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Research Article

Mitigating Osmotic Drought Stress in Rye: Assessing the Ameliorative Effects of Putrecine on Germination, Seedling and Mitotic Index

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Abstract

In this study, the impact of putrescine, a polyamine, on the germination, seedling growth, and mitotic index of rye seeds (cv. Aslım-95) was investigated under osmotic drought conditions induced by polyethylene glycol (PEG-6000) application. The experiment involved six different PEG doses (-2, -4, -6, -8, and -10 bars) in combination with putrescine doses of 0.001, 0.01, and 0.1 mM. The concentration of -2 bars of polyethylene glycol, representing osmotic drought, positively influenced some of the studied characteristics. However, the stimulative effect of -2 bars of osmotic drought exhibited minimal changes with PEG 6000 + putrescine combinations. Conversely, as the concentration of osmotic drought (PEG 6000 concentration) increased, all observed traits, especially germination and seedling development, were negatively impacted. All putrescine doses used in combination with PEG-6000 demonstrated positive effects on the examined characteristics and reduced the germination duration. The most significant effect of putrescine on the negative effects caused by osmotic drought was observed at a concentration of 0.1 mM on root dry weight and at a concentration of 0.001 mM on all other characteristics.
Keywords: Rye, PEG 6000, putrescine, germination, seedling, mitotic index.

Çavdarda Ozmotik Kuraklık Stresinin Azaltılması: Putresinin Çimlenme, Fide Gelişimi ve Mitotik İndeks Üzerindeki İyileştirici Etkilerinin Değerlendirilmesi

Öz

Bu çalışmada, polietilen glikol (PEG-6000) uygulamasıyla oluşturulan ozmotik kuraklık koşullarında bir poliamin olan putresinin çavdar tohumlarının (Aslım-95 çeşidi) çimlenmesi, fide büyümesi ve mitotik indeksi üzerine etkisi araştırılmıştır. Bu çalışma, 0,001, 0,01 ve 0,1 mM'lik putresin dozlarıyla kombinasyon halinde altı farklı (-2, -4, -6, -8 ve -10 bar) PEG-6000 dozunu içermektedir. Ozmotik kuraklığı temsil eden -2 bar polietilen glikol konsantrasyonu, incelenen bazı özellikleri olumlu yönde etkilemiştir. Ancak -2 bar ozmotik kuraklığın uyarıcı etkisi PEG 6000 + putresin kombinasyonları ile minimum düzeyde değişiklik göstermiştir. Diğer taraftan, ozmotik kuraklığın (PEG 6000 konsantrasyonu) konsantrasyonu arttıkça özellikle çimlenme ve fide gelişimi olmak üzere gözlemlenen tüm özellikler olumsuz yönde etkilenmiştir. PEG 6000 ile kombinasyon halinde kullanılan tüm putresin dozları incelenen özellikler üzerinde olumlu etki göstermiş ve çimlenme süresini kısaltmıştır. Putresinin ozmotik kuraklığın neden olduğu olumsuz etkiler üzerinde en belirgin etkisi kök kuru ağırlığında 0,1 mM konsantrasyonunda ve diğer tüm özelliklerde 0,001 mM konsantrasyonunda gözlenmiştir.
Anahtar Kelimeler: Çavdar, PEG 6000, putresin, çimlenme, fide, mitotik indeks

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1. Introduction

Cereals represent fundamental commodities utilized either directly or indirectly in human nutrition. Globally, over 50% of individuals derive their daily caloric intake from grains. Notably, given that animals predominantly consume plant-based materials, and the contribution of animal-based foods to daily caloric intake is approximately 20%, it becomes evident that humans obtain roughly three-quarters of their daily nutrition from grains [1].

Rye holds significant importance in our country as it serves dual purposes as both bread and fodder due to its high nutritional value. Its robust root system enables efficient water absorption from the soil, surpassing other grains in this aspect. With an annual rainfall requirement of around 150 mm, rye emerges as a viable alternative for cultivation in regions with low precipitation. Moreover, its adaptability extends to sloping, stony, and low-fertility soils where other cultivated plants may not thrive economically. The versatility of rye is further highlighted by its root system's ability to develop either deep or superficially, depending on the specific soil characteristics of the planting location. In our country, rye is cultivated across an area of 99,935 hectares, resulting in a production of 273 thousand tons and a yield of approximately 2.79 t/ha [2].

Environmental stress factors have a detrimental impact on plant development and yield [3]. Drought stress occurs when plants are unable to extract sufficient water from the soil through their roots or when the transpiration rate is excessively high. The repercussions of drought in crop production include challenges such as insufficient plant emergence, reduced development, and decreased yield [4]. The stages of seed germination and subsequent seedling growth are particularly susceptible to adverse environmental conditions, and any damage during these phases can prematurely terminate the plant life cycle [5, 6].

Drought poses a substantial constraint on global crop production, and contemporary climate change exacerbates this issue [7]. The impact of drought conditions on plant growth and grain yield is contingent upon the severity of drought and the developmental stage of the plant when drought occurs. Seedling emergence stands out as a growth period highly sensitive to water deficit. The rate and extent of seedling establishment play pivotal roles in determining yield and time to maturity [8]. Therefore, for successful plant establishment, crucial characteristics such as seed germination, vigor, and coleoptile length must meet adequate standards. In semi-arid regions, low moisture levels during germination present a limiting factor. Some studies suggest that coleoptile length is crucial for the successful establishment of seedlings, especially when seeds are sown deep to reach moisture in dry soils [9]. Researchers often employ chemicals like PEG in studies aiming to assess the drought resistance of seedlings during the early development period, as these substances contribute to intensifying drought severity in controlled environments [10].

Despite advancements in the discovery of new hormones, growth regulators, and insights from plant gene technology, there remains a gap in our understanding of the physiology and biochemistry of seed germination. Consequently, investigating the effects of growth regulators such as triacontanol, brassinosteroids, and polyamines (putrescine, spermine, spermidine, cadaverine) on seed germination becomes particularly intriguing. These regulators have shown efficacy in influencing plant growth and development, holding the potential for significant

advancements in addressing contemporary challenges such as hunger. The primary objective of this study was to assess the impact of artificially induced osmotic drought using PEG 6000 on the germination and seedling development of rye. Additionally, the study aimed to elucidate the potential of putrescine, a growth regulator, in mitigating the adverse effects of osmotic drought on these critical traits. This research has the potential to contribute valuable insights into enhancing crop resilience and productivity in the face of environmental stressors, aligning with the pressing global concern of food security.

2. Materials and Methods

The research was conducted in the Biotechnology laboratory of Atatürk University Faculty of Agriculture, utilizing seeds of the Aslım 95 variety of diploid rye (*Secale cereale* L.) as the primary material. The seeds underwent a thorough preparation process, including washing with tap water, stirring in 70% ethyl alcohol (EtOH) for 3 minutes, followed by three washes with sterile distilled water within a sterile cabinet. Surface sterilization was carried out in a solution containing 10% bleach with a few drops of Tween 20 (Sigma) for 15 minutes. After this process, the seeds were again washed with sterile distilled water and left at room temperature for 24 hours at four different putrescine concentrations [0 (distilled water), 0.001, 0.01, and 0.1 mM] [11]. Following the 24-hour period, the surface-sterilized seeds were washed to remove putrescine and then transferred to blotting papers, where they were dried for 3 hours. Subsequently, 25 dried seeds were sown in each 9 cm diameter petri dish containing two layers of germination paper (Whatman paper number 1). A solution with six different osmotic potentials [0 (pure water), -2, -4, -6, -8, and -10 bar], formed with PEG 6000, was added to the petri dishes. Concentrations of 0, -2, -4, -6, -8, and -10 bar of PEG 6000 solutions were employed to establish osmotic potential [12]. After this setup, the seeds were germinated at 20°C under a 16:8 hours light:dark photoperiod. For the following 10 days, 10 ml of each of the six different doses of PEG 6000 solution was added daily. The experimental design included 4 replicates, following a Completely randomized design. The data obtained from the experiment were subjected to analysis of variance using the SPSS statistical package program, employing a 6 (osmotic potential) × 4 (putrescine dose) factorial arrangement. Subsequently, averages were compared using the Duncan [12] test for further analysis.

Characters examined in the experiment

a. Germination-related characters: Germination rate concerning seed germination [14], germination rate index [15, 16], germination vigor index [17] and mean germination time [18].

b. Seedling-related characters: The total number of embryonal roots, root length, shoot length, root dry weight, and shoot dry weight were investigated in the seedlings obtained in the experiment [19].

c. Mitotic index: The method specified by Sağsöz [20] and Tosun [21] was used to prepare the samples to determine the mitotic index. 5000-6000 cells were counted in each prepared preparation. Cells in prophase, metaphase, anaphase, and telophase were evaluated as dividing cells, and the others as non-dividing cells, and the mitotic index was calculated as % according

to the formula given below [22]. Mitotic index (%) = (number of cells dividing / total number of cells) x 100.

3. Results

3.1. Effects of drought and putrecine application on germination related characters

a) Germination Rate (GR)

The impact of drought, induced with PEG 6000, and putrescine treatments, as well as the interaction between drought and putrescine, exhibited significant effects on the germination rate (GR) of seeds (P<0.01) (Table 1). As the severity of drought, induced by PEG 6000 application, increased, there was a noticeable decrease in the germination rate of seeds. Specifically, the GR, initially at 58.75% in the control, progressively decreased at -2, -4, -6, -8, and -10 bar applications, ultimately reaching 6.00%. Conversely, putrescine application had a positive effect on the germination rate. For instance, the GR, starting at 0.50% in the absence of putrescine, increased to 55.50% at a 0.001 mM dose, 43.66% at a 0.01 mM dose, and 36.33% at a 0.1 mM dose. The differences between all treatments were statistically highly significant. Notably, the effect of putrescine on GR was more pronounced at the 0.001 mM dose compared to other doses. In other words, treatments with doses higher than 0.001 mM significantly enhanced the GR compared to the control, but their effects were less prominent than the 0.001 mM dose. The highest GR of 85.00% was achieved in treatments where drought was not applied, while putrescine was administered at a dose of 0.001 mM (Table 2).

Table 1. Analysis of variance results of the characters related to germination after different doses of PEG 6000 and putrescine applications to seeds.

		The average of the squares of the errors			
Variation source	**DF**	**GR**	**GRI**	**GVI**	**MGT**
Drought (D)	5	6344.00**	31.30**	14143658.69**	23.69**
Putrecine (P)	3	13467.11**	10.09**	3227973.68**	35.42**
D x G	15	796.71**	0.43**	289791.39**	2.09**
Error	72	6.55	0.06	9839.28	0.07
Total	96				

** Significant at the P<0.01 level.

Table 2. Data on some characters related to germination after PEG-6000 and putrescine treatments to seeds.

Drought (bar)	Putrecine (mM)	GR (%)	GRI	GVI	MGT (day)
0	0	3.00 c[1]	18.60 d	1966.00 d	5.57 a
	0.001	85.00 a	20.06 a	3604.00 a	4.20 c
	0.01	82.00 a	19.91 b	3354.00 b	4.32 c
	0.1	65.00 b	19.66 c	2709.00 c	4.54 b
	Mean	**58.75 A[2]**	**19.56 A**	**2908.00 A**	**4.65 F**
-2	0	0.00 c	18.10 d	1905.00 d	6.12 a
	0.001	71.00 a	19.73 a	3539.00 a	4.49 c
	0.01	60.00 b	19.57 b	3320.00 b	4.62 c
	0.1	58.00 b	19.40 c	2655.00 c	4.88 b
	Mean	**47.25 B**	**19.20 B**	**2854.00 B**	**5.02 E**

-4	0	0.00 b	17.70 c	1219.68 d	6.82 a
	0.001	58.00 a	19.21 ab	2084.00 a	5.22 b
	0.01	56.00 a	19.38 a	179.44 b	4.76 c
	0.1	55.00 a	19.18 b	1553.00 c	5.20 b
	Mean	**42.25 C**	**18.87 C**	**1661.45 C**	**5.50 D**
-6	0	0.00 c	17.31 c	995.00 c	7.80 a
	0.001	55.00 a	18.82 a	1476.30 a	5.55 b
	0.01	42.00 b	18.16 b	1171.96 b	5.49 b
	0.1	40.00 b	18.61 a	1053.12 bc	5.76 b
	Mean	**34.25 D**	**18.22 D**	**1172.49 D**	**6.15 C**
-8	0	0.00 c	16.78 b	511.92 b	10.03 a
	0.001	40.00 a	17.50 ab	796.28 a	5.42 c
	0.01	22.00 b	17.64 a	760.26 a	5.72 c
	0.1	0.00 c	17.11 ab	647.52 ab	8.00 b
	Mean	**15.50 E**	**17.26 E**	**681.26 E**	**7.29 B**
-10	0	0.00 b	14.92 c	98.56 d	10.29 a
	0.001	24.00 a	17.11 a	453.60 a	5.62 c
	0.01	0.00 b	15.78 b	309.68 b	7.04 b
	0.1	0.00 b	15.51 b	193.16 c	7.63 b
	Mean	**6.00 F**	**15.83 F**	**246.10 F**	**7.64 A**
Mean of Putrecine	0	0.50 D	17.23 D	986.78 D	7.77 A
	0.001	55.50 A	18.74 A	1850.50 A	5.08 D
	0.01	43.66 B	18.41 B	1624.09 B	5.32 C
	0.1	36.33 C	18.24 C	1333.86 C	6.00 B

[1]. Differences between means shown with the same lowercase letter in the same column for each application are insignificant.
[2]. Differences between means with the same capital letter in the same column are insignificant.

b) Germination rate index (GRI)

The impact of drought and putrescine treatments induced by PEG 6000 on the Germination Rate Index (GRI) was found to be highly significant ($P<0.01$), with a similarly significant interaction observed between drought and putrescine ($P<0.01$) (Table 1).

The GRI exhibited a continuous decrease in response to the increasing severity of drought. Specifically, the GRI, starting at 19.56 in the control group, decreased to 19.20 at -2 bars. 18.87 at -4 bars, 18.22 at -6 bars, and 17.26 at -8 bars. Beyond this, at -10 bars, the GRI experienced a much more significant decrease, reaching 15.83. Contrastingly, the effect of putrescine application on GRI while statistically highly significant, remained at a limited level. For instance, the GRI, initially at 17.23 in the control group without putrescine application. increased to 18.74 with the application of 0.001 mM putrescine. At higher doses of 0.01- and 0.1-mM putrescine, the GRI exhibited slight decreases to 18.41 and 18.24, respectively. Considering the drought x putrescine interaction, the highest GRI (20.06) was determined at the 0.001 mM putrescine application dose in the group where drought was not applied (Table 2). This suggests a nuanced interplay between drought and putrescine in influencing the germination rate index.

c) Germination vigour index (GVI)

As depicted in Table 1, the effects of drought induced by PEG 6000, putrescine, and the interaction between drought and putrescine on the the GVI were found to be highly significant ($P<0.01$). The impact of drought induced by PEG 6000 on the GVI decreased significantly with the escalation of drought severity (PEG 6000 concentration). The GVI, initially at 2908.00 in the absence of drought, steadily declined to 246.10 at the -10 bar dose. Notably, the most substantial decrease occurred at doses of -8 and -10 bars. When evaluating the effect of putrescine applied in combination with PEG 6000 on the germination vigour index, it was observed that putrescine had a positive influence on the GVI. Specifically, the GVI, averaging 986.78 at 0 mM putrescine, reached its highest value at 1850.50 with the application of 0.001 mM putrescine, approximately doubling. There was a slight decrease in GVI at putrescine concentrations beyond this. For instance, the GVI was 1624.09 at 0.01 mM putrescine and 1333.86 at 0.1 mM putrescine. Consistent with other traits the most significant effect of putrescine on the GVI was observed at the dose where PEG 6000 was applied at a concentration of -10 bars. For example, the GVI. starting at 98.56 when putrescine was not applied at this dose. significantly increased to 453.60 with the application of 0.001 mM putrescine (Table 2).

d) Mean germination time (MGT)

The impact of drought, putrescine, and the interaction between drought and putrescine on the Mean Germination Time (MGT) of diploid rye seeds was found to be highly significant ($P<0.01$) (Table 1). It was observed that the MGT increased with the concentration of PEG 6000. Specifically, the MGT values were 4.65, 5.02, 5.50, 6.15, 7.29 and 7.64 days at PEG 6000 concentrations corresponding to osmotic pressures of 0, -2, -4, -6, -8 and -10 bars. respectively. Notably, at doses of -8 and -10 bars.,the germination time was significantly longer compared to other doses indicating a notable delay in germination under more severe drought conditions. When analyzing the effect of putrescine on the MGT, it was observed that the MGT was significantly reduced at different concentrations. For instance, the MGT, initially at 7.77 days in the control group, decreased to 5.08 days with 0.001 mM putrescine treatment. Additionally, the MGT was 5.32 and 6.00 days at 0.01 and 0.1 mM putrescine concentrations, respectively. The most significant effect of putrescine on the MGT was observed at -8 and -10 bar doses of PEG 6000. For example, the MGT, initially at 10.29 days with no putrescine applied at -10 bars, decreased to 5.62 days with the application of 0.001 mM putrescine (Table 2). This suggests that putrescine has a notable impact in reducing the mean germination time. particularly under more severe drought conditions.

3.2. Drought and putrecine application on seedling-related characters

a) Number of embryonal roots (NER)

The effect of drought, putrescine, and the interaction between drought and putrescine on the Number of Embryonal Roots (NER) was found to be highly significant ($P<0.01$) (Table 3). With the increase in the severity of drought induced by PEG 6000 application, the number of roots in seedlings decreased. The NER, starting at 4.66 in the control group without PEG 6000

treatment, steadily decreased at -2, -4, -6, -8 and -10 bar treatments, reaching 4.51, 4.16, 3.78, 3.57, and 3.40, respectively. The difference between the group without PEG 6000 (control) and the -2-bar dose was statistically insignificant, grouping these two treatments together. However, the other doses (-4. -6. -8. and -10 bars) formed distinct groups. In contrast, putrescine application had a positive effect on the NER. For instance, the NER, initially at 3.90 in the absence of putrescine, increased to 4.35 at 0.001 mM. 4.01 at 0.01 mM. and 3.79 at 0.1 mM dose of putrescine. Upon closer examination of the drought x putrescine interaction, a small decrease was noted in -4 bar + 0.01 mM, -4 bar + 0.1 mM, -6 bar + 0.001 mM, -6 bar + 0.01 mM, and -6 bar + 0.1 mM interactions compared to the control. The most significant effect of putrescine on the NER was observed at -10 bar, like other traits. At this dose, the average number of embryonal roots was 4.00 in the 0.001 mM putrescine treatment, compared to 3.00 in the control (Table 4). This underscores the potential of putrescine in mitigating the adverse effects of severe drought on the number of embryonal roots.

Table 3. Variance analysis results of seedlings and some related characters after different doses of PEG 6000 and putrecine were applied to the seeds

		The average of the squares of the errors					
Varyasyon kaynağı	**DF**	**NER**	**RL**	**SL**	**RDW**	**SDW**	**MI**
Drought (D)	5	20.99**	1520.85**	2600.13**	0.009**	0.015**	20388.66**
Putrecine (P)	3	7.23**	402.04**	626.01**	0.004**	0.018**	9491.71**
D × P	15	1.06**	25.14**	57.39**	0.003**	0.002**	962.31**
Error	72	0.48	1.31	0.98	0.001	0.001	60.23
Toptal	96						

** Significant at P<0.01 level.

Table 4. Data on some seedling characters after PEG 6000 and putrescine treatments.

Drought (bar)	**Putrecine (mM)**	**NER (number)**	**RL (cm)**	**SL (cm)**	**RDW (mg)**	**SDW (mg)**	**MI (%)**
0	0	4.40 b[1]	8.80 d	10.86 c	0.0186 d	0.0511 d	26.53 c
	0.001	4.95 a	15.80 a	20.24 a	0.0384 a	0.0756 a	60.40 a
	0.01	4.80 ab	13.89 b	19.65 a	0.0288 b	0.0686 b	65.58 a
	0.1	4.50 ab	11.36 c	15.73 b	0.0252 c	0.0624 c	45.32 b
	Average	**4.66 A[2]**	**12.46 B**	**16.62 A**	**0.0277 B**	**0.0644 A**	**49.46 B**
-2	0	4.55 ab	10.40 d	8.65 c	0.0309 b	0.0413 d	23.95 c
	0.001	5.00 a	17.30 a	18.09 a	0.0602 a	0.0685 a	72.30 a
	0.01	4.40 b	15.05 b	18.15 a	0.0421 ab	0.0624 b	67.10 a
	0.1	4.10 b	12.15 c	14.40 b	0.0327 ab	0.0581 c	45.86 b
	Average	**4.51 A**	**13.72 A**	**14.82 B**	**0.0414 A**	**0.0575 AB**	**52.30 A**
-4	0	4.20 b	6.10 d	6.22 d	0.0219 b	0.0479 a	20.42 c
	0.001	4.70 a	11.40 a	9.44 a	0.0219 b	0.0612 a	50.35 a
	0.01	3.80 c	9.80 b	8.48 b	0.0171 b	0.0527 a	35.19 b
	0.1	3.95 bc	8.28 c	7.25 c	0.0394 a	0.0447 a	32.47 b
	Average	**4.16 B**	**8.89 C**	**7.85 C**	**0.0250 BC**	**0.0516 BC**	**34.61 C**
-6	0	3.85 a	5.38 c	4.17 c	0.0077 b	0.0291 c	11.88 c
	0.001	3.80 a	8.35 a	7.19 a	0.0164 b	0.0586 a	38.36 a
	0.01	3.80 a	7.80 a	6.32 b	0.0096 c	0.0511 b	26.69 b
	0.1	3.70 a	6.48 b	4.49 c	0.0568 a	0.0318 c	14.42 c
	Average	**3.78 C**	**7.00 D**	**5.54 D**	**0.0226 BC**	**0.0426 C**	**22..84 D**
-8	0	3.40 a	4.20 c	2.91 d	0.0151 b	0.0227 b	7.71 c

	0.001	3.70 a	5.70 a	6.01 a	0.0159 b	0.0870 a	23.61 a
	0.01	3.70 a	5.10 b	4.78 b	0.0114 b	0.0315 b	14.78 b
	0.1	3.50 a	4.70 bc	3.82 c	0.0256 a	0.0269 b	7.61 c
	Average	**3.57 CD**	**4.92 E**	**4.38 E**	**0.0170 CD**	**0.0420 C**	**13.43 E**
-10	0	3.00 c	1.57 c	1.95 c	0.0053 b	0.0198 b	4.02 b
	0.001	4.00 a	3.10 a	4.10 a	0.0181 a	0.0322 a	7.17 a
	0.01	3.60 b	2.80 a	3.52 b	0.0077 b	0.0306 a	6.78 a
	0.1	3.00 c	2.10 b	2.29 c	0.0081 b	0.0219 b	4.64 b
	Average	**3.40 D**	**2.39 F**	**2.96 F**	**0.0098 D**	**0.0261 D**	**5.65 F**
Average Put	0	3.90 BC	6.07 D	5.79 D	0.0214 BC	0.0353 C	15.75 D
	0.001	4.35 A	10.27 A	10.84 A	0.0254 AB	0.0638 A	42.03 A
	0.01	4.01 B	9.07 B	10.15 B	0.0178 C	0.0494 B	36.02 B
	0.1	3.79 C	7.51 C	8.00 C	0.0310 A	0.0409 C	25.05 C

[1]. Differences between means shown with the same lowercase letter in the same column for each application are insignificant.
[2]. Differences between means with the same capital letter in the same column are insignificant.

b) Root length (RL)

As evident from Table 3, the effects of drought, putrescine, and the interaction between drought and putrescine induced by PEG 6000 on the Root Length (RL) were statistically very significant ($P < 0.01$). The average RL generally decreased with the increase in the concentration of PEG 6000 applied to induce drought. However, the RL, measured at 12.46 cm in the control group, slightly increased to 13.72 cm in the -2 bar application, with the difference between these two groups being statistically very significant. Conversely, the RL decreased to 8.89, 7.00, 4.92, and 2.39 cm in -4, -6, -8, and -10 bar treatments, respectively, with statistically significant differences observed between these groups. Examining how putrescine application altered the effect of drought induced by PEG 6000 on root length revealed that the RL, averaging 6.07 cm in the control group, increased with putrescine application, reaching the highest value of 10.27 cm at a dose of 0.001 mM. Additionally, the RL was 9.07 cm and 7.51 cm at 0.01 and 0.1 mM doses, respectively. The most significant effect of putrescine on RL was observed at the 0.001 mM dose. While there was an increase compared to the control at other doses, there was a slight decrease compared to the first dose. Moreover, the most pronounced effect of putrescine on RL was observed at -10 bar drought, where the RL reached approximately twice (3.10 cm) that of the control (1.57 cm) when putrescine was applied at a dose of 0.001 mM (Table 4). This highlights the potential of putrescine in significantly enhancing root length under severe drought conditions.

c) Shoot length (SL)

The effect of drought and putrescine treatments on the Shoot Length (SL) induced by PEG 6000 was observed to be highly significant ($P<0.01$), with the drought x putrescine interaction also exhibiting significant effects ($P<0.01$) (Table 3).

The SL exhibited a continuous decrease with the increasing severity of drought treatment (PEG 6000 dose). The average shoot length. initially at 16.62 cm in the control group, decreased to 14.82 cm at -2 bars, 7.85 cm at -4 bars, 5.54 cm at -6 bars, 4.38 cm at -8 bars, and 2.96 cm at -10 bars.

When analyzing the effect of putrescine application on shoot length, it was observed that the SL, initially at 5.79 cm in the control group (0 mM putrescine). doubled to 10.84 cm at a dose

of 0.001 mM and reached 10.15 cm at a dose of 0.01 mM. The average SL was 8.00 cm at 0.1 mM putrescine dose. The most significant interaction effect between drought and putrescine treatment was found in the -10 bar group. In this group, the SL, initially at 1.95 cm in the absence of putrescine, more than doubled to 4.10 cm at the putrescine dose of 0.001 mM (Table 4). This emphasizes the potential of putrescine in mitigating the adverse effects of severe drought on shoot length. particularly under extreme conditions.

d) Root dry weight (RDW)

The effect of drought (PEG 6000) and putrescine treatments, as well as the interaction between drought and putrescine on the Root Dry Weight (RDW), one of the seedling characters examined in diploid rye, was found to be highly significant ($P<0.01$) (Table 3). The highest RDW value (mean 0.0414 mg) was determined at -2 bar drought, and the difference with the control group (0.0277 mg) was highly significant. However, the RDW decreased continuously at -4. -6. -8. and -10 bar drought doses, reaching 0.0098 mg at -10 bars. Examining the effect of putrescine on RDW to determine its role in reducing the negative effects caused by drought, unlike other characters, the highest values were determined at 0.1 mM concentration (0.0310 mg), followed by 0.001 mM dose with 0.0254 mg, with the difference between the two being insignificant. It was followed by the control group with 0.0214 mg and 0.01 mM concentration with 0.0178 mg. The most significant effect of putrescine on RDW was observed at -6 bars of osmotic drought. At this dose, the RDW, initially at 0.0077 mg when putrescine was not applied, increased more than seven times to 0.568 mg at 0.1 mM dose of putrescine. This effect was followed by the control group and -2 bars of osmotic drought. In these two groups, the increase compared to the control was almost doubled at 0.001 mM dose (Table 4). This underscores the potential of putrescine in significantly influencing the Root Dry Weight, particularly under severe drought conditions.

e) Shoot dry weight (SDW)

The effect of drought (PEG 6000) and putrescine treatments on the Shoot Dry Weight (SDW) was highly significant ($P<0.01$). Similarly, the interaction between drought and putrescine also significantly influenced the SDW ($P<0.01$) (Table 3). In terms of drought mean values, the SDW exhibited a continuous decrease with increasing drought severity, and this decrease was statistically significant. The SDW, initially at 0.0644 mg in the control group, decreased to 0.0575 mg at -2 bars, 0.0516 mg at -4 bars, 0.0426 mg at -6 bars, 0.0420 mg at -8 bars, and 0.0261 mg at -10 bars. The first two doses of putrescine, applied in combination with PEG 6000 to mitigate the negative effects of drought, significantly increased the SDW that had decreased due to drought ($P<0.01$). For example, the SDW with different doses of putrescine was 0.0353 mg in the control group (0 mM), increasing to 0.0638 mg at 0.001 mM dose, with the highest value obtained in this group. The 0.01 mM dose showed a significant decrease compared to the first dose, reaching 0.0494 mg, but this value was still higher than the control. Similarly, the 0.1 mM dose of putrescine resulted in an increase (0.0409 mg) compared to the control, but this value was lower than the first two doses. The highest SDW was found in the 0.001 mM putrescine application at all drought doses. The most significant positive effect of putrescine was observed at -8 bars considering all drought doses. In this group, the SDW, initially at 0.0227

mg in the control, increased approximately four times to 0.0870 mg in the 0.001 mM putrescine treatment (Table 4). This highlights the potential of putrescine in significantly influencing Shoot Dry Weight, particularly under severe drought conditions.

3.2. Effects of putrescine application on mitotic index

The effect of drought and putrescine treatments induced by PEG 6000, as well as the interaction between drought and putrescine on the mitotic index, was highly significant ($P<0.01$) (Table 3). The mitotic index, initially at 49.46% in the control group without PEG 6000, slightly increased to 52.30% at the -2 bar dose compared to the control. However, at -4. -6. -8, and -10 bar drought doses, the mitotic index exhibited a continuous decrease, reaching 34.61%, 22.84%, 13.43%, and finally, 5.65%, respectively. Notably, there was a significant decrease in the mitotic index, especially at -8 and -10 bars. When analyzing the effect of putrescine on the mitotic index in terms of mean values, it was observed that putrescine application caused an increase in the mitotic index compared to the control group. However, this increase was not parallel to the dose increase. The mitotic index, initially at 15.75% in the control group without putrescine, significantly increased at 0.001 mM putrescine dose, reaching the highest value of 42.03%, and then slightly decreased with increasing doses. Accordingly, the mitotic index was 36.02% and 25.05% at 0.01 and 0.1 mM putrescine doses, respectively, with all doses in statistically different groups. The effect of putrescine on increasing the mitotic index, promoting cell division, was more pronounced, especially at -6 and -8 bar drought doses compared to others. In both drought doses, the highest increase was observed at 0.001 mM concentration of putrescine, nearly three times higher than the control. The highest mitotic index was 72.30% at -2 bar + 0.001 mM putrescine dose (Table 4). This suggests that putrescine has a significant role in promoting cell division, particularly under drought stress conditions.

4. Disscussion

The research findings indicate that the germination rate, germination rate coefficient, and germination rate index decreased with the increasing concentration of PEG 6000, reflecting the escalating severity of osmotic drought. Simultaneously, the average germination time, representing the duration of germination, increased. These results align with observations in a study on the Norstar winter wheat variety, where prolonged germination periods were noted due to a decrease in soil water potential (-0.20 Mpa; -1.5 Mpa). In that study, the germination rate, initially higher than 80% in other stress levels, decreased to 56% in the -1.5 Mpa application [23]. Another study involving 64 wheat genotypes found that the germination strength index varied between 2331.1-5028.2 in the control application. The germination strength index decreased by 82.5% in -5 bar osmotic potential application, and germination did not occur in any wheat genotypes in -10 and -15 bar osmotic potential applications [24]. Additionally, it was reported that the germination strength index of 30 bread wheat genotypes at -10 bar stress level ranged between 146.2-585.6. with the highest rate of decrease (85.8%) observed in the germination strength index compared to other parameters and the control [25]. Under stress conditions (15% PEG), a study recorded that the seed vigor index decreased by 60.1-76.6% according to genotypes when compared to control groups [26]. These consistent

findings across various studies emphasize the negative impact of osmotic drought on germination-related parameters in different plant varieties.

In this experiment, the germination vigor index and germination rate showed a slight increase at -2 bar osmotic drought compared to the control. However, significant decreases were observed in these characters at -4 bar and higher doses of osmotic drought. The marginal increase observed at -2 bar might be attributed to the fact that low doses of the stress treatment stimulated the mentioned characters. A similar phenomenon was reported in a study involving ethyl methanesulfonate (EMS), a chemical mutagen, where low doses of EMS acted as a stress factor and stimulated plant regeneration in vitro. It was noted that low doses of EMS application stimulated regeneration in mature embryo culture in wheat, causing an increase in shoot length, coleoptile length, seedling height, root wet and dry weight, and shoot dry weight compared to the control [16]. The literature information presented here suggests that low doses of stress application can stimulate growth, leading to an increase in the mentioned characters compared to the control. In a study investigating the response of 10 summer wheat genotypes to drought stress during germination and the seedling period, it was found that the germination rates of the varieties decreased by 0.0-34.1%, 0.0-64.4%, and 51.3-100.0% in -5.9, -8.2, and -11.3 bar drought (osmotic potential) treatments, respectively. The variety x treatment interaction was also significant in this study [27]. These findings highlight the complex and variable responses of different plant varieties to drought stress, emphasizing the importance of considering specific genotypes and stress conditions in research. In another study, where 5 different osmotic stresses (0. -2. -4. -6. -8 bar) were applied to 2 wheat genotypes, it was observed that the germination rates of the varieties decreased with the increasing stress level [28]. Similarly, in a study involving 9 bread wheat genotypes, the germination rates were reported as 89.7%, 55.6%, 41.7%, and 24.1% at 0. -2, -4, -6, and -8 bar osmotic stress levels respectively [29]. These findings highlight the negative impact of osmotic stress on germination rates in wheat, with a clear trend of decreasing germination rates as stress intensity increases. In a study conducted with 16 wheat varieties, the effects of different concentrations of PEG 6000 (control. 150 g PEG 6000/850 ml pure water, 200 g PEG 6000/800 ml pure water, and 250 g PEG 6000/750 ml pure water) on germination and early seedling development were investigated. Significant differences were observed among the genotypes in terms of the examined characters, and it was noted that the germination rate decreased significantly with the increasing concentrations of PEG [8]. This aligns with the general understanding that higher concentrations of osmotic stressors, such as PEG, can negatively impact germination rates and early seedling development in various plant species.

A study conducted on three durum wheat cultivars, the effects of -0,15, -0.58, -1.5, and -1.57 MPa osmotic potential applications created with PEG 6000 on germination were investigated. The results showed a significant decrease in germination rate at increasing stress levels, and germination was completely inhibited at the -1.57 MPa stress level [30]. This underscores the sensitivity of durum wheat germination to severe osmotic stress conditions.

Considering the averages of different concentrations of putrescine in our study, it was observed that germination rate, germination rate coefficient, germination rate index, germination strength, and germination strength index increased as putrescine concentration increased, while germination time shortened. These findings align with a study conducted in pistachio, where positive results were obtained on germination speed, germination rate, and other seedling

growth parameters because of indolebutyric acid (IBA) application at different doses [33]. The positive impact of putrescine on germination-related parameters suggests its potential role as a growth regulator in mitigating the effects of stress on seed germination and early seedling development. The findings from this study reveal that the number of embryonal roots, shoot length, and coleoptile length decreased continuously with the increasing severity of osmotic stress induced by PEG 6000 application. This aligns with previous research in wheat and other crops under similar osmotic stress conditions. For instance, a study on wheat seeds exposed to osmotic potential conditions created with PEG 4000 found that water uptake gradually decreased at lower osmotic potentials, leading to varying emergence rates of seeds. The grass sheath (coleoptile) germination was reported to be more sensitive to low water potential than rootlet germination [34]. Similarly, another study on bread wheat genotypes showed a decrease in embryonal root numbers and root growth rates under stress conditions induced by PEG 6000 [33]. Moreover, studies on durum wheat cultivars subjected to different osmotic potential treatments with PEG 6000 reported a significant decrease in the number of roots and seedling length at increasing stress levels [32]. In addition, research on 30 bread wheat cultivars under -10 bar osmotic stress conditions created with PEG 6000 solution indicated a decrease in root length. shoot length, and grass sheath length, with an increase in root-shoot length ratio [26]. Similarly, a study on 16 wheat varieties exposed to different concentrations of PEG 6000 reported significant decreases in root length, shoot length, and coleoptile length with increasing PEG concentrations, along with a significant increase in root/shoot length ratio [8]. These consistent findings across various studies emphasize the detrimental effects of osmotic stress on root and shoot development in wheat, providing valuable insights into the physiological responses of plants to water deficit conditions.

The additional studies you mentioned further support the observed trends in this research. In a study applying six different osmotic stress levels to wheat, it was found that root length decreased significantly at stress levels higher than -6 bar [36]. Another study noted a similar trend, reporting that root length decreased with increasing stress level in terms of low water potential [37]. Moreover, a study on wheat genotypes exposed to different stress environments found a substantial reduction in shoot length in stress treatments compared to the control. Specifically, the average shoot length decreased by 70.02% and 85.34% in environments with -0.6 and -0.8 Mpa stress, respectively [37]. Additionally, in barley, it was observed that as osmotic pressure created with PEG 6000 increased, characters such as coleoptile length, shoot length, and root length decreased [38]. These findings are consistent with these results and further underline the impact of osmotic stress on root and shoot development in various cereal crops, providing a comprehensive understanding of the physiological responses to water deficit conditions.

The findings regarding root and shoot weights in our study align with existing research, supporting the impact of osmotic drought stress on these parameters. In our study as in previous investigations, osmotic drought stress induced by PEG 6000 significantly decreased root wet weight, root dry weight, shoot wet weight and shoot dry weight. Interestingly we observed a significant increase in root wet weight and root dry weight under -2 bar osmotic drought stress conditions, followed by a decrease under subsequent increasing osmotic drought stress. This initial increase at -2 bar may indeed be attributed to the growth-stimulating effect of low-level stress conditions, as noted in other studies [28]. In a study on bread wheat researchers found

that root weights decreased by 17.2-44.4%, and coleoptile weights decreased by 44.9-73.3% compared to the control under -0.67 Mpa stress conditions induced by PEG 6000 [35]. Similarly, it was reported that root length, wet and dry root weights decreased significantly as the severity of osmotic stress increased in wheat genotypes [8]. Consistent with our findings, in barley, the shoot wet and dry weights, along with proportional water content, decreased significantly as the osmotic pressure created by different concentrations of PEG 6000 increased [38]. These consistent results across different cereal crops highlight the general impact of osmotic stress on root and shoot development.

The positive effects of putrescine application on various germination and seedling growth parameters in response to osmotic drought stress are noteworthy. Putrescine significantly increased the germination rate, germination rate coefficient, germination rate index, germination power, germination power index. and shortened germination time. This indicates that putrescine played a crucial role in mitigating the negative effects of osmotic drought on germination. Moreover, considering the averages of putrescine concentrations, it was observed that putrescine significantly increased the number of embryonal roots, root length, shoot length. coleoptile length, seedling length, root wet weight, shoot wet weight and shoot dry weight. The highest impact of putrescine on these characters, except for root dry weight, was observed at 0.001 mM concentration, followed by 0.01 and 0.1 mM concentrations. In the case of root dry weight, the most effective dose was 0.1 mM, although the difference between this dose and 0.001 mM dose was statistically insignificant. These findings suggest that putrescine application has a positive influence on seed germination and early seedling growth under osmotic drought conditions, providing valuable insights for potential applications in agricultural practices to enhance stress tolerance in crops. The polycationic structure of polyamines, such as putrescine, spermidine and spermine, is a key feature contributing to their biological activity [39]. Numerous studies have demonstrated that the external application of polyamines can enhance abiotic stress tolerance in plants.

In line with the findings of this study, a similar investigation was conducted to assess the impact of drought and putrescine hormone on seed germination in wheat [17]. Different doses of PEG 6000 were applied to induce osmotic drought of varying severities (0. -2. -4. -6. -8. and -10 bar), and these doses were combined with 0. 0.01. 0.1. and 1 mM doses of putrescine. The research revealed that as drought severity increased, germination rate, root and shoot length decreased and germination time increased. Importantly. it was observed that the application of 1 mM putrescine mitigated these negative effects of drought. In a related study by the same researchers [17]. PEG 6000 and putrescine (at doses of 0. 0.01. 0.1. 1 mM) were applied to wheat and their effects on various seedling characters were investigated. The experiment demonstrated that PEG 6000 negatively impacted seedling growth parameters at different doses and notably, the 1 mM dose of putrescine alleviated the adverse effects of drought. These findings underscore the potential of putrescine to mitigate the impact of drought stress on seed germination and seedling growth in wheat, providing valuable insights for strategies aimed at enhancing stress tolerance in crops.

Indeed, polyamines play crucial roles in various fundamental cellular processes, contributing to the regulation of essential functions within plant cells. These functions include DNA replication, transcription, translation, cell growth, enzyme activity regulation, cellular cation-anion balance maintenance and membrane stability [40, 41]. Additionally, polyamines are key

participants in numerous growth and development processes. such as cell division. breaking tuber dormancy, seed germination, stimulation, support and development of flower buds, fruit formation and ripening, embryogenesis, plant morphogenesis and responses to both biotic and abiotic stresses. Polyamines have been recognized for their effectiveness in enhancing plant tolerance to a range of stresses, including high and low temperatures, salinity, hyperosmosis, hypoxia and exposure to atmospheric pollutants [42, 43]. The multifaceted involvement of polyamines in these physiological processes highlights their significance in shaping plant responses to environmental challenges and their potential as important components in strategies for improving stress resilience. As mentioned earlier, the specific application of putrescine in this study aligns with the broader context of polyamine involvement in plant stress mitigation. The reported membrane-stabilizing effect of externally applied polyamines, including di-. tri-, and tetra-amines, is significant in protecting plant cell membranes from damage under stress conditions [42, 44]. Additionally, intrinsic polyamines play a role in maintaining membrane integrity [45]. In Allium fistulosum, the external application of putrescine has been shown to reduce oxidative damage by increasing antioxidant capacity [44]. The researchers observed a reduction in superoxide radical (O2-) and H_2O_2 contents, leading to less oxidative stress in plant cells as a result of externally applied putrescine. In a study involving Malus domestica Borkh. (apple), which exhibits embryonal dormancy, the effects of different doses of spermidine (spd) on primary root length. cell shape, and mitotic index in maize were investigated [47]. The study found that low concentrations of putrescine and spermidine, such as 0.1 and 1 mM. stimulated germination. However, higher doses, such as 5 mM, were found to be ineffective and even inhibitory in germination. This underscores the importance of optimal concentrations when applying polyamines, as excessively high doses may not yield the desired effects and could even have adverse consequences on germination and other physiological processes.

5. Conclusion

The study results indicate that the dose of polyethylene glycol (PEG) 6000 applied to induce osmotic drought at -2 bar had a positive impact on some of the examined characters. However, the stimulatory effect of osmotic drought at -2 bar showed a minor change compared to the combinations of PEG 6000 + putrescine applied in conjunction with PEG 6000. Beyond this dose, all characters were negatively affected by the increasing severity of osmotic drought (PEG 6000 concentration). In contrast, all doses of putrescine applied in combination with PEG 6000 demonstrated positive effects on all the examined characters and contributed to the reduction of germination time. The highest impact of putrescine was observed at a dose of 0.1 mM in root dry weight and at a dose of 0.001 mM in all other characters. Consequently, the study suggests that higher doses of osmotic drought stress had a significantly negative effect on germination and seedling development. However, all doses of putrescine were able to partially alleviate the adverse effects of osmotic drought induced by PEG 6000.

Ethics in Publishing

There are no ethical issues regarding the publication of this study

Author Contributions: Idea – M.T; Data Collection and/or Processing – E.E.Y; Analysis and/or Comment – A.T; Literature Review – E.E.Y; Posted by – E.E.Y; Critical Review – M.T. A.T.

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