Influence of Polyethylene Glycol (PEG 6000) Generate Osmotic Stress on Seed Germination of Different Wheat (*Triticum aestivum* L.) Genotypes

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Abstract: *The yield of most crops relies on the intensity of drought and the stage of plant growth period where it occurs. The crop growth stage as seed germination is very sensitive to water deficit. To evaluate and observe the effects of polyethylene glycol on seed germination in six wheat (Triticum aestivum L.) genotypes is our primary objective. Wheat genotypes (cv. IBWSN-1010, IBWSN-1025, TD-1, ESW-9525, Khirman, and Chakwal-86) were screened for their response at the seedling stage under different water stress treatments (control 0.0, T1 -0.5, T2 -0.75, and T3 -1.0 MPa) in laboratory conditions. The results revealed that genotype Khirman showed the best performance for radicle length, coleoptile potassium (K^+) %, radicle potassium (K^+) %, and radicle calcium(Ca^{2+}) % whereas, Chakwal-86 showed the maximum seed germination and coleoptile length under the highest osmotic stress levels (-1.0MPa). Among the tested genotypes, Khirman and Chakwal-86 exhibited the best performance, whereas,TD-1, ESW-9525, and IBWSN-1025 showed moderately tolerant under water stress conditions. The genotype with improved traits may be used as parents in wheat breeding for drought conditions. We concluded that the findings may be helpful and fruitful for the selection of drought stress in wheat under the deliberated traits.*
Keywords: **Wheat, Water stress, In-vitro screening, Drought stress, Ionic content, PEG 6000, Osmotic stress**

1. INTRODUCTION

Water scarcity is one of the most key environmental factors and is a global constraint restricting plant survival in infertile regions [1,2]. It is predicted by the year 2025 there will be an absolute water shortage and the world population will have to face an extreme water-stressed environment [2]. The effects of drought on crop yield lead to the severity and ultimately affect each stage of plant growth. Moreover, the germination potential, coleoptile length and vigor are reduced [3, 4]. Wheat (*Triticum aestivum* L.) is the existent cereal crop in Pakistan [5, 6]. Wheat is an important staple food nutritionall over the worldand Pakistan devoted more than 9.046 million hectares for wheat cultivation with yearly production of 24.032 million tons [7]. The most threatening problem in wheat production is the shortage of water at the seedling stage, mid-season water stress, terminal stress or a combination of any two or three [8,9]. Seed germination and seedling growth characters are very important factors in determining yield [10]. Wheat seed germination is the first critical phase most affected by drought [11]. Plants tolerant to drought can be acquired by applying polyethylene-glycol (PEG) or mannitol [4, 12]. Conditions of water stress can be created in the laboratory using an osmotic medium. Polyethylene glycol6000 is chemically an inert and non-toxic chemical substance with high molecular weight. Selection of drought tolerance at the early seedling stage is frequently accomplished using simulated drought induced by chemicals like (PEG 6000) identification of wheat genotype that can tolerate limited. Water condition is vital to boost wheat production which can be achieved only by exploring maximum genetic potential from the available germplasm of wheat. The study of character organization for early seedling traits of various wheat genotypes under water stress conditions is also important for understanding yield-limiting factors. Therefore, the current study was designed to identify wheat genotype which could tolerate well under PEG 6000 induced osmotic stress conditions. The objective of this study was to screen out wheat (*Triticum aestivum* L.) genotypes under water stress conditions at the seedling stage using PEG 6000 with different concentrations.

2. MATERIALS AND METHODS

A laboratory experiment was conducted at Plant Physiology Division, Nuclear Institute of Agriculture (NIA), Tandojam, Sindh Pakistan during 2019. The purpose was to observe the effects of water stress using polyethylene glycol on seed germination of seedling growth parameters in wheat; the treatment included a factorial combination using a completely randomized design (CRD) with three replications. In the present study, we have used seeds of six wheat genotypes (cv. IBWSN-1010, IBWSN-1025, TD-1, ESW-9525, Khirman, and Chakwal-86). Grains of six genotypes were subjected to four stress levels of PEG6000 (control 0.0, T1 -0.5, T2 -0.75 and T3 -1.0 MPa).Polyethylene Glycol 6000 was prepared by dissolving the required amount of PEG in distilled water at 30°C. Wheat grains were disinfected with 10% sodium hypochlorite solution for 30 seconds. After the treatment, the grains were washed two times with distilled water. Twenty grains from each genotype were sown in glass bowls (15 and 10 cm intensive) in respective treatment from PEG6000. The glass bowls were placed in an incubator under a photoperiod (4.9 Î ¼ mol m^{-2} s^{-1}) for 20 days. Grains were considered germinated when they exhibited radical extension of>3 mm. Every 24 hours after soaking, germinated grains were counted daily the experiment to determine the following seedling parameters. Seed germination %, coleoptile and radical length (cm), coleoptile and radical potassium K^+ (%) and coleoptile and radical calcium Ca^{2+}(%) were also observed from coleoptile and radical of wheat genotype with the help of a flame photometer [13].

$$\text{Germination Percentage (\%)} = \frac{\text{Number of Seeds Germinated}}{\text{Total Number of Seeds Germinated}} \times 100$$

The data recorded were subjected to analysis of variance to discriminate the superiority of treatment means and LSD test were applied by the following method [14] to compare the means.

3. RESULTS AND DISCUSSION

The ability of the six wheat genotypes under water-stressed, induced by PEG 6000 during the early seedling stage was assessed under an in-vitro environment. Statistical analysis data revealed that different six wheat genotypes depicted performance differently under the different PEG 6000 treatments. There was a significant two-way interaction (drought level and genotype) (P≤ 0.01) for all seed germinations and seedling traits. Data pertaining to the effect of PEG 6000 induced stress on i.e., Seed germination (%), coleoptile and radical length (cm), coleoptile and radical potassium (K^+)% content and coleoptile and radical calcium (Ca^{2+}) % content are shown in Table 1.

Table 1. Mean squares from analysis of variance of germination and seedling traits of wheat genotype under osmotic stress Polyethylene glycol (PEG 6000)

Source of variation	Germination Traits							
	DF	Seed germination	Coleoptile length	Radical length	Coleoptile K^+	Radical K^+	Coleoptile Ca^{2+}	Radical Ca^{2+}
Replications	2	114.93	2.47	66.19	0.01088	0.00111	0.01942	0.0017
Treatments	3	3320.37**	128.22**	8187.65**	2.1955**	0.32288**	0.56984**	0.07825*
Genotype	5	1220.56**	31.49**	2298.43**	0.74908*	0.14684*	0.65473**	0.01648**
G x T	15	622.04**	10.93**	494.15**	0.39261**	0.0604**	0.07713**	0.02033**
Error	40	28.96	3.99	125.3	0.09586	0.01358	0.01489	0.00325

**= significant at P<0.01probability level, *= Significant at P<0.05probability level and ns. = Non-significant

3.1. Seed Germination (%)

The performance of the six wheat genotypes under chemical desiccation, induced by PEG6000 during the early seedling stage was assessed under in-vitro conditions. Data are relevant to the effect of osmotic stress induced by PEG 6000 on seed germination percentage (%) as presented in Figure1. At the control level, seed germination percentage was highest (100%) in all wheat genotypes except Khirman, which showed 96.67% seed germination. The germination percentage of seed was decreased gradually with increasing osmotic stress level by using PEG 6000in all genotypes except TD-1, which showed an increasing trend in germination percentage with increasing osmotic stress level. However, Khirman and Chakwal-86 showed the maximum seed germination under increased osmotic stress (-1.0MPa) and the values were 95% and 98.33%, respectively. Similarly, under the higher osmotic stress level (-1.0MPa), TD-1 also showed higher seed germination (71.67%). Delayed and reduced germination can result from water stress at the germination stage or it may retard germination completely. However, once a seed gets at a sufficient level of hydration it will be prevented toward full germination [15, 16] concluded that osmotic stresses reduce seed germination and seedling growth under osmotic stress conditions. Osmotic stress decreases water potential gradient between seeds and their surrounding environment, hence, [16] reported that it can be a cause of a reduction in seed germination. Exploration of genetic variation among the genotype that could be useful to develop a new genotype that can be adopted in arid and a semiarid region was suggested by [17,18].

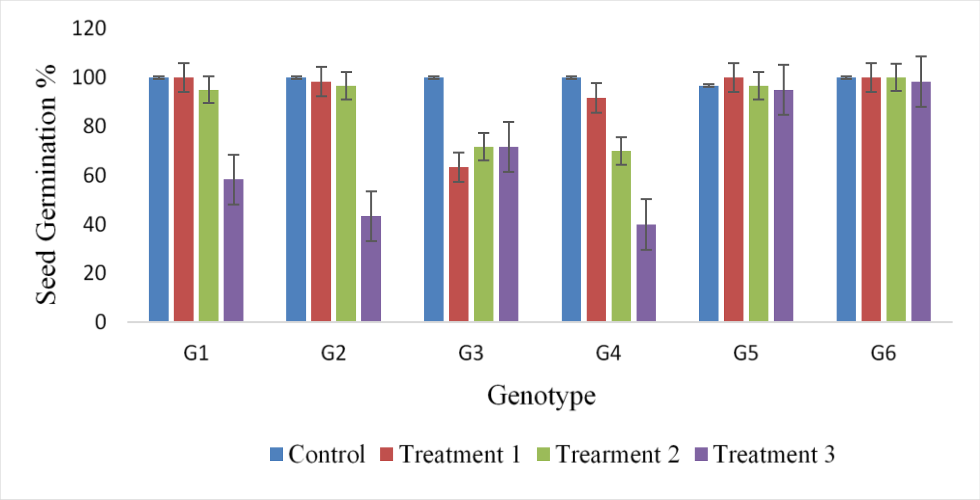

Figure 1. Effect of different levels of PEG 6000 on Seed Germination (%)of six wheat genotypes at seedling stage. **Abbreviations:** G1= IBWSN-1010, G2= IBWSN-1025, G3= TD-1, G4= ESW-9525, G5= Khirman, and G6= Chakwal-86

3.2. Coleoptile and Radical length (cm)

Coleoptile and radicle length influenced together correspondingly by osmotic stress levels(Figure 2).It was observed that the coleoptile and radicle growth lengths decreased with different osmotic stress treatments (Control, T1, T2, and T3) using PEG 6000. However, among the all-genotypes maximum coleoptile length was recorded in Khirman (T1=0.323cm, T2=0.265cm) followed by Chakwal-86 (T1=0.247cm, T2=0.163cm) under all the treatments except T3, whereas,the maximum coleoptile length recorded in Chakwal-86 (T3=0.182cm). Moreover,the minimum coleoptile length was observed in IBWSN-1010 (0.053cm) under maximum osmotic stress treatment (-1.0 MPa). However, it was observed that with the increase of osmotic stress levels the coleoptile length was decreased steadily for all genotypes except IBWSN-1025 andChakwal-86.

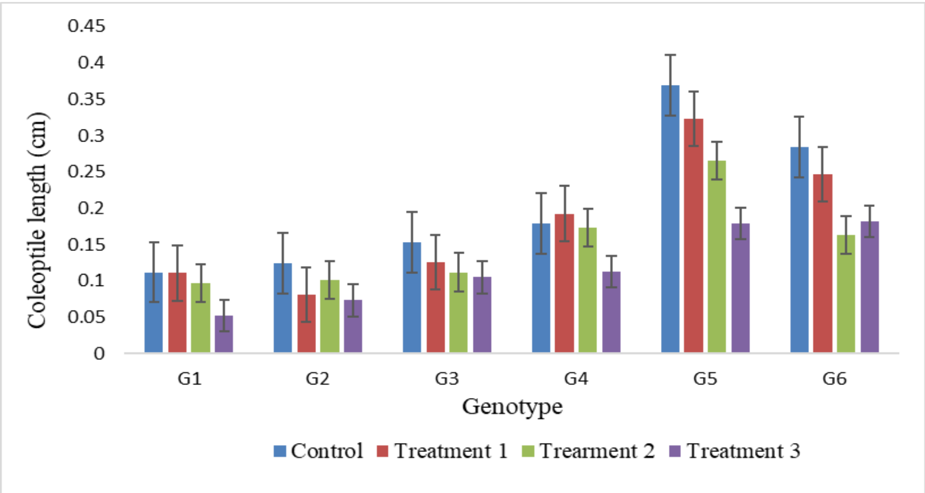

Figure 2. Effect of different levels of PEG 6000 on Coleoptile length (cm) of six wheat genotypes at seedling stage. **Abbreviations:** G1= IBWSN-1010, G2= IBWSN-1025, G3= TD-1, G4= ESW-9525, G5= Khirman, and G6= Chakwal-86

Similarly, the maximum radicle length was observed in genotypes Khirman and Chakwal-86 compared to other genotypes for all treatments (Figure 3). The maximum radicle length was recorded in genotypes Khirman (0.398cm) and Chakwal-86 (0.295cm) at osmotic stress -0.50MPa. Moreover, the minimum radical length was observed in IBWSN-1010(0.068 cm) under maximum osmotic stress treatment (-1.0 MPa). This reveals that as the coleoptile length was increased the radicle length also increased correspondingly. Reduction in the coleoptile and radical length under a water stress environment result of an inhibition of cell division and elongation reported by [19]. The decreasing trend in coleoptile and radical growth was also reported by [20, 21, 22, 23] under water stress.

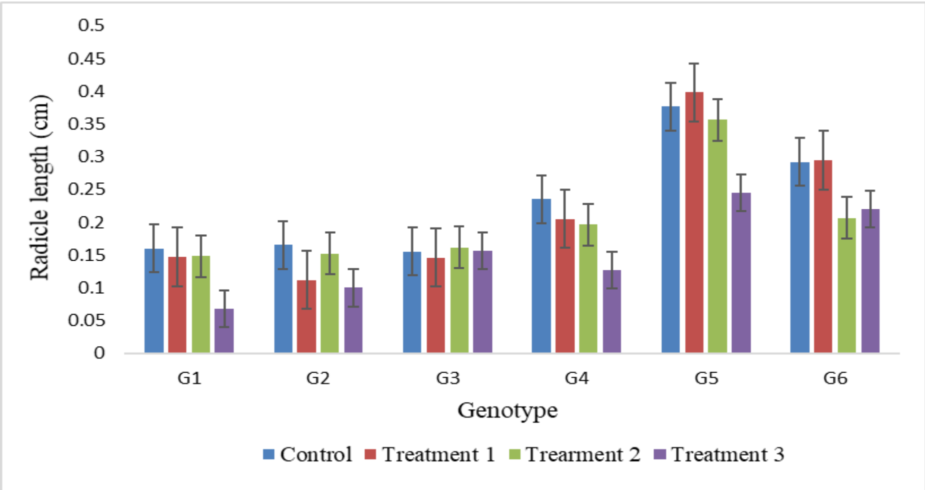

Figure 3. Effect of different levels of PEG 6000 on Radical length (cm) of six wheat genotypes at seedling stage. **Abbreviations:** G1= IBWSN-1010, G2= IBWSN-1025, G3= TD-1, G4= ESW-9525, G5= Khirman, and G6= Chakwal-86

3.3. Coleoptile and Radical Potassium (K^+) %

The coleoptile is dependent on different ionic nutrients as potassium. The six genotypes were observed differently with potassium concentrations (Figure 4). The mean value for coleoptile potassium (K^+)concentration was recorded in four different treatments of PEG 6000 viz. control, T1 (-0.5MPa), T2 (-0.75MPa), and T3 (-1.0 MPa). Among the all-genotypes greater coleoptile potassium % was observed in Khirman, TD-1, and ESW9525and the values were 1.05, 1.03, and 0.93 %, respectively under control conditions. In case of T1 (-0.5MPa), the highest Coleoptile potassium (K^+) % was recorded in genotypes ESW-9525 (1.82%), followed by TD-1, Khirman, IBWSN-1025, IBWSN-1010, and Chakwal-86. While, Chakwal-86 showed the highest coleoptile potassium with the value of 1.89%, followed by Khirman, ESW-9525, IBWSN-1010, TD-1, and IBWSN-1025 atosmotic stress level -0.75MPa(T2). However, the least potassium concentration was observed in IBWSN-1010 (0.21%) and maximum in Khirman (1.66%) genotypes under maximum osmotic stress -1.0MPa (T3).

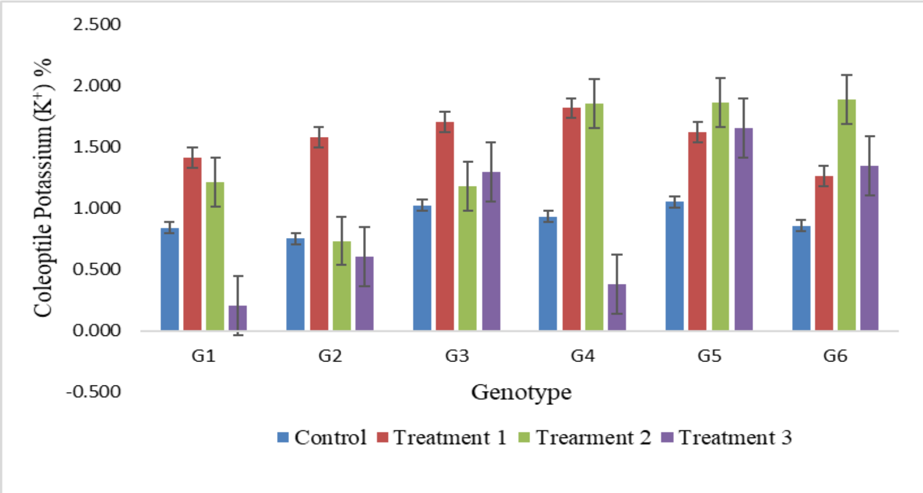

Figure 4. Effect of different levels of PEG 6000 on Coleoptile Potassium (K^+) % of six wheat genotypes at seedling stage. **Abbreviations:** G1= IBWSN-1010, G2= IBWSN-1025, G3= TD-1, G4= ESW-9525, G5= Khirman, and G6= Chakwal-86

On the other hand, radicle potassium showed variably under different water stress conditions (Figure 5). IBWSN-1010 showed little effect among the four treatments as no such significant variation cannot be observed. However, the maximum radicle potassium was observed in TD-1 under control and T1 with the values of 0.53 and 0.70%, respectively. Moreover, Chakwal-86 exhibited better performance under T2 osmotic stress conditionsandKhirman showed the maximum radicle potassium % under the highestosmotic stress level -1.0MPa (T3). This might be explained that higher K^+ concentration in plant growing medium offered more opportunity for radicals absorbing K^+ cellular membrane recovery enhanced K^+ conservation in plant tissues. The results are supported by the research carried out by [24, 25], in these studies, the adequate external K^+ significantly increased K^+ contents in both coleoptile and radical of PEG6000stress.

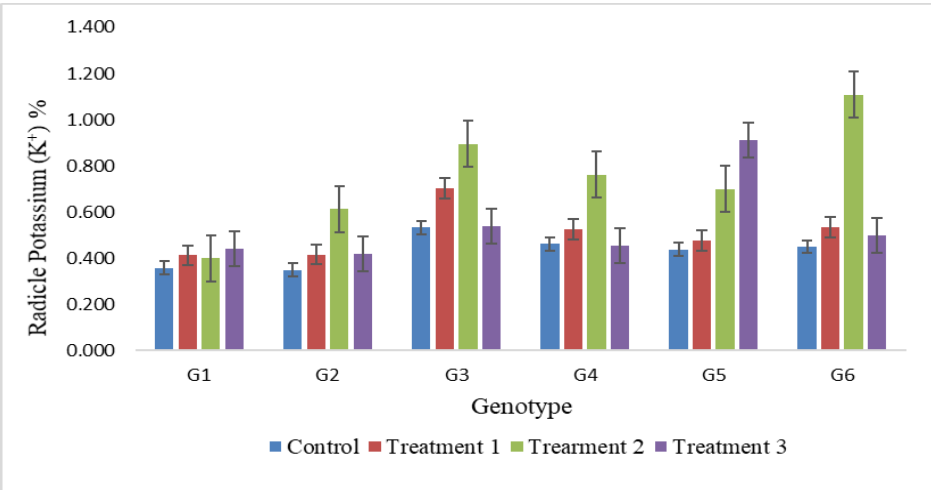

Figure 5. Effect of different levels of PEG 6000 on Radical Potassium (K^+) %of six wheat genotypes at seedling stage. **Abbreviations:** G1= IBWSN-1010, G2= IBWSN-1025, G3= TD-1, G4= ESW-9525, G5= Khirman, and G6= Chakwal-86

3.4. Coleoptile and Radical Calcium (Ca^{2+}) %

The coleoptilecalcium varied significantly among the six genotypes using different treatments of PEG 6000 concentrations (Figure 6). The maximum coleoptile calcium was observed in the genotypesTD-1 and IBWSN-1010 (1.21 and 1.05 %, respectively) under control condition. In case ofT1 (-0.5MPa), the highest Coleoptile calcium (Ca^{2+}) % was observed in IBWSN-1025 (1.53%), followed by TD-1 and IBWSN-1010. However, for T2 (-0.75 MPa), maximum coleoptile calcium recorded in IBWSN-1025 (1.31%), followed by IBWSN-1010 and TD-1.Under the highest osmotic stress -1.0MPa (T3), Coleoptile Ca^{2+}percentage had accumulated more in genotype TD-1 (1.13%), while the least accumulation of Ca^{2+} was observed in ESW-9525 (0.33%).

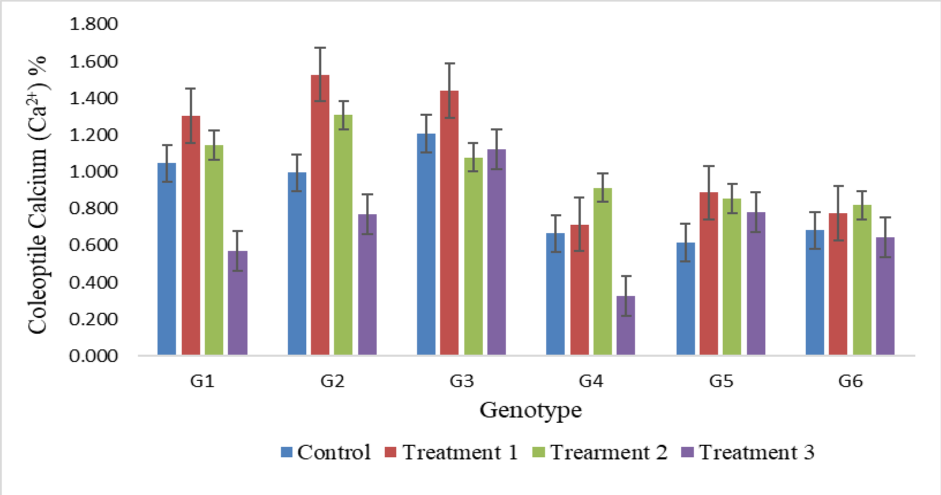

Figure 6. Effect of different levels of PEG 6000 on Coleoptile Calcium (Ca^{2+}) % of six wheat genotypes at seedling stage. **Abbreviations:** G1= IBWSN-1010, G2= IBWSN-1025, G3= TD-1, G4= ESW-9525, G5= Khirman, and G6= Chakwal-86

Similarly, the accumulation of calcium in the radicle varied significantly (Figure 7). In case of T1 (-0.5MPa), the highest radicle calcium % was in TD-1 (0.65%), followed by ESW-9525 and IBWSN-1025. Correspondingly, the maximum accumulation ofcalcium in the radicle was observed in Chakwal-86 (0.85%) at T2 (0.75 MPa) concentration level of PEG6000.However, it was observed that Khirmanhad tremendous accumulation of calcium in the radicleunder the highest osmotic stress -1.0MPa (T3) with the value 0.66%, followed by IBWSN-1025 and TD-1, while the minimum accumulation of radical Ca^{2+}% was foundin genotype ESW-9525 (4.0%). The study depicted that accumulation of radical for coleoptile calcium there was a varietal difference. The same results were found by [26] in their study, they mentioned that PEG 6000 ratio was not significantly affected on radical K^+/Ca^{2+} by osmotic stress.

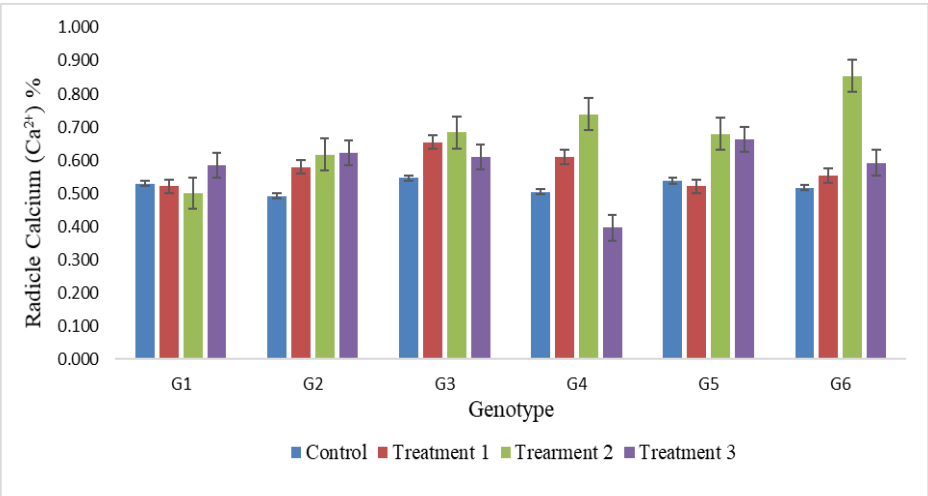

Figure 7. Effect of different levels of PEG 6000 on Radical Calcium (Ca^{2+}) % of six wheat genotypes at seedling stage. **Abbreviations:** G1= IBWSN-1010, G2= IBWSN-1025, G3= TD-1, G4= ESW-9525, G5= Khirman, and G6= Chakwal-86

4. Conclusion

Plants have developed biochemical and physiological approaches to tolerate water deficit environments. Osmotic stress significantly reduced the Seed germination%, coleoptile/radical length (cm), coleoptile K^+, radical K^+, coleoptile Ca^{2+}. Among the tested genotypes, Khirman and Chakwal-86 exhibited the best performance under drought conditions, whereas, TD-1, ESW-9525 and IBWSN-1025 showed moderately tolerant under water stress conditions. The genotype with improved traits may be used as parents in wheat breeding for moisture stress conditions. So, it is suggested that the findings may be helpful and fruitful for the selection of drought stress in wheat under the deliberated traits.

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