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Effect of Putrescine Application and Drought Stress on Germination of Wheat (*Triticum aestivum* L.)

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ABSTRACT: One of the important food and essential crops is wheat (*Triticum aestivum* L.). Drought stress as a major detrimental factor leads to ultimately reduce crop yield in arid and semiarid regions. In order to study the effects of different concentrations of putrescine on the germination stage of wheat under drought stress, this experiment was carried out as factorial based on completely randomized design with four replications. Final germination percentage (FGP), mean germination time (MGT), coefficient velocity germination (CVG), rate index (RI) germination rate index (GRI) and seedling vigour index (SVI) data were obtained. Analysis of variance indicated that putrescine, osmotic potential and their interaction were significant for the trait mentioned above. When PEG 6000 increases. FGP, CVG, RI, GRI and SVI significantly decreased, whereas the MGT increased. In addition, putrescine, particularly the concentration of 1 mM, decreased the adverse effect of drought created by PEG 6000. In other words, it elevated the rate of FGP, CVG, RI, GRI and SVI, and lowered MGT. The results suggested that putrescine increased the osmotic stress tolerance of wheat germination stage.

Key Words: Drought, germination, putrescine, wheat

Buğdayın (*Triticum aestivum* L.) Çimlenmesine Kuraklık Stresi ve Putresin Uygulamasının Etkisi

ÖZET: Buğday (*Triticum aestivum* L.) dünyada insanların beslenmesinde en önemli besin kaynaklarından biridir. Kuraklık stresi, kurak ve yarı kurak bölgelerde verimde çok önemli düşüşlere neden olmaktadır. Bu araştırma, kuraklık stresinde putresinin farklı konsantrasyonlarının buğdayın tohum çimlenmesi üzerine olan etkilerini belirlemek amacıyla yapılmıştır. Araştırma, tam şansa bağlı deneme planda faktöriyel düzende 4 tekrarlı olarak yürütülmüştür. Denemede söz konusu uygulamaların çimlenme oranı (FGP), ortalama çimlenme zamanı (MGT), çimlenme gücü katsayısı (CVG), hız indeksi (RI), çimlenme hızı indeksi (GRI) ve fide gücü indeksi (SVI) verileri alınmıştır. İncelenen bu özellikler üzerine kuraklığın. putresinin ve bunlara ait interaksiyonun etkisi çok önemli olmuştur. Araştırmada PEG 6000'in konsatrasyonu, diğer bir ifadeyle kuraklığın şiddeti artıkça FGP, CVG, RI, GRI ve SVI çok önemli derecede azalmış, buna karşın MGT uzamıştır. Diğer taraftan, putresinin özellikle 1 mM'lık konsantrasyonu, Polyethylene glycol (PEG) 6000 ile oluşturulan kuraklığın olumsuz etkilerini azaltmıştır. Diğer bir ifadeyle, çimlenme oranını, kök ve sürgün uzunluğunu artırmış; çimlenme süresini ise kısaltmıştır. Sonuç olarak, putresin buğdayın çimlenme döneminde ozmotik strese toleransı artırdığı gözlenmiştir.

Anahtar Kelimeler: Kuraklık, çimlenme, putresin, buğday

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is a main crop global and is grown on about 713 million hectare in a range of agricultural region (FAO, 2013). Drought stress is the most significant environmental stress in agriculture globally and thus improved yields under water limitation are targeted increasingly in plant breeding studies (Cattivelli et al., 2008). Drought is one of the most important problems for agriculture universally. During growth and development, it restricts nutrient uptake and reduces metabolism, which is reflected in reduced crop quality and yield. It is one of the main causes of crop reduce worldwide, which commonly reduces the average yield for many crop plants. Reaction to drought stress are specific and genotype dependent (De Leonardis et al., 2007). Also, the nature response of plants of drought is influenced by the severity and duration of water loss (Pinheiro and Chaves, 2011), the stage and age of development at the point of drought exposure (De Leonardis et al., 2007), as well as the cell type and organ experiencing water loss (Pastori and Foyer, 2002).

Polyethylene glycol is used widely in laboratory experiments because of its simulating environmental conditions (Kaufman and Eckard, 1971). PEG has high molecular weight, it cannot be transmitted through cell wall and used for regulating water potential in germination laboratories. Germination rate is major index for the evaluation of drought tolerance, generally in stress condition genotype with high germination rate, and has more chance for growth. Duman (2006) reported that drought stress decrease the germination percent, root length and shoot length. Kumar and Singh (1998) state that germination parameters obtained in low water potential are accepted as an effective method in the determination of drought resistance genotypes. Wheat genotypes with high seedling vigour index and root length are able to germinate and form seedling within 10 bar PEG 6000 solution as drought-tolerant genotypes (Dhanda et al., 2004).

Polyamines (PAs) are ubiquitous polycationic compounds that mediate essential aspects of cell growth, differentiation and cell death in the organisms. Generally occurring PAs in higher plant include; putrescine, spermidine and spermine which are protonated at cytoplasmic pH. PAs are group of phytohormone like aliphatic amine natural compounds with aliphatic nitrogen structure and present in almost all living organisms including plants (Gill and Tuteja, 2010). Exogenous application of polyamines improved tolerance against several abiotic stresses (Amri and Mohammadi, 2012; Nayyar et al., 2005; Sharma et al., 1997; Yang et al., 2007). Positive response of exogenously applied polyamines has been reported in olive, rice, soybean, alfalfa and pomegranate

The purpose of this study was to investigate the effects of the putrescine on germination indexes of wheat under drought stress.

MATERIAL AND METHODS

Plant material and experimental trial

In order to evaluate the response of polyamine and drought stress, four bread wheat genotypes (*Triticum aestivum* L.) namely; cv. Kırmızı Kılçık, Hawk, Pehlivan and Müfitbey were used. The experiment was carried out in seed technology laboratory of Ataturk University as factorial experiment with completely randomized design of four replications. The factors included four levels of putrescine (0.01, 0.1 and 1 mM and distilled water as control) and six levels of PEG 6000 osmotic potential (-2, -4, -6, -8 and -10 bar and distilled water as control). The genotypes were exposed to different concentrations of putrescine for 24 hours at room temperature. Different osmotic potentials were prepared according to Michel and Kaufmann (1973) in order to dissolve the required amount of PEG in distilled water at 25°C. seeds were surface sterilized in 70% (v/v) ethanol for 3 min, rinsed twice with sterile distilled water, incubated further in commercial bleach (5% sodium hypochlorite) for 25 minute, and rinsed twice in sterile distilled water. In this study, 25 seeds from each genotype were germinated on two layers of filter paper in 9-cm petri dishes with respective treatment from PEG 6000 solution. The petri dishes covered to prevent the loss of moisture by evaporation were kept in 16:8 h light: dark photoperiod and germinated at 25±1 °C for 10 days.

Computation and data analysis

Germinated seeds were counted daily at the specified time and this task continued for 10 consecutive days. Seeds with at least 1 mm radicle length were considered germinated.

At the end of this period, parameters including final germination percentage (FGP), mean germination time (MGT), coefficient velocity germination (CVG), rate ındex (RI), germination rate index (GRI) and seedling vigour index (SVI) were conclude.

FGP is the final germination percent

$$FGP = Ng/N_1 \times 100$$

Where Ng is total germinated seeds, N_1 is the total number of seeds tested (Czabator, 1962).

Means time to germination is an index for germination rate (Ellis and Roberts, 1981).

$$MTG = \Sigma(nT)/\Sigma n$$

Where ‘n’ is number of germinated seeds in day T. Σn is total germinated seeds. T day of counting.

Coefficient of the velocity of germination is an index for germination speed (Maguire, 1962).

$$CVG = \sum Ni/\sum NiTi \times 100$$

Where Ti is the number of days from the start of the experiment and Ni is the number of germinated seeds per day (Maguire, 1962).

Rate Index (RI) was calculated as described in the Association of Official Seeds Analysts by the following formula (AOSA, 1983):

RI=number of germinated seed/Days of first count +…..+ no. of germinated seed / Days of final count.

Speed of germination is an index for the germination rate (GRI) was calculated according to the equation:

$$GR = \Sigma(N_i)/(T_i)$$

Where N_i is the number of germinated seeds per day and T_i. is the number of days from the start of the experiment (Maguire, 1962).

Seedling Vigour Index (SVI) was assessed using the equation (Abdul-Baki and Anderson, 1970)

SVI= (mean of shoot length + mean of radicle length) × total germination percentage

Analysis of variance carried out by SAS/PC statistical program was used for all computations (SAS, 1996) and Least Significant Difference (LSD) tests were used to measure the statistical differences between treatment methods and controls ($P < 0.01$).

RESULTS

Final Germination Percent (FGP)

The changes in final germination percentages are accepted as an important germination indicator under stress condition. With regard to germination percentages, analysis of variance (Table 1) showed that there were significant differences among genotypes, different levels of PEG 6000 and putrescine based on FGP. The results of mean comparison of FGP for genotypes showed that the highest mean FGP was observed in Hawk genotype with 97.75 %, whereas the lowest FGP was in Pehlivan genotype with 95.54% (Table 2). Based on drought application, control (0 concentration) treatment gave the highest mean FGP (99.44%), whereas the lowest mean FGP was observed in the treatment of -10 bar with 94.63% (Table 2). When means comparison for the highest FGP at different levels of putrescine treatments was considered. 1mM application resulted in 98.21% which was higher than the control (no Putrescine) with 96.25% (Table 2). With the increased, putrescine application from 0.01 to 1 mM. FGP also increased.

Analysis of variance indicated that there were not any significant two and three way interactions between drought application, genotype and putrescine (Table 1). Responses of different genotype to drought stress varied. Each genotype has different response to drought application which makes genotype × drought interaction significant. The highest FGP (100%) was observed in Pehlivan and Hawk genotypes with control (0 bar) treatment. Whereas, the lowest FGP (92.75) was Pehlivan with -10 bar. There was a slight increase of FGP in control (0 bar) treatment. However, in the other PEG 6000 treatment (-2, -4, -6, -8 and -10 bar), there was a trend that FGP was decreasing while osmotic potential simulated by PEG 6000 was increasing (Table 2).

Although the interaction between drought × putrescine was not significant, putrescine treatments had positive effect reducing the effect of drought. Genotypes gave different response to putrescine levels for the trait mentioned above. In all genotypes, putrescine treatments increased the FGP. The highest average FGP (98.33%) was in Kırmızı kılçık genotype with the application of 1 mM putrescine hormone while the lowest average FGP (94%) was in Pehlivan genotype with 0 and 0.01 mM putrescine concentrations. As a result, an increase in the application of putrescine concentrations resulted in the increase of FGP.

Table 1. Analysis of variance of different drought levels and putrescine at the germination stage of four genotypes of wheat

Sources of Variation	FGP	MGT	CVG	RI	GRI	SVI
F value (Drought)	15.76**	679.64**	533.23**	15.76**	1295.58**	2611.14**
F value (Genotype)	7.44**	223.79**	134.73**	7.44**	556.72**	552.47**
F value (Putrescine)	5.20**	20.97**	27.04**	5.20**	112.53**	55.31**
F value (D×G)	1.27	38.54**	13.70**	1.27	42.25**	49.61**
F value (D×P)	0.64	2.05*	2.07*	0.64	8.78**	10.56**
F value (G×P)	1.15	10.64**	8.85**	1.15	18.58**	1.27
F value (D×P×G)	0.49	1.62*	1.94**	0.49	6.94**	2.04**
LSD (Drought)	1.674	0.176	3.949	0.0076	0.6125	66.477
LSD (Genotype)	1.367	0.1437	3.225	0.0062	0.5	54.278
LSD (Putrescine)	1.367	0.1437	3.225	0.0062	0.5	54.278
LSD (D×G)	-	0.351	7.90	-	1.225	132.954
LSD (D×P)	-	0.267	5.99	-	1.225	132.954
LSD (G×P)	-	0.218	6.45	-	1	-
LSD (D×P×G)	-	0.534	15.80	-	2.45	265.90

*. **: Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Table 2. Mean comparison of different drought levels and putrescine of germination indices parameters of four genotypes of wheat

Parameters	Drought (bar)	0 mM Putrescine					0.01 mM Putrescine					0.1 mM Putrescine					1mM Putrescine					Putrescine Mean				
		1*	2*	3*	4*	Mean	1	2	3	4	Mean	1	2	3	4	Mean	1	2	3	4	Mean	1	2	3	4	Mean
Final Germination Percentage (FGP)	0	100.00	100.00	100.00	97.00	99.25	100.00	100.00	100.00	97.00	99.25	100.00	100.00	100.00	99.00	99.75	99.00	100.00	100.00	99.00	99.50	99.75	100.00	100.00	98.00	99.44
	-2	99.00	97.00	99.00	98.00	98.25	98.00	98.00	94.00	98.00	97.00	99.00	100.00	98.00	98.00	98.75	100.00	100.00	99.00	99.00	99.50	99.00	98.75	97.50	98.25	98.38
	-4	97.00	98.00	96.00	98.00	97.25	99.00	97.00	93.00	98.00	96.75	99.00	99.00	95.00	98.00	97.75	100.00	99.00	99.00	98.00	99.00	98.75	98.25	95.75	98.00	97.69
	-6	96.00	97.00	90.00	98.00	95.25	97.00	97.00	93.00	96.00	95.75	98.00	98.00	95.00	98.00	97.25	100.00	99.00	98.00	98.00	98.75	97.75	97.75	94.00	97.50	96.75
	-8	94.00	96.00	90.00	96.00	94.00	96.00	97.00	91.00	99.00	95.75	94.00	95.00	95.00	97.00	95.25	95.00	97.00	97.00	98.00	96.75	94.75	96.25	93.25	97.50	95.44
	-10	93.00	96.00	89.00	96.00	93.50	96.00	95.00	93.00	97.00	95.25	95.00	95.00	94.00	92.00	94.00	96.00	96.00	95.00	96.00	95.75	95.00	95.50	92.75	95.25	94.63
	Mean	**96.50**	**97.33**	**94.00**	**97.17**	**96.25**	**97.67**	**97.33**	**94.00**	**97.50**	**96.63**	**97.50**	**97.83**	**96.17**	**97.00**	**97.13**	**98.33**	**98.50**	**98.00**	**98.00**	**98.21**	**97.50**	**97.75**	**95.54**	**97.42**	**97.05**
Mean Germination Time (MGT)	0	1.14	1.10	1.16	1.09	1.12	1.11	1.11	1.13	1.08	1.11	1.12	1.14	1.17	1.11	1.14	1.11	1.11	1.07	1.04	1.08	1.12	1.12	1.13	1.08	1.11
	-2	1.15	1.43	2.25	1.23	1.51	1.12	1.22	1.53	1.22	1.27	1.12	1.46	1.25	1.23	1.27	1.08	1.12	1.05	1.16	1.10	1.12	1.31	1.52	1.21	1.29
	-4	1.17	1.27	2.35	2.42	1.80	1.18	1.45	2.37	2.29	1.83	1.22	1.65	1.62	2.49	1.74	1.10	1.59	1.15	2.09	1.48	1.17	1.49	1.87	2.32	1.71
	-6	1.47	2.91	3.61	2.84	2.71	1.31	2.44	2.90	2.75	2.35	2.08	2.34	2.19	2.79	2.35	1.30	2.56	1.67	2.77	2.07	1.54	2.56	2.59	2.79	2.37
	-8	1.64	3.83	3.99	4.28	3.44	1.78	2.79	4.09	4.23	3.22	2.07	2.85	2.81	4.26	3.00	1.46	2.87	2.36	3.42	2.53	1.74	3.08	3.32	4.05	3.05
	-10	2.85	4.26	4.70	6.26	4.52	2.45	4.65	4.74	6.12	4.49	2.47	4.70	4.19	6.35	4.43	2.46	4.51	3.65	6.36	4.25	2.55	4.53	4.32	6.27	4.42
	Mean	**1.57**	**2.47**	**3.01**	**3.02**	**2.52**	**1.49**	**2.28**	**2.79**	**2.95**	**2.38**	**1.68**	**2.36**	**2.21**	**3.04**	**2.32**	**1.42**	**2.29**	**1.83**	**2.81**	**2.09**	**1.54**	**2.35**	**2.46**	**2.95**	**2.33**
Coefficient Velocity Germination (CVG)	0	87.80	91.06	86.46	91.76	89.27	90.11	90.56	89.55	92.41	90.66	89.34	87.95	85.49	89.84	88.16	90.19	90.56	93.48	96.12	92.59	89.36	90.03	88.75	92.53	90.17
	-2	87.83	70.05	46.06	82.07	71.50	89.26	81.87	65.62	81.78	79.63	89.67	69.36	81.11	81.58	80.43	92.72	89.57	95.19	86.24	90.93	89.87	77.71	72.00	82.92	80.62
	-4	85.48	80.12	42.62	42.29	62.63	85.48	68.90	42.55	44.12	60.26	82.59	60.90	62.65	40.90	61.76	91.17	63.79	86.93	48.56	72.61	86.18	68.43	58.69	43.97	64.32
	-6	70.42	35.09	27.75	35.48	42.18	76.64	41.24	34.90	36.53	47.33	57.30	42.80	46.38	36.52	45.75	77.09	39.18	62.58	47.49	56.59	70.36	39.58	42.90	39.00	47.96
	-8	64.38	26.50	25.18	23.40	34.87	58.27	36.27	24.50	23.73	35.69	55.58	35.45	35.80	23.84	37.67	69.46	35.75	42.40	43.81	47.85	61.93	33.49	31.97	28.69	39.02
	-10	35.86	23.57	21.36	16.23	24.26	41.87	21.72	21.45	16.39	25.36	40.79	21.30	23.87	15.77	25.43	41.89	22.22	27.71	15.77	26.90	40.10	22.20	23.60	16.04	25.48
	Mean	**71.96**	**54.40**	**41.57**	**48.54**	**54.12**	**73.61**	**56.76**	**46.43**	**49.16**	**56.49**	**69.21**	**52.96**	**55.88**	**48.08**	**56.53**	**77.09**	**56.84**	**68.05**	**56.33**	**64.58**	**72.97**	**55.24**	**52.98**	**50.53**	**57.93**
Rate Index (RI)	0	0.4545	0.4545	0.4545	0.4409	0.4511	0.4545	0.4545	0.4545	0.4409	0.4511	0.4545	0.4545	0.4545	0.4500	0.4534	0.4500	0.4545	0.4545	0.4500	0.4523	0.4534	0.4545	0.4545	0.4455	0.4520
	-2	0.4500	0.4409	0.4500	0.4455	0.4466	0.4455	0.4455	0.4273	0.4455	0.4409	0.4500	0.4545	0.4455	0.4455	0.4489	0.4545	0.4545	0.4500	0.4500	0.4523	0.4500	0.4489	0.4432	0.4466	0.4472
	-4	0.4409	0.4455	0.4364	0.4455	0.4420	0.4500	0.4409	0.4227	0.4455	0.4398	0.4500	0.4500	0.4318	0.4455	0.4443	0.4545	0.4500	0.4500	0.4455	0.4500	0.4489	0.4466	0.4352	0.4455	0.4440
	-6	0.4364	0.4409	0.4091	0.4455	0.4330	0.4409	0.4409	0.4227	0.4364	0.4352	0.4455	0.4455	0.4318	0.4455	0.4420	0.4545	0.4500	0.4455	0.4455	0.4489	0.4443	0.4443	0.4273	0.4432	0.4398
	-8	0.4273	0.4364	0.4091	0.4364	0.4273	0.4364	0.4409	0.4136	0.4500	0.4352	0.4273	0.4318	0.4318	0.4409	0.4330	0.4318	0.4409	0.4409	0.4455	0.4398	0.4307	0.4375	0.4239	0.4432	0.4338
	-10	0.4227	0.4364	0.4045	0.4364	0.4250	0.4364	0.4318	0.4227	0.4409	0.4330	0.4318	0.4318	0.4273	0.4182	0.4273	0.4364	0.4364	0.4318	0.4364	0.4352	0.4318	0.4341	0.4216	0.4330	0.4301
	Mean	**0.4386**	**0.4424**	**0.4273**	**0.4417**	**0.4375**	**0.4439**	**0.4424**	**0.4273**	**0.4432**	**0.4392**	**0.4432**	**0.4447**	**0.4371**	**0.4409**	**0.4415**	**0.4470**	**0.4477**	**0.4455**	**0.4455**	**0.4464**	**0.4432**	**0.4443**	**0.4343**	**0.4428**	**0.4411**
Germination Rate Index (GRI)	0	23.55	23.65	23.60	22.50	23.33	24.69	23.90	23.49	21.88	23.49	21.63	23.57	24.16	23.18	23.13	23.75	24.08	22.60	22.23	23.16	23.41	23.80	23.46	22.45	23.28
	-2	22.49	19.16	16.69	11.43	17.44	24.50	22.38	19.53	20.43	21.71	23.90	23.58	22.55	20.73	22.69	23.77	20.16	20.55	22.43	21.73	23.66	21.32	19.83	18.75	20.89
	-4	23.05	14.21	12.60	13.58	15.86	23.69	17.29	14.36	18.91	18.56	24.75	20.75	20.65	13.46	19.90	23.48	21.23	18.85	15.56	19.78	23.74	18.37	16.61	15.38	18.53
	-6	22.36	7.69	8.66	7.61	11.58	22.38	10.97	10.83	11.91	14.02	21.67	8.93	17.85	8.89	14.33	22.31	11.78	17.20	15.49	16.69	22.18	9.84	13.63	10.97	14.16
	-8	16.36	6.95	7.38	7.08	9.44	21.31	10.42	6.57	7.45	11.44	17.18	11.41	12.04	6.14	11.69	20.31	10.46	11.48	8.32	12.64	18.79	9.81	9.37	7.25	11.30
	-10	6.86	4.63	6.29	3.53	5.33	13.92	6.28	6.42	5.73	8.09	14.41	6.33	9.75	4.24	8.68	12.51	6.28	7.61	5.42	7.96	11.93	5.88	7.52	4.73	7.51
	Mean	**19.11**	**12.71**	**12.54**	**10.95**	**13.83**	**21.75**	**15.21**	**13.53**	**14.38**	**16.22**	**20.59**	**15.76**	**17.83**	**12.77**	**16.74**	**21.02**	**15.67**	**16.38**	**14.91**	**16.99**	**20.62**	**14.84**	**15.07**	**13.25**	**15.95**
Seedling Vigourity Index (SVI)	0	2381.75	2076.50	1787.50	1963.08	2052.21	2705.50	2626.75	1883.50	1925.75	2285.38	2763.75	2827.25	2273.50	2207.45	2517.99	2777.46	2881.00	2470.50	2541.46	2667.61	2657.12	2602.88	2103.75	2159.44	2380.79
	-2	2261.19	1244.33	1329.75	1434.72	1567.50	2206.85	1203.00	1102.67	1355.43	1466.99	2377.22	1748.23	1309.35	1465.08	1724.97	2552.75	2058.25	1399.29	1864.49	1968.70	2349.50	1563.45	1285.27	1529.93	1682.04
	-4	1670.74	790.25	348.63	713.72	880.84	1746.22	683.32	363.16	472.17	816.22	1743.01	757.36	373.88	563.95	859.55	2140.75	1016.09	459.22	775.05	1097.78	1825.18	811.76	386.22	631.22	913.60
	-6	1281.91	305.94	9.00	139.57	434.11	1091.86	112.68	159.74	125.63	372.48	1314.05	164.23	164.18	206.88	462.34	1431.25	333.32	218.26	217.50	550.08	1279.77	229.04	137.80	172.40	454.75
	-8	449.68	108.19	9.00	103.92	167.70	494.50	106.63	9.50	117.55	182.05	533.88	108.14	9.10	131.69	195.70	610.93	118.10	9.70	125.35	216.02	522.25	110.27	9.33	119.63	190.37
	-10	9.30	9.60	8.90	9.60	9.35	9.50	9.50	9.40	9.20	9.40	9.60	9.50	9.30	9.70	9.53	9.60	9.60	9.50	9.60	9.58	9.50	9.55	9.28	9.53	9.46
	Mean	**1342.43**	**755.80**	**582.13**	**727.44**	**851.95**	**1375.74**	**790.31**	**588.00**	**667.62**	**855.42**	**1456.92**	**935.79**	**689.89**	**764.13**	**961.68**	**1587.12**	**1069.39**	**761.08**	**922.24**	**1084.96**	**1440.55**	**887.82**	**655.27**	**770.36**	**938.50**

***1. Kırmızı kılçık 2. Hawk 3. Pehlivan and 4. Müfitbey, respectively.**

Means Time to Germination (MGT)

In the present study, analysis of variance (Table 1) showed that there were significant differences among genotypes, different levels of PEG 6000 and putrescine based on MGT. The results of mean comparison of MGT for genotypes indicated that the highest mean MGT was observed in Müfitbey genotype with 2.95 days, whereas the lowest MGT was in Kırmızı kılçık genotype with 1.54 days (Table 2). Based on the drought application, -10 bar treatment gave the highest mean MGT (4.42 day), whereas the lowest mean MGT was observed in the treatment of control with 1.11 days (Table 2). When means comparison for the lowest MGT at different levels of putrescine treatments was considered, 1mM application of putrescine resulted in 2.09 days which is higher than the control (no putrescine) with 2.52 days (Table 2). With the increased putrescine application from 0.01 to 1 mM, MGT also decreased.

Analysis of variance showed that there were significant two-way interactions between drought × genotype ($P \leq 0.01$), drought × putrescine ($P \leq 0.05$) and genotype × putrescine ($P \leq 0.01$) (Table 1). Responses of different genotype to drought stress varied. Each genotype has different response to drought application which makes genotype × drought interaction significant. The highest MGT (6.27 day) was observed in Müfitbey genotype with -10 bar treatment. Whereas, the lowest MGT (1.12 day) was in Kırmızı kılçık and Hawk with control (0 bar). There was a clear increase of MGT with the increased PEG 6000 concentration treatment. There was a trend that MGT was increasing while osmotic potential simulated by PEG 6000 was increasing (Figure 1a).

Although the interaction between drought × putrescine was significant, putrescine treatments has positive effect reducing the effect of drought. When concentration of putrescine increased, the adverse effect of drought simulated by PEG 6000 application decreased. Application of the particular combination concentrations of 1 mM putrescine and control (0 bar) PEG 6000 concentration to compare other different level combinations of putrescine and PEG 6000 applications resulted in the lowest (1.08 day) means time to germination formation. It was also observed that with the increasing concentration of putrescine from 0.01 mM to 1 mM in seed application, there was a significant decrease in the effect of PEG 6000 (Figure 1b).

Genotypes gave different responses to putrescine levels for the trait mentioned above. In all genotypes, putrescine treatments decreased the MGT. The highest average MGT (3.04 day) was in Müfitbey genotype with the application of 0.1 mM putrescine hormone while the lowest average MGT (1.42 day) was in Kırmızı kılçık genotype with 1 mM putrescine concentration. As a result, an increase in the application of putrescine concentrations resulted in the decrease of MGT (Figure 1c).

A significant three way interaction among drought × putrescine × genotype was observed significantly at the 0.05 level. The result verified that the highest MGT was in the combination of -10 bar PEG 6000 and 1 mM putrescine in Müfitbey genotype with 6.36 days, also the lowest MGT was in the combination of control (0 bar) PEG 6000 and 1 mM putrescine in Müfitbey genotype with 1.04 days (Figure 1d).

Coefficient of velocity of germination (CVG)

CVG is an index for germination speed. In the present study, analysis of variance (Table 1) indicated that there were significant differences among genotypes, different levels of PEG 6000 and putrescine based on CVG. The results of mean comparison of CVG for genotypes showed that the highest mean CVG was observed in Kırmızı kılçık genotype with 72.97, whereas the lowest CVG was in Müfitbey genotype with 50.53 (Table 2). Based on the drought application, control (0 bar) treatment gave the highest mean CVG (90.17), whereas the lowest mean CVG was observed in the treatment of -10 bar with 25.48 (Table 2). When means comparison for the highest CVG at different levels of putrescine treatments was considered, 1mM application of putrescine resulted in 64.58 which is higher than the control (no putrescine) with 54.12 (Table 2). With the increased, putrescine application from 0.01 to 1 mM. CVG also increased.

Analysis of variance presented that there were significant two-way interactions between drought × genotype ($P \leq 0.01$), drought × putrescine ($P \leq 0.05$) and genotype × putrescine ($P \leq 0.01$) (Table 1). Responses of different genotype to drought stress varied. Each genotype has different response to drought application which makes genotype × drought interaction significant. The highest CVG (92.53) was observed in Müfitbey genotype with control (0 bar) treatment. Whereas, the lowest CVG (16.04) was Müfitbey with -10 bar. There was a clear decrease of CVG with the increased PEG 6000 concentration treatment. There was a trend that CVG was decreasing while osmotic potential simulated by PEG 6000 was increasing (Figure 2a).

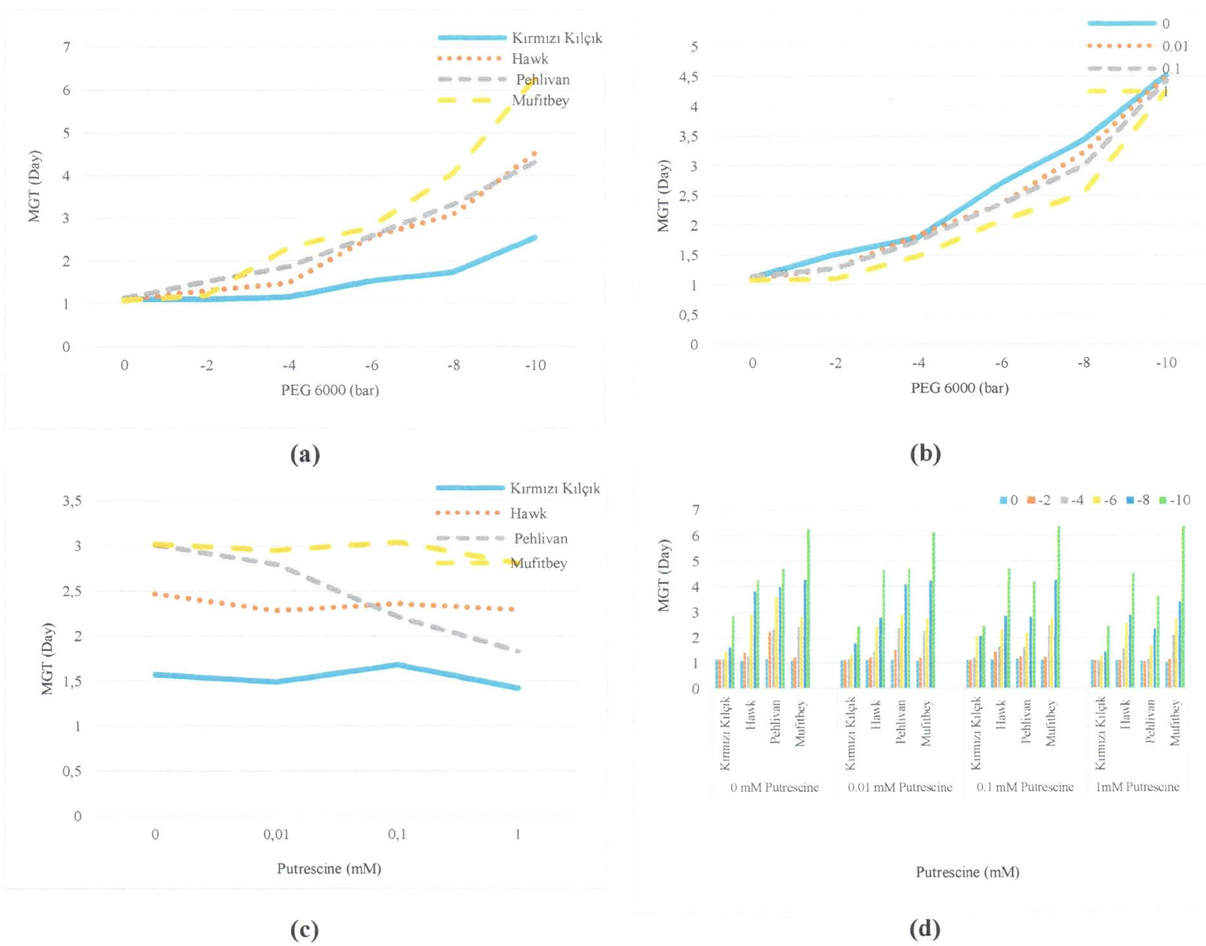

Figure 1. Means comparison of the interactions for MGT. a) genotype × drought. b) drought × putrescine c) genotype × putrescine and d) genotype × putrescine × drought

Although the interaction between drought × putrescine was significant, putrescine treatments has a positive effect reducing the effect of drought. When the concentration of putrescine increased, adverse effect of drought simulated by PEG 6000 application decreased. Application of the particular combination concentrations of 1 mM putrescine and control (0 bar) PEG 6000 concentration to compare the other different level combinations of putrescine and PEG 6000 applications resulted in the highest (92.59) coefficient of velocity of germination formation. It was also observed that with the increasing concentration of putrescine from 0.01 mM to 1 mM in seed application, there is a significant decrease in effect of PEG 6000 (Figure 2b).

Genotypes gave different responses to putrescine levels for the trait mentioned above. In all genotypes, putrescine treatments increased the CVG. The highest average CVG (77.09) was in Kırmızı kılçık genotype with the application of 1 mM putrescine hormone while the lowest average CVG (41.57) was in Pehlivan genotype with the control (0 putrescine) treatment. As a result, an increase in the application of putrescine concentrations resulted in the increase of CVG (Figure 2c).

A significant three way interaction among drought × putrescine × genotype was observed significantly at the

0.01 level. The result verified that the highest CVG was in the combination of control (0 bar) PEG 6000 and 1 mM putrescine in Müfitbey genotype with 96.12, also the lowest CVG was in the combination of highest PEG 6000 concentration (-10 bar) and 1 mM 0.1 and 1mM putrescine in Müfitbey genotype with 15.77 (Figure 2d).

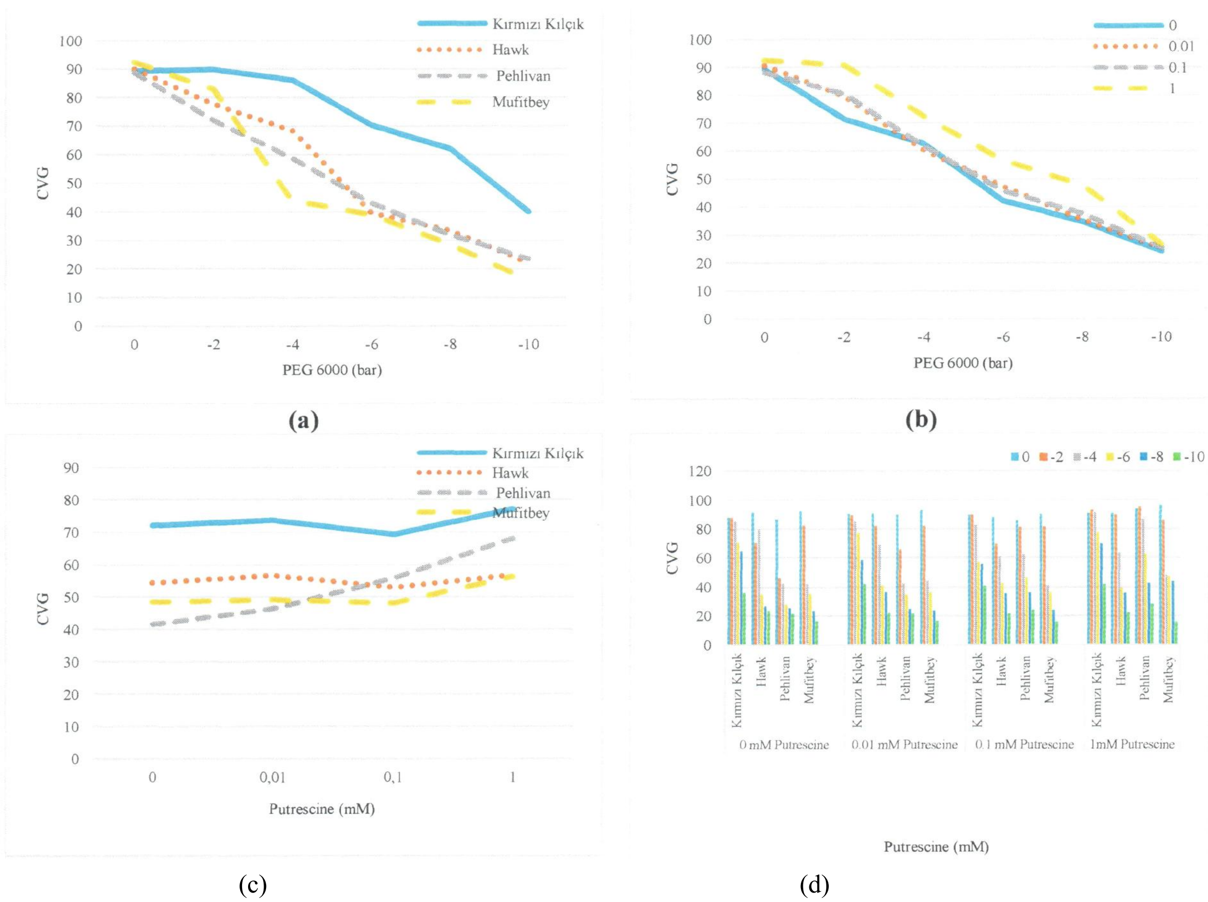

Figure 2. Means comparison of the interactions for CVG. a) genotype × drought. b) drought × putrescine c) genotype × putrescine and d) genotype × putrescine × drought

Rate Index (RI)

Analysis of variance (Table 1) showed that there were significant differences among genotypes, different levels of PEG 6000 and putrescine based on RI. The results of mean comparison of RI for genotypes indicated that the highest mean RI was observed in Hawk genotype with 0.4443, whereas the lowest RI was in Pehlivan genotype with 0.4343 (Table 2). Based on drought application, control (0 bar) treatment gave the highest mean RI (0.4520), whereas the lowest mean RI was observed in the treatment of -10 bar with 0.4301 (Table 2). When means comparison for the highest RI at different levels of putrescine treatments was considered 1mM application of resulted in 0.4464 which is higher than control (no putrescine) with 04375 (Table 2). While with increased putrescine application from 0.01 to 1 mM, RI was also increased.

Investigation of variance presented that there were not a significant two and three-way interactions between drought × genotype, drought × putrescine, genotype × putrescine and drought × putrescine × genotype (Table 1).

Germination Rate Index (GRI)

Analysis of variance (Table 1) showed that there were significant differences among genotypes, different levels of PEG 6000 and putrescine based on GRI. According to the results of mean comparison of GRI for the genotypes presented, the highest mean GRI was detected in Kırmızı kılçık genotype with 20, whereas the lowest GRI was in Hawk genotype with 14.84 (Table 2). Based on the drought application, control (0 bar) treatment gave the highest mean GRI (23.28), whereas the lowest mean GRI was observed in the treatment of -10 bar with 7.51 (Table 2). When means comparison for the highest GRI at different levels of putrescine treatments was considered. 1mM application of putrescine resulted in 16.99 which is higher than the control (no putrescine) with 13.83 (Table 2). With the increased putrescine application from 0.01 to 1 mM. GRI also increased.

Analysis of variance presented that there were significant two-way interactions between drought × genotype ($P \leq 0.01$), drought × putrescine ($P \leq 0.01$) and genotype × putrescine ($P \leq 0.01$) (Table 1). Responses of different genotype to drought stress varied. Each genotype has different response to drought application which makes genotype × drought interaction significant. The highest GRI (23.80) was observed in Hawk genotype with the control (0 bar) treatment. Whereas, the lowest GRI (4.73) was in Müfitbey with -10 bar. There was a clear decrease of GRI with the increased PEG 6000 concentration treatment. There was a trend that GRI was decreasing while osmotic potential simulated by PEG 6000 was increasing (Figure 3a).

Although the interaction between drought × putrescine was significant, putrescine treatments has a positive effect reducing the effect of drought. When the concentration of putrescine increased, the adverse effect of drought simulated by PEG 6000 application decreased. Application of the particular combination concentrations of 1 mM putrescine and control (0 bar) PEG 6000 concentration to compare the other different level combinations of putrescine and PEG 6000 applications resulted in the highest (23.16) germination rate index formation. It was also observed that with the increasing concentration of putrescine from 0.01 mM to 1 mM in seed treatment, there was a significant decrease in effect of PEG 6000 (Figure 3b).

Genotypes gave different responses to putrescine levels for the trait mentioned above. In all genotypes, putrescine treatments increased the GRI. The highest average GRI (21.02) was in Kırmızı kılçık genotype with the application of 1 mM putrescine hormone while the lowest average GRI (10.95) was in Müfitbey genotype with the control (0 putrescine) treatment. As a result, an increase in the application of putrescine concentrations resulted in the increase of GRI (Figure 3c).

A significant three way interaction among drought × putrescine × genotype was observed significantly at the 0.01 level. The result verified that the highest GRI was in the combination of control (0 bar) PEG 6000 and 1 mM putrescine in Kırmızı kılçık genotype with 23.77, also the lowest GRI was in the combination of highest PEG 6000 concentration (-10 bar) and 1 mM putrescine in Müfitbey genotype with 5.42 (Figure 3d).

Seedling Vigour Index (SVI)

With regard to analysis of variance, Table 1 showed that there were significant differences among genotypes, different levels of PEG 6000 and putrescine based on SVI. According to the results of mean comparison of SVI for the genotypes presented, the highest mean SVI was detected in Kırmızı kılçık genotype with 1440.55, whereas the lowest SVI was in Pehlivan genotype with 655.27 (Table 2). Based on the drought application, control (0 bar) treatment gave the highest mean SVI (2380.79), whereas the lowest mean SVI was observed in the treatment of -10 bar with 9.46 (Table 2). When means comparison for the highest SVI at different levels of putrescine treatments was considered 1mM application resulted in 1084.96 which was higher than the control (no putrescine) with 851.95 (Table 2). With the increased putrescine application from 0.01 to 1 mM. SVI also increased.

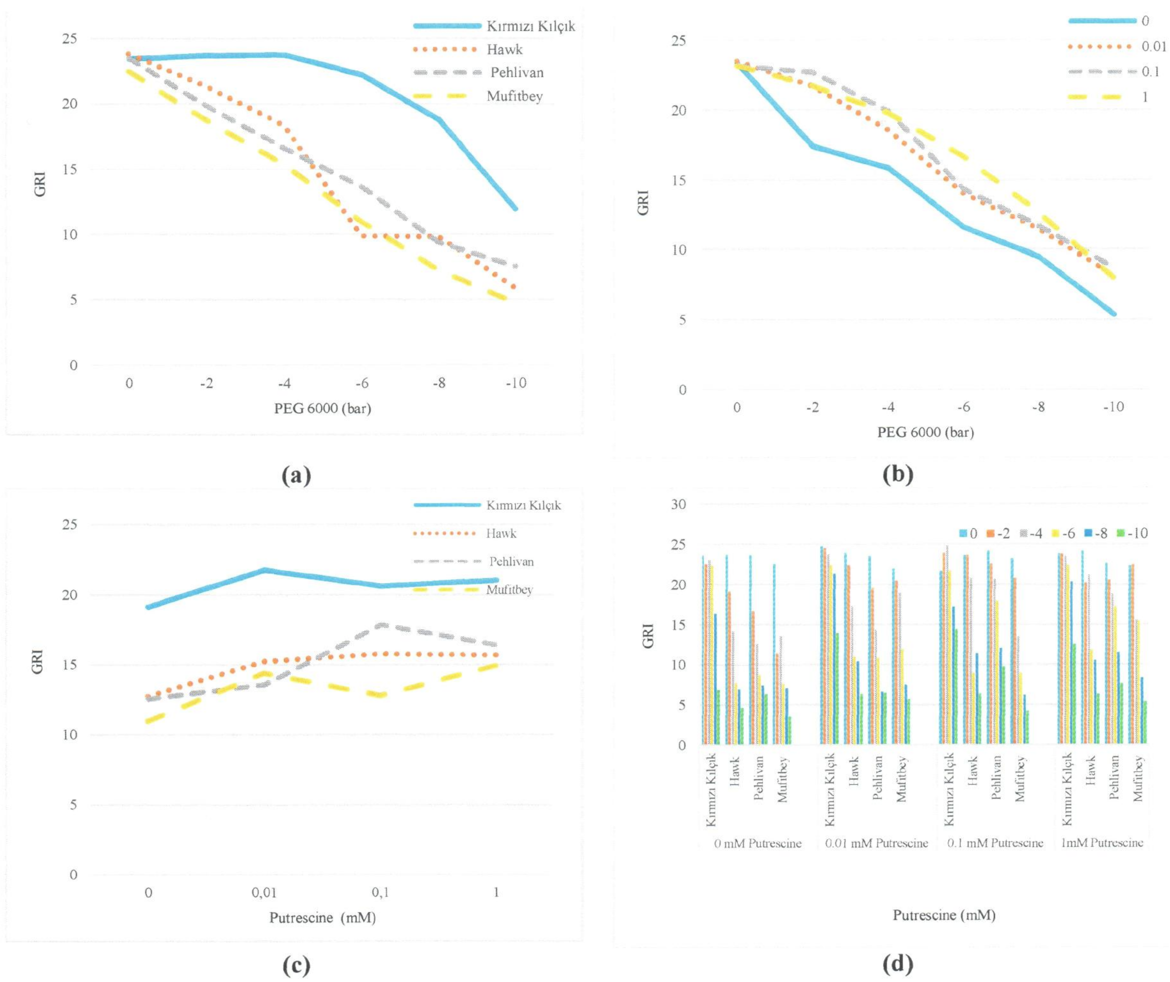

Figure 3. Means comparison of the interactions for GRI. a) genotype × drought. b) drought × putrescine c) genotype × putrescine and d) genotype × putrescine × drought

Analysis of variance presented that there were significant two-way interactions between drought × genotype ($P \leq 0.01$) and drought × putrescine ($P \leq 0.01$) (Table 1). Responses of different genotype to drought stress varied. Each genotype has different response to drought application which makes genotype × drought interaction significant. The highest SVI (2657.12) was observed in Kırmızı kılçık genotype with the control (0 bar) treatment. Whereas, the lowest SVI (9.28) was in Pehlivan genotype with -10 bar. There was a clear decrease of SVI with the increased PEG 6000 concentration treatment. There was a trend that SVI was decreasing while osmotic potential simulated by PEG 6000 was increasing (Figure 4a).

Interaction of drought × putrescine was significant, putrescine treatments has positive effect reducing the effect of drought. When concentration of putrescine increased, adverse effect of drought simulated by PEG 6000 application was decreased. Application of the particular combination concentrations of 1 mM putrescine and control (0 bar) PEG 6000 concentration to compare the other different level combinations of putrescine and PEG 6000 applications resulted in the highest (2667.61) seedling vigour index formation. It was also observed that with the increasing concentration of putrescine from 0.01 mM to 1 mM in seed treatment, there was significant decrease, in effect of PEG 6000 (Figure 4b).

Genotypes gave different response to putrescine levels for trait mentioned above but this interaction was not significant. In all genotypes, putrescine treatments increased the SVI. The highest average SVI (1587.12) was in Kırmızı kılçık genotype with the application of 1 mM putrescine hormone while the lowest average SVI (582.13) was in Pehlivan genotype with the control (0 putrescine) treatment. As a result, an increase in the application of putrescine concentrations resulted in the increase of SVI (Figure 4c).

A significant three way interaction among drought × putrescine × genotype was observed significantly at the 0.01 level. The result verified that the highest SVI was in the combination of control (0 bar) PEG 6000 and 1 mM putrescine in Kırmızı kılçık genotype with 2777.46, also the lowest SVI was in the combination of highest PEG 6000 concentration (-10 bar) and without putrescine in Pehlivan genotype with 8.9 (Figure 4d).

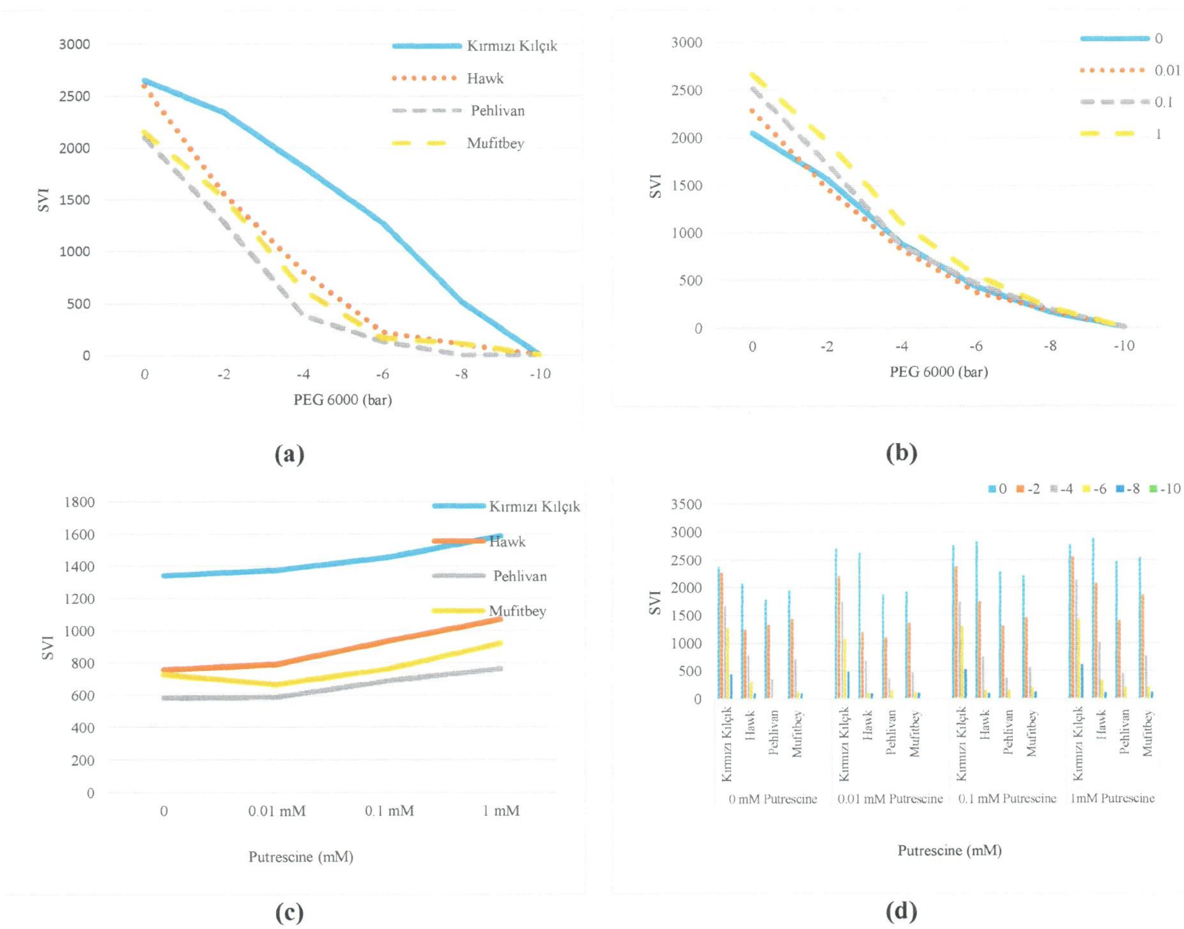

Figure 4. Means comparison of the interactions for SVI. a) genotype × drought. b) drought × putrescine c) genotype × putrescine and d) genotype × putrescine × drought

DISCUSSION

Bread wheat (*Triticum aestivum* L.) is the main food of people (Ghanifathi et al., 2011). One of the most important problem in the world wide is drought stress. Seriously reducing the global crop production (Pan et al., 2002). Drought parameters have been used for screening the drought tolerant genotypes. Development at the germination stage have been adopted a suitable growth stage for testing the drought stress tolerance in wheat. It could be assumed that the presence of increased concentrations of osmotic potential during the growth of germination stage inhibits the developmental traits and survival of wheat. Selection of drought tolerance at early seedling stage is frequently accomplished using the simulated drought induced by chemicals like PEG 6000 (Almaghrabi, 2012). Our results demonstrated that the trait such as the final germination percentage (FGP), mean germination time (MGT), coefficient velocity germination (CVG), rate index (RI), germination rate index (GRI), seedling vigour index (SVI) among genotypes, different levels of PEG 6000 and putrescine were significant. Our results show that while PEG 6000 osmotic potential increases. FGP, CVG, RI, GRI and SVI significantly decreased, whereas the MGT increased. In addition, putrescine, particularly the concentrations of 1 mM, decreased the adverse effect of drought created by PEG. These findings are also in agreement with Almaghrabi (2012) and (Jahanian et al., 2012). Changes in the final germination percentages are accepted as an important germination indicator under stress condition. Our results showed that drought stress at the germination stage delayed and lowered the germination or may prevent germination completely. Akbari and Abadi (2007) stated that in canola seeds that were under drought stress, more time was needed for germination. Seedling growth characters and seed germination are extremely important factors in determining plant produce (Rauf et al., 2007). Duman (2006) reported that drought stress decrease the germination percent, root length and shoot length in lettuce. Sapra et al. (1991) and Dhanda et al. (2004) similarly detected significant differences in germination percentages of wheat genotypes under drought stress conditions. Lafond and Fowler (1989) showed retarded germination and decreased germination percentage under drought stress and stated that germination percentage of above 80% under low stress levels decreased to 56% under -1.5 MPa treatment. Almansouri et al. (2001) emphasise significant decreases in germination percentages of wheat genotypes with increasing osmotic stress levels (-0.15. -0.58. -1.05 and -1.57 MPa). They indicated the negative impacts of PEG treatment on germination. Germination rates of 30 wheat genotypes under -10 bar osmotic stress treatments varied between 14.3 - 64.6% and decreased by 63.3% compared to control treatment genotype averages (Dhanda et al., 2004). It was clear that each genotype, with a lower MGT and higher GI, GRI, CVG, FGP will have higher strength and speed of germination. If these characteristics were under intended drought stress, it would be indicative of sensitivity and tolerance of genotypes to drought stress at the germination phase. Drought stress affects plant growth in many aspects and causes a decrease and delay in germination, decrease the shoot growth and biomass production.

Heikal et al. (1982) stated that amount of water absorption by seeds as well as the final germination percentage and the rate of seed germination were considerably reduced with the rise of osmotic stress level of grain growth. Plumule length and seed vigour index are among other germination traits that are most sensitive to drought stress (Dhanda et al., 2004). Blum et al. (1980) investigated the response of 10 spring wheat genotypes to drought stress during germination and seedling, germination rates of genotypes decreased with the increasing osmotic potation. Similar to the findings of the present study, researchers also indicated the significance of ‘genotype x stress’ interaction describing the mean germination rates of wheat genotypes under 0. -2. -4, and -8 bar osmotic stress levels respectively as 89.7, 55.6, 41.7 and 24.1%. In the same research, germination was not observed under -10 and -15 bar osmotic potential treatments and the seeds were observed to be hard and they did not absorb water at the end of the study. Dhanda et al. (2004) stated the germination rate of wheat genotypes under -6 bar stress level as between 14.3-64.6% and seed vigour indexes as between 146.2-585.6%. Baloch et al. (2012) also showed that seed vigour indexes of genotypes under stress conditions decreased by 60.1-76.6% compared to the control treatment. Heikal et al. (1982) stated that germination rate is an index for the assessment of drought tolerance valuation, as variety with high germination rate in stress condition has more chance for growth.

Some researchers investigated that putrescine stimulates the germination of seeds in several species; for instance *Cucumis melo* (Korkmaz et al., 2005), *Hordeum vulgare* (Locke et al., 2000), *Oryza sativa* (Prakash and Prathapasenan, 1988), *Citrullus lanatus* (Korkmaz et al., 2004), and *Medicago sativa* (Zeid and Shedeed, 2006). Meskaoui and Trembaly (2009) suggested that putrescine are either essential plant growth regulators (PGRs) or secondary messengers of PGRs. Zapata et al. (2008) and Goyal and Asthir (2010) indicated that polyamine hormones are small and biologically active molecules involved in various physiological processes and play an integral role under different environmental stress conditions. Exogenous spermine was found to reduce the adverse effects of salinity stress on some plants (Ali et al., 2009; Ibrahim, 2004; Yamaguchi et al., 2006). Gupta and Gupta (2011) stated that polyamines were found to enhance the productivity in wheat under drought stress conditions. The polyamines accumulated in the embryos of *Oryza sativa* and *Avena fatua,* but their contents in endosperms were low. Zeid (2004) stated that exogenous putrescine treatment (0.01 mM) under normal and NaCl-induced stress conditions increased the germination and growth of bean may be due to the activation of amylase and protease during germination. One of the plant adaptations to drought stress is the increased content of organic solutes such as sugars and amino acids for osmoregulation (Zeid, 2004).

Application of exogenous application of polyamine hormone such as spermidine and putrescine could reduce the effects of drought stress. Polyamines are involved in plant defence to environmental stresses. Exogenous spermidine and putrescine application lessened the growth inhibition of wheat and improved the grain yield of plants under drought stress. Results suggested that putrescine facilitated the osmotic stress tolerance of wheat germination stage. Our results demonstrated that whereas PEG 6000 osmotic potential increases. FGP, CVG, RI, GRI, and SVI significantly decreased, and the MGT increased. In addition putrescine, particularly the concentrations of 1 mM, decreased the adverse effect of drought created by PEG. In other words, it elevated the rate of FGP, CVG, RI, GRI and SVI lowered the mean duration of germination. It has been showed that application of putrescine is also an effective approach for enhancing drought stress tolerance of wheat for enhanced productivity.

Acknowledgements

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