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Effect of Application of Putrescine on Seedling Growth and Cell Division of Wheat (*Triticum aestivum* L.) under Drought Stress

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Abstract: Wheat has been a staple crop for thousands of years and is of massive economic importance worldwide. The study was conducted to evaluate the effect of drought and putrescine hormone on the seedling growth parameters and cell division of wheat genotypes. Wheat genotypes seeds (Kırmızı Kılçık, Hawk, Pehlivan and Müfitbey) were primed with four levels of putrescine (0.01, 0.1 and 1 mM and distilled water as control), then kept under drought stress induced by polyethylene Glycol (PEG 6000) at different concentrations [0 (distilled water), -2, -4, -6, -8 and -10 bar] for 10 days. Experiment was arranged as factorial in a completely randomized design with four replications. At the end of 10 days, root number, root, coleoptile and shoot length and mitotic index (MI) data were obtained. Analysis of variance indicated that genotype, putrescine, osmotic potential and their interaction were significant. While PEG 6000 osmotic potential increases, root number, root, coleoptile and shoot length and mitotic index (MI) significantly decreased. In addition, particularly 1 mM putrescine has decreased the adverse effect of drought created by PEG 6000. Based on the comparison of genotypes, Kırmızı Kılçık was selected as tolerant to drought stress whereas Pehlivan was identified as susceptible.

Keywords: Cell division, Drought stress, Putrescine, Wheat

Kuraklık Stresi Altındaki Buğdayın (*Triticum aestivum* L.) Fide Gelişimi ve Hücre Bölünmesi Üzerine Putresin Uygulamasının Etkisi

Özet: Buğday binlerce yıldan beri en önemli bitki olmuş ve dünya çapında büyük bir ekonomik öneme sahiptir. Bu araştırma, kuraklık ve putresin hormonunun buğdayda fide büyüme parametreleri ve hücre bölünmesi üzerine olan etkilerini belirlemek amacıyla yapılmıştır. Buğday genotiplerinin (Kırmızı Kılçık, Hawk, Pehlivan ve Müfitbey) tohumlarına 4 farklı putresin konsantrasyonunda (0, 0.01, 0.1 ve 1 mM) ön uygulama yapılmış ve daha sonra PEG 6000 ile oluşturulmuş 6 farklı ozmotik potansiyelde [0 (distile su) , -2, -4, -6, -8 ve -10 bar] 10 gün süreyle bekletilmiştir. Araştırma, tam şansa bağlı deneme planına göre 4 tekrarlı olarak yürütülmüştür. On gün sonunda, kök sayısı, kök uzunluğu, koleoptil uzunluğu, sürgün uzunluğu ve hücre bölünmesine ait veriler elde edilmiştir. İncelenen özellikler üzerine genotipin, kuraklığın, putresinin ve bunlara ait interaksiyonun etkisi çok önemli olmuştur. Araştırmada PEG 6000'in konsantrasyonu, diğer bir ifadeyle kuraklığın şiddeti artıkça kök sayısı, kök uzunluğu, koleoptil uzunluğu, sürgün uzunluğu ve hücre bölünmesi çok önemli derecede azalmıştır. Diğer taraftan, putresinin özellikle 1 mM'lık dozu, PEG 6000 ile oluşturulan kuraklığın olumsuz etkilerini azaltmıştır. Genotipler arasında karşılaştırma yapıldığında, Kırmızı Kılçık kuraklık stresine en dayanıklı, Pehlivan ise en duyarlı genotip olarak belirlenmiştir.

Anahtar kelimeler: Buğday, Çimlenme, Hücre bölünmesi, Kuraklık ve Putresin

Introduction

Wheat (*Triticum aestivum* L.) is a major crop worldwide and is grown on about 713 million hectare in a range of environments (FAO 2013). Universally, wheat production must continue to increase 2% annually until 2020 to meet the future demands of the population and for prosperity growth (Abdel Ghany et al. 2004). Drought is one of the most important problems for agriculture universally. During growth and development, it confines nutrient uptake and reduces metabolism, which is reflected in reduced crop

quality and yield. Responses to drought stress are genotype specific and often species specific (De Leonardis et al. 2007). Furthermore, the nature of drought answer of plants is influenced by the severity and duration of water loss (Pinheiro and Chaves 2011), the age and phase of development at the point of drought stress exposure (De Leonardis et al. 2007), as well as the organ and cell type experiencing water shortages (Pastori and Foyer 2002). Polyethylene glycol (PEG) is used widely in laboratory experiments because it simulates environmental conditions. PEG has high molecular weight; it cannot be transmitted through cell wall and used for regulating water potential in germination laboratories. For creating drought stress, PEG 6000 is more suitable than smaller one (PEG 4000), because germination percent in solution made by PEG 6000 is equal to water potential in soil (Kaufman and Eckard 1971).

In response to the abiotic stress, cellular polyamine content often changes. Polyamines are ubiquitous polycationic compounds that mediate fundamental aspects of cell growth, differentiation, and cell death in eukaryotic and prokaryotic organisms. In plants, polyamines are implicated in a variety of growth and developmental processes, in addition to abiotic and biotic stress responses (Baron and Stasolla 2008). Polyamines, important growth regulatory polycationic molecules, are long established to be involved in a wide range of plant growth and development process such as embryogenesis, root development, flowering, tuber formation, senescence and fruit ripening (Srivastava et al. 2007). Exogenous application of polyamines improved tolerance against several abiotic stresses (Cakmak and Atici 2009). Polyamines are involved in a multitude of developmental processes and stress response in plants. Basra et al. (1997) reported treatment of polyamines either prior to heat shock or during heat shock period itself enhanced the recovery of growth of both roots and hypocotyls of *Vigna radiate* seedlings. Positive response of exogenously applied polyamines has been reported in olive, rice, soybean, alfalfa and pomegranate (Sharmaet al. 1997; Nayyar et al. 2005; Yang et al. 2007; Elias 2012). Polyamines were found to enhance the productivity in wheat under water stress conditions (Gupta and Gupta 2011).

Here, we further investigated the effect of the putrescine on seedling growth stage of wheat under drought stress, as well as its effect on cell division.

Materials and Methods

Plant Material and Laboratory Experiment

In order to estimate 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 wheat genotypes were exposed to different concentrations of putrescine for 24 hours at room temperature. The seeds were kept in different concentrations of PEG 6000 osmotic potential medium. Different osmotic potentials were prepared according to Michel and Kaufmann (1973) in order to dissolve the needed amount of PEG 6000 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. The petri dishes were 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. Root, coleoptile and shoot length were measured using a ruler.

Determinate the mitotic index

To determinate the mitotic index (MI) primary ten root tip were set up for each treatment. The initiated roots were collected when they were about 1 - 2 cm long, between 9.00 am and 12.00 noon when mitotic activities are believed to be high.The roots were treated with Carnoy's fixative in 1:3 (v/v) acetic acid/ethanol for 24 h before using them for mitotic studies. The fixed roots were hydrolysed in 1 mol/L hydrochloric acid at 60 ^{0}C for 15 min, then washed with distilled water, stained with feulgen, and squashed. For MI, dividing cells were counted out from 5000 to 6000 cells and the data were expressed in percent. The effects of drought and putrescine treatment and control on mitotic index were observed under light microscope to score the cell cycle phases (prophase, metaphase, anaphase, and telophase). The

best slides were photographed using Olympus BX51 microscope, attached with Canon EOS1100D digital camera. For mitotic data to be taken from 400X microscope fields, at least 10 slides were prepared for each treatment and control. Mitotic index was calculated for each treatment and the control, using the following formula:

Mitotic index = (Number of dividing cells/Total number of cells counted) x 100.

Statistical analysis

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

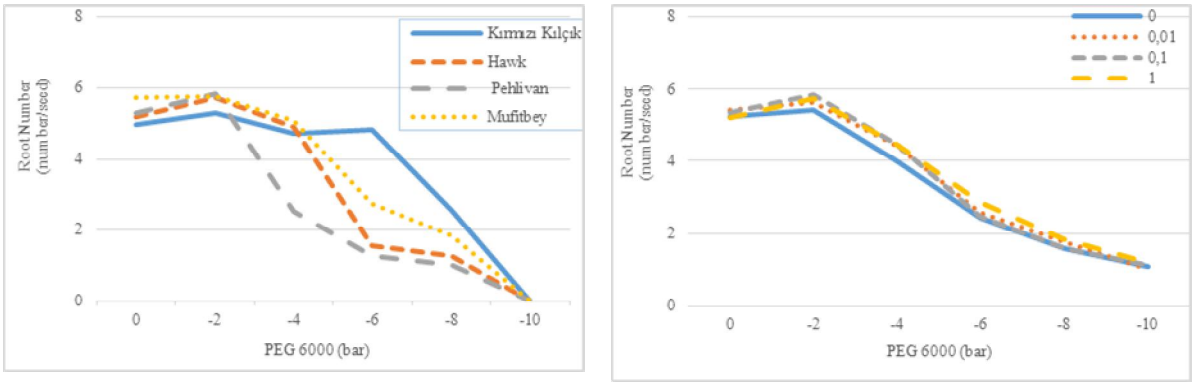
Results

Root Number

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 root number. The results of mean comparison of root number for genotypes showed that the highest mean root number was observed in Kırmızı Kılçık genotype with 3.97 number/seed, whereas the lowest root number was in genotype Pehlivan with 2.83 number/seed (Table 2). Based on drought application, control (0 concentration) treatment gave the highest mean root number (5.31 number/seed), whereas the lowest mean root number was observed in the treatment of -10 bar (Table 2). When means comparison for the highest root number at different levels of putrescine treatments was considered, 1mM application of putrescine resulted in 3.55 number/seed which is higher than control (no putrescine) with 3.28 number/seed (Table 2). While putrescine application increased from 0.01 to 1 mM, root number also increased.

Analysis of variance showed that there were significant two-way interactions between drought × genotype ($P\leq 0.01$) and genotype × putrescine ($P\leq 0.05$). Other interactions that were drought × putrescine and drought × putrescine × genotype were not significant (Table 1).

Responses of different genotypes to drought stress varied. Each genotype has different response to drought application which makes genotype × drought interaction significant. The highest root number (5.85 number/seed) was found to be observed in Pehlivan genotype with -2 bar treatment. Whereas, the lowest root number (1 number/seed) was in Pehlivan and Müfitbey with -10 bar. There was a slight increase of root number in -2 bar treatment. However, in other PEG 6000 treatments (-4, -6, -8 and -10 bar) except -2 bar, there was a trend that root number was decreasing while osmotic potential simulated by PEG 6000 was increasing (Figure 1a).



(a) **(b)**

Figure 1. Means comparison of the interactions for root number: a) genotype × drought and b) drought × putrescine

Table 1. Analysis of variance of different drought levels and putrescine of seedling growth parameters and cell division of four genotypes of wheat

Sources of variations	Root Number	Root Length	Coleoptile Length	Shoot Length	MI (%)
F value (Drought)	785.15**	1532.73**	1112.14**	2019.41**	2364.07**
F value (Genotype)	80.47**	465.39**	355.76**	284.89**	1691.45**
F value (Putrescine)	4.04**	45.01**	16.28**	26.65**	79.44**
F value (D×G)	34.26**	33.97**	43.14**	38.44**	121.93**
F value (D×P)	1.06ns	7.47**	3.74**	6.94**	6.83**
F value (G×P)	1.95*	2.41*	2.45*	3.11**	20.41**
F value (D×P×G)	1.09ns	2.49**	0.80ns	1.82**	6.16**
Drought LSD	0.2518	0.3855	0.1615	0.4475	0.555
Genotype LSD	0.2056	0.3148	0.1319	0.3653	0.532
Putrescine LSD	0.2056	0.3148	0.1319	0.3653	0.532
D×G LSD	0.50	0.77	0.323	0.895	1.109
D×P LSD	-	0.77	0.323	0.895	1.109
G×P LSD	0.31	0.48	0.20	0.731	0.906
D×P×G LSD	-	1.54	-	1.790	2.219

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

Although the interaction between drought × putrescine was not significant, putrescine treatments have positive effect reducing the effect of drought. When 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 -2 bar PEG 6000 concentration to compare other different level combinations of putrescine and PEG 6000 applications resulted in the highest (5.84 number/seed) root 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, affecting PEG 6000 (Table 2).

Genotypes gave different response to putrescine levels for the trait mentioned above. In allgenotypes, putrescine treatments increased the root number. The highest average root number (4.19 number/seed) was in Kırmızı Kılçık genotype with the application of 1 mM putrescine hormone while the lowest average root number (2.73) was in Pehlivan genotype with 1 mM putrescine concentration. As a result, an increase in the application of putrescine concentrations resulted in the increase of root number (Figure 1b).

Root Length

Analysis of variance (Table 1) showed that root length was significantly affected by the differences among genotypes, different levels of PEG 6000 and putrescine. The results of mean comparison of root length for genotypes showed that the highest mean root length was observed in Kırmızı Kılçık genotype with 7.72 cm, whereas the lowest root length was in Pehlivan genotype with 3.38 cm (Table 2).

Based on drought application, control (0 concentration) treatment gave the highest mean root length (9.79 cm), whereas the lowest mean root length with 0.1 cm was observed in the treatment of -10 bar (Table 2). When means comparison for the highest root length at different levels of putrescine treatments was considered, 1mM application of putrescine resulted in 5.89 cm which is higher than the control (no putrescine) and 0.01 mM with 4.68 cm (Table 2). With the increased putrescine application from 0.01 to 1 mM, root number also increased.

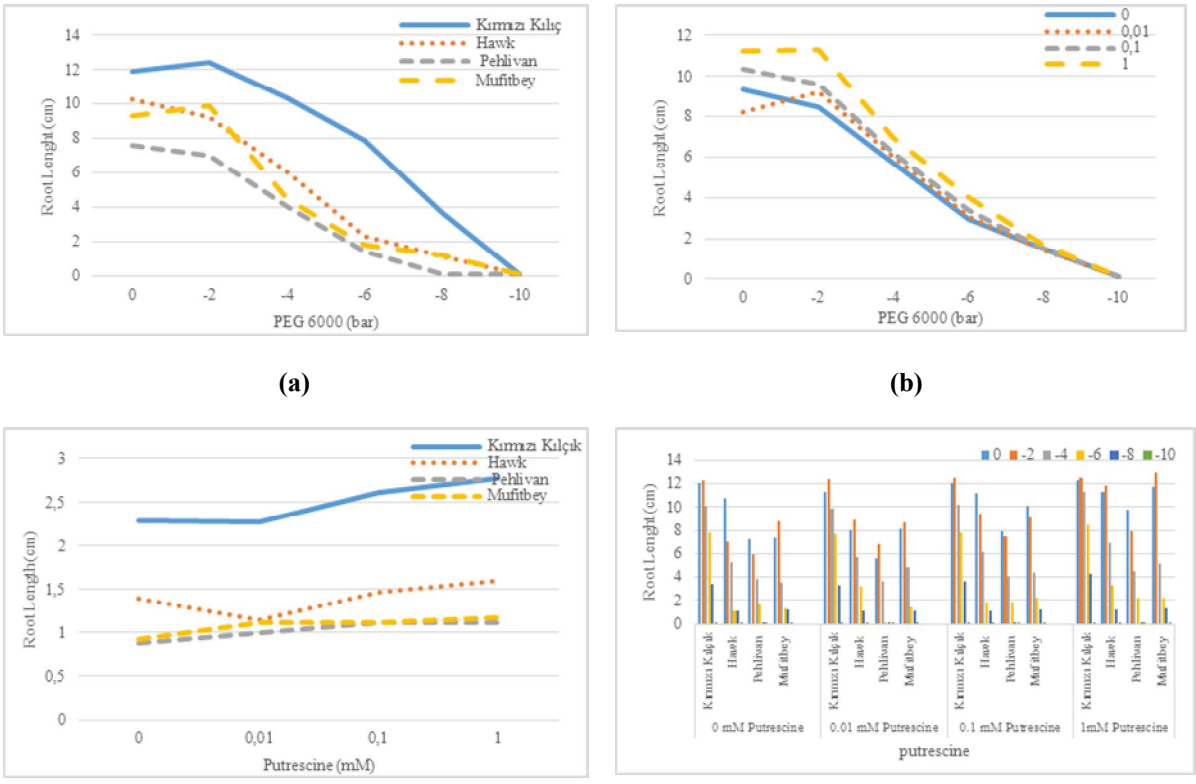
A significant two-way interaction [(drought × genotype, drought × putrescine, ($P \leq 0.01$) and genotype × putrescine ($P \leq 0.05$)] and three interactions [drought × putrescine × genotype ($P \leq 0.01$)] were observed (Table 1). Responses of different genotypes to drought stress varied. Each genotype has different response to drought application which makes genotype × drought interaction significant. The highest root

length (12.4 cm) was observed in Kırmızı Kılçık genotype with -2 bar treatment. Whereas, the lowest root length (0.1 cm) was in all genotypes with -10 bar. There was a slight increase of root length in -2 bar treatment. However, in other PEG 6000 treatments (-4, -6, -8 and -10 bar) except -2 bar, there was a trend that root length was decreasing while osmotic potential simulated by PEG 6000 was increasing (Figure 2a).

Interaction between drought × putrescine was significant; putrescine treatments had positive effect reducing the effect of drought. When the concentration of putrescine increased, adverse effect of drought simulated by PEG 6000 treatment decreased. Application of the particular combination concentrations of 1 mM putrescine and -2 bar PEG 6000 concentration to compare other different level combinations of putrescine and PEG 6000 applications resulted in the highest (11.29 cm) root formation (Figure 2b). 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, affecting PEG 6000 (Table 2).

Genotypes gave different response to putrescine levels for the trait mentioned above. In all genotypes, putrescine treatments increased the root length. The highest average root length (8.14 cm) was in Kırmızı Kılçık genotype with the application of 1 mM putrescine hormone while the lowest average root length (2.72 cm) was in Pehlivan genotype with 0.01 mM putrescine concentration. As a result, an increase in the application of putrescine concentrations resulted in the increase of root length (Figure 2c).

A significant three way interaction among drought × putrescine × genotype ($P \leq 0.01$) was observed. The result verified that the highest root was in the combination of -2 bar and 1 mM Put in Kırmızı Kılçık genotype, and the lowest root was in Mufitbey genotype (Figure 2d).



(a) **(b)**

(c) **(d)**

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

Coleoptile Length

Analysis of variance exposed that coleoptile length was significantly affected by genotype, drought stress and putrescine (Table 1).

The results of mean comparison of coleoptile length for the genotypes showed that the highest mean coleoptile length was observed in Kırmızı Kılçık genotype with 2.49 cm, whereas the lowest coleoptile length of 1.03 cm was in Pehlivan genotype (Table 2).

Based on drought application, control (0 concentration) treatment gave the highest mean coleoptile length (3.73 cm), whereas there was no coleoptile formation in the treatment of -10 bar (Table 2). When means comparison for the highest coleoptile length at different levels of putrescine treatments was considered, 1mM application of putrescine resulted in 1.67 cm which is higher than the control (no putrescine) with 1.37 cm (Table 2). With the increased putrescine application from 0.01 to 1 mM, root number also increased.

A significant two-way interaction [(drought × genotype, drought × putrescine (P≤ 0.01) genotype × putrescine (P≤ 0.05)] has been recorded (Table 1). Responses of different genotypes to drought stress varied. Each genotype has different response to drought application which makes genotype × drought interaction significant. The highest coleoptile length (4.13 cm) was observed in Kırmızı Kılçık genotype with the control (0 bar) treatment. Whereas, there was no coleoptile formation in all genotypes with the treatment of -10 bar. There was a slight increase of coleoptile length in -2 bar treatment. However, in other PEG 6000 treatments (-4, -6, -8 and -10 bar) except -2 bar, there was a trend that coleoptile length was decreasing while osmotic potential simulated by PEG 6000 was increasing (Figure 3a).

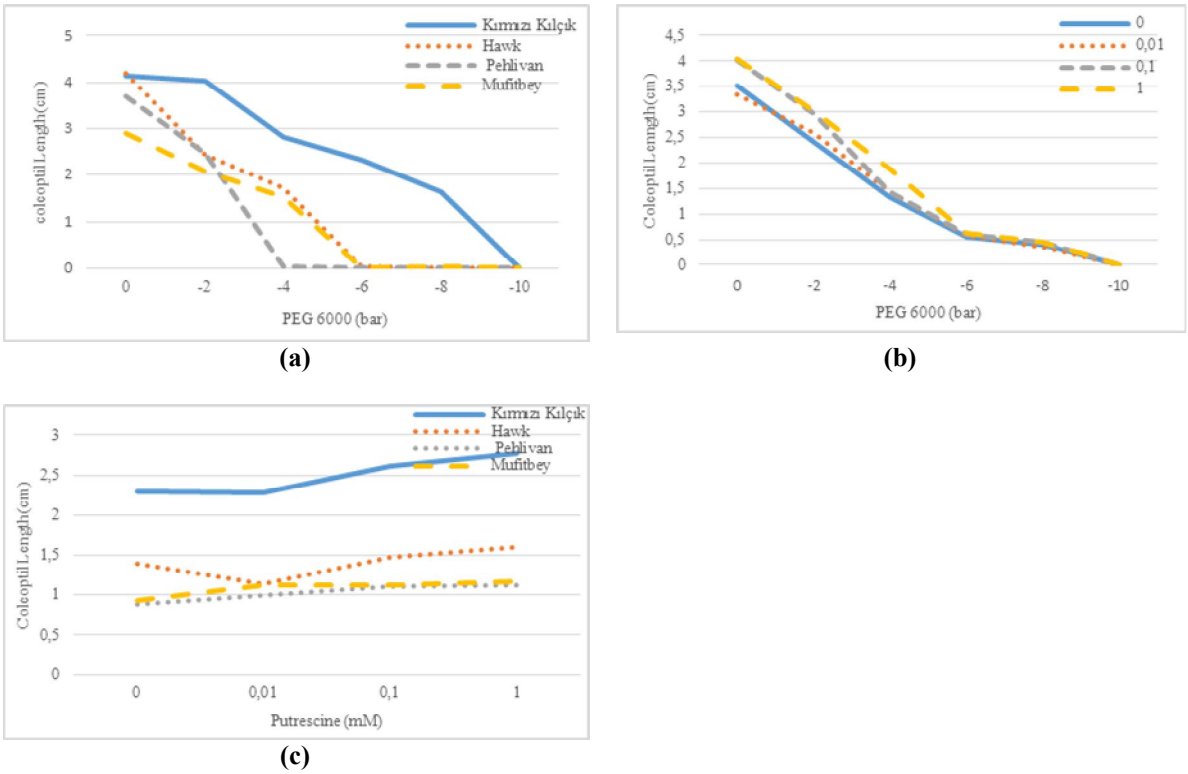


Figure 3. Means comparison of the interactions for coleoptile length. a) genotype × drought, b) drought × putrescine and c) genotype × putrescine × drought.

Although the interaction between drought × putrescine was significant, putrescine treatments have positive effect reducing the effect of drought. When the concentration of putrescine increased, adverse

effect of drought simulated by PEG 6000 treatment decreased. Application of the particular combination concentrations of 1 mM putrescine and -2 bar PEG 6000 concentration to compare other different level combinations of putrescine and PEG 6000 applications resulted in the highest (3.01 cm) root formation after the control treatment (Figure 3b). 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, affecting PEG 6000 (Table 2).

Genotypes gave different responses to putrescine levels for the trait mentioned above. In all genotypes, putrescine treatments increased the coleoptile length. The highest average coleoptile length (2.78 cm) was in Kırmızı Kılçık genotype with the application of 1 mM putrescine hormone while the lowest average coleoptile length (0.88 cm) was in Pehlivan genotype with no putrescine concentration. As a result, an increase in application of putrescine concentrations resulted in the increase of coleoptile length (Figure 3c). Three - way interaction among drought × putrescine × genotype was not significant (Table 2).

Shoot Length

Analysis of variance (Table 1) showed that shoot length was significantly affected by genotypes, different levels of PEG 6000 and putrescine. The results of mean comparison of shoot length for genotypes showed that the highest mean shoot length was observed in KırmızıKılçık genotype with 6.87 cm, whereas the lowest shoot length was in genotype Pehlivan with 3.27 cm (Table 2).

Based on drought application, control (0 concentration) treatment gave the highest mean shoot length (14.15 cm), whereas there was no shoot formation in the treatment of -10 bar (Table 2). When means comparison for the highest shoot length at different levels of putrescine treatments was considered, 1mM application of putrescine resulted in 5.03 cm which is higher than the control (no putrescine) and 0.01 mM with 3.97 cm (Table 2). With the increased putrescine application from 0.01 to 1 mM, root number also increased.

Significant two and three-way interactions [(drought × genotype, drought × putrescine, genotype × putrescine and drought × putrescine × genotype ($P \leq 0.01$)] were observed (Table 1).Responses of different genotypes to drought stress varied. Each genotype has different response to drought application which makes genotype × drought interaction significant. The highest shoot length (15.72 cm) was observed in Hawk genotype with the control (0 bar) treatment. Whereas, there was no shoot formation in any of the genotypes with the treatment of -10 bar. There was a trend that shoot length was decreasing while osmotic potential simulated by PEG 6000 was increasing (Figure 4a). Interaction between drought × putrescine was significant, putrescine treatments has positive effect reducing the effect of drought. When the concentration of putrescine increased, adverse effect of drought simulated by PEG 6000 treatment decreased. Application of the particular combination concentrations of 1 mM putrescine and -2 bar PEG 6000 concentration to compare other different level combinations of putrescine and PEG 6000 applications resulted in the highest (8.47 cm) root formation after control (Figure 2b). 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, affecting PEG 6000 (Table 2).

Genotypes gave different responses to putrescine levels for the trait mentioned above. In all genotypes, putrescine treatments increased the shoot length. The highest average shoot length (7.84 cm) was in Kırmızı Kılçık genotype with the application of 1 mM putrescine hormone while the lowest average shoot length (2.83 cm) was in Pehlivan genotype without putrescine concentration. As a result, an increase in the application of putrescine concentrations resulted in the increase of shoot length (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 shoot was in the combination of control and 1 mM putrescine in Hawk genotype also the lowest shoot was in Mufitbey genotype (Figure 4d).

Table 2. Mean comparison of different drought levels and putrescine of seedling growth parameters and cell division 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
Root Number (number)	0	4.67	5.41	5.25	5.58	5.23	4.91	5.00	5.75	6.00	5.41	5.00	5.33	5.25	5.88	5.36	5.33	5.00	5.00	5.55	5.22	4.98	5.19	5.31	5.75	5.31
	-2	5.29	5.67	5.50	5.25	5.42	5.25	5.58	6.00	5.75	5.64	5.30	5.83	6.00	6.25	5.84	5.42	5.83	5.92	5.88	5.76	5.31	5.73	5.85	5.78	5.67
	-4	4.83	4.91	2.33	3.92	3.99	4.75	5.17	2.50	5.25	4.41	4.79	4.41	3.14	5.55	4.47	5.00	5.08	2.16	5.63	4.47	4.74	4.89	2.53	5.08	4.31
	-6	4.44	1.00	1.50	2.75	2.42	4.75	2.00	1.25	2.25	2.56	4.83	1.00	1.00	3.00	2.46	4.92	2.25	1.33	2.98	2.87	4.83	1.56	1.27	2.74	2.60
	-8	2.48	1.33	1.00	1.42	1.56	2.50	1.33	1.00	2.17	1.75	2.58	1.00	1.00	1.65	1.56	2.75	1.33	1.00	2.23	1.83	2.58	1.25	1.00	1.86	1.67
	-10	1.38	1.00	1.00	1.00	1.09	1.08	1.00	1.00	1.00	1.02	1.41	1.00	1.00	1.00	1.10	1.75	1.00	1.00	1.00	1.19	1.41	1.00	1.00	1.00	1.10
	Mean	**3.85**	**3.22**	**2.76**	**3.32**	**3.28**	**3.87**	**3.35**	**2.92**	**3.73**	**3.47**	**3.98**	**3.10**	**2.90**	**3.89**	**3.47**	**4.19**	**3.42**	**2.73**	**3.88**	**3.55**	**3.97**	**3.27**	**2.83**	**3.70**	**3.44**
Root Length (cm)	0	12.02	10.71	7.25	7.38	9.34	11.24	8.03	5.59	8.07	8.23	12.11	11.21	7.94	10.11	10.34	12.25	11.28	9.73	11.73	11.25	11.90	10.31	7.63	9.32	9.79
	-2	12.27	6.98	5.91	8.81	8.49	12.42	8.88	6.82	8.71	9.21	12.45	9.31	7.50	9.13	9.60	12.47	11.87	7.90	12.93	11.29	12.40	9.26	7.03	9.89	9.65
	-4	10.07	5.31	3.82	3.54	5.68	9.87	5.69	3.64	4.88	6.02	10.16	6.17	4.05	4.37	6.19	11.30	6.91	4.52	5.19	6.98	10.35	6.02	4.01	4.49	6.22
	-6	7.77	1.15	1.68	1.29	2.97	7.71	3.16	0.10	1.42	3.09	7.82	1.71	1.77	2.16	3.36	8.43	3.25	2.24	2.22	4.03	7.93	2.32	1.45	1.77	3.36
	-8	3.44	1.12	0.10	1.22	1.47	3.33	1.12	0.10	1.08	1.41	3.59	1.12	0.10	1.23	1.51	4.28	1.17	0.10	1.28	1.71	3.66	1.13	0.10	1.20	1.52
	-10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
	Mean	**7.61**	**4.23**	**3.14**	**3.72**	**4.68**	**7.44**	**4.50**	**2.72**	**4.04**	**4.68**	**7.70**	**4.94**	**3.57**	**4.52**	**5.18**	**8.14**	**5.76**	**4.10**	**5.57**	**5.89**	**7.72**	**4.86**	**3.38**	**4.46**	**5.11**
Coleoptile Length (cm)	0	3.85	4.42	3.14	2.72	3.53	3.68	3.52	3.49	2.66	3.34	4.39	4.42	4.11	3.14	4.01	4.61	4.43	4.07	3.05	4.04	4.13	4.20	3.70	2.89	3.73
	-2	3.58	2.36	2.17	1.56	2.42	3.58	1.86	2.53	2.42	2.60	4.43	2.75	2.56	2.13	2.97	4.51	2.77	2.53	2.22	3.01	4.03	2.43	2.45	2.08	2.75
	-4	2.44	1.60	0.00	1.28	1.33	2.66	1.46	0.00	1.66	1.44	2.73	1.64	0.00	1.37	1.43	3.44	2.23	0.13	1.77	1.89	2.82	1.73	0.03	1.52	1.52
	-6	2.24	0.00	0.00	0.00	0.56	2.29	0.00	0.00	0.00	0.57	2.44	0.00	0.00	0.00	0.61	2.36	0.10	0.00	0.00	0.62	2.33	0.03	0.00	0.00	0.59
	-8	1.63	0.00	0.00	0.00	0.41	1.45	0.00	0.00	0.00	0.36	1.67	0.00	0.00	0.10	0.44	1.75	0.05	0.00	0.00	0.45	1.63	0.01	0.00	0.03	0.42
	-10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Mean	**2.29**	**1.40**	**0.88**	**0.93**	**1.37**	**2.28**	**1.14**	**1.00**	**1.12**	**1.38**	**2.61**	**1.47**	**1.11**	**1.12**	**1.58**	**2.78**	**1.60**	**1.12**	**1.17**	**1.67**	**2.49**	**1.40**	**1.03**	**1.09**	**1.50**
Shoot Length (cm)	0	15.04	15.56	11.59	12.06	13.56	12.58	12.74	12.29	12.20	12.45	15.53	17.06	14.80	12.65	15.01	15.81	17.53	14.97	13.95	15.57	14.74	15.72	13.41	12.71	14.15
	-2	10.03	5.05	5.36	5.01	6.36	10.45	3.93	6.62	5.95	6.74	11.81	8.51	6.46	5.85	8.15	13.06	8.71	6.22	5.90	8.47	11.34	6.55	6.16	5.68	7.43
	-4	7.56	1.60	0.00	1.28	2.61	7.37	2.36	0.00	2.37	3.03	7.45	1.64	0.00	1.37	2.61	10.11	3.39	0.13	2.74	4.09	8.12	2.25	0.03	1.94	3.08
	-6	3.39	0.00	0.00	0.00	0.85	5.60	0.00	0.00	0.00	1.40	5.74	0.00	0.00	0.00	1.43	5.89	0.10	0.00	0.00	1.50	5.15	0.03	0.00	0.00	1.29
	-8	1.81	0.00	0.00	0.00	0.45	1.45	0.00	0.00	0.00	0.36	1.99	0.00	0.00	0.10	0.52	2.19	0.05	0.00	0.00	0.56	1.86	0.01	0.00	0.03	0.47
	-10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Mean	**6.30**	**3.70**	**2.83**	**3.06**	**3.97**	**6.24**	**3.17**	**3.15**	**3.42**	**4.00**	**7.08**	**4.53**	**3.54**	**3.33**	**4.62**	**7.84**	**4.96**	**3.55**	**3.76**	**5.03**	**6.87**	**4.09**	**3.27**	**3.39**	**4.41**
Mitotic index (MI)	0	18.5	18.9	17.8	11.1	16.6	27.6	20.7	18.4	12.8	19.9	29.6	19.2	18.3	12.5	19.9	31.3	19.7	18	13.5	20.6	26.8	19.6	18.1	12.5	19.3
	-2	20.6	19.1	18.5	11.4	17.4	27.4	18.2	17.8	12	18.8	27.8	19.8	18	12.7	19.6	28.5	20.6	18.3	13.9	20.3	26.1	19.4	18.1	12.5	19
	-4	18.3	10.5	8.3	10.1	11.8	19.9	11	9	10.2	12.5	23.9	11.3	9.9	10.2	13.8	24.8	12.6	10.2	10.7	14.6	21.7	11.4	9.4	10.3	13.2
	-6	17.8	9.7	0	10.1	9.4	17.9	10	9.3	10.1	11.8	21.2	10.3	9.5	10.3	12.6	25.3	10.7	9.6	10.5	14	20.5	10.2	7.1	10.3	12
	-8	17.1	7.8	0	0	6.2	18.7	8.6	0	0	6.8	19.2	9.1	0	0	7.1	20.3	9.3	0	0	7.4	18.8	8.7	0	0	6.9
	-10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Mean	**15.4**	**11**	**7.4**	**7.1**	**10.2**	**18.6**	**11.4**	**9.1**	**7.5**	**11.6**	**20.3**	**11.6**	**9.3**	**7.6**	**12.2**	**21.7**	**12.1**	**9.3**	**8.1**	**12.8**	**19**	**11.5**	**8.8**	**7.6**	**11.7**

1. Kırmızı Kılçık 2. Hawk 3. Pehlivan and 4. Müfitbey respectively

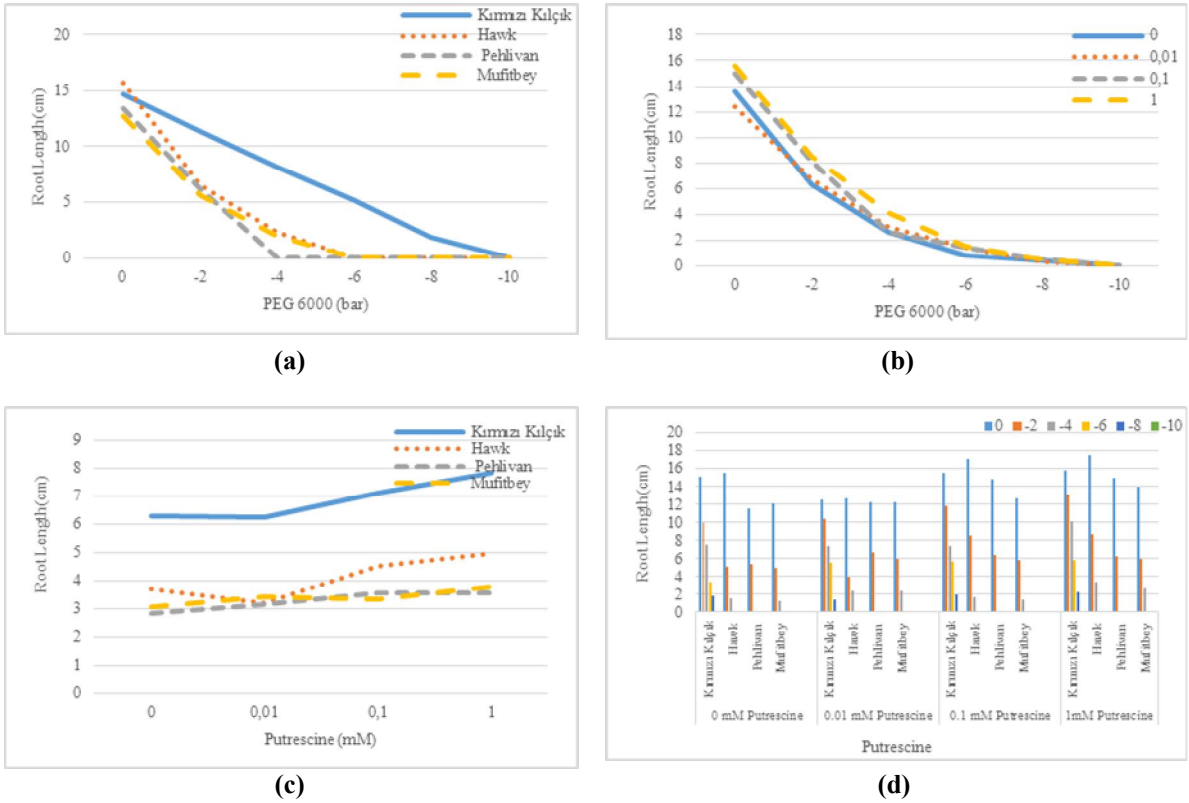


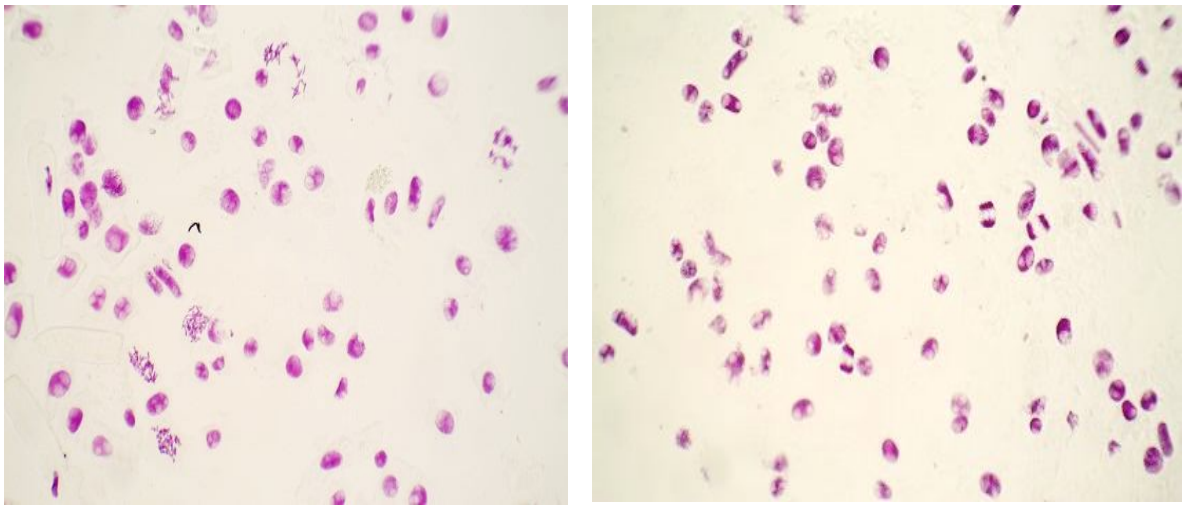
Figure 4. Means comparison of the interactions for shoot length. a) genotype × drought, b) drought × putrescine c) genotype × putrescine and d) genotype × putrescine × drought.

Mitotic index (MI)

The effects of putrescine and PEG 6000 on cell division of wheat root tip cells were presented in Figure 5. In the present study, analysis of variance (Table 1) showed that there were significant (P≤ 0.01) genotypes, different levels of PEG 6000 and putrescine based on mitotic index (MI). The results of mean comparison of mitotic index for the genotypes showed that the highest mean mitotic index was observed in Kırmızı Kılçık genotype with 19%, whereas the lowest mitotic index was in Pehlivan genotype with 7.6% (Table 2).

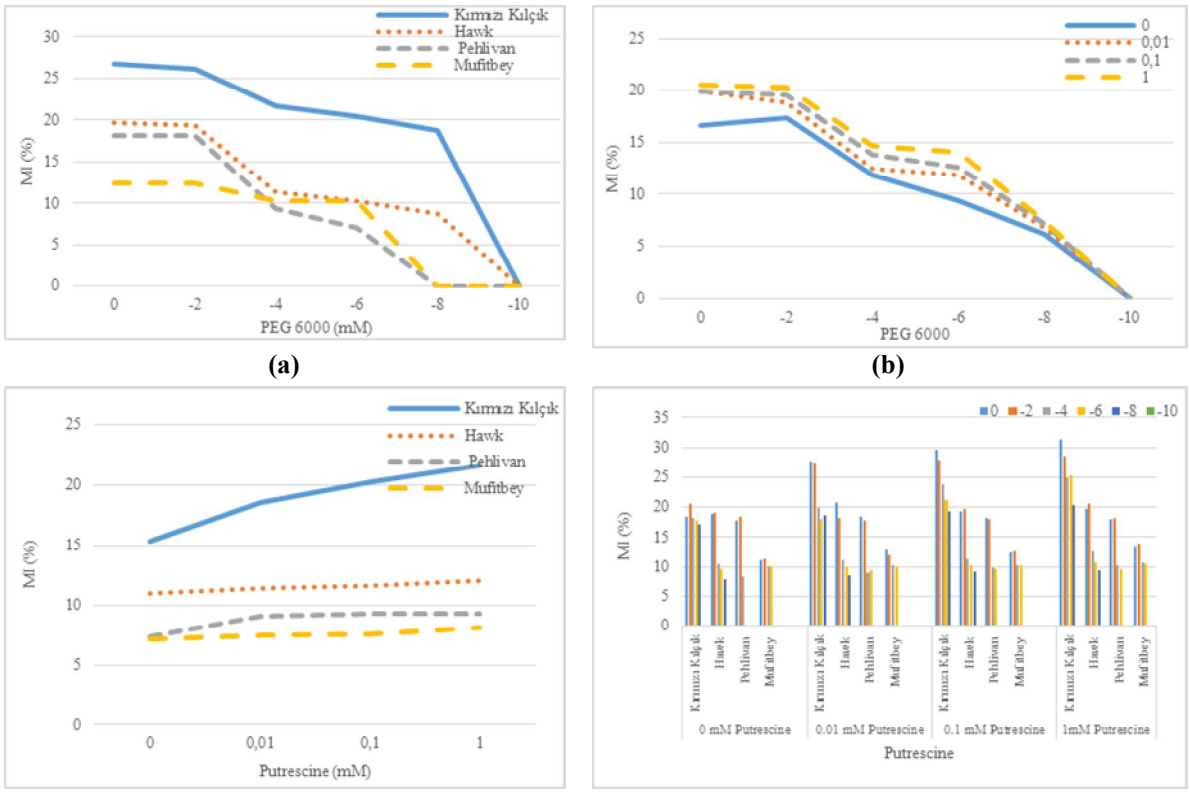
Based on drought application, control (0 concentration) treatment gave the highest average mitotic index 19.3%, whereas there was no mitotic index in the treatment of -10 bar (Table 2). When means comparison for the highest mitotic index at different levels of putrescine treatments was considered, 1mM application of putrescine resulted in 12% MI which is higher than the control (Table 2). With the increased putrescine application from 0.01 to 1 mM, MI also increased. Significant two and three-way interactions [(drought × genotype, drought × putrescine, genotype × putrescine and drought × putrescine × genotype (P≤ 0.01)] were observed (Table 1). The highest mitotic index (26.8 %) was observed in Pehlivan genotype with the control (0 bar) treatment. Whereas, there was no mitotic index formation in any of the genotypes with -10 bar. However, in other PEG 6000 treatments (-2, -4, -6, -8 and -10 bar), there was a trend that mitotic index was decreasing while osmotic potential simulated by PEG 6000 was increasing (Figure 6a). Interaction between drought × putrescine was significant regarding MI; putrescine treatments have positive effect reducing the effect of drought in MI trait. When the concentration of putrescine increased, adverse effect of drought simulated by PEG 6000 treatment decreased. Application of the particular combination concentrations of 1 mM putrescine and -2 bar PEG 6000 concentration to compare other different level combinations of putrescine and PEG 6000 applications resulted in the highest (20.3%) MI after the control (Figure 5b). 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, affecting PEG 6000 based on MI (Table 2).



(a) **(b)**

Figure 5. Cell division in wheat root tip cells induced by putrescine and PEG 6000.



(a) **(b)**

(c) **(d)**

Figure 6. Means comparison of the interactions for mitotic index. a) genotype × drought, b) drought × putrescine c) genotype × putrescine and d) genotype × putrescine × drought.

Genotypes gave different response to putrescine levels for the trait mentioned above. In all genotypes, putrescine treatments increased the mitotic index. The highest average mitotic index (21.7 %) was in Kırmızı Kılçık genotype with the application of 1 mM putrescine hormone while the lowest average mitotic index (7.1 cm %) was in Müfitbey genotype with no putrescine concentration. As a result, an

increase in the application of putrescine concentrations resulted in the increase of mitotic index (Figure 6c). A significant three way interaction among drought × putrescine × genotype ($P \leq 0.01$) was observed significantly at the 0.01 level. The highest mitotic index (MI)was in the combination of control and 1 mM putrescine in Kırmızı Kılçık genotype, and the lowest mitotic index (7.8) was in the combination of control and without putrescine treatment in Hawk genotype (Figure 6d).

Discussion

Drought stress is a major growth limiting factor for wheat plant. Abiotic stress such as drought stress is a complicated phenomenon which includes osmotic stress, specific ion effect, nutrient deficiency etc., thereby affecting various physiological and biochemical mechanisms associated with plant growth and development (Sairam et al. 2002). Drought parameters have been used for screening drought tolerant genotypes. Development at seedling stage have been adopted a suitable growth stage for testing the drought tolerance in wheat (Almaghrabi 2012). It could be conjectured that the presence of increased concentrations of osmotic potential (PEG 6000) during the growth of seedling inhibits the developmental traits and survival of wheat. Our results show that drought stress leads to decrease in root number, root length, coleoptile length, shoot length and mitotic index (MI) confirming the reported results by Sairam et al. (2002); Almaghrabi (2012); Abdel-Fattah et al. (2013) and Radhouane (2007). Responses of different genotypes to drought stress varied. Our consequence displayed that root parameters were affected by genotypes, different levels of PEG 6000 and putrescine. Among studied seedling parameters, the root traits were the most sensitive parameter and affected primarily under stress condition. Dhanda et al. (2004); Rauf et al. (2007); Baloch et al. (2012) stated that embryonic root lengths reveal significant information about drought resistances of wheat genotypes under osmotic stress conditions. Reduction in the root length under stress may be due to an inhibition of elongation and cell division (Fraser et al.,1990). With regard to shoot lengths, differences between wheat genotypes analysis of variance showed that shoot length was affected by genotypes, different levels of PEG 6000 and putrescine. Jajarmi (2009) indicated the shoot length as the most susceptible plant characteristic to drought and reported significant decreases in shoot lengths especially after higher concentrations of (-6 bar) stress levels. Dhanda et al. (2004) and Rauf et al. (2007) indicated significant differences in shoot lengths parameters of genotypes under stress conditions and stated decreased shoot lengths with increasing stress levels. Duman (2006) reported that drought stress decreased the germination percent, root length and shoot length. Baloch et al. (2012) stated that shoot length was highly susceptible to stress conditions compared to control treatment, they reported 57.5 - 68.4% decrease in the shoot lengths with stress treatments. Rauf et al. (2007) stated that seed germination and seedling growth characters are extremely important factors in determining the yield. Dhanda et al. (2004) stated that shoot length and seed vigour index are among the most sensitive to drought stress, followed by root and coleoptiles length. Almaghrabi (2012) reported that root length parameter was decreased significantly by increasing the PEG concentration. Reduction in the root length under drought stress may due to an barrier of cell division (Fraser et al. 1990).

In our study, analysis of variance showed that there were significant differences among genotypes, different levels of PEG 6000 and putrescine in terms of mitotic index. Based on drought application, control treatment gave the highest mean mitotic index, whereas there was no mitotic index in the treatment of -10 bar. In the present study, the mitotic index decreased in response to an increase in concentrations of the PEG 6000 in wheat crop compared to the control. Depressive effect of the osmotic potential may be due to the interference of osmotic potential in the normal process of mitosis by reducing the number of dividing cells. The inhibition in the mitotic index (MI) may be due to the interference of PEG 6000 in the normal sequence of cell division, which prevents or reduces the number of cells entering the prophase stage. Mitotic index is an important parameter to be determined as an alternative for the screening of root growth inhibition (Yumurtaci et al. 2007). Increased mitotic index is attributed to the formation of aberrant cells (Patel and Patel 2013). Karmakar et al. (2014) stated that the inhibition of root growth can be attributed to the inhibition of mitosis (reduced MI%) and the evacuation in biomass production due to the abnormality of vascular bundle formation revealed from the root anatomy concerned with the transport chain. According to Zidan et al. (1990), inhibition of root growth in maize under salinity is due to the reduction in the length of root tip elongation zone and decline in cell division rate; and also the accumulation of proline in roots under stress condition is clearly associated with the reduction in the root growth and decrease in mitotic index with the increase in NaCl concentration.

Polyamines such as putrescine, spermidine and spermine are small biologically active molecules involved in different physiological processes and they play an integral role under various environmental stress conditions such as drought etc. (Todorova et al. 2007; Zapata et al. 2008). Our results demonstrated that genotypes gave different responses to putrescine levels. The application of putrescine decreased the negative effect of drought stresses. Our result was consistent with the findings of Ozhan and Hajibabaei (2013). Moreover, Ethylene inhibits shoot and root growth in stress condition but putrescine treatment also decreases ethylene biosynthesis and directly antagonizes several ethylene –mediated responses in many terrestrial plants (Mattoo and White 1991), and delays senescence of wheat seedling (Felix and Harr 1987). In addition, Behera et al. (2000) stated that polyamines are involved in abiotic stress tolerance in plants.

When the concentration of putrescine increased, adverse effect of drought simulated by PEG 6000 treatment decreased. In other words, 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, affecting PEG 6000. Increased polyamine levels in stressed plants are of adaptive significance because of their involvement in the regulation of cellular ionic environment, maintenance of membrane integrity, prevention of chlorophyll loss and stimulation of protein, nucleic acid and protective alkaloids (Hocking and Stapper 2001). Kuehn et al. (1990) stated that simulative effect of polyamine on growth and yield component may be due to the effect of putrescine which serves as specific protective agent in plants adapted to extreme environment. Locke et al. (2000) showed that exogenous polyamines at 1µM concentration stimulated the growth of barley seedling. Moreover, Mansour et al. (2002) indicated that polyamines pre-treatment (2.5 mM putrescine, 5 mM spermidine and 2.5 mM spermine) induced growth of wheat plants. Zeid (2004) offered that exogenous putrescine treatment (0.01 mM) increasing germination and growth of bean under normal and NaCl-induced stress conditions may be due to the activation of amylase and protease during germination.

In this study, particularly 1 mM putrescine has decreased the adverse effect of drought created by PEG 6000. Based on the comparison of genotypes, Kırmızı Kılçık was selected as tolerant to drought stress whereas genotype Pehlivan was identified as susceptible.

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References

Abdel-Fattah GM, Ibrahim AH, Al-Amri SM, Shoker AE (2013). Synergistic effect of arbuscular mycorrhizal fungi and spermine on amelioration of salinity stress of wheat (*Triticum aestivum* L. Cv. Gimiza 9). Australian Journal of Crop Science, AJCS 7(10):1525-1532.

Abdel Ghany H, Nawar A, Ibrahim M, El-Shamarka Selim SAM, Fahmi A (2004). Using tissue culture to select for drought tolerance in bread wheat. In: New directions for a diverse planet: Proceedings of the 4th International Crop Science Congress.–Brisbane, Australia.

Almaghrabi OA (2012). Impact of drought stress on germination and seedling growth parameters of some wheat cultivars. *Life Sci J*, 9(1): 590-598.

Baloch MJ, Dunwell J, Khakwani AA, Dennett M, Jatoi WA, Channa SA (2012). Assessment of wheat cultivars for drought tolerance via osmotic stress imposed at early seedling growth stages. Journal of Agricultural Research, 50 (3): 299-310.

Baron K, Stasolla C (2008). The role of polyamines during in vivo and in vitro development. In Vitro Cellular and Developmental Biology - Plant, 44(5): 384-395. DOI 10.1007/s11627-008-9176-4.

Basra RK, Basra AS, Malik C, Grover I (1997). Are polyamines involved in the heat-shock protection of mung bean seedlings? Botanical Bulletin of Academia Sinica, 38: 165-169.

Behera U, Chougule B, Thakur R, Ruwali K, Bhawsar R, Pandey H (2000). Influence of planting dates and nitrogen levels on yield and quality of durum wheat (*Triticum durum*). Indian Journal of Agricultural Sciences, 70(7): 434-436.

Buchanan BB, Gruissem W, Jones RLB (2000). Biochemistry and molecular biology of plants. American Society of Plant Physiologists, Rockville, Maryland, USA.

Cakmak T, Atici O (2009). Effects of putrescine and low temperature on the apoplastic antioxidant enzymes in the leaves of two wheat cultivars. Plant Soil Environ, 55(8): 320-326.
Chattopadhyay MK, Tabor CW, Tabor H (2002). Absolute requirement of spermidine for growth and cell cycle progression of fission yeast (*Schizosaccharomyces pombe*). Proceedings of the National Academy of Sciences, 99(16): 10330-10334.
De Leonardis AM, Marone D, Mazzucotelli E, Neffar F, Rizza F, Fonzo N, Di Cattivelli L, Mastrangelo AM (2007). Durum wheat genes up-regulated in the early phases of cold stress are modulated by drought in a developmental and genotype dependent manner. Plant science, 172(5): 1005-1016.
Dhanda S, Sethi G, Behl R (2004). Indices of drought tolerance in wheat genotypes at early stages of plant growth. Journal of Agronomy and Crop Science, 190(1): 6-12.
Duman I (2006). Effects of seed priming with peg or k3po4 on germination and seedling growth in lettuce. Pakistan Journal of Biological Sciences, 9(5): 923-928.
El-Bassiouny HMS and MA Bekheta (2001). Role of putrescine on growth, regulation of stomatal aperture, ionic contents and yield by two wheat cultivars under salinity stress. Egyptian J. Physiol. Sci., 2– 3: 239–258.
Elias A (2012). Effects of timing of drought stress on pomegranate seedlings (*Punica granatum* L. Cv 'atabaki') to exogenous spermidine and putrescine polyamines. African Journal of Microbiology Research, 6(25). DOI 10.5897/ajmr11.1355.
FAO (2013). http://www.fao.org/publications/sofa/2013/en/.
Felix H, Harr J (1987). Association of polyamines to different parts of various plant species. Physiologia Plantarum, 71(3): 245-250.
Fraser TE, Silk WK, Rost TL (1990). Effects of low water potential on cortical cell length in growing regions of maize roots. Plant Physiology, 93(2): 648-651.
Gupta S, Gupta N (2011). Field efficacy of exogenously applied putrescine in wheat (*Triticum aestivum*) under water-stress conditions. Indian Journal of Agricultural Sciences, 81(6): 516.
Hocking P, Stapper M (2001). Effects of sowing time and nitrogen fertiliser on canola and wheat, and nitrogen fertiliser on Indian mustard. Ii. Nitrogen concentrations, n accumulation, and n fertiliser use efficiency. Crop and Pasture Science, 52(6): 635-644.
Jajarmi V (2009). Effect of water stress on germination indices in seven wheat cultivar. In: Proceedings of World Academy of Science, Engineering and Technology. Citeseer: pp: 105-106.
Karmakar NA, Chakravarty BPK, Das PK (2014). Response of fenugreek (*Trigonella foenum-graecum* L.) seedlings under moisture and heavy metal stress with special reference to antioxidant system. African Journalof Biotechnology, 13(3): 434-440.
Kaufman MR, Eckard AN (1971). Evolution of stress control by polyethylene. Glycols by analysis of gelation. Physiology, 47: 453-456.
Kuehn GD, Bagga S, Rodriguez-Garay B, Phillips G (1990). Biosynthesis of uncommon polyamines in higher plants and their relationship to abiotic stress responses. Plant Physiol, 94, Pp 855-857.
Locke JM, Bryce JH, Morris PC (2000). Contrasting effects of ethylene perception and biosynthesis inhibitors on germination and seedling growth of barley (*Hordeum vulgare* L.). J Exp Bot, 51(352): 1843-1849.
Mansour M, Al-Mutawa M, Salama K, Hadid AA (2002). Salt acclimation of wheat salt sensitive cultivar by polyamines. In: Prospects for saline agriculture. Springer: pp: 155-160.
Mattoo AK, White WB (1991). The plant hormone ethylene. In: Regulation of ethylene biosynthesis, A. K. Mattoo and J. C. Suttle, (Eds.). CRC Press, Inc., Boca Raton, Florida: pp: 21-42.
Michel BE, Kaufmann MR (1973). The osmotic potential of polyethylene glycol 6000. Plant Physiology, 51(5): 914-916.
Nassar A, El-Tarabily K, Sivasithamparam K (2003). Growth promotion of bean (*Phaseolus vulgaris* L.) by a polyamine-producing isolate of streptomyces griseoluteus. Plant Growth Regulation, 40(2): 97-106.
Nayyar H, Satwinder K, Kumar S, Singh K, Dhir K (2005). Involvement of polyamines in the contrasting sensitivity of chickpea (*Cicer arietinum* L.) and soybean (*Glycine max* L. *merrill*.) to water deficit stress. Botanical Bulletin of Academia Sinica, 46.sayfa eksik
Ozhan N, Hajibabaei M (2013). Germination responses of wheat tajan cultivar to gibberlic acid and polyamines under salinity stress. International Research Journal of Applied and Basic Sciences, 4(6): 1617-1623.
Pastori GM, Foyer CH (2002). Common components, networks, and pathways of cross-tolerance to stress. The central role of "redox" and abscisic acid-mediated controls. Plant Physiology, 129(2): 460-468..

Patel KP, Patel KM (2013). Cytological changes in *Trigonella foenum-graecum* (L.) under the cadmium stress. Journal of Life Sciences and Technologies Vol, 1(1).sayfa eksik

Pinheiro C, Chaves M (2011). Photosynthesis and drought: Can we make metabolic connections from available data, J Exp Bot, 62(3): 869-882.

Radhouane L (2007). Response of tunisian autochthonous pearl millet (*Pennisetum glaucum* L.) to drought stress induced by polyethylene glycol (peg) 6000. African Journal of Biotechnology, 6(9).

Rauf M, Munir M, UL Hassan M, Ahmad M Afzal M 2007. Performance of wheat genotypes under osmotic stress at germination and early seedling growth stage. African journal of biotechnology, 6(8). 971-975

Sairam RK, Rao KV, Srivastava G (2002). Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Science, 163(5): 1037-1046..

Sharma KR, Leung PS, Zaleski HM (1997). Economic analysis of size and feed type of swine production in Hawaii. Swine Health Prod., 5(3): 103-110.

Srivastava A, Chung SH, Fatima T, Datsenka T, Handa AK, Mattoo AK (2007). Polyamines as anabolic growth regulators revealed by transcriptome analysis and metabolite profiles of tomato fruits engineered to accumulate spermidine and spermine. Plant biotechnology, 24(1): 57-70.

Talaat IM, Bekheta M, Mahgoub MH (2005). Physiological response of periwinkle plants (*Catharanthus roseus* L.) to tryptophan and putrescine. Int. J. Agric. Biol, 7(2): 210-213.

Theiss C, Bohley P, Voigt J (2002). Regulation by polyamines of ornithine decarboxylase activity and cell division in the unicellular green alga chlamydomonas reinhardtii. Plant Physiology, 128(4): 1470-1479.

Todorova D, Sergiev I, Alexieva V, Karanov E, Smith A, Hall M (2007). Polyamine content in *Arabidopsis thaliana* (L.) heynh during recovery after low and high temperature treatments. Plant Growth Regulation, 51(3): 185-191.

Yang J, Zhang J, Liu K, Wang Z, Liu L (2007). Involvement of polyamines in the drought resistance of rice. J Exp Bot, 58(6): 1545-1555.

Yumurtaci A, Vardar F, Uenal M (2007). Inhibition of barley root growth by actinomycin d: Effects on mitotic activity, protein content and peroxidase activity. Fresenius Environmental Bulletin, 16(8): 917-921.

Zapata PJ, Serrano M, Pretel MT, Botella MA (2008). Changes in free polyamine concentration induced by salt stress in seedlings of different species. Plant growth regulation, 56(2): 167-177..

Zeid IM (2004). Response of bean (*Phaseolus vulgaris*) to exogenous putrescine treatment under salinity stress. Pakistan Journal of Biological Sciences, 7(2): 219-225.

Zidan I, Azaizeh H, Neumann PM (1990). Does salinity reduce growth in maize root epidermal cells by inhibiting their capacity for cell wall acidification, Plant Physiology, 93(1): 7-11.