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IMPACTS OF POLYAMINES ON THE MORPHO-PHYSIOLOGICAL CHARACTERS AND PROLINE CONTENT OF THE TWO DİFFERENT BARLEY CULTIVARS IN DROUGHT CONDITIONS

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Abstract

Impacts of the exogenously applied polyamines (PAs) on some physiological features and proline content in drought-resistant barley cultivars (*Hordeum vulgare* cv. Bülbül-89) and drought-susceptible (*Hordeum vulgare* cv. Burakbey) were comparatively examined under PEG-6000-simulated drought stress conditions. It was found that drought stress application had adverse impact on all physiological parameters at least 40% compared to that of the control group. It also dramatically increase the proline content of the cultivars. However, exogenously applied PAs (especially Spm on both barley cultivars) under drought stress conditions showed positive effect on all physiological parameters studied. The proline content increased in the presence of all PAs (especially Spd) in drought-susceptible Burakbey cultivar. If in Bülbül-89, polyamine that increases the proline content was Spd only. Among polyamines Cad was found as the polyamine that provided generally the least successful on all parameters.

Introduction

Drought is one of the most important environmental stresses which seriously threating plant productivity, and with climate change, this threat is increasing worldwide (Li *et al.* 2019). About 45% of the world's land surface is considered to be arid or semiarid. Over 2 billion people live in these areas where 25% of the world's population is located (National Geographic, 2023). However, 90% of the arid areas in the world are seen in developing countries (FAO 2019). Agricultural regions affected by drought can experience yield loss up to 50% or more. The vast majority of Türkiye is under the influence of the semi-arid climatic conditions (51 million hectares, 37% of Türkiye's territory).

Drought stress causes damage to plants at the cellular, tissue and organ level, preventing plants from getting water. Many physiological and biochemical processes essential for plant growth and development are affected by water deficit condition, and plants consequently exhibit various defense mechanisms against drought stress at the molecular, cellular and whole plant levels (Zhang *et al.* 2018). Physiological responses of plants to stress are effective in maintaining structural integrity and maintaining the functionality of the plant by developing resistance to dehydration through the accumulation of certain metabolites such as proline and glycine betaine, to ensure the survival of the plant (Annunziata *et al.* 2019). Reddy *et al.* (2004) reported that proline may protect proteins structure by maintaining their structural stability and act as free radical scavenger. There are numerous studies about the biological and metabolic functions of proline accumulation in drought and other stresses (Solanki *et al.* 2014, Bandurska *et al.* 2017, Dien *et al.* 2019).

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Polyamines (PAs) are aliphatic polycations ubiquitously present in all tissues and all cell types examined in animals and plants. Putrescine (Put), spermidine (Spd) and spermine (Spm) are the main polyamines found in plant and mammalian cells. Other than these PAs, flowering plants also synthesize cadaverine, 1,3-diaminopropane, and other modified forms. In plants, PAs function in the regulation of many physiological (rhizogenesis, somatic embryogenesis, pollen formation, flowering and fruit abscission, dormancy and senescence) functions and are associated with defense mechanisms in environmental stresses such as osmotic stress, mineral nutrient deficiency, high and low temperature, salt and drought stresses (Sairam and Tyagi 2004). Also, in plants PAs have specific and protective roles against abiotic stresses in a large range of processes including seed germination, seedling growth, mitotic activity and chromosomal aberrations (Çavuşoğlu *et al.* 2007, Tabur and Demir 2010). Changes in the content of PAs may both promote and control the accumulation of proline under salt stress conditions (Hayat *et al.* 2012). Increased proline content or polyamine biosynthesis in transgenic plants has been associated with tolerance to abiotic stresses (Kasukabe *et al.* 2004).

One of the most important way to reduce the harmful effects of abiotic stresses on plant cultivation is to produce plant varieties resistant to these stress factors. Therefore, various plant hormones, growth regulators and vitamins, which have protective function have been used in numerous studies to diminish or minimise harmful effects of abiotic stresses (Çavuşoğlu *et al.* 2017, Noman *et al.* 2018, Özmen and Tabur 2020). However, the correlation between these protectors used and tolerance of abiotic stresses is quite complex and not always apparent in plants. For this reason, this work was designed to compare the effects of the exogenously applied polyamines on some physiological features and proline content, in both drought susceptible and drought resistant cultivars of barley under PEG-6000-simulated drought stress.

Materials and Methods

The seeds of *Hordeum vulgare* cv. Bülbül-89 and *Hordeum vulgare* cv. Burakbey were obtained from the Ankara Field Crops Research Institute and from the Aegean Agricultural Research Institute (in Turkey), respectively. Spermine (Spm), spermidine (Spd), putresin (Put) and cadaverine (Cad) used in the experiment were obtained from Fluka and Sigma-Aldrich firms and Polyethyleneglycol (PEG-6000) was obtained from Merck firm. As a result of repeated preliminary studies, the most appropriate PEG-6000 concentration was found as 24% for Bülbül 89 and 22% for Burakbey (with a germination rate below 50%), and polyamine concentrations to be applied were determined as 10 µM.

Before the seeds were planted in petri dishes, they were subjected to surface sterilization in 1% sodium hypochlorite solution for 10 minutes and pretreated in distilled water (control) and in the medium with PAs, for 24 hours in room temperature. Then, the seeds were sown in petri dishes containing 10 ml of distilled water and PEG-6000 (at the appropriate concentration for each cultivar), and left to germinate in the incubator at 20°C. Physiological measurements were made after 7 days of germination. After determination of the final germination percentages, the coleoptile emergence percentages and radicle numbers were also recorded, and the coleoptile and radicle lengths of the seedlings were measured in mm, and in addition, the fresh weights in mg/seedling were determined. All experiments were repeated three times.

After the germinated seeds are transferred to peat soil in 9x5 viols, they were grown to the climate cabin for 21 days (one seed for each viol). The control group plants were irrigated every three days with 7 ml of distilled water while those of drought-treated were irrigated with 7 ml PEG 6000 at the appropriate concentration for each cultivar on the tenth day. Samples of both plant groups were taken from the climate cabin at the end of the 21st day, stored at -80°C by disintegrating with liquid nitrogen and then proline content were determined (Bates *et al.* 1973). Then after, 0.5 g fresh leaf samples were first homogenized with 3% sulfosalicylic acid. Acid ninhydrin and glacial acetic acid were added to the filtered homogenate, and they were taken to the ice bath after standing in a water bath at 100°C for 1 hr. Next mixture was extracted with toluene, the absorbance of the toluene fraction aspirated from the liquid phase was read on the spectrophotometer at a wavelength of 520 nm. Calculations were done as μmol g^{-1} with the help of the calibration curve created with the proline standard. Statistical evaluation of all parameters was carried out according to Duncan's (1955) multiple range test using SPSS 25 program.

Results and Discussion

Effects of exogenously applied polyamines on the physiological properties and proline content of the seedlings belonging to both barley cultivars germinated under stress-free and drought stress conditions were investigated and results are presented in Tables 1-2 and Fig. 1. After seven-days of germination, the germination rate of the Bülbül-89 seeds belonging to the control group was found as 97%. Drought stress applied alone (24% PEG-6000) decreased the germination percentage by approximately 50% compared to the control group. All of the polyamines studied under stress-free conditions caused a statistically insignificant decrease in germination percentage compared to the control group, but they showed close results to each other. All polyamines applied exogenously to Bülbül-89 seeds germinated under drought stress significantly encouraged germination. It was found that Spm showed the most successful effect in improving the negative effect caused by stress with 74% germination rate, although Cad, Spd and Put showed close results to each other (Table 1). On the other hand, in Burakbey seeds, the control group germination percentage was recorded as 90%. PEG-6000 application (22%) alone showed a 50% decrease as in Bülbül-89 seeds. Put application increased the germination percentage by 3%, Cad and Spm application showed a decrease of ~ 5%, as well as Spd application the same effect as the control. However, these values were statistically insignificant. All plant growth regulators applied, also in Burakbey seeds stimulated germination in drought stress conditions. The highest germination rate was recorded by Put, with an increase of ~50% (Table 2).

In addition, drought stress had an inhibitory effect of at least 40% on all other growth parameters studied in both cultivars. Among these parameters, the most negative effect of drought stress was recorded in the coleoptile percentage of Bülbül-89 with a rate of 95%. As compared with the control group, exogenous PAs pretreatment in conditions without stress has been statistically ineffective on these parameters in both Bülbül-89 and Burakbey cultivars. However, considering the numerical value, in Bülbül-89, Put and Spm showed the most successful effect on fresh weight (302,5 mg) and the radicle number (4.83), respectively. After 24% PEG-6000 application, in Bülbül-89 cultivar, the most effective polyamine to overcome the negative effect of drought has been Spm in all growth parameters studied while Cad is the most unsuccessful. If in Burakbey, after 22% PEG-6000 application, polyamine which showed the most positive affect on all growth parameters except coleoptile percentage was Spm, as in Bülbül-89. In addition, in Burakbey cultivar Cad maintained its unsuccessful on the growth parameters such as the radicle length and radicle number while Spm displayed the least success in the coleoptile percentage, and Spd in the coleoptile length.

According to the data obtained from the study, none of the polyamine applied under stress-free conditions had a stimulate effect on the physiological parameters studied here [except for the effect of Spd on the coleoptile length in Burakbey cultivar, and the effect of all polyamines (especially Put) on the fresh weight in Bülbül-89]. In other words, it was found that all polyamines applied showed statistically the same effects on both cultivars as the control group. In the absence of any stress conditions, the growth regulators given to the plants in exogenous may have positive effects on seed germination, seedling growth and cell division, as well as causing negative effects (Çavuşoğlu *et al.* 2007; Tabur and Demir 2010). Çavuşoğlu *et al.* (2007) reported that polyamines exogenously added in barley plants do not have an inhibitory effect on the germination rate.

Table 1. Effects of exogenous PAs treatments on some growth parameters of *H. vulgare* L. Bülbül-89.

Polyamines (10µM)	Germination %	Fresh weight (mg/seedling)	Radicle length (mm)	Radicle number	Coleoptile %	Coleoptile length (mm)
Control (Distilled water)	97.00±2.00[d*]	271.25±5.85[c]	145.69±9.41[b]	4.63±0.23[a]	97.00±2.00[b]	99.27±3.66[a]
Cad	96.00±3.27[d]	289.75±6.65[cd]	140.06±6.09[b]	4.77±0.09[a]	96.00±3.27[b]	90.66±3.21[a]
Spd	93.00±5.03[d]	288.00±27.73[cd]	146.03±13.51[b]	4.64±0.12[a]	93.00±5.03[b]	94.13±10.15[a]
Put	93.00±5.03[d]	302.50±17.33[d]	134.14±8.78[b]	4.82±0.13[a]	93.00±5.03[b]	97.95±5.84[a]
Spm	92.00±5.66[d]	291.00±6.06[cd]	141.89±4.64[b]	4.83±0.15[a]	92.00±5.65[b]	95.13±4.11[a]
PEG 6000 (%24)	47.00±6.00[a]	160.75±10.53[b]	27.31±3.56[a]	2.23±0.19[b]	2.00±2.31[a]	5.25±6.08[b]
PEG+Cad	67.00±5.03[bc]	123.25±15.84[a]	23.61±5.14[a]	1.71±0.18[a]	2.00±4[a]	2.63±5.25[b]
PEG+Spd	65.00±6.00[b]	140.50±8.39[ab]	29.96±7.08[a]	2.06±0.51[ab]	3.00±3.8[a]	6.75±7.81[b]
PEG+Put	65.00±3.83[b]	145.50±13.33[ab]	29.84±0.19[a]	1.87±0.30[ab]	2.00±2.3[a]	5.00±5.77[b]
PEG+Spm	74.00±6.93[c]	142.75±16.44[ab]	30.35±3.21[a]	2.19±0.32[b]	5.00±3.8[a]	11.15±9.46[b]

*Values with insignificant difference (P ≤ 0.05) for each column are indicated with same letters (± Standard deviation). As test solution, PEG-6000 concentration was determined as 24% with a germination rate below 50% and polyamine concentrations to be applied were 10 µM. Seeds were pretreated in distilled water (control) and in the medium with PAs for 24 hours in room temperature and sown in petri dishes containing 10 ml of distilled water and 24% PEG-6000. Physiological measurements were made after 7 days. All data are a mean of three replicates.

Table 2. Effects of exogenous PAs treatments on some growth parameters of *H. vulgare* L. Burakbey.

Polyamines (10µM)	Germination %	Fresh weight (mg/seedling)	Radicle length (mm)	Radicle number	Coleoptile %	Coleoptile length (mm)
Control (Distilled water)	90.00±5.16[c*]	254.00±18.82[c]	126.37±18.37[b]	5.38±0.17[c]	90.00±5.16[b]	78.84±6.48[b]
Cad	85.00±10.52[c]	261.25±21.52[c]	138.73±16.17[b]	5.44±0.23[c]	85.00±10.52[b]	84.05±3.60[bc]
Spd	89.00±3.83[c]	263.50±17.06[c]	139.62±13.79[b]	5.43±0.16[c]	89.00±3.83[b]	87.66±4.38[c]
Put	93.00±6.00[c]	249.00±10.17[c]	139.23±14.16[b]	5.31±0.14[c]	93.00±6.00[b]	79.69±2.74[b]
Spm	86.50±7.55[c]	260.00±12.33[c]	133.91±16.08[b]	5.40±0.05[c]	86.50±7.55[b]	84.95±6.39[bc]
PEG 6000 (%22)	48.00±3.27[a]	145.75±13.45[ab]	38.19±4.08[a]	3.79±0.25[ab]	29.00±5.03[a]	18.64±3.26[a]
PEG+Cad	69.00±6.00[b]	136.00±8.49[a]	35.00±4.97[a]	3.42±0.24[a]	34.00±8.33[a]	17.29±2.65[a]
PEG+Spd	73.00±5.03[b]	142.50±10.34[ab]	39.34±3.91[a]	3.66±0.16[ab]	40.00±5.66[a]	16.67±1.15[a]
PEG+Put	75.00±5.03[b]	125.50±9.00[a]	38.15±1.98[a]	3.45±0.20[a]	34.00±6.93[a]	17.16±1.75[a]
PEG+Spm	51.00±11.49[a]	158.00±2.16[b]	42.91±6.19[a]	3.89±0.48[b]	32.00±13.47[a]	21.54±4.03[a]

*Values with insignificant difference (P ≤ 0.05) for each column are indicated with same letters (± Standard deviation). As test solution, PEG-6000 concentration was determined as 24% with a germination rate below 50% and polyamine concentrations to be applied were 10 µM. Seeds were pretreated in distilled water (control) and in the medium with PAs for 24 hours in room temperature and sown in petri dishes containing 10 ml of distilled water and 24% PEG-6000. Physiological measurements were made after 7 days. All data are a mean of three replicates.

Similarly, Palavan *et al.* (1990) stated that Spm, Spd and Put are not effective in germinating wheat seeds, while Mutlu and Bozcuk (2000) observed that Put and Spm were ineffective in sunflower seeds. However, most of the polyamine applications were generally found to be successful in improving the inhibitory effect caused by drought stress on all the growth parameters mentioned in both cultivars (Tables 1 and 2). For example; in the drought-resistant Bülbül-89 cultivar, it was determined that Spm among the applied polyamines reduces the negative effect of stress on the germination percentage, radicle length, coleoptile length and coleoptile percentage. Whereas, it was noted that none of the polyamines exhibited a curative effect on fresh weight and the radicle number in this cultivar. On the other hand, in the drought-sensitive Burakbey cultivar, polyamines studied under drought stress conditions generally showed a stimulating effect that can be considered statistically significant. In this context, it was determined that Spm had the most positive effect on fresh weight, radicle length, radicle number, coleoptile length, Put's germination percentage, and Spd's coleoptile percentage. Farooq *et al.* (2009) reported that the adverse effect caused by drought stress on the fresh weight, germination percentage, radicle length was improved with Spd exogenously added in rice plant. Zeid and Shedeed (2006) stated that Put treatment against drought stress promotes germination percentage and radicle length in alfalfa plant. Aydin *et al.* (2016) proved that putresin used against drought stress in wheat increases germination percentage, radicle number, radicle length and coleoptile length.

At the end of the 21st day, proline content in leaves of drought-resistant Bülbül-89 grown at control conditions (irrigated every three days with 7 ml of distilled water) was determined as 20.06 µmol g^{-1}, and 240.7 µmol g^{-1} in drought-susceptible Burakbey. While all polyamines applied to the control group of both cultivars significantly decreased the amount of proline, the decrease in Burakbey cultivar was higher (Fig. 1).

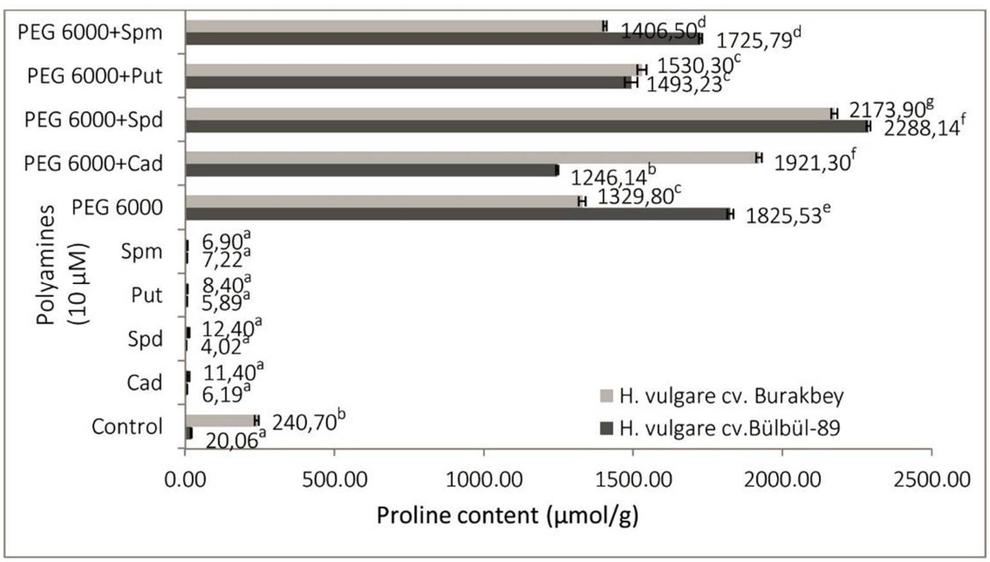

Fig 1. The effect of polyamines on proline content in leaves of *H. vulgare* L. Bülbül-89 and *H. vulgare* L. Burakbey cultivars under PEG-6000-simulated drought stress. *Values with insignificant difference ($P \leq 0.05$) for each column are indicated with same letters (± Standard deviation). As test solution, PEG-6000 concentration was determined as 24% for Bülbül 89 and 22% for Burakbey and polyamine concentrations to be applied were 10 µM. Seeds were pretreated in distilled water (control) and in the medium with PAs for 24 hrs in room temperature and sown in Petri dishes containing 10 ml of distilled water and PEG-6000. Physiological measurements were made after 7 days. All data are a mean of three replicates.

As a result of drought stress application in Bülbül-89 cultivar, the amount of proline increased significantly (90 fold) compared to control and reached 1825.5 µmol g^{-1}. If in Burakbey cultivar, drought stress applied alone increased 6-fold the amount of proline and it was detected as 1329.8 µmol g^{-1}. In Bülbül-89 cultivar, after the polyamine application in drought stress conditions, Spd displayed a statistically significant increase in the amount of proline as compared alone 24% PEG-

6000 application. Whereas other polyamines studied decreased the amount of proline and Cad (1246,14 μmol g^{-1}) was determined as the polyamine that provided the most reduction on this parameter. In Burakbey cultivar, after the polyamine application in drought stress conditions, all polyamines studied showed remarkably increase in the amount of proline and the most successful effect was again achieved by Spd. The results obtained for this cultivar were statistically rather significant (Fig. 1).

Results of the present study have indicated that all polyamines applied to the control group (in non-stress conditions) of both cultivars significantly decreased the amount of proline, especially in Burakbey cultivar decreased more. This decrease in the amount of proline suggests to the fact that all polyamines (especially Spd in Bülbül-89 and Spm in Burakbey) contribute to the resistance of these barley cultivars.

Whereas, accumulation of proline in leaves of both plants under drought stress compared to control Bülbül-89 under drought stress conditions proves that this cultivar's drought stress tolerance is higher. Because accumulation of proline is believed to play an adaptive role in plants during drought stress. According to Blum and Ebercon (1976) a positive correlation between magnitude of proline accumulation and drought tolerance an index for determining drought tolerance potential of different genotypes. The current results indicate a powerful positive correlation between proline accumulation and drought tolerance in both barley cultivars. In addition, the present results expressed that Burakbey cultivar, which are more sensitive to drought than Bülbül-89, gained a better tolerance to drought stress due to application of PAs at appropriate doses. Handa *et al.* (2018) reported that PAs positively impact cellular functions especially in plant development and responses to extreme environments. Also these researchers suggested that one or more PAs are commonly found in genomic and metabolomics studies using plants, particulary during different abiotic stresses. Alcázar *et al.* (2010) noted that PAs are molecules with regulatory functions that tolerate abiotic stresses, including drought and salinity in plants. Application of PEG-6000 at concentrations of 24 and 22%, respectively increased the proline accumulation 90 fold in drought-resistant Bülbül-89 and 6 fold in drought-susceptible Burakbey (Fig. 1). These results are in agreement with the findings of Szabados and Savouré (2010), Solanki *et al.* (2014), Dien *et al*. (2019).

Considering the data obtained from the present study, it has been determined that drought stress application had adverse impact on all physiological parameters at least 40% compared to the control group. However, exogenously PAs applied under drought stress conditions have enabled development under these adverse conditions. Furthermore, the increase in proline content, which plays an important role in response to stress, was also achieved in the presence of PAs, it has proved that PAs can be used to eliminate or improve the negative effect of drought stress on plants. Especially, while Spm and Spd were had the best effect on all parameters studied Cad was PA that the least successful. In this context, it is thought that using appropriate doses of PAs will potentially beneficial for tolerance gain of drought-susceptible plant species such as Burakbey cultivar against various abiotic stresses.

Considering the physiological results of the present study, it may be concluded that the most successful polyamine is Spm in both cultivars studied in alleviating the inhibitory effect caused by drought stress. Only in Burakbey cultivar, the polyamine that had the most positive effect were Put on the germination percentage, and Spd on the coleoptile percentage. In addition the effect of polyamines may differ according to the plant species examined, polyamine concentrations applied and pretreatment forms. The present work will provide new insight into the process of drought tolerance in barley; aiding breeding programs aimed at increased resistance and shed light on similar subsequent studies.

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