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Research Article

Effects of Osmotic Drought and GA_3 Applications on Germination, Seedling Growth and Mitotic Index of Rye

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Abstract

In this study, effect of gibberellic acid (GA_3) and putrescine of polyamines on germination, seedling growth and mitotic index were analyzed on rye seeds cv. Aslım-95 under osmotic drought conditions formed by using polyethylene glycol (PEG) 6000 application. In order to form osmotic drought, test was conducted by combining 6 different doses of PEG as 0, -2, -4, -6, -8 and -10 including the control (0) and the doses of GA_3 as 0, 300, 600, 900 mM. The concentration of -2 bar of polyethylene glycol (PEG) 6000 that was conducted so as to form osmotic drought influenced positively some characters that were examined in the study. However, the incentive effect of -2 bar of osmotic drought showed few change in compliance with PEG 6000+GA_3 combinations. On the other hand, following that concentration, when osmotic drought level, that is PEG 6000 concentration, became higher, all the characters were influenced in a negative way. All the doses of GA_3 that was used in combination with PEG 6000 showed positive effects on the examined characters, and shortened the germination time, that is, germinating duration. The biggest effect was observed in the concentration of 900 mM which is the highest concentration of GA_3. As a result, especially high doses of osmotic drought stress affect germination and seedling growth in a very negative way; It was determined that all doses of GA_3 could partially compensate for the effect of osmotic drought induced by PEG 6000.

Keywords: Rye, PEG 6000, GA_3, germination, seedling, mitotic index

Ozmotik Kuraklık Ve Giberellik Asit Uygulamalarının Çavdarda Çimlenme, Fide Gelişimi ve Mitotik İndeks Üzerine Etkileri

Özet

Çavdarın Aslım-95 çeşidine ait tohumların kullanıldığı bu çalışmada polietilen glikol (PEG) 6000 uygulaması ile oluşturulan ozmotik kuraklık ve giberellik asit (GA_3) uygulamalarının çimlenme, fide gelişimi ve mitotik indeks üzerine olan etkileri incelenmiştir. Denemede ozmotik kuraklık oluşturmak amacıyla PEG 6000'in 0, -2, -4, -6, -8 ve -10 bar olacak şekilde, kontrol (0) dahil 6 farklı dozu, GA3'ün 0, 300, 600, 900 mM'lık dozları ile kombinasyon halinde uygulanmıştır. Ozmotik kuraklık oluşturmak amacıyla uygulanan PEG 6000'in -2 bar'lık dozu denemede incelenen bazı karakterleri olumlu yönde etkilemiştir. Ancak -2 bar'lık ozmotik kuraklığın teşvik edici etkisi PEG 6000 ile kombine halde uygulanan PEG 6000 + GA_3 kombinasyonlarına göre küçük değişim göstermiştir. Diğer taraftan bu dozdan sonra ozmotik kuraklığın şiddeti, yani PEG 6000'in konsantrasyonu arttıkça tüm karakterler olumsuz yönde etkilenmiştir. PEG 6000 ile kombine halde uygulanan GA_3'ün tüm dozlarının incelenen karakterler üzerine etkisi olumlu yönde olmuş ve average çimlenme zamanını yani çimlenme süresini kısaltmıştır. GA_3'ün en büyük etkisi en yüksek dozu olan 900 mM'lık konsantrasyonunda tespit edilmiştir. Sonuç olarak, ozmotik kuraklık stresinin özellikle yüksek dozlarının çimlenme ve fide gelişimini çok belirgin şekilde olumsuz yönde etkilediği ve tüm GA_3 dozlarının PEG 6000 ile oluşturulan ozmotik kuraklığın etkisini kısmen telafi edebildikleri belirlenmiştir.

Anahtar Kelimeler: Çavdar, PEG 6000, GA_3, çimlenme, fide, mitotik indeks

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1. Introduction

Grains are the main products used directly or indirectly in human nutrition. In the world, more than 50% of people's daily calories come from grains. Since animals are mostly fed with plant materials and the share of animal foods in daily calorie supply is about 20%, humans get about 3/4 of their daily nutrients from grains. [1]. Our country uses Rye as bread and fodder due to its high nutritional value. Since the root system is strong, the rye plant can take the necessary water from the soil more easily than other grains. Since annual precipitation is around 150 mm, it is a plant that should be evaluated in regions with low precipitation. In addition, it can be easily grown on inclined-stony soils with low yield power, where it is not possible to grow other cultural plants economically. The development of the root system towards the depths or outcrops according to the soil characteristics ensures that the adaptation limits are wide. In our country, the cultivation area of rye is 99755 ha, its production is 200 thousand tons, and its yield is about 200 kg/da [2]. Environmental stress factors negatively affect plant growth and yield [3]. Plants encounter drought stress when their roots cannot get enough water from the soil or when the transpiration rate is too high. Two main effects of drought are important in crop production, the first of which is the inability to provide sufficient plant emergence, and the second is the decrease in development and yield [4]. Seed germination is one of the most sensitive stages of the plant life cycle. Both the seed during the germination phase and the seedling formed after germination is extremely sensitive to adverse environmental conditions. If damaged, the plant life cycle may end before it even starts [5]. Drought, one of the environmental factors affecting germination, is a and significant problem that severely restricts crop production worldwide. Today, global climate change makes this situation even more serious [6]. The effect of arid conditions on plant growth and grain yield depends on the severity of the drought and the development period of the plant at the time of the drought. Seedling emergence is one of the growth periods sensitive to water deficiency. The rate and degree of the seedling plant; are critical factors that determine the yield and maturity time [7]. Therefore, for a good plant establishment, the characteristics such as seed germination, density and shoot length should be sufficient. In semi-arid regions, low humidity during germination is a germination limiting factor. Some researchers have reported that coleoptile and shoot length are important for the success of seedling establishment in deeply sown seeds, especially in order to reach moisture in dry soils [8]. The molecular weight of Polyethylene Glycol 6000 (PEG 6000) is small enough to affect the osmotic potential but too large to be absorbed by the plant, so it is not expected to penetrate plant tissues quickly [9]. Chemicals such as PEG are frequently used in the environment to stimulate drought in studies to be carried out in terms of drought resistance during the early development period of seedlings [10]. Despite the discovery of new hormones and growth regulators in plants, and new information especially in gene technology and basic research, it is seen that there little needs to be more information about the physiology and biochemistry of seed germination even today. For this reason, the study on the effects of traditional but preserving hormones such as gibberellin (GA3), kinetin, benzyladenine and ethylene, which have been found in recent years and learned to be effective in plant growth and development, is very interesting and hopeful in today's world where hunger is one of the important problems. thought to lead to positive developments. The aim of this research is to

determine the effects of artificially induced osmotic drought on germination, seedling growth and mitotic index in rye, and also to determine the effects of gibberellic acid, one of the growth regulators, in compensating for the effects of osmotic drought on the specified properties.

2. Materials and Method

In the research carried out in the Atatürk University Faculty of Agriculture Biotechnology laboratory, seeds of diploid rye (*Secale cereale* L.) Aslım 95 variety were used as material. The variety has been developed for dry areas and has good feed properties and high yield potential. It is very tolerant of winter, drought, diseases and nutrient deficiency. It gives better results than barley and wheat in low soil fertility, exposed and sloping area. After washing the seeds with tap water, they were mixed in 70% ethyl alcohol (EtOH) for 3 minutes, washed three times with sterile distilled water in a sterile cabinet, and surface sterilization was performed by mixing in 10% bleach containing a few drops of Tween 20 (Sigma) for 15 minutes. After surface sterilized seeds were washed with sterile distilled water, they were kept in 4 different GA3 concentrations [0 (pure water), 300, 600, and 900 mM] at room temperature for 24 hours [11]. At the end of this period, the seeds were washed with water to remove GA3 and the seeds were transferred to drying papers and dried for 3 hours. The dried seeds were sown as 25 seeds in each petri dish with a diameter of 9 cm with two layers of germination paper (whatman paper number 1). Then in the petri dishes with 6 different osmotic potentials [0 (pure water), - 2, -4, -6, -8, and -10 bar], 10 ml of each solution was added. In order to create osmotic potential, PEG 6000 solutions were prepared and used according to Michel and Kaufman [12]. After this process, the seeds were germinated at 20°C in the 16:8 hour light:dark photoperiod. After the seeds were placed in the germination medium, 10 ml of 6 different doses of PEG 6000 solution were added every day for 10 days. The data obtained from the trial, which was established with 4 replications according to a completely random trial design, were subjected to variance analysis using the SPSS statistical package program in 6 (osmotic potential) × 4 (GA3 dose) factorial order and the averages were compared with the Duncan test.

Characters examined in the experiment

a. Germination related characters: Germination rate in relation to seed germination ([13], germination rate index ([14], germination power [15], germination power index ([16], germination percentage ([12] and average germination time ([17] detected.

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 [18].

c. Mitotik indeks: The method specified by Sağsöz [19] and Tosun [20] was used 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 [21].

Mitotic index (%) = (number of cells dividing / total number of cells) x 100

3. Results and Discussion

3.1 Effects of drought and GA3 application on germination related characters

a) Germination Rate (GH)

The effects of drought (created with PEG 6000) and GA_3 applications and drought x GA3 interaction on the germination rate of seeds were very significant (P<0.01) (Chart 1). As the severity of drought induced by PEG 6000 application increased, the germination rate of seeds decreased. As a matter of fact, the germination rate, which was 56.50% in the control group (without PEG 6000 applied), decreased continuously at -2, -4, -6, -8, and -10 bar applications and finally decreased to 5.50%. On the other hand, GA_3 application increased the germination rate. For example, germination rate, which was 1.00% when GA_3 was not applied, increased to 36.16% at 300 mM dose, 44.50% at 600 mM dose, and 54.50% at 900 mM dose. Differences between all treatments were statistically very significant. Since the effect of both GA3 and drought on seed germination rate is very important, the drought x GA3 interaction was also very important. The highest germination rate with 80% was obtained from the treatments where drought was not applied, whereas GA3 was applied at 600 and 900 mM doses (Chart 2).

Chart 1. Analysis of variance results of germination-related characters after different doses of PEG 6000 and GA3 applications to seeds

		Error Mean Squares					
Variation source	**DF**	**GR**	**GRİ**	**GP**	**GPİ**	**GP**	**AGT**
Drought (D)	5	6333,76**	35.71**	13838.66**	11175907.47**	8623.76**	20,56**
GA_3 (G)	3	12993,50**	10,02**	1362,88**	2166152,12**	60,16**	31,91**
D × G	15	748,96**	0,39**	422,22**	170393,19**	185,10**	1,98**
Error	72	13,389	0,08	24	18509,94	20,611	0,07
Total	96						

** Significant at the P<0.01 level.

Chart 2. Data on some germination-related characters after PEG 6000 and GA3 applications to seeds

Drought (bar)	**GA_3 (mM)**	**GR (%)**	**GRİ**	**GP (%)**	**GPİ**	**GP (%)**	**AGT (gün)**
0	0	4,00 c[1]	18,55 c	97,00	1866,90 d	98,00 b	5,52 a
	300	62,00 b	19,56 b	100,00	2383,00 c	100,00 a	4,67 b
	600	80,00 a	19,87 a	100,00	2721,00 b	100,00 a	4,36 c
	900	80,00 a	19,86 a	100,00	3396,00 a	100,00 a	4,42 c
	Average	**56,50 A[2]**	**19,46 A**	**99,25 A**	**2587,00 B**	**99,50 A**	**4,74 F**
-2	0	2,00 c	18,26 b	100,00	2032,00 c	100,00	5,93 a
	300	59,00 b	19,35 a	97,00	2521,00 bc	100,00	4,98 b
	600	61,00 b	19,49 a	99,00	2796,00 b	100,00	4,77 bc
	900	70,00 a	19,68 a	100,00	3250,00 a	100,00	4,56 c
	Average	**48,00 B**	**19,20 B**	**99,00 A**	**2649,00 A**	**100,00 A**	**5,06 E**
-4	0	0,00 c	17,83 c	94,00 ab	1049,00 c	100,00	6,62 a
	300	54,00 b	19,11 b	92,00 b	1363,23 b	99,00	5,24 b
	600	60,00 a	19,50 a	98,00 a	1641,42 a	99,00	4,65 c
	900	60,00 a	19,14 b	90,00 b	1837,18 a	97,00	5,11 b
	Average	**43,50 C**	**18,89 C**	**93,50 B**	**1475,32 C**	**98,75 A**	**5,40 D**
-6	0	0,00 c	17,29 c	67,00 b	890,00 c	100,00 a	7,84 a
	300	42,00 b	18,60 a	83,00 a	1066,90 b	94,00 b	5,63 b

	600	44,00 b	18,18 b	74,00 ab	1052,88 b	82,00 a	5,35 c
	900	58,00 a	18,97 a	82,00 a	1352,64 a	96,00 a	5,39 bc
	Average	**36,00 D**	**18,26 D**	**76,50 C**	**1096,47 D**	**93,00 B**	**6,05 C**
-8	0	0,00 c	16,17 c	20,00 c	438,90 b	66,00 ab	9,95 a
	300	0,00 c	16,53 bc	44,00 b	539,00 b	70,00 ab	7,69 b
	600	22,00 b	17,43 a	63,00 a	712,08 a	72,00 a	5,70 c
	900	37,00 a	17,17 ab	54,00 ab	679,54 a	61,00 b	5,39 c
	Average	**14,75 E**	**16,82 E**	**45,25 D**	**594,49 E**	**67,25 C**	**7,18 B**
-10	0	0,00 b	14,71 c	1,00 c	97,72 d	28,00 c	9,92 a
	300	0,00 b	15,53 b	34,00 b	165,00 c	44,00 b	7,42 b
	600	0,00 b	15,61 b	38,00 b	259,60 b	44,00 b	6,95 b
	900	22,00 a	16,89 a	51,00 a	405,33 a	59,00 a	5,62 c
	Average	**5,50 F**	**15,68 F**	**31,00 E**	**218,75 F**	**43,75 D**	**7,48 A**
Average GA_3	0	1,00 D	17,14 D	63,16 C	941,36 D	82,00 B	7,63 A
	300	36,16 C	18,11 C	75,00 B	1204,97 C	84,50 AB	5,94 B
	600	44,50 B	18,35 B	78,66 A	1384,92 B	82,83 AB	5,30 C
	900	54,50 A	18,62 A	79,50 A	1674,09 A	85,50 A	5,08 D

[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 effect of drought and GA3 applications created with PEG 6000 on the germination rate index was very significant (P<0.01), and the drought x GA3 interaction was also found to be very significant (P<0.01) (Chart 1). The germination rate index decreased continuously depending on the severity of drought application (dose of PEG 6000). The germination rate index, which was 19.46 in the control group, was 19.20 at -2 bar, 18.89 at -4 bar and 18.26 at -6 bar. In the applications above this dose, the germination rate index decreased more significantly and became 16.82 at -8 bar and 15.68 at -10 bar. On the other hand, although the effect of GA3 application on the germination rate index was statistically very significant, it remained at a limited level. As a matter of fact, according to the average data, the germination rate index, which was 17.14 in the control group without GA3, became 18.11, 18.35 and 18.62, with a small increase at doses of 300, 600 and 900 mM, respectively. Considering the drought x GA3 interaction, the highest germination rate index (19.87) was determined in the combination where drought was not applied (0 bar) x GA3 was applied at a dose of 600 mM (Chart 2).

c) Germination Power (GP)

The effects of drought (created with PEG 6000), GA3 and drought x GA3 interaction on germination vigor were statistically significant (P<0.01) (Chart 1). It was observed that the germination power decreased continuously as the concentration of PEG 6000 applied to create drought was increased. For example, germination power, which was 99.25% in the control group, decreased as the concentration of PEG 6000 increased and decreased to 31.00% at -10 bar. This decrease in germination power was relatively less at -2 and -4 bar doses where the effect of drought was lower; however, it appeared much more prominently at -6, -8 and -10 bar. On the other hand, when the negative effect of drought induced by different concentrations of PEG 6000 on germination power changed when GA3 was applied, the average germination power, which was 63.16% in the control group without GA3, increased with GA3 application. , was 75.00%, 78.66% and 79.50% at 600 and 900 mM doses, respectively. However, the most pronounced effect of GA3 on germination power was observed at the dose of -10 bar, where

drought was most severe. As a matter of fact, the germination power of 1.00% when GA3 is not applied at this dose; It showed a very significant increase with GA3 application and increased to 51.00% at 900 mM dose (Chart 2).

d) Germination strength index (GSI)

As can be seen from Chart 1, the effects of drought, GA3, and drought x GA3 interaction created with PEG 6000 on the germination strength index, which is one of the parameters related to germination, were very significant ($P<0.01$). It was observed that the effect of drought induced by PEG 6000 on the germination power index decreased significantly due to the increase in the severity of the drought (PEG 6000 concentration), and the germination strength index, which was 2587.00 on average when drought was not applied, decreased to 218.75 at -10 bar dose. The germination power index slightly increased at -2 bar (2649.00) compared to the control (2587.00), but then decreased steadily as drought severity increased. However, the greatest decrease occurred at doses of -8 and -10 bar. When the effect of gibberellic acid (GA3) applied in combination with PEG 6000 on the germination power index was evaluated, it was seen that it had a positive effect compared to the applied dose, that is, it increased the germination power index. As a matter of fact, the germination power index, which was 941.36 on average at a 0 mM dose of GA3, increased continuously as the GA3 concentration increased (300, 600 and 900 mM) and increased to an average of 1674.09 at the highest dose. As with other properties, the most significant effect of GA3 on the germination power index was observed at the dose of PEG 6000 at a concentration of -10 bar. For example, the germination power index, which was 97.72 when GA3 was not applied at this dose, increased significantly to 405.33 at 900 mM GA3 dose (Chart 2).

e) Germination Percentage (GP)

The effect of PEG 6000 and GA3 applications on germination percentage in diploid rye seeds and drought x GA3 interaction were very significant ($P<0.01$) (Chart 1).

As the applied PEG 6000 concentration increased to 0, -2, -4, -6, -8 and -10 bar, the germination percentage decreased in general, but at -2 bar there was a small increase compared to the control. Accordingly, the average germination percentage of 99.50% in the control group increased to 100.00% with a small increase at -2 bar, and decreased to 98.75% with a slight decrease at -4 bar. differences between them were statistically insignificant. On the other hand, germination percentages at -6, -8 and -10 bar were 93.00%, 67.25% and 43.75% on average. As it can be understood from these data, the most significant decrease in germination percentage occurred at doses of -8 and -10 bar. GA3 application slightly increased the germination average and the most significant difference statistically compared to the control was between the control group (mean 82.00%) and 900 mM concentration (85.50%). Again, the most significant effect of GA3 in compensating for the negative effect of drought induced by PEG 6000 appeared at -10 bar. The germination percentage, which was 28.00% when GA3 was not applied at this concentration (control group), showed a very significant increase with GA3 application and increased to 59.00%, exceeding twice the control at 900 mM dose (Chart 2).

f) Average germination time (AGT)

The effect of drought, GA3 and drought x GA3 interaction on the mean germination time of seeds in diploid rye was very significant ($P<0.01$) (Chart 1). It was observed that the germination time increased as the concentration of PEG 6000 increased. Accordingly, the mean germination time at PEG 6000 concentrations applied to create an osmotic pressure of 0, -2, -

4, -6, -8 and -10 bar is respectively 4,74, 5,06, 5,40, 6,05, 7,18 and 7.48 days. As can be seen from the data presented here, the germination time was significantly longer, especially at doses of -8 and -10 bar, compared to other doses. On the other hand, when the effect of GA3 on the mean germination time was examined, it was determined that the germination time was significantly shortened at different concentrations. For example, the mean germination time of 7.63 days in the control group decreased to 5.94 days in the application of 300 mM GA3. In addition, the mean germination time at 600 and 900 mM GA3 concentrations was 5.30 and 5.08 days, respectively. On the other hand, the most significant effect of gibberellic acid (GA3) on the mean germination time was observed at -8 and -10 bar doses of PEG 6000. For example, the mean germination time at -10 bar, which was 9.92 days without GA3 application, decreased to 5.62 days when 900 mM GA3 was applied (Chart 2).

3.2 Drought and GA3 application on seedling-related characters

a) Total number of embryonal roots (TNER)

As can be seen from Chart 3, the effect of drought, GA3 and drought x GA3 interaction on total embryonal root number was very significant (P<0.01). As the severity of the drought induced by PEG 6000 application increased, the root number of the seedlings decreased. As a matter of fact, the root number, which was 4.45 in the control group (without PEG 6000 application), decreased continuously in the applications of -2, -4, -6, -8 and -10 bar and was 4.33, 4.08, 3.83, decreased to 3.61 and 3.26. On the other hand, GA3 application increased the number of roots, which increased from 3.64 in the control group to 3.80, 4.04 and 4.24 at doses of 300, 600 and 900 mM, respectively. Since PEG 6000 and GA3 applicaFtions had very significant effects separately, the effect of drought x GA3 injection on root number was also very significant (P< 0.01) (Chart 3). The highest number of inbryonal roots was obtained with 900 mM dose of GA3 in the control group, where PEG 6000 was not applied, with 4.70 units (Chart 4).

Chart 3. Analysis of variance results of seedlings and some related characters after applying different doses of PEG 6000 and GA3 to seeds

		Error Mean Squares					
Variation source	**DF**	**TNER**	**RL**	**SL**	**RDW**	**SDW**	**Mİ**
Drought (D)	5	8,38**	194,20**	576,94**	0,006**	0,008**	7789,61**
GA_3 (G)	3	16,25**	1365,18**	1906,82**	0,013**	0,013**	23427,74**
D × G	15	0,59**	15,65**	31,60**	0,001**	0,00001**	726,80**
Error	72	0,37	1,33	0,71	0,001	0,000019	49,25
Total	96						

** Significant at the P<0.01 level.

Chart 4. Data on some characteristics of seedlings after PEG 6000 and GA3 applications to seeds

Drought	**GA_3 (mM)**	**TNER (number)**	**RL (cm)**	**SL (cm)**	**RDW (mg)**	**SDW (mg)**	**Mİ (%)**
0	0	4,30	8,73 c[1]	10,32 d	0,0247 b	0,0594 c	34,27 c
	300	4,40	10,70 b	13,13 c	0,0189 c	0,0637 b	44,49 b
	600	4,40	11,30 b	15,91 b	0,0383 a	0,0694 a	63,96 a
	900	4,70	14,50 a	19,46 a	0,0368 a	0,0718 a	62,22 a
	Average	**4,45 A[2]**	**11,30 A**	**14,70 A**	**0,0296 B**	**0,0660 A**	**51,24 B**
-2	0	4,30 ab	11,03 c	9,29 d	0,0276 b	0,0426 d	30,49 c
	300	4,00 b	11,81 bc	13,40 c	0,0358 b	0,0549 c	45,25 b
	600	4,40 a	12,65 b	15,31 b	0,0396 ab	0,0581 b	69,64 a
	900	4,65 a	14,64 a	17,86 a	0,0719 a	0,0614 a	73,36 a

	Average	**4,33 A**	**12,53 A**	**13,96 B**	**0,0437 A**	**0,0542 B**	**54,68 A**
-4	0	3,45 c	5,03 d	5,46 d	0,0232 b	0,0395 d	22,21 c
	300	4,00 b	7,20 c	6,57 c	0,027 a	0,0514 c	30,99 b
	600	4,40 a	8,60 b	7,98 b	0,0224 b	0,0580 b	33,50 b
	900	4,50 a	9,75 a	9,19 a	0,0268 a	0,0621 a	49,78 a
	Average	**4,08 B**	**7,64 B**	**7,30 C**	**0,0248 BC**	**0,0527 C**	**34,12 C**
-6	0	3,35 b	4,79 c	4,11 d	0,0127 b	0,0319 d	10,36 d
	300	3,80 a	6,03 b	5,32 c	0,0181 ab	0,0427 c	13,90 c
	600	4.00 a	6,30 b	6,54 b	0,0163 ab	0,0479 b	25,83 b
	900	4,20 a	6,80 a	7,29 a	0,0281 a	0,0596 a	31,24 a
	Average	**3,83 C**	**5,98 C**	**5,81 D**	**0,0188 CD**	**0,0455 D**	**20,33 D**
-8	0	3,35 c	3,70 b	2,95 d	0,0096 b	0,0295 d	5,90 c
	300	3,40 bc	3,20 c	4,50 c	0,0081 b	0,0338 c	7,62 c
	600	3,80 ab	4,10 ab	5,79 b	0,0123 b	0,0391 b	13,96 b
	900	3,90 a	4,50 a	6,64 a	0,0265 a	0,0474 a	22,15 a
	Average	**3,61 D**	**3,87 D**	**4,97 E**	**0,0141 DE**	**0,0374 E**	**12,41 E**
-10	0	3,10 b	1,55 c	1,94 b	0,0044 b	0,0214 c	2,79 c
	300	3,20 ab	1,55 c	2,20 b	0,0058 b	0,0296 b	4,68 b
	600	3,25 ab	2,10 b	3,80 a	0,0107 a	0,0313 b	6,57 a
	900	3,50 a	2,70 a	4,17 a	0,0067 ab	0,0374 a	7,21 a
	Average	**3,26 E**	**1,97 E**	**3,03 F**	**0,00691 E**	**0,0299 F**	**5,32 F**
Average GA_3	0	3,64 D	5,80 D	5,68 D	0,0170 B	0,0373 D	17,67 D
	300	3,80 C	6,74 C	7,52 C	0,0189 B	0,0460 C	24,48 C
	600	4,04 B	7,50 B	9,22 B	0,0232 B	0,0506 B	35,57 B
	900	4,24 A	8,81 A	10,77 A	0,0328 A	0,0566 A	40,49 A

[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)

The effects of drought (created with PEG 6000), GA3, and drought x GA3 interaction on root length were statistically significant ($P<0.01$) (Chart 3). It was observed that the average root length generally decreased as the concentration of PEG 6000 applied to create drought was increased. However, root length, which was measured as 11.30 cm in the control group, increased to 12.53 cm with a slight increase in the application of -2 bar. Still, the difference between these two groups was statistically insignificant. On the other hand, root length decreased to 7.64, 5.98, 3.87 and 1.97 cm, respectively, at -4, -6, -8 and -10 bar applications, and the differences between these groups were statistically significant. On the other hand, when the effect of drought created by applying PEG 6000 on root length was examined, how GA3 application changed; Root length, which was 5.80 cm in the control group, increased with GA3 application and increased to an average of 6.74, 7.50 and 8.81 cm at doses of 300, 600 and 900 mM, respectively. However, the most significant effect of GA3 on root length was seen at the dose of -4 bar. Root length, which was 5.03 cm when GA3 was not applied at this dose, increased to 9.75 cm by approximately doubling at 900 mM dose of GA3 (Chart 4).

c) Shoot length (SL)

The effect of drought and GA3 applications created with PEG 6000 on shoot length was very significant (P<0.01), and the drought x GA3 interaction was also found to be very important (P<0.01) (Chart 3). Shoot length decreased continuously depending on the severity of drought application (dose of PEG 6000). The average shoot length of 14.70 cm in the control group increased to 13.96 cm at -2 bar, 7.30 cm at -4 bar, 5.81 cm at -6 bar, and -8 bar' It decreased to 4.97 cm at -10 bar, and to 3.03 cm at -10 bar. On the other hand, when the effect of GA3 application on shoot length was examined, it was observed that the shoot length, which was 5.68 cm on average in the control group (0 mM), increased with GA3 application and reached 10.77 cm by almost doubling at the 900 mM dose. According to the drought x GA3 interaction, the highest shoot length was 19.47 cm with 0 bar drought and 900 mM GA3 application. The most significant effect of drought x GA3 application was determined in the -8 bar group. As a matter of fact, the shoot length, which was 2.96 cm when GA3 was not applied in this group, increased approximately 2.5 times and reached 6.64 cm at the 900 mM GA3 dose (Chart 4).

d) Root dry weight (RDW)

The effect of drought (PEG 6000) and GA3 applications and drought x GA3 interaction on root dry weight, which is one of the seedling characters examined in diploid rye, was very significant (P<0.01) (Chart 3). The highest value for root dry weight (mean 0.0473 mg) was determined at -2 bar drought and the difference between the control group (0.0296 mg) was very significant. On the other hand, dry root weights decreased continuously at -4, -6, -8 and -10 bar drought doses and finally decreased to an average of 0.0069 mg at -10 bar. When the effect of gibberellic acid (GA3) applied on the root dry weight was examined to determine the situation of reducing the negative effect caused by drought, it was seen that although 300 and 600 mM doses of GA3 slightly increased the root dry weight compared to the control group, there was no statistically significant difference. As a matter of fact, the average dry root weight of 0.0170 mg in the control group increased to 0.0189 mg with a small increase at the 300 mM dose and to 0.0232 mg at the 600 mM dose. In contrast, the effect of the 900 mM dose was very pronounced and increased to approximately twice that of the control group (0.0328 mg). The greatest effect of GA3 on root dry weight occurred at -2 bar osmotic drought. In this group, the root dry weight, which was 0.0276 mg in the control, increased two and a half times to 0.0719 mg at the 900 mM dose (Chart 4).

e) Shoot dry weight (SDW)

The effects of drought (PEG 6000) and GA3 treatments on shoot dry weight were very significant (P<0.01). Likewise, drought x GA3 interaction affected shoot dry weight very significantly (P<0.01) (Chart 3). According to the average drought values, as the severity of drought increased, shoot dry weight decreased continuously and this decrease was statistically significant. Depending on the increasing drought severity, shoot dry weight, which was 0.0660 mg in the control group, was 0.0542 mg at -2 bar, 0.0527 mg at -4 bar, 0.0455 mg at -6 bar, and -8 bar 0.0374 mg and decreased to 0.0299 mg at -10 bar. Each application was in a different statistical group. GA3, which was applied in combination with PEG 6000 to reduce the negative effect of drought, positively affected the shoot dry weight, which decreased with the effect of drought, by increasing it at a very significant (P<0.01) level. The effect of different doses of gibberellic acid (GA3) on shoot dry weight was 0.0373 mg in the control group (0 mM); It increased at doses of 300, 600 and 900 mM to 0.0460 mg, 0.0506 mg and 0.0566 mg,

respectively, and the difference between all groups was statistically significant. The highest shoot dry weight was determined in 900 mM GA3 application at all drought doses (Chart 4).

Effects of GA3 application on mitotic index

The effect of drought x GA3 interaction with drought and GA3 applications created with PEG 6000 on the mitotic index was very significant ($P<0.01$) (Chart 3). The mitotic index, which was 51,24% in the control group without PEG 6000, increased slightly to 54.68% at the -2 bar dose compared to the control group. At 0, -4, -6, -8 and -10 bar drought doses, the mitotic index decreased continuously and reached 51.24%, 34.12%, 20.33%, 12.41%, and finally 5.32%, respectively. Has been found to reduce. As it can be understood from these data, there was a great decrease in the mitotic index, especially at -8 and -10 bar. On the other hand, when the effect of GA3 on the mitotic index was examined in terms of mean values, it was determined that the mitotic index increased as the GA3 dose increased. As a matter of fact, the mitotic index, which was 17.67% in the control group in which GA3 was not applied, was 24.48%, 35.57%, and 44.05% at the 300, 600 and 900 mM doses of GA3, respectively, and all doses were in different statistical groups. Has received. As can be seen from the relevant Chart, the mitotic index-increasing effect of GA3, that is, promoting cell division, was clearly observed at doses of -8 and -10 bar, especially when drought was severe. For example, the mitotic index, which was 5.91% when GA3 was not applied at -8 bar, increased to 22.15% with an increase of approximately 4 times at the 900 mM dose. On the other hand, the highest mitotic index of 91.71% was determined at -2 bar + 900 mM GA3 dose (Chart 4).

According to the results obtained from the research, the germination rate and germination rate index required by PEG 6000, in other words, it got rid of the burden of osmotic drought, and the average germination time, that is, the germination time, was prolonged. Similar to the findings obtained from this study; Lafond and Fowler [22] found that the germination time of Norstar winter wheat variety was prolonged due to the decrease in soil water potential (-0.20 Mpa; -1.5 Mpa), the germination percentage higher than 80% at other stress levels was -1.5 Mpa. They found that it decreased to 56% in the application. Taşkesenligil [23], In his study conducted on 64 varieties of wheat genotypes, the germination power index varied between 2331.1 and 5028.2 in the control application, the germination power index decreased by 82.5 in the application of -5 bar osmotic potential, and in the applications of -10 and -15 bar osmotic potential, no wheat genotypes were found. found that germination did not occur. Dhanda et al. (2004), They stated that the germination power index of 30 bread wheat genotypes at -10 bar stress level was between 146.2-585.6, and the highest decrease rate (85.8%) was in the germination power index compared to the control when compared to the control. Baloch et al. [24] reported that the seed vigor index decreased by 60.1-76.6% under stress conditions (15% PEG) compared to the control genotypes. Another situation detected in this experiment is that the germination power index and germination percentage slightly increased in -2 bar osmotic drought compared to the control. However, very significant decreases were observed in these characters at doses of -4 bar and higher. Here, a slight increase compared to the control may be due to the low-dose stress application promoting the indicated characters. For example, Pius et al. [25] reported that ethyl methanesulfonate (EMS), a chemical mutagen, is one of many stress factors and stimulates plant regeneration in vitro. As it can be understood from the literature information given here, it can be said that the application of low-dose stress promotes growth,

therefore, there is a slight increase in these characters compared to the control. In a study investigating the response of 10 summer wheat genotypes to drought stress during germination and seedling periods; In -5.9, -8,2 and -11.3 bar drought (osmotic potential) applications, the germination percentages of the varieties were 0.0-34.1%, 0.0-64.4% and 51.3%, respectively, compared to the control. They determined that it decreased by 100.0 and the variety x application interaction was important [26].

Gholamin et al. [27] applied 5 different (0, -2, -4, -6, -8 bar) osmotic stresses to 2 wheat genotypes, and they determined that the germination percentages of the varieties decreased with the increase in the stress level. Similarly, Rezzaq et al. [28], as an average of 9 bread wheat cultivars, germination rates at 0, -2, -4, -6 and -8 bar osmotic stress levels were 89.7%, 55.6%, 41.7% and 24.1%, respectively. they have determined.

Rauf et al. [7], in their study with 16 wheat cultivars, determined that different concentrations formed with PEG 6000 (control, 150 g PEG 6000/850 ml distilled water, 200 g PEG 6000/800 ml distilled water and 250 g PEG 6000/750 ml distilled water) investigated the effect of germination and early seedling growth. Significant differences were found between the genotypes in terms of the investigated characters, and it was determined that the germination percentage decreased significantly due to increasing PEG concentrations. Almansouri et al. [29] investigated the effects of -0.15, -0.58, -1.5 and -1.57 Mpa osmotic potential applications created with PEG 6000 on germination in 3 durum wheat cultivars. It was determined that the germination rate decreased significantly at increasing stress levels, and germination was completely inhibited at the stress level of -1.57 Mpa.

Considering the averages of different concentrations of GA3, it was determined that as the concentration of GA3 increased, the germination rate, germination rate index, germination power and germination power index increased, while the germination time was shortened. Similar to the findings obtained from the research, positive results were obtained on germination rate, germination percentage and other seedling growth parameters as a result of the application of GA3 and indolbutyric acid (IBA) at different doses in a study conducted on pistachios [30]. Again, in a study conducted by Hızarcı [31], it was revealed that the germination rate of the seeds increased and the germination time shortened as a result of different doses of GA3 and folding application. On the other hand, Erdemli [32] stated that the application of different doses of GA3 (50, 100, 200, 400 ppm) in sunflower did not have a significant effect on the germination percentage, but it shortened the average germination time.

As the severity of osmotic stress induced by PEG6000 application increased, embryonal root number and shoot length decreased continuously. In contrast, a very small increase in root length was observed at -2 bar osmotic drought stress compared to control, but very significant decreases were observed at higher doses. Similar to the results obtained from this study, Naylor and Gurmu [33] investigated rootlet and coleoptile growth in wheat seeds under the osmotic potential conditions created with PEG 4000, and since water uptake gradually decreases at lower osmotic potentials, -4.5 and -7.3 bar different Seed emergence rates varied between 40-90% and 15-80% in osmotic potential applications. Researchers also reported that grass sheath (coleoptile) emergence is more sensitive to low water potential than rootlet emergence. Likewise, in a study conducted by Barutçular et al. [34] on 14 bread wheat genotypes, under stress conditions created with PEG 6000 (-0.67 Mpa), embryonal root number was 18.9-40.0%, and root growth rate was 6.1%. It was determined that it decreased by 62.0. Almansouri et al.

[28] investigated the effects of -0.15, -0.58, -1.5 and -1.57 Mpa osmotic potential applications created with PEG 6000 in 3 durum wheat cultivars on germination. They stated that germination was completely inhibited at the stress level of -1.57 Mpa. Dhanda et al. [35] reported that root length decreased 53.8%, shoot length 63.9%, grass sheath length 40.2% compared to control, as an average of 30 bread wheat varieties, under -10 bar osmotic stress conditions created with PEG 6000 solution. They found that the rate of length increased by 40.0%. Rauf et al. [7], in their study with 16 wheat cultivars, determined that different concentrations formed with PEG 6000 (control, 150 g PEG 6000/850 ml distilled water, 200 g PEG 6000/800 ml distilled water and 250 g PEG 6000/750 ml distilled water) investigated the effect of germination and early seedling growth. They reported that root length and shoot length decreased significantly, while the root/stem length ratio increased significantly depending on increasing PEG concentrations. Taşkesenligil [22], in his study conducted on 64 wheat genotypes, showed that the total embryonal root length of the varieties was between 14.84-38.13 cm in the control application, the shoot length was between 7.99-19.55 cm, in the germination medium at -5 bar osmotic potential, the shoot It was determined that the lengths of the embryos varied between 0.07-4.73 cm and the embryonal root lengths between 2.91-13.92 cm. The investigator reported that the total embryonal root length and shoot length decreased by 67.4% and 90.7%, respectively, when the mean of genotypes was compared with the control conditions and the application of -5 bar osmotic potential. Jajarmi [36], who applied 6 different (control, -3, -6, -9, -12 and -15 bar) osmotic stress levels in wheat, noted that root length decreased significantly at stress levels higher than -6 bar. Likewise, Landraces et al. [37] determined that root length decreased as the stress level increased in terms of low water potential. The researchers determined that the average shoot length of the wheat genotype was 9.44 cm, 2.83 cm and 1.38 cm in 0, -0.6 and -0.8 Mpa stress environments, respectively, and the shoot length was 70%, respectively, in stress applications compared to the control. They stated that they decreased by .02 and 85.34%. Again, as the osmotic pressure created by PEG 6000 in barley increased (0, -0.45, -0.77, -1.03 Mpa), it was determined that there was a decrease in characters such as coleoptile length, shoot length and root length [38].

Considering the average doses in gibberellic acid (GA3) application, it was determined that the number of embryonal roots, root length and shoot length increased significantly as the concentration of GA3 increased. The stimulating effect of GA3 was realized at doses of -8 and -10 bar, especially where osmotic drought stress was severe. This shows that GA3 has an important role in reducing the negative effect of osmotic stress created by PEG 6000.

Besnard-Wibant et al. [39] reported that gibberellic acid promoted cell division in the apical meristem of Silene armaria seedlings. Liu and Loy [40] found that gibberellic acids increased cell proliferation in subapical stem meristems of watermelon seedlings. It has been emphasized that gibberellins are effective in growth and development events by increasing cell elongation [41]. Güçlü [42] investigated the drought and heat resistance levels of different wheat genotypes and determined that the DNA structures that were damaged after drought stress application were repaired after gibberellic acid application and stated that this situation occurred more in drought-resistant varieties.

According to the findings obtained from this study, osmotic drought stress induced by PEG 6000 significantly reduced root dry weight and shoot dry weight, as in the previously described characters. However, root dry weight increased significantly under -2 bar osmotic drought stress

conditions, whereas it decreased under subsequent increasing osmotic drought stress. Here, the observed increase in osmotic pressure of -2 bar relative to control may have resulted from the growth-stimulating effect of low-level stress conditions, as previously explained. In a study conducted by Barutçular et al. [33], it was determined that root weights decreased by 17.2-44.4% and coleoptile weights by 44.9-73.3% compared to the control under the stress conditions of -0.67 Mpa created with PEG 6000. has been done. Again, Rauf et al. [7] determined that root length, fresh and dry root weights decreased significantly as the severity of osmotic stress increased in wheat genotypes. Likewise, as the osmotic pressure created by different concentrations of PEG 6000 in barley (control, -0.45, -0.77, -1.03 Mpa) increased, it was noted that there was a significant decrease in shoot fresh and dry weights and proportional water content [37].

In order to determine the effect of gibberellic acid application on osmotic drought stress created by using PEG 6000, 4 different concentrations of GA3 including control were tried. Considering the average of the doses administered, there was a statistically significant difference in dry weight only at the highest dose of 900 mM compared to the control; On the other hand, it was observed that all GA3 doses caused very significant increases in shoot dry weight compared to the control.

Considering the averages of PEG 6000 doses, when evaluated in terms of mitotic index, it was observed that the mitotic index was slightly higher than the control at -2 bar osmotic drought stress. As noted earlier, a low-intensity drought stress may have stimulated cell division. On the other hand, the mitotic index decreased significantly at -4 bar and higher doses, especially at -8 and -10 bar. It has been reported that mitotic activity is decreased in the stem cells of sunflower [43] and corn plant [44] and wheat leaves [45] germinated under water stress conditions due to heat stress, and this decrease causes a decrease in the number of regenerated cells. In osmotic drought conditions created with PEG 6000, the highest mitotic index average in wheat was determined as 19.3% at 0 bar application of drought, and cell division was not observed at -10 bar application of drought [46].

When the average doses of gibberellic acid (GA3), which is one of the plant growth regulators, were examined, it was determined that GA3 application increased the mitotic index very significantly. This increase was parallel to the increase in GA3 dose. The most significant effect of GA3 on mitotic index was much more pronounced in severe osmotic drought stress of -8 and -10 bar. Similar to the findings obtained from this study [47], the inhibitory effect of salt stress [0.30, 0.35, 0.40, 0.45 mM] on the mitotic index in barley pretreated with combinations of plant growth regulators was found to be GA3 (900 mM).) combinations, combinations without GA3 were not successful.

4. Conclusion

-2 bar dose of polyethylene glycol (PEG) 6000 applied to create osmotic drought positively affected some of the characters examined in the trial. However, the stimulating effect of -2 bar osmotic drought showed little change compared to PEG 6000 + GA3 combinations applied in combination with PEG 6000. On the other hand, after this dose, as the severity of osmotic drought, that is, the concentration of PEG 6000, increased, all characters were negatively affected. All doses of GA3 applied in combination with PEG 6000 had a positive effect on the

investigated characters and shortened the average germination time, that is, the germination time. The greatest effect of GA3 was detected at the highest dose of 900 mM. As a result, it was determined that especially high doses of osmotic drought stress negatively affected germination and seedling growth and all GA3 doses were able to partially compensate for the effect of osmotic drought induced by PEG 6000.

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