lead to drought, which is a major limiting factor for optimal plant growth and crop yield [10,11]. The physiological and biochemical changes in pigeon-pea are impacted by water deficiency caused by Polyethylene glycol (PEG). Two levels of stress were applied to the plants, namely moderate (- 0.51 MPa) and cruel (-1.22 MPa). A PEG nutrient solution was used to irrigate the seedlings grown in pots that were 14 days old, and the osmotic potential was gradually reduced by -0.04 MPa. As a result of the drought stress, the Relative Water Content (RWC) decreased significantly. The presence of higher levels of free proline during the water stress conditions indicated that proline is a compatible osmolyte that is commonly used under such circumstances [12].

Polyethylene Glycol, commonly known as PEG, is a synthetic, water-soluble polymer. It is widely employed in various industrial applications, including as an emulsifier in food and cosmetics, and as an excipient in pharmaceuticals [13]. However, its role in agriculture,

particularly in seed germination and seedling development, is increasingly being acknowledged. PEG acts as an osmotic agent, simulating water-deficit conditions in experimental setups [14]. Its application is particularly valuable for assessing the drought tolerance of different plant species, including maize varieties. The polymer's high water-holding capacity makes it a suitable medium for mimicking water stress conditions without the confounding effects of nutrient deficiency or salt stress [15].

In maize varieties, PEG has been shown to have a dual role. On one hand, it can adversely affect germination rates and seedling growth by inducing osmotic stress, thus simulating conditions of low water availability [16]. On the other hand, PEG treatment can serve as a selective pressure that helps in identifying drought-tolerant genotypes within a varieties collection [17]. To summarize, PEG is not just an industrial polymer; it is a versatile tool in agronomic research. Its ability to simulate drought conditions provides a valuable means of screening for drought-resistant maize varieties, thus contributing to the development of more resilient agricultural systems.

The study [18] focused on using PEG as a selective pressure for identifying drought-tolerant maize genotypes. They used PEG concentrations of 15% and 25% to induce moderate and high osmotic stress, respectively. The study concluded that certain maize genotypes showed better germination and seedling survival rates under PEG-induced stress, thus identifying them as potentially drought-tolerant. In this study [19] the researchers examined the impact of PEG on maize germination under controlled conditions. They used different concentrations of PEG solutions, including 10%, 20%, and 30%, to mimic varying levels of water stress. The results indicated that as the PEG concentration increased, the germination rate and seedling vigor decreased. This study highlighted the utility of PEG in simulating drought-like conditions and its impact on maize germination.

To induce drought stress in maize, the use of high molecular weight osmotic substances such as polyethylene glycol (PEG) has been previously reported [20,21]. The present study also employed PEG to induce drought stress in maize, with the aim of investigating the physiological and hormonal changes in response to this stress. The study seeks to address the limited knowledge on the hormonal response of maize to drought stress. Specifically, this research will examine the effects of various concentrations of PEG on the germination and seedling characteristics of maize varieties, including biomass, shoot and root length, germination percentage, and mean germination time. By gaining a deeper understanding of the mechanisms of drought tolerance in maize, this study can contribute to improving maize production in water-limited regions.

2. MATERIALS AND METHODS

The current investigation was carried out over 45 days. The purpose of this study was to investigate the way two distinct types of maize that were under drought stress responded to various dosages of polyethylene glycol (PEG). In July 2022, the experiment was carried out at the National Agriculture Research Center's Fodder and Forage Laboratory in Islamabad's Crop

Science Institute (CSI). In an experimental framework employing a Factorial Randomized Complete Block Design. The site's coordinates were 33.6982 latitude and 73.0393 longitudes. On July 22, 2022, two separate hybrid types of maize—Maize OPV-3 and SARGODHA-202—were planted. The experimental setup had 24 experimental units, each of which included one pot with the following measurements: 30 cm in height, 33 cm at the top, and 25 cm at the base. Each pot held 10 seeds and was filled with 4.5 kg of dry sand. Treatments T0 (control), T1 (10% PEG), T2 (20% PEG), and T3 (30% PEG) were all different from one another. Each treatment had three replications, each with a single pot.

2.1. Doses of Polyethylene Glycol (PEG)

The different doses of PEG were prepared by taking three beakers of 500 ml and measuring the weight of PEG with a weighing balance. Different doses were prepared according to the test requirements (10%, 20%, and 30%). Distilled water was added to each beaker according to the requirements (10% PEG add 270 ml water, 20% PEG add 240 ml water, 30% PEG add 210 ml water), and PEG was added. The solution was mixed well with the help of a stirrer, and when the solution was ready to apply, 50 ml was put in each test tube and applied to each experimental unit.

Table 1. presents the application timing and dose-dependent effects of polyethylene glycol (PEG).

Date	T ₀	T ₁	T ₂	T ₃
22-06-2022 (On sowing)	0% PEG (0g PEG of 300ml solution) and 50 ml solution add in per pot.	10% PEG (30g PEG of 300ml solution) and 50 ml solution add in per pot.	20% PEG (60g PEG of 300ml solution) and 50 ml solution add in per pot.	30% PEG (90g PEG of 300ml solution) and 50 ml solution add in per pot.
26-06-2022 (During seedling)	0% PEG (0g PEG of 300ml solution) and 50 ml solution add in per pot.	10% PEG (30g PEG of 300ml solution) and 50 ml solution add in per pot.	20% PEG (60g PEG of 300ml solution) and 50 ml solution add in per pot.	30% PEG (90g PEG of 300ml solution) and 50 ml solution add in per pot.

2.2. Statistical Data Analysis

The 2016 version of Microsoft Excel was used to gather and record the data for this investigation. After that, Statistic version 8.1 was used to do a statistical analysis of variance (ANOVA). The researchers used the least significant difference (LSD) approach at a 5% probability level, as advised by Steele and Torrie (1980) [22], to ascertain if there were significant differences between treatment means.

The objective of the current study was to determine the way various PEG dosages affected the growth and development of two maize types under drought stress. Multiple characteristics, including germination percentage (GP %), shoot length (SL), shoot moisture content (SMC %), root moisture content (RMC %), crop growth rate, and mortality percentage, were examined in the experiment, which used a totally randomized design with three replications. To find significant

differences between treatment means, the least significant difference (LSD) approach was used at a 5% probability level. The experimental units were subjected to different PEG doses to evaluate the effect of PEG on mitigating the impact of drought stress on the growth and development of the maize varieties.

2.3. Germination Percentage (GP %)

The no. of seeds that germinated was divided by the total no. of seeds. The quotient obtained was multiplied by 100 to determine the germination percentage (GP %).

2.4. Mortality Percentage (MP %)

To determine the mortality percentage (MP %), the number of deceased plants was divided by the number of germinated plants, and the outcome was multiplied by 100.

The formula [23] to calculate the Mortality percentage (MP %) is:

$$\text{MP\%} = (\text{Number of dead plants} / \text{Number of germinated plants}) \times 100$$

2.5. Root Length (SL)

The experiment involved utilizing root length, measured in millimeters, as a metric to evaluate plant growth. The measurement process was executed by employing a ruler or caliper to gauge the distance between the plant's base and the root's tip.

2.6. Shoot Length (SL)

In the study, the length of the shoots was measured in millimeters as a way to assess the growth of the plant. To measure the shoot length, a ruler or caliper was used to determine the distance from the base of the plant to the tip of the shoot. The measurement was taken in millimeters because it is a precise unit of measurement that allows for accurate comparisons between different samples.

2.7. Shoot Moisture Content (SMC %)

The fresh weight and dry weight of the samples were measured.

The shoot moisture content percentage (SMC %) was calculated using the formula [24]: $(\text{Fw of the shoot} - \text{Dw of the shoot}) / \text{Fw of shoot} \times 100$

2.8. Root Moisture Content (RMC %)

The root moisture content percentage (RMC %) was determined using the formula [25]: $(\text{Fw of root} - \text{Dw of the root}) / \text{Fw of root} \times 100$

Fw represents the fresh weight and Dw represents the dry weight.

2.9. Crop Growth Rate (CGR)

In 1952, a definition was proposed by Watson for Crop Growth Rate (CGR) as a metric for the rise in dry matter production per unit of land area over a specified time period. The measure was typically expressed in grams per meter squared per day ($\text{gm m}^{-2} \text{day}^{-1}$). The CGR calculation involved the following formula:

$$\text{CGR} = (\text{W2} - \text{W1}) / (\text{t2} - \text{t1})$$

Where, W1 and W2 represented the dry weight of the aerial plant per unit area gained at time t1 and t2, respectively. This formula allowed for a precise calculation of CGR in a given period, providing valuable insights into crop development and production. The effectiveness of this formula has been widely recognized by professionals and scientists in the field of agriculture, allowing for more accurate assessments of crop growth rates and helping farmers optimize their agricultural practices.

3. RESULTS

The study aimed to investigate the effect of different concentrations of Polyethylene glycol (PEG) on two varieties of maize (Sargodha 202 and OPV-3) regarding different parameters. The experiment had four treatments: T0 (control), T1 (10% PEG), T2 (20% PEG), and T3 (30% PEG), and each treatment was tested on both varieties.

Table 2. "A statistical analysis of parameter variations in two varieties of maize crop under PEG treatment".

Treatments	Varieties	Germination %age	Mortality %age	Root length (RL) (CM)	Shoot length (SL) (CM)	Shoot moisture content %age	Root moisture content %age	Crop growth rate (CGR) (g)
T0	Variety 1	96.6 a	6.6 c	17.3 a	58.0 a	81.0 a	76.0 a	0.151 a
T0	Variety 2	93.3 a	10.0 c	16.0 b	54.0 b	77.3 b	72.3 b	0.144 b
T1	Variety 1	93.3 a	3.3 c	15.0 c	52.6 c	77.3 b	72.3 b	0.144 b
T1	Variety 2	90.0 a	6.6 c	12.6 d	47.6 d	71.3 c	66.3 c	0.133 c
T2	Variety 1	90.0 a	10.0 c	12.3 d	47.3 d	72.6 c	67.6 c	0.135 c
T2	Variety 2	76.6 bc	23.3 ab	8.6 e	43.0 e	71.0 c	66.0 c	0.132 c
T3	Variety 1	86.6 ab	13.3 bc	7.6 f	42.3 e	68.0 d	63.0 d	0.127 d
T3	Variety 2	73.3 c	26.6 a	5.0 g	38.6 f	62.6 e	57.6 e	0.116 e

Note: The values in the tables are presented in centimeters (cm), percentage (%) and grams (g). The letters (a, b, c, d) represent the statistical significance of the values, with a indicating the highest value in each column.

3.1. Germination Percentage

After two days of planting, both maize varieties and treatments exhibited germination. The results showed that an increase in PEG content led to a decrease in the germination percentage of both maize varieties. Variety 1 (Maize Sargodha 202) displayed better germination rates than Variety 2 (Maize OPV-3) in all treatments. The analysis of variance ($p < 0.05$) revealed a significant variation in the germination percentage among PEG treatments. For both maize

varieties, the highest germination percentage was observed in the control group (T0) observed in Table 2.

3.2. Mortality Percentage

The mortality rate in plant research refers to the percentage of seeds that failed to germinate or died during the study. The study showed that both the type of seeds and the treatments applied had a significant impact on the mortality rate. Variety 1 (Sargodha 202) had a lower mean mortality rate than variety 2 (OPV-3) (Table 3). Moreover, the mortality rate in both varieties increased significantly with the increase in PEG concentration. The control group (T0) had the lowest mortality rate of 8.3%, while the highest rate of 19.5% was observed in T3 (30% PEG) shown in (Table 2).

3.3. Root Length

Based on the study, it was observed that the roots of maize of both varieties shrank significantly as the levels of Polyethylene Glycol (PEG) increased. Variety 1 exhibited a greater root length than variety 2 in all treatments. At T0, variety 1's root length was 17.3 cm, which was significantly longer than variety 2's 16 cm root length. At T1, variety 1's root length was 15 cm, while variety 2's root length was 12.6 cm. At T2, variety 1's root length was 12.3 cm, while variety 2's root length was 8.6 cm. lastly, at T3; variety 1's root length was 7.6 cm, compared to variety 2's root length of 5 cm shown in (Table 2).

3.4. Shoot Length

The current investigation aimed to explore the impact of water stress induced by polyethylene glycol (PEG) on two distinct cultivars of maize, denoted as variety 1 and variety 2. The study measured the shoot length of both varieties in four different treatments, namely T0, T1, T2, and T3. The results showed that variety 1 had significantly longer shoots than variety 2 in all treatments. The shoot length of variety 1 was 58 cm in T0 and 52.6 cm in T1, and it gradually decreased to 42.3 cm in T3. Similarly (Table 3) Showed variety 2's shoot length was 54 cm at T0 and 47.6 cm at T1, and it decreased to 38.6 cm at T3. The statistical analysis confirmed that there were significant differences ($p < 0.05$) between the two varieties and the treatments.

3.5. Shoot Moisture Content %Age

The conducted research revealed that the percentage of shoot moisture content was significantly influenced by treatments, varieties, and their interactions. The statistical analysis of variance (ANOVA) confirmed that the main effects of treatments, varieties, and their interaction were all significant at an alpha level of 0.05. The results showed that increasing concentrations of PEG led to a decrease in shoot moisture content percentage. T3, which had the highest PEG concentration of 30%, showed the greatest reduction in shoot moisture content percentage (Table 2).

3.6. Root Moisture Content %Age

The research conducted found that treatments, varieties, and their interactions significantly influenced the percentage of root moisture content. The statistical analysis of variance (ANOVA) confirmed that at an alpha level of 0.05, the main effects of treatments, varieties, and their interaction were all significant. The results demonstrated that the root moisture content percentage decreased with increasing concentrations of PEG. Among the treatments, T3 had the highest PEG

concentration of 30% and showed the most substantial reduction in root moisture content percentage as shown in (Table 2). These results align with previous studies that have shown a similar trend. In terms of varieties, Maize Sargodha 202 had a higher root moisture content percentage than maize opv-3. However, due to the effect of PEG, the overall root moisture content decreased, but Sargodha 202 demonstrated resistances and provided the best results. This difference may be attributed to genetic variations between the two types.

3.7. Crop Growth Rate (G)

The results of the study showed that both varieties of maize, Sargodha 202 and OPV-3, had a significant difference in their crop growth rate (g) in response to the different treatments. The crop growth rate was measured for each treatment at 20 days after pulling out the remaining plants, following an initial dry weight measurement taken 23 days after germination, where half of the plants were removed and averaged. The alpha value used was 0.05%. For variety 1, the highest crop growth rate was recorded for treatment T0 (control) at 0.151g, followed by T2 (0.135g), T1 (0.144g), and T3 (0.127g). For variety 2, the highest crop growth rate was observed for treatment T0 (0.144g), followed by T2 (0.132g), T1 (0.133g), and T3 (0.116g) (Table 2). Comparing the two varieties, Sargodha 202 had a higher crop growth rate than OPV-3 in all treatments. Furthermore, the differences in crop growth rates between the varieties were most pronounced in treatments T2 and T3, where Sargodha 202 had a higher crop growth rate than OPV-3 observed in (Table 3).

Table 3. "Quantitative assessment of agronomic parameters for diverse varieties of maize (*Zea mays L.*) Crop".

Varieties	Germination %age	Mortality %age	Root length (CM)	Shoot length (CM)	Shoot moisture content %age	Root moisture content %age	Crop growth rate (G)
Variety 1	91.66 a	16.66 a	13.08 a	50.08 a	74.75 a	69.75 a	0.139 a
Variety 2	83.33 b	8.33 b	10.58 b	45.83 b	70.58 b	65.58 b	0.131 b

Note: The values in the tables are presented in centimeters (cm), percentage (%) and grams (g). The letters (a, b, c, d) represent the statistical significance of the values, with a indicating the highest value in each column.

Table 3 presents a comparison test of various parameters for two maize varieties - Sargodha 202 and OPV-3. The table shows the percentage of germination and mortality for each variety, along with the length of the root and shoot, moisture content in both the root and shoot, and crop growth rate. The results show that Sargodha 202 has a higher germination percentage of 91.66%, as compared to OPV-3, which has a germination percentage of 83.33%. Additionally, Sargodha 202 has a longer root and shoot length, at 13.08cm and 50.08cm respectively, while OPV-3 has a shorter root and shoot length of 10.58cm and 45.83cm respectively. Regarding moisture content, Sargodha 202 has higher shoot and root moisture content than OPV-3. The crop growth rate, which is an indicator of the plant's growth rate, is also higher for Sargodha 202 than OPV-3. In conclusion, the comparison of parameters in Table 3 suggests that Sargodha 202 has better growth characteristics and performs better than OPV-3. However, further research is required to validate

these findings, and environmental factors such as soil, temperature, and rainfall could also impact the plant's growth. All the graphs also support to Table 3 and above discussion.

Distribution of Parameters for Sargodha 202 and OPV-3 with Mean Lines and Data Points

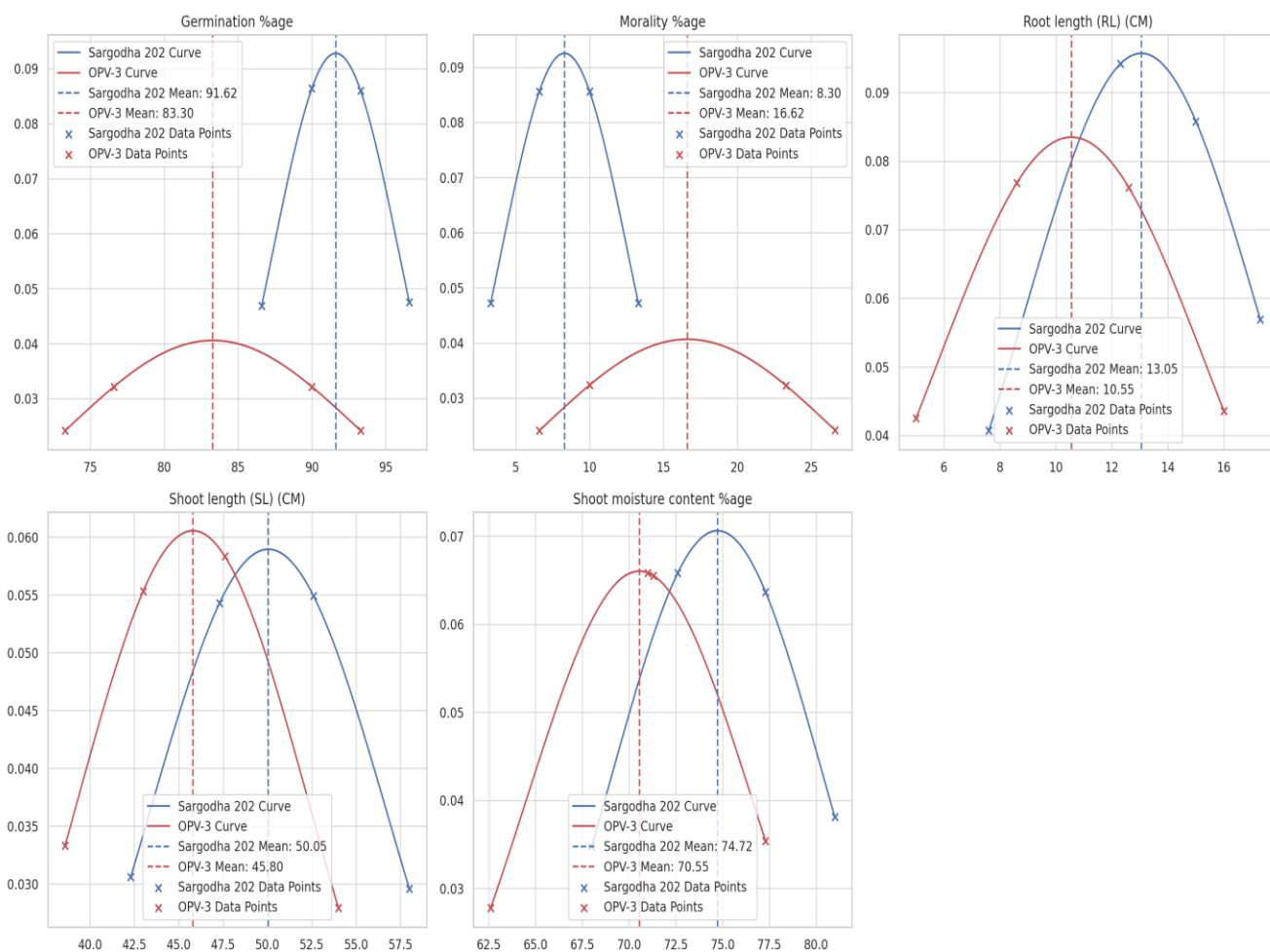


Figure 1. Comparative distribution analysis of agronomic parameters for different maize (*Zea mays L.*) varieties.

The graph compares key growth parameters for two maize varieties. Sargodha 202 generally shows higher mean values for germination, root and shoot length, and moisture content. OPV-3 has a higher mean morality percentage, suggesting it may be less resilient to stress conditions. The dashed lines represent the average performance for each variety, and individual data points show the range of observed values. In summary, Sargodha 202 appears to have better growth and resilience characteristics based on these parameters.

4. DISCUSSIONS

The results of germination are consistent with previous studies. [26] reported a negative effect of water deficit on germination and seedling growth, while [27,28] found that the Seimareh cultivar had higher germination rates than the Leucurum cultivar. In our study, we observed negative effects of PEG and drought stress on germination, but the Sargodha 202 variety showed

significant resistance. The effects of salt (NaCl) and polyethylene glycol (PEG) investigate on the germination of three sunflower cultivars under different temperature regimes. They found that both NaCl and PEG inhibited germination, with PEG having a greater impact. The adverse effects of PEG were attributed to osmotic stress rather than the accumulation of specific ions. These findings suggest that PEG increases plant mortality instead of promoting growth. Therefore, it is important to choose drought-resistant varieties and use a low dosage of PEG to minimize negative impacts on maize seedlings. Research has also shown that PEG can decrease plant root growth, as demonstrated by [27] who found significant effects of stress levels on radicle and plumule growth. While the variety only affected plumule length. Specifically, the Seimareh genotype had longer radicles compared to Leucurum, while the latter had longer plumules. Our study similarly found that both PEG and drought stress negatively affected radicle and plumule length, but the Sargodha 202 variety demonstrated greater resistance than the other variety, and the difference was statistically significant. The response of plants to drought stress can be inferred from their root length, and previous studies by [28,29] focusing on four corn hybrids, showed that hybrid golden west and KSC 704 had the highest root, shoot, and seedling length, respectively, under drought stress conditions.

These findings are consistent with previous research that has shown a similar trend. Maize Sargodha 202 had a higher shoot moisture content percentage compared to maize opv-3. However, the overall shoot moisture content decreased due to the effect of PEG, but Sargodha 202 exhibited resistance and provided the best results. This variation may be attributed to genetic differences between the two types. A previous study [12] observed significant differences in relative water content (RWC) between young leaves (26 days old) under stress (S1) and the control (C1). The stressed plants (S2) showed a sharp decrease in RWC in old leaves (46 days old) compared to the control (C2). Both control groups (C1 and C2) exhibited more than 90% of RWC. The S1 plants had a 25% lower RWC than C1, while S2 plants of the same age group had a 56% decrease in RWC compared to C2. The study [21] investigated the differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. under conditions of water stress mediated by polyethylene glycol (PEG). The authors reported that on day 14, *P. acutifolius* exhibited an increase in root and shoot dry weight, whereas *P. vulgaris* exhibited a decrease. The study found that treating two types of beans, *P. acutifolius* and *P. vulgaris*, with PEG had different effects on their relative water content (RWC). The impact of PEG treatment on the relative water content (RWC) of *Phaseolus acutifolius* and *Phaseolus vulgaris* was investigated. Results revealed that PEG treatment did not have a significant effect on the RWC of *P. acutifolius*, whereas it decreased the RWC of *P. vulgaris*. Additionally, *P. acutifolius* exhibited higher stomatal conductance, which is the rate at which water vapor is released from the stomata, under water stress induced by PEG treatment, in comparison to *P. vulgaris*. Moreover, *P. acutifolius* had a lower constitutive level of lipid peroxidation, a process that damages cell membranes, when compared to *P. vulgaris*. This difference remained consistent throughout the experiment. These results suggest that *P. acutifolius* is better adapted to tolerate water stress than *P. vulgaris*, possibly due to its ability to maintain a higher RWC and stomatal conductance, and lower lipid peroxidation levels.

In a study by [12] significant differences were observed in relative water content (RWC) between young leaves (26 days old) under stress (S1) and the control (C1). The stressed plants

(S2) showed a sharp decrease in RWC in old leaves (46 days old) compared to the control (C2). Both control groups (C1 and C2) had over 90% RWC. The S1 plants had 25% lower RWC than C1, while the S2 plants of the same age group had a 56% decrease in RWC compared to C2. The study [30] proposed that drought stress negatively affects growth specification parameters like crop growth rate (CGR), relative growth rate (RGR), and net assimilation rate (NAR). Their research findings supported this, showing a reduction in these growth parameters under drought stress. Researcher [31] conducted a study and found that exposure to polyethylene glycol (PEG) has a negative impact on various growth parameters, including 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). However, the study also suggests that the negative impact of PEG-induced osmotic stress on these growth parameters can be reduced with the presence of calcium (Ca) and magnesium (Mg) nutrients.

5. CONCLUSION

In this study, we examined how different concentrations of Polyethylene Glycol (PEG) affected two varieties of maize. We found that the concentration of PEG had a notable impact on the germination percentage, mortality rate, root length, and shoot length of both maize varieties. Increasing the PEG concentration resulted in a decrease in germination percentage, root length, and shoot length, while it increased the mortality rate in both varieties. Additionally, we observed that variety 1 (Maize Sargodha 202) demonstrated better tolerance to PEG-induced stress compared to variety 2 (Maize OPV-3), as shown by higher germination percentage, root length, and shoot length. Based on our findings, we recommend selecting resilient varieties with strong resistance to drought stress and using a low dosage of PEG to mitigate the negative effects of drought stress on maize seedlings. These results have significant implications for enhancing maize production in areas prone to drought. Variety Sargodha 202 exhibits high resistance under drought stress conditions and is thus recommended for cultivation in rainfed regions susceptible to crop damage caused by unpredictable rainfall patterns.

Highlights

This study is original in its investigation of the effect of Polyethylene Glycol (PEG) concentration on two specific maize varieties (Sargodha 202 and OPV-3) under drought stress. Previous studies may have explored the impact of PEG on other crops or different varieties, but this research focuses specifically on these two maize varieties.

REFERENCES

1. Adom, K. K., & Liu, R. H. (2002). Antioxidant activity of grains. *Journal of Agricultural and Food Chemistry*, 50(21), 6182-6187.

2. FAOSTAT. (2021). Crops. Food and agriculture organization of the United Nations. Retrieved from <http://www.fao.org/faostat/en/#data/QC>
3. FAO. (2017). The Future of Food and Agriculture: Trends and Challenges. Rome Retrieved from <https://www.fao.org/3/I8429EN/i8429en.pdf>
4. Pakistan Agricultural Research Council. (2020). Maize. Retrieved from <http://www.parc.gov.pk/index.php/en/maize>
5. Chavan, M. M., Jain, S. K., Pawar, A. B., Gurav, A. A., & Mhade, R. R. (2022). A review: Silage preparation and silo management techniques.
6. Nuss, E. T., & Tanumihardjo, S. A. (2010). Maize: A paramount staple crop in the context of global nutrition. *Comprehensive Reviews in Food Science and Food Safety*, 9(4), 417-436. <https://doi.org/10.1111/j.1541-4337.2010.00117.x>
7. Dale, E., & Niernberger, F. (1982). Economic models of dry corn milling: Part I-model design and system specifications. *Association of Operative Millers Technical Bulletin*, 56(3), 994-1002.
8. WHO, FAO, UNICEF, GAIN, MI, & FFI. (2009). Recommendations on wheat and maize flour fortification. Meeting report: Interim consensus statement. Geneva, Switzerland: World Health Organization.
9. Orhun, G. E. (2013). Maize for life. *International Journal of Food Science and Nutrition Engineering*, 3(2), 13-16.
10. Hirt, H., & Shinozaki, K. (2003). Plant responses to abiotic stress. Berlin Heidelberg: Springer-Verlag.
11. Szilagyi, L. (2003). Influence of drought on seed yield components in common bean. *Bulgarian Journal of Plant Physiology*, 2003(spl issue), 320-330.
12. Kumar, R. R., Karajol, K., & Naik, G. (2011). Effect of polyethylene glycol induced water stress on physiological and biochemical responses in pigeonpea (*Cajanus cajan* L. Millsp.). *Recent Research in Science and Technology*, 3(1), 148-152.
13. Aldaais, E. A. (2023). Meta-Analysis of Polyethylene Glycol and Cellulose-based Polymers in Vaccine and Drug Delivery: A Comprehensive Review. *Preprints 2023*, 2023080228. <https://doi.org/10.20944/preprints202308.0228.v1>
14. Ramappa, S., Joshi, M. A., Krishna, H., Dunna, V., Jain, N., Sreevathsa, R., & Devate, N. B. (2023). Unravelling the Genetic Basis of Moisture Deficit Stress Tolerance in Wheat for Seedling Vigour-Related Traits and Root Traits Using Genome-Wide Association Study. *Genes*, 14(10), 1902.
15. Funami, T., & Nakauma, M. (2023). How to assess consumer perception and food attributes of novel food structures using analytical methodologies. In *Food Structure Engineering and Design for Improved Nutrition, Health and Well-Being* (pp. 365-398).

16. Badr, A., El-Shazly, H. H., Tarawneh, R. A., & Börner, A. (2020). Screening for drought tolerance in maize (*Zea mays* L.) varieties using germination and seedling traits under simulated drought conditions. *Plants*, 9(5), 565.
17. Basha, P. O., Sudarsanam, G., Reddy, M. M. S., & Sankar, S. (2015). Effect of PEG induced water stress on germination and seedling development of tomato varieties. *Inter J. Recent Sci. Res*, 6(5), 4044-4049.
18. Han, W., Tanveer, M., Jiang, L., & Wang, L. (2022). Pre-soaking foxtail millet seeds with oligosaccharides enhances germination and seedling growth under PEG-induced osmotic stress. *Seed Science and Technology*, 50(3), 381-386.
19. Pei, Z. F., Ming, D. F., Liu, D., Wan, G. L., Geng, X. X., Gong, H. J., & Zhou, W. J. (2010). Silicon improves the tolerance to water-deficit stress induced by polyethylene glycol in wheat (*Triticum aestivum* L.) seedlings. *Journal of plant growth regulation*, 29, 106-115.
20. Landjeva, S., Neumann, K., Lohwasser, U., & Börner, A. (2008). Molecular mapping of genomic regions associated with wheat seedling growth under osmotic stress. *Biologia Plantarum*, 52(2), 259-266. <https://doi.org/10.1007/s10535-008-0056-x>
21. Türkan, I., Bor, M., Özdemir, F., & Koca, H. (2005). Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. *Plant Science*, 168(1), 223-231. <https://doi.org/10.1016/j.plantsci.2004.07.032>
22. Steel, R.G.D. and Torrie, J.H. (1980) Principles and procedures of statistics. A biometrical approach, 2nd Edition, McGraw-Hill Book Company, New York.
23. De Silva, Y. S. K., Rajagopalan, U. M., Kadono, H., & Li, D. (2022). Effects of microplastics on lentil (*Lens culinaris*) seed germination and seedling growth. *Chemosphere*, 303, 135162.
24. Radi, H., Bouchiha, F., El Maataoui, S., Oubassou, E. Z., Rham, I., Alfeddy, M. N., ... & Mazri, M. A. (2023). Morphological and physio-biochemical responses of cactus pear (*Opuntia ficus indica* (L.) Mill.) organogenic cultures to salt and drought stresses induced in vitro. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 1-14.
25. Williams, A., Langridge, H., Straathof, A. L., Muhamadali, H., Hollywood, K. A., Goodacre, R., & de Vries, F. T. (2022). Root functional traits explain root exudation rate and composition across a range of grassland species. *Journal of Ecology*, 110(1), 21-33.
26. Van, D. B. L., & Zeng, Y. (2006). Response of South African indigenous grass species to drought stress induced by polyethylene glycol (PEG) 6000. *South African Journal of Botany*, 72(2), 284-286. <https://doi.org/10.1016/j.sajb.2005.07.006>
27. Gholamin, R., Khayatnezhad, M., Jamaati-e-Somarin, S., & Zabihi-e-Mahmoodabad, R. (2010). Effects of polyethylene glycol and NaCl stress on two cultivars of wheat (*Triticum durum*) at germination and early seeding stages. *American-Eurasian Journal of Agricultural & Environmental Sciences*, 9(1), 86-90.

28. Sajjad ul. Z, Fatima Z, Ijaz SM, Abbas R, Bilal M, Subhan M, Hussain S. 2023 Influence of zinc on germination of maize (*Zea mays*) varieties under saline stress condition. *Discovery*; 59: e54d1227
29. Khayatnezhad, M., Gholamin, R., Jamaatie-Somarin, S., & Zabihi-Mahmoodabad, R. (2010). Effects of peg stress on corn cultivars (*Zea mays* L.) at germination stage. *World Applied Sciences Journal*, 11(5), 504-506.
30. Mostafavi, K., Geive, H. S., Dadresan, M., & Zarabi, M. (2011). Effects of drought stress on germination indices of corn hybrids (*Zea mays* L.). *International Journal of AgriScience*, 1(1), 10-18.
31. Hossain, M., Khatun, A., Talukder, M., Dewan, M., & Uddin, M. (2010). Effect of drought on physiology and yield contributing characters of sunflower. *Bangladesh Journal of Agricultural Research*, 35(1), 113-124. <https://doi.org/10.3329/bjar.v35i1.5872>
32. Bibi, S., Ullah, S., Hafeez, A., Khan, M., Javed, M., Ali, B., & Wahid, N. (2022). Exogenous Ca/Mg quotient reduces the inhibitory effects of PEG induced osmotic stress on *Avena sativa* L. *Brazilian Journal of Biology*, 84, e264642-e264642. <https://doi.org/10.1590/1519-6984.264642>

UNDER PEER REVIEW