Polyamines protect mung bean [*Vigna radiata* (L.) Wilczek] plants against drought stress

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Introduction: Drought is a major environmental factor that limits crops productivity. To cope with the adverse effects of drought, plants have evolved various adaptive mechanisms, such as accumulation of osmolytes. Polyamines (PAs) play an important role in the plant growth and development and response to abiotic stresses. This experiment was conducted to investigate the role of exogenous PAs on drought tolerance of mung bean. *Methods:* Mung bean seeds were soaked in 0 or 100 µM putrescine (Put), spermidine (Spd), spermine (Spm), or their mixture for 10 hr. Then, treated seeds were sown in the field and were exposed to drought stress condition. *Results:* The growth attributes including shoot length, biomass, leaf area index (LAI), and grain yield markedly reduced due to drought stress. Drought stress also decreased soluble protein content, relative water content (RWC), chlorophyll value, stomatal conductance (g_s), and net photosynthetic rate (P_N) but increased malondialdehyde (MDA), total soluble sugars (TSS), and proline contents. Nonetheless, seed priming with the mixture of PAs alleviated adverse effects of drought stress and improved all growth attributes. Exogenous application of PAs also increased soluble protein content, RWC, chlorophyll value, P_N, and more increased TSS and proline contents but decreased g_s and MDA level. *Discussion:* These results indicated that seed treatment with PAs especially combined treatment of Put + Spd + Spm could enhance drought tolerance of mung bean plants through the accumulation of osmoprotectants, improving water status, chlorophyll value, and P_N as well as reduction of oxidative damage.

INTRODUCTION

Mung bean [*Vigna radiata* (L.) Wilczek] is an important warm season grain legume with short duration, wide adaptability, high quality of protein, nitrogen fixation capability, ability to prevent soil erosion, and suitable for various cropping systems. However, this crop is susceptible to drought stress, especially during the reproductive phase (Ranawake et al., 2011; Sadeghipour, 2009).

Drought is a major environmental factor that limits crops growth and productivity. To cope with the adverse effects of drought, plants have evolved various adaptive mechanisms, one of which is the accumulation of osmolytes. In addition to osmoregulation, protection of proteins, enzymes, and membranes, scavenging reactive oxygen species (ROS), and stabilizing cellular components are the predominant functions of osmolytes. These compatible solutes include amino acids, quaternary amines, polyamines (PAs), polyols, or sugars (Mofizur Rahman & Hasegawa, 2012).

The most abundant PAs in plants including diamine putrescine (Put), triamine spermidine (Spd), and tetraamine spermine (Spm) are small ubiquitous polycationic nitrogenous compounds, which are present in the free forms, soluble conjugated to small molecules, and insoluble-bound to macromolecules. Since PAs are positively charged at physiological pH, they can bind to different anionic macromolecules (DNA, RNA, nucleic acids, proteins, and membrane phospholipids), thus regulating growth and development processes of plants, such as cell division, differentiation, proliferation and death, seed germination, breaking of tuber dormancy, gene expression, DNA and protein synthesis, flower bud initiation, leaf senescence, morphogenesis, embryogenesis, and fruit ripening (Alcázar et al., 2010; Gill & Tuteja, 2010; Shi et al., 2010). It has been demonstrated that PAs are also involved in the tolerance of plants to abiotic stresses because the accumulation of PAs is enhanced under stressful conditions (Fariduddin et al., 2013; Pal et al., 2015). It is noted that PAs can improve plant

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tolerance to abiotic stresses such as drought (Fu et al., 2014; Gupta et al., 2012; Nayyar & Chander, 2004), salinity (López-Gómez et al., 2017), heat (Todorova et al., 2016), chilling (Zeng et al., 2016), waterlogging (Yiu et al., 2009), and heavy metal (Xu et al., 2011b). There are also reports that show the exogenous application of PAs is an effective approach for improvement of crop tolerance to drought stress. Exogenous application of PAs alleviated the adverse effects of osmotic stress by modulating the antioxidant system and decreasing the H_2O_2 and water loss, therefore enhanced drought tolerance in maize seedlings (Kutlu Calişkan et al., 2017). Drought tolerance of wheat plants increased by exogenous application of PAs through improving water status, accumulated osmoprotectants, and PAs and upregulated PAs biosynthetic genes (Ebeed et al., 2017). Drought stress severely reduced the rice growth, whereas PAs application increased drought tolerance by improving membrane properties, photosynthesis, water use efficiency, leaf water status, production of free proline, anthocyanins, and soluble phenolics (Farooq et al., 2009b). In *Ctenanthe setosa*, increasing drought tolerance by PAs application was related to osmotic adjustment via the increase in proline, reducing sugars and soluble protein contents (Saruhan et al., 2006).

Limited studies have been conducted on the effects of exogenously PAs on the mung bean drought tolerance. In this study, it was investigated if PAs pretreatment has a protective role against drought stress in mung bean plants, what is their mechanism of action?

MATERIALS AND METHODS

Growth conditions, plant materials, and treatments

This study was carried out in the research field of the Yadegar-e-Imam Khomeini (RAH) Shahre Rey Branch, Islamic Azad University (51°28′ E, 35°35′ N, 1,000 m AMSL), Tehran, Iran, in 2015. This region is located in an arid climate. The mean annual rainfall and temperature are 201.7 mm and 20.4 °C. Meteorological data of the growing season of mung bean were presented in Table 1. The soil characteristics were as follows: pH 7.7, EC, 2.2 dS/m, organic carbon, 2.6%; N, 0.22%; P and K, 17.9 and 378 mg/kg, respectively, and texture, clay loam. This experiment was laid out in a randomized complete block design with four replications. Each replication consisted of nine plots with four planting rows with length and distance of 5 and 0.5 m, respectively. Two irrigation regimes were used in this

study including irrigation after 60 and 120 mm evaporation from a class A evaporation pan as normal and drought stress conditions, respectively. Seeds of mung bean (cv. Partow) were surface sterilized using 5% sodium hypochlorite solution for 5 min and then rinsed three times with sterile distilled water. The seeds were then soaked in water (control), or 100 μM aqueous solution of Put, Spd, Spm, or their mixture (control, drought stress, drought + Put, drought + Spd, drought + Spm, drought + Put + Spd, drought + Put + Spm, drought + Spd + Spm, and drought + Put + Spd + Spm) for 10 hr. Afterward, air-dried seeds were sown on the planting rows manually in 3-cm depth of soil on May 17, 2015. All plots were irrigated immediately after sowing, but subsequent irrigations were carried out according to the irrigation regimes. After thinning at the three-leaf stage, the distance of seedlings on rows was 10 cm.

Determination of growth attributes

At the physiological maturity, shoot length was recorded. Leaf area of plants was also calculated using a leaf area meter (CI-202, CID Bio-Science, USA). Moreover, plants in 2 m^2 of the second row of each plot were harvested, sun-dried, and then grain yield per unit area was determined. Later, above ground plant parts were oven-dried at 80 °C for 48 hr and biomass per unit area was calculated.

Physiological traits assessment

At the early flowering stage, physiological and biochemical traits were estimated. To determine the relative water content (RWC), 10 disks (1 cm in diameter) from the middle portion of the youngest fully expanded leaves were collected, immediately weighed to record the fresh weight (FW), then rehydrated in petri dishes containing distilled water for 24 hr under dim light and room temperature to get the turgid weight (TW) and subsequently the disks were oven-dried at 70 °C for 48 hr to record the dry weight (DW). RWC was calculated as: RWC (%) = (FW − DW)/(TW − DW) × 100.

Chlorophyll value of the youngest fully expanded leaves was measured using a chlorophyll meter (Chlorophyll Content Meter, CL-01, Hansatech Instruments Ltd., England).

Stomatal conductance (g_s) and net photosynthetic rate (P_N) were measured on sunny days between 10:00 and 11:00 hr on the youngest fully expanded leaves using a portable photosynthesis system (Handheld Photosynthesis System, CI-340, CID Bio-Science, USA), with open system mode. The constant conditions in the leaf chamber (6.25 cm^2) were maintained as follows: CO_2 inflow concentration (390 μmol/mol), photosynthetic photon flux density 1,000 μmol · m^{-2} · s^{-1}, chamber temperature 27 ± 2 °C, and relative humidity 55% ± 4%.

Biochemical traits assessment

Lipid peroxidation was estimated in terms of malondialdehyde (MDA) content according to the method of Heath and Packer (1968). Leaf samples (500 mg) were homogenized in 10 ml of trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000 rpm for 5 min. Four ml (0.5%) of

Table 1. Monthly meteorological data for the growing season of mung bean (2015)

Parameters	Mean temperature (°C)	Mean humidity (%)	Sunshine duration (hr)	Precipitation (mm)
May	25.3	20.5	329.6	2.8
June	31.2	21.5	353.2	0.4
July	31.8	21.0	370.6	0.4
August	30.1	32.5	319.3	0

thiobarbituric acid in 20% TCA was added to a 1 ml aliquot of the supernatant. The mixture was heated at 95 °C for 30 min and then cooled rapidly in an ice bath. After centrifugation at 10,000 rpm for 10 min, the absorbance was recorded at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The MDA content was calculated using the absorption coefficient of 155 mM$^{-1}\cdot$cm^{-1} and expressed as nmol/g$\cdot$FW.

For proline estimation, 500 mg of the fresh leaf was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate filtered, following the method of Bates et al. (1973). Two ml of acid ninhydrin (prepared by warming 1.2 g of ninhydrin in 30 ml of glacial acetic acid) was added to 2 ml of filtrate in a digestion tube and placed in a boiling water bath for 90 min. The reaction was terminated in an ice bath. Four ml of toluene was added to the reaction mixture and agitated vigorously for 30 min. The chromophore containing toluene was aspirated from the aqueous phase and the absorbance read at 520 nm. The proline concentration was determined using a standard curve and expressed as μmol/g$\cdot$FW.

Total soluble sugars (TSS) were extracted by overnight submersion of 200 mg oven-dried leaves in 10 ml of 80% (v/v) ethanol at 25 °C with periodic shaking and centrifuged at 600 rpm. The supernatant was evaporated until completely dried and then dissolved in a known volume of distilled water to be ready for determination of soluble sugars (Homme et al., 1992). TSS was analyzed by reacting of 0.1 ml of ethanolic extract with 3.0 ml freshly prepared anthrone (150 mg anthrone + 100 ml of 72% H_2SO_4) in boiling water bath for 10 min and reading the cooled samples at 625 nm using Spekol Spectrocolorimeter (Yemm & Willis, 1954).

Total soluble protein content of the oven-dried leaves was determined according to the method described by Bradford (1976). Leaf samples (200 mg) were homogenized in 5 ml phosphate buffer (pH 7.0). The crude homogenate was centrifuged at 13,000 rpm for 15 min at 4 °C. The supernatant was separated and the final volume was made up to 10 ml using a phosphate buffer. About 1 ml of this protein extract was added to 5 ml of coomassie brilliant blue reagent (G-250) and mixed thoroughly. Absorbance was recorded by spectrophotometer at 595 nm using bovine serum albumin as the standard.

Statistical analysis

All data were analyzed using the MSTAT-C statistical software and means were compared by the least significant difference test at $p \leq .05$.

RESULTS

Growth attributes

Drought stress significantly reduced the growth attributes of mung bean plants, where shoot length, biomass, leaf area index (LAI), and grain yield were decreased by 41%, 46%, 54%, and 55%, respectively, compared to the control. However, seed priming with PAs alleviated the adverse effects of drought stress and improved all growth attributes under stress conditions. Among PAs treatments, the combined treatment of Put + Spd + Spm was the most effective (Table 2).

Physiological traits

Relative Water Content (RWC). The RWC of mung bean leaves was decreased with drought stress. A 27% decline was observed compared with the control. Under drought stress conditions, treatment of seeds with Put, Spd, and Spm separately had no significant effect on RWC but the mixture of PAs, especially Put + Spd + Spm, markedly increased RWC (Table 3).

Chlorophyll value. Drought stress markedly declined the chlorophyll value by 40% as compared to the control. However, seed treatment with combined treatments of PAs increased the chlorophyll value. Under drought stress conditions, exogenous application of Put, Spd, and Spm individually had no significant effect but combined treatments of PAs especially Put + Spd + Spm was the most effective in improvement of chlorophyll value (Table 3).

Stomatal conductance (g_s) and net photosynthetic rate (P_N). The highest g_s and P_N were observed in control plants, while drought stress significantly decreased these attributes. Drought stress reduced g_s and P_N by 20% and 42%, respectively, compared with the control. Seed priming with the mixture of Put + Spd + Spm reduced g_s under drought stress conditions and other PAs treatments had no significant effect. Concerning P_N, combined treatments of PAs

Table 2. Effects of seed pretreatment by polyamines on growth attributes of mung bean under drought stress

Treatments	Shoot length (cm)	Biomass (g/m^2)	Leaf area index	Grain yield (g/m^2)
Control	62.8 ± 3.14[a]	317.1 ± 15.84[a]	2.89 ± 0.11[a]	115.7 ± 5.77[a]
Drought	37.3 ± 1.86[f]	171.0 ± 8.53[d]	1.32 ± 0.05[f]	52.3 ± 2.62[d]
Drought + Put	39.3 ± 1.96[ef]	200.8 ± 10.0[cd]	1.56 ± 0.06[ef]	58.9 ± 2.92[cd]
Drought + Spd	40.1 ± 2.00[def]	205.6 ± 10.24[cd]	1.58 ± 0.06[de]	59.7 ± 2.96[cd]
Drought + Spm	42.6 ± 2.13[cde]	209.8 ± 10.44[cd]	1.60 ± 0.06[de]	61.8 ± 3.06[cd]
Drought + Put + Spd	44.9 ± 2.24[cd]	211.9 ± 10.57[c]	1.79 ± 0.07[cde]	64.7 ± 3.22[cd]
Drought + Put + Spm	45.3 ± 2.26[cd]	215.2 ± 10.73[c]	1.82 ± 0.07[cd]	65.4 ± 3.23[cd]
Drought + Spd + Spm	47.3 ± 2.36[c]	222.3 ± 11.10[c]	1.90 ± 0.08[c]	69.9 ± 3.47[c]
Drought + Put + Spd + Spm	52.7 ± 2.63[b]	267.6 ± 13.31[b]	2.17 ± 0.09[b]	87.1 ± 4.33[b]
LSD	5.1	40.1	0.25	16.5

Note. Data are mean ± SE ($n = 4$). Means within a column followed by the same letter(s) are not significantly different at $p \leq .05$ level using LSD. Put: putrescine; Spd: spermidine; Spm: spermine; LSD: least significant difference.

Table 3. Effects of seed pretreatment by polyamines on physiological traits of mung bean under drought stress

Treatments	RWC (%)	Chlorophyll (SPAD unit)	g_s (μmol $\cdot$ m^{-2} $\cdot$ s^{-1})	P_N (μmol $\cdot$ m^{-2} $\cdot$ s^{-1})
Control	93.23 ± 3.79[a]	35.77 ± 1.07[a]	117.84 ± 5.85[a]	15.28 ± 0.92[a]
Drought	67.92 ± 3.01[d]	21.38 ± 0.64[d]	94.11 ± 4.70[c]	8.90 ± 0.53[d]
Drought + Put	70.78 ± 3.10[d]	23.50 ± 0.70[cd]	92.39 ± 4.62[c]	9.65 ± 0.58[cd]
Drought + Spd	71.00 ± 3.12[d]	23.76 ± 0.71[cd]	92.00 ± 4.60[c]	9.87 ± 0.59[cd]
Drought + Spm	71.69 ± 3.11[cd]	23.97 ± 0.72[cd]	91.89 ± 4.59[c]	10.11 ± 0.61[cd]
Drought + Put + Spd	77.55 ± 3.30[cd]	26.86 ± 0.80[c]	90.25 ± 4.51[c]	10.61 ± 0.64[c]
Drought + Put + Spm	78.20 ± 3.34[c]	27.10 ± 0.81[c]	89.30 ± 4.46[c]	10.98 ± 0.66[c]
Drought + Spd + Spm	78.60 ± 3.31[c]	27.44 ± 0.82[c]	87.88 ± 4.39[c]	11.19 ± 0.67[c]
Drought + Put + Spd + Spm	85.96 ± 3.59[b]	31.55 ± 0.95[b]	84.55 ± 4.23[b]	12.85 ± 0.77[b]
LSD	6.94	4.02	8.87	1.48

Note. Data are mean ± *SE* ($n = 4$). Means within a column followed by the same letter(s) are not significantly different at $p \leq .05$ level using LSD. RWC: relative water content; g_s: stomatal conductance; P_N: net photosynthetic rate; Put: putrescine; Spd: spermidine; Spm: spermine; LSD: least significant difference.

noticeably improved this trait under drought stress (Table 3). In the other words, under stress conditions, the minimum g_s and the maximum P_N were recorded from combined treatment of Put + Spd + Spm.

Biochemical traits

MDA content. Table 4 shows that MDA content as an indicator of membrane lipid peroxidation increased significantly during drought stress by 95% as compared to nonstress conditions. However, just the application of combined treatments of PAs diminished the adverse effect of drought stress on membrane lipid peroxidation and consequently reduced the MDA content. The mixture of Put + Spd + Spm was the most effective.

Proline content. Proline content was increased due to drought stress (Table 4). This increase was about 31% over control plants. A further increase was obtained in proline content as a result of PAs treatments, particularly combined treatments under drought stress conditions. The mixture of Put + Spd + Spm was the most effective.

TSS content. Drought stress elevated the TSS content by 25% in comparison with the control. Pretreatment of seeds with PAs had a positive effect on TSS by more increase in their content under drought stress conditions. Although all PAs treatments had significant effects on increasing the TSS

content, whereas combined treatment of Put + Spd + Spm was the most effective (Table 4).

Total soluble protein content. Total soluble protein content was declined under drought stress by 28% as compared to the control. Seed priming with combined treatments of PAs particularly Put + Spd + Spm noticeably raised total soluble protein content under drought conditions, so that it was higher than the control treatment. However, Put, Spd, and Spm treatments alone had no significant effect on total soluble protein content (Table 4).

DISCUSSION

In this study, drought stress intensively reduced growth attributes of mung bean plans, including shoot length, biomass, LAI, and grain yield. These results are in conformity with previous findings (Sadeghipour, 2009; Sheteawi & Tawfik, 2007). On the other hand, seed priming with PAs especially the mixture of Put + Spd + Spm alleviated the adverse effects of drought and improved all mentioned growth attributes. Similarly, the positive effects of exogenously PAs on the growth and tolerance to abiotic stresses in different plant species have been reported by others (Farooq et al., 2009b; López-Gómez et al., 2017; Xu et al., 2011a).

Table 4. Effects of seed pretreatment by polyamines on biochemical traits of mung bean under drought stress

Treatments	MDA (nmol/g^{-1} FW)	Proline content (μmol/g FW)	TSS (mg/g DW)	Soluble protein (mg/g DW)
Control	10.83 ± 0.76[d]	9.84 ± 0.64[d]	25.32 ± 1.87[e]	20.77 ± 1.49[ab]
Drought	21.11 ± 1.47[a]	12.87 ± 0.84[c]	31.65 ± 2.32[d]	15.02 ± 1.08[c]
Drought + Put	19.52 ± 1.37[a]	14.10 ± 0.92[bc]	37.44 ± 2.77[c]	16.13 ± 1.16[c]
Drought + Spd	19.12 ± 1.34[a]	14.44 ± 0.94[bc]	37.50 ± 2.59[c]	16.30 ± 1.17[c]
Drought + Spm	19.00 ± 1.33[a]	14.68 ± 0.95[bc]	37.71 ± 2.79[c]	16.98 ± 1.22[c]
Drought + Put + Spd	16.31 ± 1.14[b]	15.49 ± 1.00[b]	41.00 ± 3.01[bc]	18.10 ± 1.30[bc]
Drought + Put + Spm	16.43 ± 1.15[b]	15.77 ± 1.02[b]	41.73 ± 3.06[bc]	18.55 ± 1.33[bc]
Drought + Spd + Spm	16.11 ± 1.13[b]	15.94 ± 1.04[b]	41.94 ± 3.08[b]	19.36 ± 1.39[b]
Drought + Put + Spd + Spm	13.57 ± 0.95[c]	18.20 ± 1.18[a]	46.77 ± 3.44[a]	22.80 ± 1.64[a]
LSD	2.46	2.13	4.32	2.95

Note. Data are mean ± *SE* ($n = 4$). Means within a column followed by the same letter(s) are not significantly different at $p \leq .05$ level using LSD. MDA: malondialdehyde; TSS: total soluble sugars; DW: dried weight; FW: fresh weight; Put: putrescine; Spd: spermidine; Spm: spermine; LSD: least significant difference.

RWC is one of the most important indicators of water status in plants, which is related to water uptake from roots and transpiration rate from leaves. Reduction of RWC under water stress conditions has been reported in many plant species (Farooq et al., 2009a; Mofizur Rahman & Hasegawa, 2012). Due to some PAs properties such as hydrophilicity, which help to maintain the turgor pressure and water content of the cells, they are also often considered as compatible solutes under drought stress conditions (Kusnetsov & Shevyakova, 2009). The results of this study showed that the RWC of mung bean leaves was declined under drought stress while, seed priming with combined treatments of PAs, especially Put + Spd + Spm markedly increased RWC under drought conditions. In accordance with the present findings, treatment with PAs enhanced tolerance to water stress in chickpea (Nayyar & Chander, 2004) and soybean (Radhakrishnan & Lee, 2013) by improving the RWC. According to the results of this experiment, the exogenous application of PAs via increasing proline and TSS levels enhanced osmotic adjustment as well as by stomatal closure and maintained plant water; as a result, improved RWC of mung bean leaves under drought stress.

The reduction in chlorophyll content under drought stress is well known. Chlorophyll loss under drought stress might be the result of inhibition of chlorophyll synthesis as well as chlorophyll photooxidation and degradation (Mofizur Rahman & Hasegawa, 2012). In this study, chlorophyll value was decreased by drought stress, while seeds soaking in combined treatments of PAs especially Put + Spd + Spm increased chlorophyll value under drought conditions. Thus, it can be concluded that PAs protect chlorophyll by improving its biosynthesis and reducing its degradation under stressful conditions. In agreement with the present results, PAs application enhanced chlorophyll content of different plant species under stressful conditions (Radhakrishnan & Lee, 2013; Puyang et al., 2016; Yamaguchi et al., 2007; Yiu et al., 2009). PAs quickly penetrate in the chloroplast and protect the photosynthetic system from the harmful effects of stress. In this regard, the roles of PAs can be considered the reduction of oxidative stress, protection of pigment-protein complexes and thylakoid membranes, regulation of chlorophyll biosynthesis and degradation pathways, and controlling the enzymes related to those pathways and maintenance of cellular pH (Nahar et al., 2016).

In this study, drought induced reduction in g_s and P_N of mung bean leaves. On the other hand, pretreatment of mung bean seeds with the mixture of Put + Spd + Spm further reduced g_s but improved P_N under drought conditions. In agreement with these findings, there are several reports that PAs strongly induced closure of stomata (Bitrián et al., 2012; Fu et al., 2014; Shi et al., 2010). PAs modulate the activity of Ca_2^+-permeable channels in the guard cells and raise cytoplasmic Ca_2^+ concentration, consequently inactivate the K^+ inward rectifier at the plasma membrane, which could stimulate closure of stomata (Yamaguchi et al., 2007). In addition, PAs modulate abscisic acid (ABA) biosynthesis in response to abiotic stresses, which eventually induced stomatal closure (Alcázar et al., 2010). In accordance with the results of the present experiment, there are several

documents that show PAs application improved photosynthesis under stressful conditions (Gupta et al., 2012; Todorova et al., 2016; Yiu et al., 2009; Zhang et al., 2016). The conjugation of PAs to photosynthetic complexes and proteins leads to enhanced photosynthetic activity under stress conditions (Pal et al., 2015). Similarly, PAs synthesized in the chloroplasts, apparently stabilize photosynthetic complexes of thylakoid membranes under stress conditions (Kusnetsov & Shevyakova, 2009). In this study, exogenous PAs further decreased g_s but improved P_N under drought stress. This event suggested that, first, closure of stomata prevented water loss more than the reduction of CO_2 intake; second, although the inflow of CO_2 into the leaves was reduced, other factors involved in photosynthesis were improved, which enhanced photosynthesis. In conformity with these findings, it was also reported that, under drought stress conditions, exogenous PAs application on rice improved the photosynthetic attributes as well as water use efficiency, particularly net photosynthesis although g_s was decreased (Farooq et al., 2009b). Totally, in this study, under drought stress conditions, seed treatment with PAs improved P_N of mung bean leaves through enhanced chlorophyll value, maintenance of plant water, and reducing membrane lipid peroxidation.

Reactive aldehyde forms, such as MDA, is the final product of membrane lipid peroxidation, considered as an appropriate indicator for assessment of oxidative damage by ROS (Mofizur Rahman & Hasegawa, 2012; Radhakrishnan & Lee, 2013). In this study, MDA content in the mung bean leaves was markedly raised under drought stress. On the other hand, priming of seeds with the mixture of Put + Spd + Spm noticeably declined oxidative damage and MDA content. This indicates that PAs act as a membrane stabilizer in mung bean plants subjected to drought stress. Similarly, reduction in oxidative injury and MDA content by exogenously PAs has been reported under drought (Nayyar & Chander, 2004), salinity (López-Gómez et al., 2017), chilling (Zeng et al., 2016), waterlogging (Yiu et al., 2009), heavy metal (Xu et al., 2011b), and heat stress (Todorova et al., 2016). The important effect of PAs on reducing oxidative damage and MDA content under drought stress conditions could be ascribed to its roles as inhibitor the auto-oxidation of metals, which in turn impairs the supply of electrons for the generation of ROS, ROS scavengers, enhancer antioxidant systems, as well as bind to phospholipids and membrane stabilizers (Liu et al., 2015; Nahar et al., 2016; Nayyar & Chander, 2004; Shi et al., 2010;).

In many plant species, increased proline accumulation is correlated with improved stress tolerance. Proline as an amino acid contributes to osmotic adjustment, scavenging free radicals, stabilizing subcellular structures (e.g., membranes and proteins), and buffering cellular redox potential under stress conditions (Ashraf & Foolad, 2007). The studies have shown that there is a close correlation between the increased content of cellular proline and PAs. It is found that difluoromethyl arginine, which blocks Put synthesis from arginine, also inhibits proline accumulation (Kusnetsov & Shevyakova, 2009). In this study, the proline level was raised due to drought stress; however, PAs treatments, particularly combined treatments, and especially Put + Spd + Spm treatment more raised the proline content

under drought stress conditions. Similarly, the positive effect of exogenous application of PAs on proline content under drought conditions has been observed in wheat (Ebeed et al., 2017) and *C. setosa* (Saruhan et al., 2006). Therefore, stimulation of proline accumulation caused by exogenous PAs could be considered as a substantial strategy for improving drought tolerance.

Short-term water stress inhibits starch synthesis more than sucrose synthesis and also enhances the conversion of starch to sucrose. Therefore, plants exposed to drought stress increase TSS levels in their leaves as osmoregulators (Mofizur Rahman & Hasegawa, 2012). Soluble sugars may act as osmoprotectants under water stress as well as being sources of carbon for maintenance and regrowth during recovery (Chaves et al., 2002). During drought stress, sugars can replace water; thereby keeping the membrane surface hydrated and also protecting the membrane from fusion by maintaining space between phospholipids molecule (Iqbal et al., 2016). In this study, TSS contents in mung bean leaves were increased by drought stress. Mung bean seeds soaked with all PAs treatments further raised the TSS levels under drought conditions. Combined treatment of Put + Spd + Spm was the most effective. These results are in consistent with Ebeed et al. (2017), who reported that under drought stress, soluble sugars were significantly increased in shoots of wheat compared to well-irrigated plants, and PAs applications further markedly elevated the soluble sugars.

In general, proteins in the plant tissues decrease during water deficiency due to protein degradation or their suppressed synthesis; however, the synthesis of some proteins namely stress-induced proteins, such as dehydrins, late embryogenesis-abundant proteins, desiccation stress proteins, proteins those respond to ABA, proteases, enzymes required for the biosynthesis of various osmoprotectants, and detoxification enzymes, may be induced or upregulated (Farooq et al., 2009a; Mofizur Rahman & Hasegawa, 2012). Therefore, the increase or decrease of total proteins in plants subjected to drought stress is related to genotype and stress severity. In this study, total soluble protein content was reduced as a result of the drought. Nonetheless, the exogenous mixture of PAs especially Put + Spd + Spm elevated protein content under drought stress conditions. Similar to these findings, increases in the soluble protein content by PAs application under drought stress have been reported by other researchers (Radhakrishnan & Lee, 2013; Saruhan et al., 2006). Due to PAs cationic nature, they interact with negatively charged macromolecules, such as nucleic acids, phospholipids, and proteins. These ionic interactions, which are reversible, leading to the stabilization of DNA, tRNA, membranes, and some proteins. PAs binding to proteins or nucleic acids not only protects them from degradation but also provides a molecule with the most stable conformation under stress conditions. PAs also could inhibit DNA methylation, which permits the expression of specific genes responsible for the synthesis of stress proteins (Bachrach, 2005; Kusnetsov & Shevyakova, 2009).

In conclusion, seed priming with PAs could effectively improve mung bean growth and tolerance to drought stress through inducing accumulation of osmoprotectants, stabilization of cell membranes, improved plant water status, chlorophyll value, as well as P_N. Among the PAs treatments, the combined treatment of Put + Spd + Spm was the most effective. However, further researches are needed to determine the appropriate concentrations of exogenously applied PAs under drought stress conditions in various plant species.

CONCLUSION FOR FUTURE BIOLOGY

Drought is a major threat to plant productivity in agricultural systems worldwide. Therefore, crops with high tolerance to drought stress should be developed to feed the increasing world population. PAs are involved in many metabolic processes of plants, ranging from cell division to abiotic stresses tolerance. In this study, drought had negative effects on the growth and grain yield of mung bean plants. However, seed priming with PAs improved the drought tolerance of this crop. In the future, the exogenous application of PAs may be used as an effective approach for increasing the drought tolerance of mung bean. Furthermore, the genetic manipulation of mung bean with genes encoding enzymes of PAs biosynthetic pathways may provide better drought stress tolerance to this crop. Moreover, the similar experiments are needed for other crops.

Data Accessibility: All data is provided in full in "Results" section of this paper.

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