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EXOGENOUS SPERMIDINE ALLEVIATES NEGATIVE EFFECTS OF DROUGHT STRESS ON ANTIOXIDANT ENZYME ACTIVITY, CELL CYCLE REGULATION, ENDOGENOUS POLYAMINES AND TOTAL PROTEIN AMOUNT IN *HORDEUM VULGARE* L.

SIGNEM ONEY-BIROL[1], SELMA TABUR[2] AND SERKAN OZMEN[2*]

[1]Department of Moleculer Biology and Genetics, Faculty of Arts and Science, Burdur Mehmet Akif Ersoy University, 15030-Burdur, Turkey
[2]Department of Biology, Faculty of Engineering and Natural Sciences, Süleyman Demirel University, 32260-Isparta, Turkey
**Corresponding author's email: serkanozmn@yahoo.com*

Abstract

Effects of exogenous spermidine (Spd) (10µM) application on the biochemical enzymes and molecular effects in the Burakbey cultivar of barley were investigated both normal (distilled water) and drought (-6,30 mPA PEG 6000) conditions. It is obvious that drought stress applied to barley cultivars negatively affected plant metabolism and inhibited plant growth when all other parameters were taken into account. Under normal conditions, Spd showed an impact as if drought stress were applied to the plant at a low rate. Exogenous Spd application under drought stress alleviated the harmful effects of drought stress by showing encouraging effects on cell cycle, total protein content, malondialdehyde (MDA), and ascorbate peroxidase (APX) levels. Superoxide dismutase (SOD) and catalase (CAT) enzyme levels in the same group increased further after exogenous Spd application and formed a response to drought stress. As a result, it has been revealed that the application of exogenous Spd in barley can increase endogenous polyamine, antioxidant capacity, promotion of cell division, and protein content, as well as ameliorate the detrimental effects of drought stress on plant metabolism.

Key words: Biochemical activity, Cell cycle, Endogenous polyamines.

Introduction

Climates are changing day by day and plants are exposed to the stresses created by changing environments (Kour *et al*., 2020). Drought, caused by global climate change and disruption of ecological balance has become a serious ecological problem facing all humanity and a key factor that restricts the development of agricultural production (Batool *et al*., 2020; Seleiman *et al*., 2021; Yang *et al*., 2021). The effects of drought stress are highly variable and generally have negative effects on the physiological, morphological, ecological, biochemical and molecular properties of plants (Bhargava & Sawant, 2013, Roy *et al*., 2021; Saleem *et al*., 2023). In addition, drought stress may adversely affect plant growth, quantity and production levels (Salehi-Lisar & Bakhshayeshan-Agdam, 2016). Reactive oxygen species (ROS) are produced as a response mechanism to drought stress in plants. These species interact directly with membrane lipids and a large number of macromolecules, causing significant damage to plants, resulting in reduced plant growth and productivity (Yang *et al*., 2009). In addition to this situation, drought stress has detrimental effects on those processes such as stomatal conductivity, cell turgidity, sweating, photosynthesis, respiration, proline, and soluble sugar production (Velázquez-Márquez *et al*., 2015; Emami Bistgani *et al*., 2017; Falqueto *et al*., 2017; Al-Selwey *et al*., 2023a and 2023b; Seleiman *et al*., 2023). Therefore, it is highly important to increase the resistance of plants to drought stress and to regulate their vital activities under drought stress (Talaat & Shawky, 2016).

Polyamines are a class of biogenic amines that are organic polycations having variable hydrocarbon chains and two or more primary amino groups (Mattoo *et al*., 2015). Diamine putrescine (Put), triamine spermidine (Spd), tetraamine spermine (Spm) are the most common polyamine varieties found in living organisms. Polyamines play an important role in physiological processes such as organogenesis, embryogenesis, flower development, leaf aging, pollen tube growth, fruit development, response to abiotic and biotic stresses, as well as regulating basic cellular processes such as cell division, gene expression, DNA, protein synthesis, and apoptosis (Alcázar *et al*., 2010; Tiburcio *et al*., 2014; Özmen *et al*., 2022a).

Polyamines, which have an important role in plant growth and development, are synthesized from arginine and ornithine by the enzymes arginine decarboxylase (ADC) and ornithine decarboxylase (ODC). Agmatine synthesized from arginine is converted to Put, then Spd and Spm are formed as a result of adding aminopropyl moieties catalyzed by Spd and Spm Synthesis to the skeleton structure of Put. The aminopropyl groups included in the structure of Spd and Spm are derived from the methionine formed as a result of the reaction catalyzed by S-adenosyl methionine (SAM) and SAM decarboxylase (SAMD) (Pál *et al*., 2018).

One of the three main polyamines, Spd which is applied exogenously against environmental stresses causes an increase in photosynthetic pigments, cell membrane stability, leaf water content, and decreases the number of chromosomal abnormalities, is the type of polyamine most commonly associated with stress tolerance in plants (Shen *et al*., 2000). In other words, application of Spd exogenously provide the continuity of function under stress and showed healing effects on the negative effects of stress in plants (Tabur & Demir, 2010; Liu *et al*., 2016; Mustafavi *et al*., 2016; Özmen *et al*., 2022b). The increase in polyamine levels in various plants during the adaptation phase to stress revealed that polyamines were involved in this process. Polyamine levels that vary in the presence of stress depend

on a variety of factors such as plant type, stress duration, tolerance, or sensitivity to stress (Amri *et al*., 2011).

Therefore, this study was designed to reveal the effects of exogenous Spd application on reducing the harmful effects of drought stress on endogenous polyamine, cell cycle, total protein amounts, and some biochemical substances such as SOD, CAT, APX, MDA in Burakbey cultivar of barley.

Material and Methods

Plant growth and drought treatment: Barley seeds (*Hordeum vulgare* cv. Burakbey) were obtained from Aegean Agricultural Research Institute Directorate, Turkey. Spd (124-20-9) was used as a growth regulator in the study, and PEG 6000 (25322-68-3), which showed the effect of drought stress, were purchased from Merck. After the preliminary studies, it was determined that the PEG 6000 concentration, which reduced the germination percentage below 50%, was -6.30 mPa to show the impact of drought stress in barley seeds. The next step after the definition of the PEG 6000 concentration, Spd concentration that best improved the negative effect of stress on the germination was determined as 10 µM. All germination experiments were carried out in the climate cabinet set at 22°C in dark ambient conditions. While cell cycle analyzes were made from the root tips obtained from the seeds kept in the climate cabinet after 7 days, samples from leaf tissue were used for all the remaining analyses. The seeds germinated in the incubator for a week were transferred to the peat soil in 9x5 viols and then grown in the climatic cabinet for 21 days. Plants were grown for 14 days in a growth chamber maintained at 10,000 lux light for 16 hours daily with 25/18◦C (day/night). The growing section in control group, plants were irrigated with 7 mL of distilled water per pod every three days, while the drought-treated plant group was irrigated with 7 mL of -6.30 MPa PEG 6000 solution under the same conditions. Samples of both groups were taken from the climate cabinet at the end of the 21st day and leaves were analyzed after they were grinded in liquid nitrogen for further experiments and stored at -86°C.

Flow cytometric assay: In this study, cell cycle determination studies were carried out with the assistance of Capal Petr from the Institute of Experimental Botany of the Czech Academy of Sciences. Quantification of cellular DNA and analysis of the cell cycle were determined by CyFlow Space flow cytometer (Sysmex Partec). In order to analyze the differences in the cell cycle samples from 3 different plants from each group were taken and the results were compared. Cell cycle analyzes were performed according to the method of Dolozel & Bartos (2005). The root tips of the seven days old barley plants were cut (c. 2.5 cm) and placed on the petridishes having ice. The root tips taken on ice were cut into small pieces in 5 mL of Otto I solution with the help of a scalpel. Broken pieces were filtered with the help of 33 µm mesh and 10 mL of Otto II solution was added, they were analyzed in flow cytometry and the cell cycle was determined. The changes between the cell cycle phases with the Flowing Software program were determined by comparing the results obtained from the samples after reading an equal number of nuclei in each suspension.

Antioxidant activity assay: To determine the amount of endogenous polyamines, samples taken from leaf tissues were analyzed in HPLC according to the method of Anlı *et al*., (2004), 2.5 g of leaf tissue was homogenized with 25 mL of 0.4 M perchloric acid on ice. The obtained homogenates were centrifuged at 3°C 10000 rpm for 10 min. and filtered with a 0,45 µm filter. In the 400 µL of the filtered sample 400 µL of Na_2CO_3 and 400 µL of dansyl chloride were added and kept in a 40ºC water bath for half an h. 200 µL of Na-L-glutamate monohydrate was added to the mixture, which was kept in the water bath, under the same conditions for 1 more h. Finally, 1 mL CH_3CN was added to the samples taken from the water bath and centrifuged at 3000 rpm for 15 min and the samples taken in the upper phase were analyzed in HPLC.

Total protein: To determine the total protein amount, 0,3 g of leaf tissue was homogenized with 4 mL of 50 mM potassium phosphate baffur solution (pH: 7.0) containing 2 mM Na-EDTA and 1% PVP, then centrifugre at 4°C at 10.000 rpm for 10 min. The resulting supernatants were used for protein analysis Ozden *et al*., (2009). Total protein amounts were determined from the mixture by applying the protocol of the Bio-Rad extraction kit (Bradford, 1976).

Biochemical analysis

MDA: The measurement of the amount of MDA for the specification of the lipid peroxidation level was made according to the Li (2000) method. According to this method, 200 mg of fresh leaf tissue was crushed in a mortar with 5 mL of TCA solution, the homogenates were centrifuged at 1000 rpm for 25 min. After 2 mL of each concentration of the supernatants was taken, 2 mL of 0.6% TBA solution in 10% TCA was added, and the mixture was boiled in a steam bath for 15 min. and then instant cooling was applied in an ice bath. Finally, the material was centrifuged at 4000 rpm for 10 min, the absorbance of the supernatant was determined at 600, 532 and 450 nm levels in spectrophotometry. As a result, MDA calculation is; Calculated according to 6.45 $(A_{532} – A_{600}) – 0.56\ A_{450}$.

SOD: To determine the SOD enzyme levels, the enzyme extracts were prepared at +4ºC. After washing the fresh leaves in 0.2 g distilled water, they were homogenized with 5 mL of cold Sodium Phosphate buffer (50mM, pH 7.8) in a mortar. The homogenates were centrifuged at 10,500 rpm for 20 min., the mixture was obtained according to the method of Beauchamp & Fridovich (1971). The resulting 3 mL mixture was kept in 2X15W fluorescent lamps for 10 min, then kept in the dark for 10 min. and measured at 560 nm.

CAT: Measuring catalase enzyme levels was done according to the Beers & Sizers (1952) method. Enzyme extracts and preparation processes were done at +4 degrees. Fresh leaves were washed in 0.2 g distilled water, homogenized with 5 mL cold Sodium Phosphate buffer (50mM, pH 7.8) in a mortar, the homogenates were centrifuged at 10,500 rpm for 20 min. The Cat level was determined by calculating the change in the result of the 4-minute kinetic reading at 240 nm in the centrifuged samples.

APX: For the quantification of APX enzyme amount, fresh leaves were washed in 0.2 g distilled water and homogenized with 5 mL cold Sodium Phosphate buffer (50mM, pH 7.8) in mortar. After the homogenates were centrifuged at 10,500 rpm for 20 min., the extract was prepared according to the method of Kato & Shimuzu (1987), and the measurement was made by calculating the change in the kinetic reading at 290 nm for 4 min.

Stastistical analysis

Statistical results were obtained as a result of three replications of the samples obtained from three different tissues for all analyzes of all parameters examined in the study. In order to determine the statistical differences, SPSS program (IBM Corp, Armonk, NY USA) was used and Duncan test analysis of the program was performed. In addition, correlation and PCA analyzes were performed using the same program.

Results

Change values between cell cycle phases are shown in Figure 1. The density of the cells were 20.93% arrested in G_1 phase, in the S phase 61.42%, and in the G2 phase 17.65% in the control group. After treatment with exogenous Spd alone, an increase c. 50% was detected in the G_1 phase of the cell cycle compared to the distilled water medium. In the S phase, there was a decrease of c. 50%. This showed that it prevented cell division as a result of the accumulation of cells in the G_1 phase. In the G_2 phase, an increase of almost 100% was observed after the application of Spd and the cell density of 34.47% was determined. The results obtained after the application of drought stress were found similar to exogenous Spd treatment alone. Accordingly, while the cell accumulation in the G_1 stage was increased over 100% in the application of drought stress, this increase was also statistically significant. Cells in the S phase of the cell cycle showed a 45% reduction due to the inhibitory effect caused by drought. While the results obtained from the G_2 phase after drought stress application did not differ statistically, they showed relative values compared to the control group. Exogenous Spd application against drought stress clearly showed ameliorative effect on cell cycle parameter. After exogenous Spd application under drought stress, there was a decrease in cell densities in the G_1 stage, while there was an increase in the cell densities in the S and G_2 stages compared to the concentration applied drought stress alone. Especially the decrease of approximately 75% in the G_1 phase and the increase of more than 100% in the G_2 phase revealed that the Spd application under drought stress was quite significant both numerically and statistically (Fig. 2).

According to the HPLC analysis, it was found that the amount of Put, which was one of the endogenous polyamine varieties, was higher than the other polyamines with a level of 120.95 µg/g in the control group barley seedlings. The amounts of endogenous Spd and Cad in the control group were close to each other and these amounts were 4,90 and 4,25 µg/g, respectively. Spm, another type of polyamine, had the least amount of endogenous polyamine content in the control group with the amount of 1,00 µg/g. After the exogenous Spd application to the control group, all endogenous polyamine amounts were increased and all of these increases were statistically significant. When the increases in the amount of endogenous polyamines were examined, the polyamine variety showing the most rise was Spm with a value of 3,25 µg/g, which increased proportionally more than 200%. Drought caused a disproportionate change in the content of polyamines in plant metabolism. Accordingly, drought stress caused a 55% decrease in the amount of endogenous Cad and a 75% decrease in the amount of Put, while the endogenous amounts of Spm and Spd showed close values under drought stress and increased up to 14 µg/g levels. Exogenous Spd application under drought stress caused a decrease in all endogenous polyamine amounts compared to the drought stress control group, in other words, exogenous Spd application under stress showed a mitigating effect on the negative effects of stress (Fig. 3).

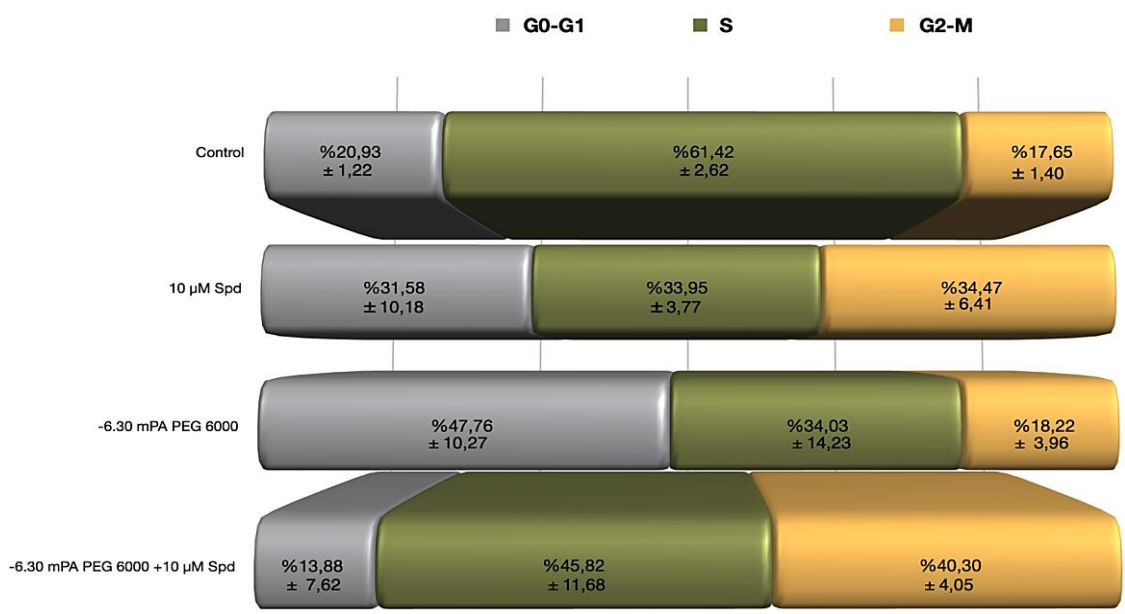


Fig. 1. Cell cycle change showing the effect of exogenous Spd on *H. vulgare* L. cv. Burakbey seeds germinated in control group (distilled water) and drought stress environment (-6,30 mPa PEG 6000). The pretreatment process of seeds was performed by soaking 24 h in constant volumes of distilled water (control) or Spd. ± Standard deviation.

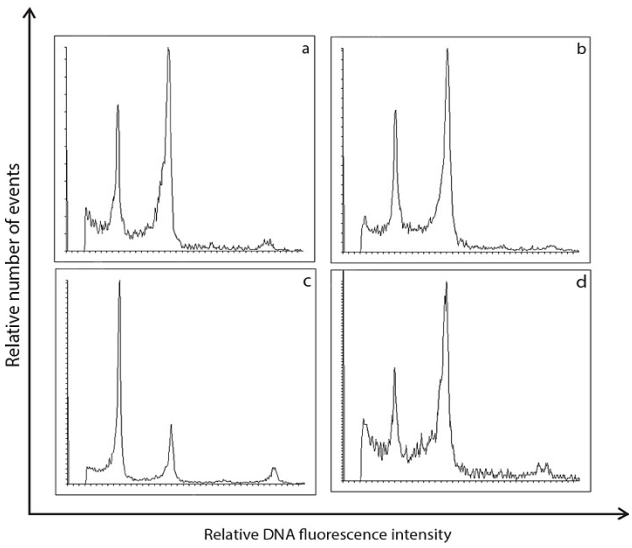
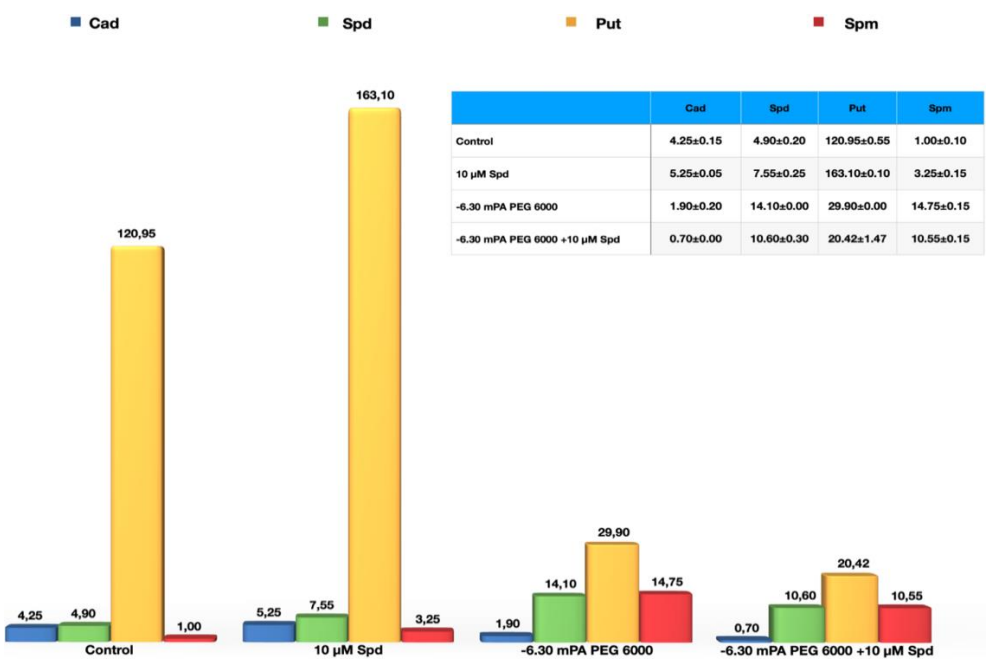


Fig. 2. Flow cytometry plots of separation of cells in mitotic phases of the cell cycle. (a: Control, b: 10 µM Spd, c: -6.30 mPa PEG 6000, d: -6.30 mPa PEG 6000+10 µM Spd.)

The effect of exogenous Spd application and drought stress on total protein amount are given in Table 1. In the control group, the total protein amount of *H. vulgare* cv. Burakbey was found to be 0.385 mg/mL, while it was determined that there was an increase in the total amount of protein in the remaining study groups. It has been determined that exogenous Spd application applied in a nonstress environment has an encouraging effect on the total protein amount. As a result of stress application to the *H. vulgare* the total protein amount was increased approximately 3.5 times compared to the control group and was measured as 1.314 mg/mL. After the application of exogenous Spd under drought stress, the total protein amount was increased more than the stress environment control and it was revealed that it had the highest value among all the concentrations studied with a total protein amount of 1.726 mg/mL.

The effects of exogenous Spd application on MDA, SOD, CAT and APX activities are shown in Table (1). It was determined that both exogenous Spd application alone and drought stress were increased SOD levels compared to the control group. While the mean SOD level in the control group was measured as 0.37 µMol/L^{-1}, after application of alone exogenous 10 µM Spd and -6.30 mPa PEG 6000, SOD levels were determined to be 0.38 µMol/L^{-1}, and 0.41 µMol/L^{-1}, respectively. In exogenous spd application under drought stress, SOD was increased by 30% compared to the drought stress control group and increased to 0.50 µMol/L^{-1}. When the change in MDA levels were examined, the mean MDA activity in the control group was measured as 0.12 µMol/L^{-1} and it was determined that the activity was increased at all other study concentrations. According to these results, MDA levels were determined as 0.16 µMol/L^{-1}after exogenous Spd application alone, 0.41 µMol/L^{-1}after -6.30 mPa PEG 6000 application, and 0.21 µMol/L^{-1} after exogenous Spd application under drought stress. The results clearly showed that the application of exogenous Spd under drought stress reduced the MDA level by approximately 50%, creating a response to drought stress. Considering the change in CAT levels, it was determined that exogenous Spd application alone had the highest CAT level with the amount of 14.86 µmol min^{-1}/mg. Drought stress and exogenous Spd application under drought stress showed an increase in CAT activity compared to the control group and increased to 12.09 µmol min^{-1}/mg and 13.39 µmol min^{-1}/mg, respectively. While the amount of APX in the control group was measured as 109.26 µmol min^{-1}/mg, the amount of APX was increased after drought stress and reached 121.50 µmol min^{-1}/mg. Exogenous Spd applied to the control group and under drought stress had a decreasing effect on the amount of APX compared to their control groups, and the value of 89.21 µmol min^{-1}/mg in the Spd concentration applied under drought stress was the lowest value among all concentrations.



	Cad	Spd	Put	Spm
Control	4.25±0.15	4.90±0.20	120.95±0.55	1.00±0.10
10 µM Spd	5.25±0.05	7.55±0.25	163.10±0.10	3.25±0.15
-6.30 mPA PEG 6000	1.90±0.20	14.10±0.00	29.90±0.00	14.75±0.15
-6.30 mPA PEG 6000 +10 µM Spd	0.70±0.00	10.60±0.30	20.42±1.47	10.55±0.15

Fig. 3. Endogenous polyamines content change showing the effect of exogenous Spd on *H. vulgare* L. cv. Burakbey seeds germinated in control group (distilled water) and drought stress environment (-6,30 mPa PEG 6000). The pretreatment process of seeds was performed by soaking 24 h in constant volumes of distilled water (control) or Spm. ± Standard deviation.

Table 1. Biochemichal characteristics change table showing the effect of exogenous Spd on *H. vulgare* L. cv. Burakbey seeds germinated in control group (distilled water) and drought stress environment (-6,30 mPa PEG). ± Standard deviation.

Treatment	Total protein amount (mg/mL)	MDA (µMol/L)	SOD (µMol/L)	CAT (µMol min^{-1}/mg)	APX (µMol min^{-1}/mg)
Control	0.39 ± 0.02	0.12 ± 0.01	0.37 ± 0.03	10.69 ± 0.69	109.26 ± 3.57
10 µM Spd	$0.88 \pm 0.07^{***}$	$0.16 \pm 0.06^{***}$	0.38 ± 0.04^{NS}	14.87 ± 3.70^{NS}	104.74 ± 8.30^{NS}
-6.30 mPa PEG	$1.31 \pm 0.12^{**}$	$0.41 \pm 0.01^{**}$	0.39 ± 0.08^{NS}	12.09 ± 0.83^{NS}	121.50 ± 8.36^{NS}
-6.30 mPa PEG+10 µM Spd	$1.73 \pm 0.07^{*}$	$0.21 \pm 0.01^{**}$	0.50 ± 0.02^{NS}	13.39 ± 0.64^{NS}	$89.21 \pm 4.88^{*}$

Table 2. Correlation of exogenous Spd application under drought stress among all investigated parameters.

	G_1	S	G_2	TP	Spm	Spd	Put	Cad	SOD	CAT	APX	MDA
G_1	1.00	-0.99^{**}	0.97^{**}	0.56	0.58	0.83^{**}	0.32	0.07	-0.19	0.83^{**}	0.54	-0.23
S		1.00	-0.98^{**}	-0.62	-0.58	-0.78^{**}	-0.29	0.13	0.18	-0.87^{**}	-0.50	0.19
G_2			1.00	0.72^{**}	0.67^{*}	0.68^{*}	0.43	-0.17	-0.06	0.91^{**}	0.56	-0.03
TP				1.00	0.33	0.01	0.26	-0.72^{*}	-0.23	0.92^{**}	0.09	0.60
Spm					1.00	0.51	0.83^{**}	0.40	0.69^{*}	0.41	0.92^{**}	-0.07
Spd						1.00	0.21	0.43	0.01	0.37	0.60	-0.70^{*}
Put							1.00	0.37	0.77^{**}	0.24	0.89^{**}	0.27
Cad								1.00	0.70^{*}	-0.56	0.61	-0.64^{*}
SOD									1.00	-0.32	0.71^{*}	0.01
CAT										1.00	0.24	0.29
APX											1.00	-0.17
MDA												1.00

*Correlation is significant at the 0.05 level.** Correlation is significant at the 0.01 level

TP: Total Protein amount, Spm: Endogenous spermine, Spd: Endogenous spermidine, Put: Endogenous putressine, Cad: Endogenous cadaverine, SOD: Superoxide dismutase, CAT: Catalase, APX: Ascorbate peroxidase, MDA: Malondialdehyde

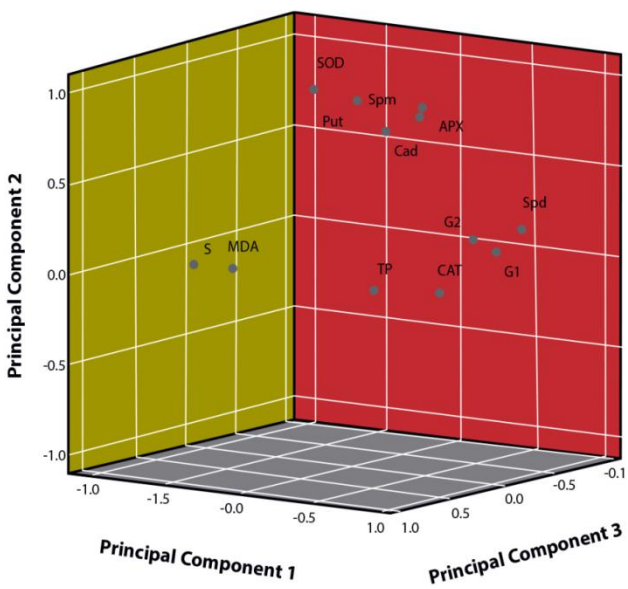


Fig. 4. Principal component analysis (PCA) biplot showing correlations between all studied parameters of Exogenous Spd application under drought stress. TP: Total Protein Content, Spm: Spermine, Spd: Spermidine, Put: Putressine, Cad: Cadaverine, SOD: Superoxide Dismutase, CAT: Catalase, APX: Ascorbate Peroxidase, MDA: Malondialdehyde.

The correlation values between all investigated parameters of exogenous Spd application under drought stress are given in Table 2 and PCA analyzes are given in Figure 4. The study showed that there were very strong relationships among many parameters. Accordingly, it was determined that the G1 phase of the cell cycle had a very strong positive correlation with the G2 phase (R^2=0.97), endogenous Spd (0.83) and CAT (0.83) enzyme activity, and a very strong negative correlation with the S phase (-0.99). The correlation values of the S phase of the cell cycle were found to be very strongly negative with the G2 phase (-0.98), endogenous Spd (-0.78) and CAT enzyme activity (-0.87), in contrast to the G1 phase. Unlike the first two phases, the G2 phase the last phase of the cell cycle, showed a very strong correlation with the total protein amount (0.72), and a strong correlation with the endogenous Spm (0.67) and Spd (0.68) content. It was revealed that the content of total protein had a positive and very strong correlation with CAT enzyme activity (0.92), and the amount of endogenous Cad (-0.72) had a strong negative correlation. Endogenous Spm content showed a positive correlation with endogenous Put content (0.83) and APX enzyme amount (0.92). The endogenous Put content, on the other hand, showed a very strong positive correlation with SOD (0.77) and APX enzyme activity (0,89). As a result, it has been determined by this study that there are very strong correlations between the interrelationships of the cell cycle's own phases and the endogenous polyamine contents, especially between the CAT enzyme activity (Table 2).

Discussion

Stress negatively affects plant growth, and plants grown under stressful conditions are morphologically smaller than plants grown under normal conditions. Although there are many reasons for this size reduction, it is usually associated with a decrease in cell number (Razem *et al*., 2006). One of the reasons that drought stress reduces the number of cells is that the cells remain

stable at the G1/S and G2/M checkpoints in the cell cycle. Another reason is that the transition of Cyclin Dependent Kinases (CDK) to M phase is slowed down due to the prolongation of the process in the S phase (De Veylder *et al*., 2007; Kitsios & Doonan, 2011). Under drought stress, growth of root tips is restricted due to the inhibition of cell division and cell cycle. Cyclins, CDK and regulatory subunits control the processes involved in the cell cycle (Skirycz *et al*., 2011). Complexes are formed by cyclin-dependent kinases with Cyclin D control G1-S transition, while complexes are formed with Cylin A and B control S-G2 and G2-M transitions (Inzé & De Veylder, 2006). Accordingly, Schupler *et al*., (1998), in wheat leaves, Setter & Flanningan (2001) reported that drought stress causes an increase in the G_1 and G_2 stages of the cell cycle in maize. In addition, Su *et al*., (2005) reported that drought stress slowed down and stopped the cell cycle in tobacco. As a result of the study, it was determined that drought stress adversely affected the cell cycle in barley plants and also showed similar results in the previous studies (Hafez & Seleiman, 2017). According to the results that the increase in S and G2 phases after exogenous Spd application under drought promotes D-type cyclins suppressed by both drought stress and A-type cyclins. Polyamines are known to govern many processes, including promoting cell proliferation and cell cycle progression (Yamashita *et al*., 2013). Parallel to these properties of polyamines, the addition of Spd in barley control group samples caused an increase in S-G2 cell cycle. Despite the inhibitory effects of drought stress on the cell cycle process, exogenous Spd application showed an increased effect on S and G2 transitions. The results clearly show that the application of exogenous Spd together with drought stress improves the disruptive effect of stress on the cell cycle.

Polyamines can be defined as substances added to the cell from the outside or found within the cell and responding to abiotic stress as plant growth and development regulators (Liu *et al*., 2016). It is known that endogenous polyamines are involved in various development processes such as translation in eukaryotic cells and maintenance of life in plant embryos (Takahashi & Kakehi, 2010), cell signaling, membrane stabilization (Tabor & Tabor, 1984), cell division, modulation of gene expression (Igarashi & Kashiwagi, 2000), cell death and apoptosis (Rangan *et al*., 2014), cell division, morphogenesis, secondary metabolism, aging (Davies, 2010). In addition, polyamines have the ability to tolerate stress in developed plants when various environmental stresses are encountered (Legocka & Sobieszczuk-Nowicka, 2012). According to Ma *et al*., (2005) reported that drought stress increased the endogenous polyamine contents in wheat. Similarly Li *et al*., (2018) found in their study that drought caused an increase in endogenous polyamine content in *Zea mays*. Unlike these results, Li *et al*., (2015) in *Agronis stolongifera*, Ebeed *et al*., (2017) in wheat stated that there was an increase in the amount of Put and Spm, and a decrease in the amount of Spd after drought stress. There are a limited number of studies examining the effects of exogenous polyamines under drought stress on endogenous polyamine contents. Exogenous Spd application in maize under drought stress caused an increase in all endogenous polyamines. In addition, while exogenous Put application in wheat increased the amount of endogenous Spm and Spd, it had a decreasing effect on the amount of endogenous Put. In the same study, exogenous Spm application increased the amount of endogenous Spd and Put and decreased the amount of endogenous Spm (Li *et al*., 2015; Ebeed *et al*., 2017). As a result of this study, drought stress caused a decrease in Put and Cad levels in barley. As a result of the decrease in the amount of Put under stress, there was an increase in the amount of Spd and Spm. The increase in Spd and Spm amounts due to the decrease in the amount of Put in barley due to the effect of drought shows consistency according to the steps in the synthesis mechanism of polyamines. This study proved once again that the change in endogenous PA levels in barley exposed to drought stress is important for polyamines to cope with harsh environmental conditions for plants. As a result of study, it was investigated how exogenous application of Spd, played an important role in the response mechanism to drought stress and normal conditions, showed changes in endogenous polyamine content and it was found that it showed healing effects against drought stress.

Polyamines in plant cells have the ability to alleviate cell damage under stress conditions by interacting with proteins, nucleic acids, membrane phospholipids and cell wall components. Evidence shows that polyamines are not only involved in plant physiological processes and development, but also play an important role in modulating defense mechanisms against environmental stresses. Genes induced under drought stress conditions protect cells in the absence of water through the production of proteins such as water channel proteins, enzymes involved in the biosynthesis of osmotic preservatives (sugars, proline, glycine-betaine), LEA proteins, chaperones, proteases (Wang *et al*., 2016). According to the results obtained from different studies the total protein amount decreased in cotton, tomato and wheat after drought stress. There was an increase in total protein amount in peanut, wheat, chickpea and rice (Jnandabhiram & Sailen Prasad, 2012; Mahlagha *et al*., 2013; Akhzari & Pessarakli, 2016). Few studies examined the effects of exogenous Spd application on total protein amount under abiotic stresses. Exogenous Spd application increased total protein under nitrate tolerance, salt stress (Yi *et al*., 2018) and $Ca(NO_3)_2$ stress (Du *et al*., 2017). In addition to these studies, studies on the effects of exogenous Spd application on total protein amount under drought stress are very limited. Shi *et al*., (2013), stated that exogenous Spd given from outside under salt and drought stress increased the total protein amount in the bermudagrass. Although the effect of drought stress on the total protein amount cannot be determined exactly, it is understood from the studies that it differs according to the type of plant applied, the parts of the plant derived from protein, and the severity of the drought. The results obtained from this study showed that the increase in total protein amount against drought stress, stress response mechanism had occurred in barley. It is concluded that Spd, which is added exogenously under drought stress, can help reduce the effect of stress and preserve the vitality of the plant under stress by changing the amount of protein involved in the stress response mechanism.

It is known that the increase in SOD activity plays an important role in the survival of plants under these conditions in the presence of oxidative stresses caused by biotic and abiotic stresses (Seleiman *et al*., 2020). Studies conducted under different stress conditions in various plants such as *Morus alba* L. (mulberry), *Cicer arietinum* L. (chickpea) and *Lycopersicon esculentum* (tomato) have reported that increases in SOD activity occur (Gapińska *et al*., 2007; Attia *et al*., 2009; Buyuk *et al*., 2012). According to the findings of our study, after applying drought stress, the amount of SOD increased and barley developed a response mechanism against drought stress. Although exogenous Spd applied alone to the control group partially increased the amount of SOD compared to the control group, exogenous Spd applied in the stressful environment considerably increased the amount of SOD compared to its drought stress control group. Drought stress and exogenous Spd application may suppress plant cells, causing lipid peroxidation and consequently increasing MDA level. Increased MDA activity against exposure to drought stress may result from oxidative damage to tissues. When the earlier studies were examined, it was found that MDA levels were increased as a result of the stress to which tomatoes, wheat, barley, and mustard were exposed (Gaur & Gupta, 1994; Nouairi *et al*., 2006; Halliwell & Gutteridge, 2015). The results obtained from this study revealed that drought stress increased the MDA level in barley plants. Addition of Spd in arid conditions improved the MDA level compared to the stressed environment controls, resulting in a decrease in lipid peroxidation. CAT and APX are important antioxidants that enable the direct conversion of H_2O_2, which is produced under stressful conditions and harmful to the plant body, to H_2O and O_2 (Gratão *et al*., 2005). Due to the increase in CAT enzyme in barley, sunflower, cabbage and corn plants under different stress conditions, the plants protected themselves against stress (Azevedo *et al*., 1998; Azpilicueta *et al*., 2007). As a result of this study, drought stress showed similar effects on both CAT and APX amounts, i.e., drought stress increased both enzymes. Accordingly, while exogenous Spd application showed an increasing effect on CAT enzyme levels compared to their own control groups, APX enzyme levels showed decreasing effects compared to their own control groups. Although it is known that drought stress causes an increase in biochemical enzyme content (Kavas *et al*., 2013; Zhang *et al*., 2017), studies have been carried out to alleviate the effect of stress with substances such as hormones and growth regulators (Zhang *et al*., 2017; Iqbal *et al*., 2018). There are limited studies on the SOD, MDA, CAT and APX contents of additional Spd application under drought stress conditions. Akter *et al*., (2018) reported that exogenous Spd increased MDA content in maize plant. Additionally, ShanLu *et al*., (2018) stated that exogenous Spd under drought decreased the amounts of MDA, SOD and POX in bamboo. The application of exogenous Spd in distiled water medium showed increasing effects on almost all biochemical enzymes, while decreasing the APX enzyme level. While there was an increase in all enzyme parameters as a result of drought stress. Exogenous application of Spd under drought stress caused a decrease in MDA and APX enzyme levels and improved the negative effect caused by stress. The increase in SOD and CAT enzyme levels in the same group revealed that exogenous Spd administration could not alleviate the effect caused by stress. With this study, the effects of exogenous Spd on MDA, SOD, CAT, and APX in *H. vulgare* cv. Burakbey plant against drought stress were explained for the first time and it was revealed that the negative effect of stress can be reduced by the use of appropriate doses of exogenous Spd.

As the air temperatures are increasing day by day, the drought stress that has occurred due to the effects of global climate change has become a vital problem to deal with. The increase in climate change is damaging the arable land around the world, and as a result, it is inevitable that there will be a shortage of agricultural products necessary for the increasing world population. For this reason, studies to grow drought-resistant plants are one of the most acceptable options in this field. For this purpose, by using barley seeds, which is an economically important model plant, the molecular and biochemical interactions of Spd application in both normal and drought conditions are comparatively explained. As a result of the study, it is explained to what extent exogenous Spd application affects total protein amount, endogenous polyamine content, cell cycle, and biochemical enzyme levels against drought stress, which has an almost lethal effect on plant development. Finally, it is concluded that the external addition of Spd, which is an important member of polyamines, greatly reduces the negative effects of drought stress on the plant and will form the basis for future studies to combat drought stress.

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