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Araştırma Makalesi (Research Article)

Effects of Peg-Induced Drought Stress on Germination and Seedling Performance of Bread Wheat Genotypes

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Abstract: This study was conducted to determine the response of some bread wheat genotypes to drought stress during germination and seedling growth stages. Two bread wheat cultivars (Karatopak and Sagittoria) and three advanced breeding lines (SERI.1B*2/3KAUZ*2BOW//KAUZ, 89N2090/WERAVER// SW91.4903 and STAR'S'KAUZ'S's) were used as the seed material. Three different doses of Polyethylene Glycol (Control, -0.6 MPa and -1.2 MPa of PEG-6000) were used to generate drought stress in germination and seedling growth stages of bread wheat. Germination experiments were carried out in petri dishes placed into an incubator with 4 replications in completely randomized factorial design. Seedling emergence experiments were carried out in plastic containers filled with a mixture of sand and peat placed into a growth cabinet with 3 replications in factorial arrangement of CRD. Germination experiments showed that genotype, PEG and genotype x PEG interactions were significant for examined traits except for germination rate. In the seedling experiments, genotype, PEG and genotype x PEG interaction were significant for all parameters, except for mean emergence time. Generally, increased doses of PEG caused remarkable decreases in all examined traits, but increase in mean germination time and mean emergence time. As the PEG doses increased, genotypes responded differently with regard to examined traits in germination and seedling emergence of bread wheat genotypes. It can be concluded that PEG-6000 was useful agent to create drought stress in germination and seedling growth of bread wheat, but greater doses and osmotic potentials lower than -1.2 MPa could be applied to better determine the drought stress tolerance of bread wheat genotypes.

PEG Kaynaklı Kuraklık Stresinin Ekmeklik Buğday Genotiplerinin Çimlenme ve Fide Gelişim Performansına Etkisi

Öz: Bu çalışma, bazı ekmeklik buğday genotiplerinin çimlenme ve fide gelişim aşamalarında kuraklık stresine tepkisini belirlemek amacıyla yapılmıştır. Tohum materyali olarak iki ekmeklik buğday çeşidi (Karatopak ve Sagittoria) ve üç adet ileri buğday ıslah hattı (SERI.1B*2/3KAUZ*2BOW//KAUZ, 89N2090/WERAVER//SW91.4903, ve STAR'S'KAUZ'S') kullanılmıştır. Ekmeklik buğdayın çimlenme ve fide gelişim aşamalarında kuraklık stresi oluşturmak için üç farklı dozda Polietilen Glikol (Kontrol, -0.6 MPa ve -1.2 MPa, PEG-6000) kullanılmıştır. Çimlendirme denemeleri tesadüf parselleri deneme deseninde faktöriyel düzende 4 tekrarlamalı olarak petri kaplarında, fide gelişimi

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Kuraklık stresi,
Fide gelişimi,
Çimlenme,
Polietilen Glikol.

denemeleri ise plastik kaplarda tesadüf parselleri deneme deseninde faktöriyel düzende 3 tekrarlamalı olarak yürütülmüştür. Ekmeklik buğday genotipleri, çimlenme ve fide gelişimi deneylerinde PEG'in neden olduğu kuraklık stresine tolerans açısından buğday genotipleri test edilmiştir. Çimlenme denemelerinin sonucunda, çimlenme oranı dışında, incelenen tüm özellikler için genotip, PEG ve genotip x PEG interaksiyonlarının önemli olduğunu belirlenmiştir. Fide gelişimi denemelerinde ise genotip, PEG ve genotip x PEG etkileşimi, ortalama çıkış süresi hariç tüm parametreler için önemli olmuştur. Genel olarak, artan PEG dozları incelenen tüm özelliklerde belirgin düşüşlere neden olmuş, ancak ortalama çimlenme süresi ve ortalama çıkış süresi uzamıştır. PEG dozları arttıkça, ekmeklik buğday genotiplerinin çimlenme ve fide gelişimindeki incelenen özelliklere göre farklı oranda tepkiler vermiştir. PEG-6000'in, ekmeklik buğdayın çimlenme ve fide gelişiminde kuraklık stresi yaratmada yararlı bir ajan olduğu sonucuna varılabilir, ancak ekmeklik buğday genotiplerinin kuraklık stres toleransını daha iyi belirlemek için -1.2 MPa'dan daha düşük ozmotik potansiyeller uygulanmalıdır.

1. Introduction

Wheat is the leading crop of Turkish agriculture in terms of the sowing area and grain production. It is cultivated across the diverse environments, ranging from warm lowlands to temperate highlands (Atak et al., 2016; Anonymous, 2017). There are some biotic and abiotic factors restricting yield in wheat growing environments. One of the major environmental stress factors in wheat growing regions adversely affecting uniform germination is drought. Wheat is mostly grown under rain-fed (dry) conditions in Turkey. In such environments, soil available moisture constitutes the primary constraint on wheat cultivation. Wheat sown in seedbeds with critical moisture because of limited rainfall at sowing period is not able to exhibit sufficient and synchronized emergence. Then significant yield losses are experienced in wheat fields. Stand establishment is required for successful crop production in stress environments. Therefore, for better stand establishment, stress-tolerant genotypes or cultivars should be used. Proper selection of wheat genotypes or growing genotypes with better adaptation to drought will of course increase the yield in wheat fields under rain-fed conditions. Germination and emergence stage of wheat as well as the other crops is the initial stage of plant growth and they constitute the most important growth stages. Some researchers have indicated the primary reason of germination failure as the inhibition of seed water uptake due to high osmotic potential of drought generated in germination environment (Sayar et al., 2010). Drought stress is responsible for either inhibition or delayed seed germination or seedling establishment (Bewley and Black, 1994; Sayar et al., 2010; Balkan and Gençtan, 2013). Although preliminary studies have been conducted about the drought tolerance of wheat and other crop cultivars by using Polyethylene Glycol (PEG-6000) as a draught stress agent, the responses of newly released wheat genotypes to drought stress haven't been fully elucidated, yet (Almonsouri et al., 2001; Giri and Schillinger, 2003; Dhanda et al., 2004; Okçu et al., 2005; Gürbüz et al., 2009; Sayar et al., 2010; Balkan and Gençtan, 2013). Responses of newly released bread wheat genotypes to drought stress are not well known and this study was conducted to determine the effects of polyethylene glycol (PEG) on germination and seedling growth of some bread wheat genotypes.

2. Materials and Methods

Seeds of bread wheat cultivars Karatopak and Sagittoria, which are recommended cultivars for the Eastern Mediterranean part of Turkey, and some breeding lines (SERI.1B*2/3KAUZ*2BOW//KAUZ, 89N2090/WERAVER//SW91.4903 and STAR'S'KAUZ'S') supplied from International Center for Agricultural Research in the Dry Areas (ICARDA), which were reported as drought tolerant lines, were used as the plant materials of the present study. Experiments were conducted in the laboratory of the Faculty of Forestry, University of Çankırı Karatekin in Turkey. Drought stress was induced by polyethylene glycol (PEG-6000) treatments. Drought stresses with different osmotic potentials of 0, -0.6 MPa and -1.2 MPa were arranged as described by Michel and Kaufmann (1973). Distilled water served as a control treatment. Four replicates of 25 pre-sterilized (with

5 % sodium hypochlorite) seeds were germinated between 2 sheets of Whatman No.1 filter papers in petri dishes (150 x 15 mm) with 10 ml of each test solution and filter papers were replaced in every other day to prevent PEG accumulation (Rehman et al., 1996). In order to prevent evaporation, the edges of the petri dishes were tightly sealed with Parafilm. The seeds were allowed to germinate at 20 ± 2 °C in the dark for 10 days (ISTA, 1996). A seed was considered as germinated when the emerging radicle elongated to 1-2 mm. Germination rate (GR) was recorded every 24 h for 4 days. Mean germination time (MGT) was calculated to assess the rate of germination (Ellis and Roberts, 1980). Germination index (GI) was calculated according to Maguire (1962). The seedlings were thinned to have 10 plantlets per petri dish after fourth day of germination. Shoot length (SL), root length (RL) and shoot fresh weight (SFW) were measured on the tenth day. Germination vigor index (GVI) was calculated by multiplying the sum of the root and shoot length by the germination percentage.

2.1. Germination Experiment and Data Analysis

Seedling growth experiments were carried out in plastic containers (97 x 165 x 90 mm) filled with a mixture of sand and peat (1:1). Containers were placed into growth cabinet with 3 replications and 20 seeds in each replication. Seeds were sown 3 cm in depth and containers irrigated with PEG-6000 solutions as to generate osmatic potentials of 0, -0.6 and -1.2 MPa in field capacity. Containers were then allowed to emerge at 25 °C and 70-80% relative humidity for 20 days. A seed/seedling was considered as emerged when the emerging radicle reached to soil surface. Emergence rate (ER) was recorded every 24 h for 12 days. Mean emergence time (MET) was calculated to assess the rate of emergence (Ellis and Roberts, 1980). Emergence index (EI) was calculated according to Maguire, 1962. The seedlings were thinned to have 10 plantlets per container. Seedling length (SLe), root length (RLe) and shoot fresh weight (SFWe) were measured on the twentieth day. Emergence vigor index (EVI) was calculated by multiplying the sum of the root and shoot length by the emergence percentage

2.2. Seedling Emergence Experiment and Data Analysis

A randomized complete design was used with a factorial arrangement of treatments (Genotype and PEG levels) with 4 replications and 25 seeds in each replicate for germination experiment and 3 replications and 20 seeds for seedling emergence experiment. Data of two experiments were analyzed by 2-way analysis of variance with MSTAT-C statistical software. Significant means were compared with Duncan's multiple range test (P < 0.05).

3. Results and Discussion

Figure 1 and Table 1 illustrates examined germination and seedling traits of bread wheat genotypes exposed to PEG-6000 induced drought stress.
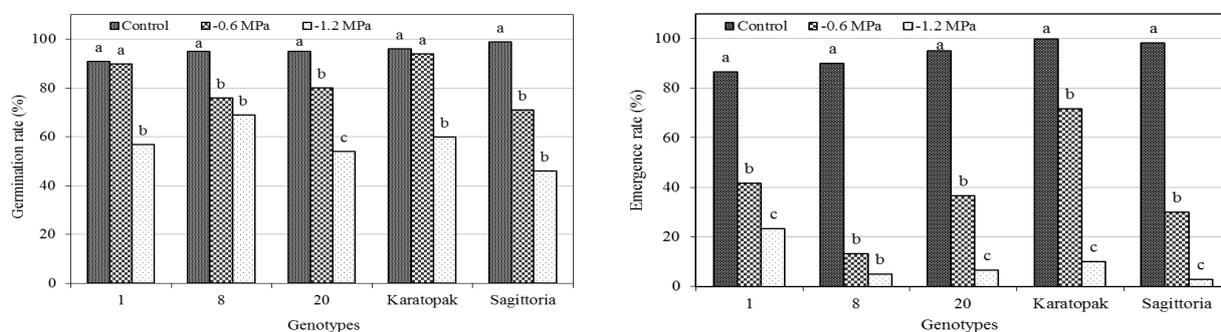

Figure 1. Effects of PEG treatments on germination rate (%) and emergence rate (%) of bread wheat genotypes (Different letters within the same column bar represent significantly different means of each genotype).
Table 1. Effects of different PEG-6000 levels on germination and seedling characteristics of bread wheat genotypes.

Genotypes	GI; Germination index				EI; Emergence index			
	Control	-0.6 MPa	-1.2 MPa	Mean**	Control	-0.6 MPa	-1.2 MPa	Mean
1	19.3 a	14.9 b	7.8 c**	14.3	7.8 a	2.7 b	1.6 c	4.0
8	22.9 a	15.1 b	9.9 c	15.9	7.6 a	0.9 b	0.3 b	2.9
20	22.6 a	12.9 b	7.8 c	14.5	8.1 a	2.5 b	0.3 c	2.6
Karatopak	22.4 a	13.4 b	6.5 c	14.1	9.2 a	4.5 b	0.3 c	4.7
Sagittoria	23.8 a	10.5 b	5.2 c	13.1	9.5 a	2.1 b	0.4 c	3.9
Mean	22.2	13.4	7.4		8.4	2.5	0.6	
	MGT; Mean germination time (day)				MET; Mean emergence time (day)			
1	1.63	1.86	2.15	1.88 ab	3.5 b	4.7 a	7.0 a	5.06
8	1.13	1.55	2.12	1.60 c	3.1 b	5.2 a	4.5 ab	4.27
20	1.19	1.92	2.20	1.77 bc	3.5 b	4.5 ab	5.0 a	4.33
Karatopak	1.20	2.07	2.50	1.92 ab	3.0 c	5.3 a	7.8 a	5.37
Sagittoria	1.10	2.27	2.93	2.10 a	2.8 c	4.8 b	6.3 a	4.63
Mean	1.25 a	1.93 b	2.38 c		3.2	4.9	6.1	
	SL; Shoot length (cm)				SLe; Shoot length (cm)			
1	10.7 a	4.2 b	0.7 c	5.7	23.4 a	20.7 ab	16.4 b	20.3
8	10.3 a	6.1b	-*	8.2	23.0 a	16.8 ab	18.7 b	19.5
20	14.5 a	-	-	14.5	25.2 a	18.6 b	11.0 c	18.3
Karatopak	14.2 a	3.8 b	-	9.0	19.9 a	17.2 a	-	18.6
Sagittoria	13.6 a	6.6 b	-	10.	23.9 a	20.0 a	7.0 b	17.3
Mean	12.7	5.2	0.7		21.1	18.8	13.4	
	RL; Root length (cm)				RLe; Root length (cm)			
1	21.6 a	5.8 b	1.0 c	10.3	15.9 a	14.1 a	15.8 a	15.3
8	18.9 a	8.5 b	-	13.7	17.1 b	15.7 ab	19.8 a	17.6
20	25.6 a	-	-	25.6	16.6 a	13.6 ab	11.6 b	13.9
Karatopak	22.6 a	4.4 b	-	13.5	16.5 a	18.4 a	-	17.5
Sagittoria	23.8 a	7.7 b	-	15.8	16.9 a	15.9 a	10.8 b	14.5
Mean	22.5	6.59	1.0		16.6	15.6	14.5	
	SFW; Shoot fresh weight (mg/plant)				SFWe; Seedling fresh weight (mg/plant)			
1	194.1 a	95.5 b	37.5 c	118.9	203.0 a	119.9 b	90.4 b	137.8
8	121.4 a	79.0 b	-	100.2	197.1 a	105.1 b	108.3 b	136.9
20	180.9 a	-	-	180.9	218.3 a	111.7 b	53.3 c	127.8
Karatopak	193.3 a	78.1 b	-	135.7	199.9 a	111.5 b	-	155.7
Sagittoria	192.5 a	77.9 b	-	135.2	230.5 a	124.0 b	49.0 c	134.5
Mean	176.6	82.6	37.5		209.8	114.5	75.3	
	GVI; Germination vigor index				EVI; Emergence vigor index			
1	2933 a	910 b	95 c	1313	2673.5 a	1211.8 b	727.8 b	1538
8	2791 a	1102 b	-	1946	3614.0 a	446.8 b	192.7 c	1418
20	3805 a	-	-	3805	3778.8 a	1163.3 b	139.7 c	1694
Karatopak	3538 a	778 b	-	2258	3650.0 a	2505.2 b	-	3078
Sagittoria	3696 a	1045 b	-	2371	4011.2 a	1115.8 b	67.8 c	1732
Mean	3353	959	95		3445.5	1288.6	282.0	

* Since data could not be obtained, means were taken over the available values.
**) Means fallowed by different letters in the same row or in the same column are significantly different (p<0.05).

G, PEG and G x PEG interaction were significant (p<0.01) for germination rate (GR). Generally decreasing GR values were observed with increasing PEG doses, but genotypes acted in a similar fashion against the increasing osmatic potentials. The greatest GR was observed in control treatment of cv. Sagittoria and the lowest GR was observed in 1.2 MPa treatment of cv. Sagittoria. When the GR values of the control and -1.2 MPa treatments were compared, the greatest decrease (53.5 %) was observed in cv. Sagittoria and the lowest decrease (27.4 %) was observed in line 8 (Figure 1). Present findings comply with the results of Almonsouri et al. (2001) reporting decreasing germination percentage and germination speed of durum wheat genotypes with increasing PEG concentrations. Similar results were also reported for germination of pea seeds exposed to PEG-induced drought stress (Okçu et al., 2005).

G, PEG and G x PEG interaction were significant (P<0.01) for emergence rate (ER). ER values decreased with increasing PEG concentrations. Present findings on ER were similar with the findings of Almonsouri et al. (2001) for durum wheat. The highest ER (100 %) was observed in control treatment of cv. Karatopak and the lowest ER (5 %) was observed 1.2 MPa treatment of line 8. When the control

and the highest doses of PEG were compared for ER, it was observed that cv. Sagittoria was the most susceptible and line 1 was the most tolerant genotype to increasing drought stress (Figure 1).

PEG and G x PEG interaction were highly significant ($p < 0.01$) whereas G was significant ($p < 0.05$) for germination index (GI), (Table 1). The highest GI (23.0) was determined in control treatment of cv. Sagittoria, while the lowest (5.2) GI was determined in 1.2 MPa treatment of cv. Sagittoria (Table 1). Increased PEG doses resulted in decreased GI. GI of cultivars was more affected from increasing PEG doses as compared to the lines. High value of GI shows the high seed quality and better performance in seedling growth of crops (Wang et al., 2004). In this sense, it could be concluded that lines had more vigorous germination as compared to the cultivars (Table 1).

G, PEG and G x PEG interaction were significant ($P < 0.01$) for emergence index (EI), (Table 1). The greatest EI (9.5) was obtained from the control treatment of cv. Sagittoria. Increased PEG doses decreased EI of genotypes. When the EI values of the control and -1.2 MPa treatments compared, the lowest decrease (79 %) was obtained from line 1 and the highest decrease (more than 95 %) was obtained from the other genotypes (Table 1). Line 1 seemed to have the most powerful emergence vigor as compared to the other genotypes.

G was significant ($p < 0.05$), PEG was highly significant ($p < 0.01$) and G x PEG was insignificant for mean germination time (MGT) (Table 1). Increased PEG doses resulted in extended MGT of the genotypes. Sagittoria, Karatopak and line 1 were lower germinated genotypes and the lines 8 and lines 20 were the faster germinated genotypes. Present findings were similar to finding of Balkan and Gençtan (2013) proposing extended MGT of wheat genotypes with increasing PEG doses.

G were not significant whereas PEG were highly significant ($p < 0.01$) and G x PEG interaction were significant ($p < 0.05$) for mean emergence time (MET). Increased PEG doses caused a remarkable increase in MET. In control treatment, MET of genotypes was 3.2 day and this time was extended to 6.1 day in -1.2 MPa treatments (Table 1).

G, PEG and G x PEG interaction were highly significant ($P < 0.01$) for shoot length (SL) in germination experiment. The reason of G x PEG interaction was no shoot formation in higher osmatic potential of PEG than -0.6 MPa in all genotypes except line 1. Even line 20 did not give any shoot formation in -0.6 MPa. Generally, SL of genotypes shortened as the PEG concentration increased. The highest SL (14.5 cm) was observed in the control treatment of line 20 and the lowest SL (0.7) was observed in -1.2 MPa treatment of line 1. Only line 1 survived and performed SL in -1.2 MPa PEG treatments. Line 20 did not even survive in -0.6 MPa PEG concentration. When the osmotic potential of growth media increased, SL of wheat genotypes shortened (Gençtan and Sağlam, 1988; Almansouri et al., 2001; Dhanda et al., 2004; Balkan and Gençtan, 2013).

G, PEG and G x PEG interaction were highly significant ($P < 0.01$) for seedling length (SLe) in seedling emergence experiment. Generally, SLe of genotypes shortened as the PEG concentration increased. The longest SLe (25.2 cm) was observed in line 20 and it was followed by cv. Sagittoria (23.9 cm). All genotypes did survive and gave SLe in -1.2 MPa treatments, but cv. Karatopak did not. It was reported that when the osmotic potential of growth media lower than -1.0 MPa, SLe of wheat seedlings were negatively affected (Sayar et al., 2010; Balkan and Gençtan, 2013).

G, PEG and G x PEG interaction were highly significant ($P < 0.01$) for root length (RL) in germination experiments. Generally, SL of genotypes diminished as the PEG concentration increased. The longest RL (25.6 cm) was observed in line 20 and it was followed by the cv. Sagittoria (23.8 cm). Only line 1 survived and performed RL in -1.2 MPa treatments. Line 20 did not even survive and showed RL in -0.6 MPa PEG concentrations. Since some genotypes did not survive and did not have formation at higher osmatic potential of PEG than -0.6 MPa, G x PEG interaction was found to be significant. Some researchers reported that when the osmotic potential of growth environment went under -1.0 MPa, RL of wheat seedlings was negatively affected (Sayar et al., 2010; Balkan and Gençtan, 2013).

G were significant ($p < 0.05$), PEG and G x PEG interaction were highly significant ($P < 0.01$) for root length (RLe) in seedling emergence experiments. Generally, RLe of genotypes decreased as the osmotic potential increased. The longest RLe (16.9 cm) was observed in cv. Sagittoria and it was followed by cv. Karatopak (16.5 cm). All genotypes did survive and gave RLe in -1.2 MPa treatments, but cv. Karatopak did not.

G, PEG and G x PEG interaction were highly significant ($P < 0.01$) for shoot fresh weight (SFW) in germination experiments. SFW of genotypes decreased as the PEG concentration increased. Lowest

SFW (35.5 mg/plant) was observed in line 1. Only line 1 survived and performed SFW in -1.2 MPa treatments. Line 20 did not even survive and gave SFW in -0.6 MPa PEG osmatic potential.

G, PEG and G x PEG interaction were highly significant (P<0.01) for seedling fresh weight (SFWe) in seedling emergence experiments. SFWe of the genotypes decreased as the PEG concentration increased. All genotypes did survive and gave SFWe in -1.2 MPa treatments, but cv. Karatopak did not.

G, PEG and G x PEG interaction were highly significant (P<0.01) for germination vigor index (GVI) in germination experiments. GVI of genotypes decreased as the PEG doses increased. The highest GVI (3805) was observed in the control treatment of line 20. But, line 20 did not survive in the other PEG treatments. Only the line 1 survived and performed GVI in -1.2 MPa treatments.

G, PEG and G x PEG interaction were highly significant (P<0.01) for emergence vigor index and (EVI) in seedling emergence experiments. EVI of genotypes decreased as the osmatic potential of PEG increased. All the genotypes did give emergence at all osmatic potentials, but Karatopak did not yield any emergence in the osmatic potential of -1.2 MPa.

As to in conclusion; generally G x PEG interactions were observed in terms of germinations and seedling characteristics of the examined bread wheat genotypes. Depending on the genotypes, all the genotypes germinated and did perform seedling growth in all PEG concentrations, but some genotypes did not survive or perform any shoot or root at higher osmatic potentials of PEG than -0.6 MPa. Line 20 cannot survive higher osmatic potential of -0.6 MPa as compared to other genotypes. Line 1 seemed to higher tolerance to drought stress then other genotypes. PEG-6000 was useful agent to generate drought stress in germination and seedling growth of bread wheat, but osmotic potentials lower than -1.2 MPa could be applied to better determine the drought stress tolerance of the genotypes.

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