DOI: 10.1007/s11099-018-0834-4 PHOTOSYNTHETICA 56 (4): 1387-1397, 2018

Leaf gas exchange and grain yield of common bean exposed to spermidine under water stress

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Abstract

Three prevalent aliphatic polyamines (PAs) include putrescine, spermidine, and spermine; they are low-molecular-mass polycations involved in many physiological processes in plants, especially, under stressful conditions. In this experiment, three bean (*Phaseolus vulgaris* L.) genotypes were subjected to well-watered conditions and two moderate and severe water-stressed conditions with and without spermidine foliar application. Water stress reduced leaf relative water content (RWC), chlorophyll contents, stomatal conductance (g_s), intercellular CO_2 concentration (C_i), transpiration rate, maximal quantum yield of PSII (F_v/F_m), net photosynthetic rate (P_N), and finally grain yield of bean plants. However, spermidine application elevated RWC, g_s, C_i, F_v/F_m, and P_N, which caused an increase in the grain yield and harvest index of bean plants under water stress. Overall, exogenous spermidine could be utilized to alleviate water stress through protection of photosynthetic pigments, increase of proline and carotenoid contents, and reduction of malondialdehyde content.

Additional key words: harvest index; number of pods; water deficit.

Introduction

Drought stress in plants is characterized by the continuous loss of water through transpiration and evaporation into the atmosphere, while the water uptake is decreased due to a reduced water content in soil (Abedi and Pakniyat 2010). The accessibility of sufficient amount of water is essential for plant growth and development. Consequently, water deficiency is a major factor limiting crop yield and biomass production. The first reaction of plants to a decline in soil moisture, before any detectable change in a leaf water potential, is stomatal closure (Chaves *et al.* 2003) as a response to drought that reduces leaf water loss. Moreover, photosynthesis is also known to be sensitive to drought stress. As water availability is reduced, many plants decline their leaf photosynthetic rate. Snider *et al.* (2014) reported that P_N was strongly influenced by g_s, indicating that g_s is a major factor governing photosynthetic responses under drought stress. Negative effects of water limitation on stomatal closure and P_N result in a lower growth capacity and decrease in biomass accumulation (Benešová *et al.* 2012). The F_v/F_m is taken as a parameter for the study of chloroplast development under normal and stress conditions (Misra *et al.* 2006). Centritto (2005) found that F_v/F_m decreased under severe water deficit. Under severe water deficit, mostly correlated with increases of leaf temperature, light and excitation energy in PSII leading to photoinhibition, which ultimately leads to the lower quantum yield of PSII (Govindjee 1999). Reductions in leaf pigments induced by drought are considered to be an oxidative stress indicator, which might be attributed to pigment photo-oxidation, chlorophyll (Chl) degradation, and/or Chl synthesis deficiency (Sánchez-Rodríguez *et al.* 2012). During water deficit, excessive generation of reactive oxygen species (ROS), such as superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$) occurs, which causes membrane lipid peroxidation leading to irreparable metabolic and structural dysfunctions and cell death (Miller *et al.* 2010). Furthermore, the increase in the concentration of organic solutes, such as proline, sucrose, and other soluble sugars may stabilize the cellular osmotic pressure under drought stress (Sucre and Suárez 2011).

Received 21 July, *accepted* 7 February 2018, *published as online-first* 21 June 2018.
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Abbreviations: Car − carotenoid; Chl − chlorophyll; C_i − intercellular CO_2 concentration; E − transpiration rate; F_v/F_m − maximum photochemical efficiency of PSII; g_s − stomatal conductance; HI − harvest index; MDA − malondialdehyde; MWD – moderate water deficit; NC – normal conditions, PAs − polyamines; P_N − net photosynthetic rate; ROS − reactive oxygen species; RWC − relative water content; SWD – severe water deficit; WUE − water-use efficiency; WUE_i − intrinsic water-use efficiency.
Acknowledgements: This research was supported by the Faculty of Agriculture, University of Tabriz, Tabriz, Iran. The authors appreciate Shiva Torabian to help in fieldwork and collecting data.

PAs, a group of low-molecular-mass polycations, are found in living cells. The common PAs in plants are spermidine, spermine, and their precursor, putrescine (Groppa and Benavides 2008). They are involved in many physiological processes, such as growth, morphogenesis, secondary metabolism, senescence, and apoptosis (Kusano *et al.* 2008). In recent years, attention has been focused on the role of polyamines in plants defense against abiotic stress (Alcázar *et al.* 2011, Kusano *et al.* 2008). Drought-tolerant plants generally increase endogenous PAs content or metabolism more than sensitive plants do in response to drought stress (Alcázar *et al.* 2011). PAs may stabilize membranes, scavenge free radicals, modulate the activities of certain ion channels, and control many aspects of DNA, RNA, and protein turnover under water stress (Groppa and Benavides 2008, Alcázar *et al.* 2011). In addition, exogenous application of PAs enhanced drought tolerance of rice (Yin *et al.* 2014), and creeping bentgrass (Shukla *et al.* 2015). Farooq *et al.* (2009) found that exogenously applied PA improves leaf water status, photosynthesis, and membrane properties, thereby enhancing the drought tolerance of rice. There are several lines of evidence supporting the relationship between PAs and photosynthesis. The conjugation of PAs to photosynthetic complexes and proteins is catalyzed by trans-glutaminase and leads to enhanced photosynthetic activity under stress conditions (Ioannidis *et al.* 2012). It is well established that exogenous PAs can improve the photosynthetic capacity by increasing the photochemical efficiency of PSII under salinity (Zhang *et al.* 2009). Duan *et al.* (2006) reported that PSII activity of wheat was preserved by spermidine during water stress. Moreover, spermidine induced an increase in both total Chl and Chl *a*/*b* ratios as compared to spermidine untreated, salt-stressed plants (Chattopadhayay *et al.* 2002). They demonstrated that at physiological concentrations, spermidine and spermine considerably prevented Chl loss and downregulated chloroplast-encoded genes. Similarly, it was reported that spermidine mitigated the degradation of Chl in salt-stressed rice (Roychoudhury *et al.* 2011). Gupta and Gupta (2011) indicated that exogenously applied putrescine enhanced a plant height, leaf area, grain number, grain mass, grain yield, and biological yield of wheat under normal as well as under water-stress conditions. Accordingly, the present study was aimed to determine the role of spermidine as a PA in drought tolerance in three common bean genotypes with reference to pigment contents, photosynthetic characteristics, and yield as well as yield components.

Materials and methods

Plants, growth conditions and treatments: The experiment was conducted on a farm in Research Center of Agricultural Science and Natural Resources, Isfahan, Iran (32°40′N, 51°48′E, and 1,590 m a. s. l.) during the bean-growing season (May−August) in 2014 and 2015. Some local climate data during the growing season are presented in the text table below. The soil type of this area is silt loam with a bulk density of 1.4 g cm^{-3} and pH 7.9. The experimental design was a randomized complete block with a split-plot arrangement of treatments with three replications. Main plot corresponded to water stress levels and subplots to three genotypes and spermidine. Fertilizer (compound fertilizer, KNO_3 and P_2O_5) was applied prior to planting to reach a local favorable nutrition. Then, field of study was ploughed three times using disc before planting. Plot dimension was 4 × 2.5 m and consisted of seven rows with 10 cm row spacing, and 25 cm distance between plants in a row. Three common bean genotypes, Akhtar (red), Pak (white), and COS12 (kidney) were obtained from the National Bean Research Station of Khomain, Iran. These are the most important genotypes in Iran, which are cultivated widely by farmers. Surface was sterilized with 2 g(Benomyl) kg^{-1} for 10 min, then rinsed with distilled water. Three water stress levels were set up based on evaporation from class A pan: irrigation after 60 mm evaporation (normal conditions, NC), and irrigation after 90 mm and 120 mm evaporation [moderate (MWD) and severe water deficit (SWD)] after emergence of the third trifoliate leaf. Two spermidine treatments [control and 1 mM spermidine (*St Louis*, MO, USA)] were sprayed after the emergence of the seventh trifoliate by backpack sprayer. As an average, each plant received 100 ml solutions each time. Weeding and routine crop management were performed manually. Thirty days after the foliar spray by spermidine (at the flowering stage), samplings for bio-molecule and pigment estimations were carried out.

Mean temperature, relative humidity and rainfall for the 2014 and 2015 growing seasons.

Month	Mean temperature [°C] First season	Relative humidity [%]	Rainfall [mm]	Mean temperature [°C] Second season	Relative humidity [%]	Rainfall [mm]
May	20.2	46	21.9	18.4	47.5	24.4
June	28.9	21.5	0	26.3	24	10.4
July	28.2	47	12.5	29.6	20.5	0
August	27.8	14.5	0	28.6	24	0
September	28.9	14.5	0	25	22.5	0

Leaf RWC, contents of Chl and Car: Representative samples were collected from top-most fully expanded leaves and quickly weighed (leaf FM). Then the samples were immediately hydrated to full turgidity for 3–4 h by floating on deionized water in a closed Petri dish under normal room light and temperature. Afterwards, hydrated samples were well dried with filter tissue paper and weighed to obtain fully turgid mass (leaf TM). Samples were oven-dried for a minimum of 24 h at 70°C to determine dry mass (leaf DM). Relative water content was calculated as RWC = [(leaf FM – leaf DM)/(leaf TM – leaf DM)] × 100. Chl and Car contents were determined according to the method described by Lichtenthaler and Wellburn (1983). Leaf discs were placed in 15-mL tubes containing 80% acetone. The tubes were kept in darkness for about 48 h until the discs were completely whitened. Absorption of the extracts at 470, 663, and 646 nm were determined using a *UV-2450* spectrophotometer (*Hitachi*, Tokyo, Japan).

Proline and malondialdehyde (MDA) contents: Proline was determined following the method of Bates *et al.* (1973). Fresh leaves were extracted in sulphosalicylic acid, an equal volume of glacial acetic acid and ninhydrin solutions were added to the extract. The sample was heated at 100°C, and then 5 ml of toluene was added. The absorbance of the toluene layer was read at 528 nm, on a spectrophotometer (*Hitachi*, Tokyo, Japan). For the MDA assay, fresh leaves were homogenized in 5 mL of 100 g(trichloroacetic acid) L^{-1} containing 250 g(thiobarbituric acid) L^{-1}, and centrifuged at 20,000 × *g* for 25 min (4°C). The mixture was heated to 95°C for 30 min, and then cooled quickly in an ice-bath. Subsequently, samples were centrifuged at 10,000 × *g* for 10 min (4°C) and the supernatant absorbance was read at 532 nm. The value for the nonspecific absorption at 600 nm was subtracted from the 532 nm reading. The concentration of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} (Rosales *et al.* 2009).

F_v/F_m and gas-exchange parameters: At the flowering stage, Chl fluorescence parameters, including maximum (F_m) and minimum (F_0) fluorescence along with the maximum efficiency of PSII [(F_m – F_0)/F_m], were measured on fully expanded, attached leaves after dark-adaptation for 20 min using a portable Chl fluorometer (*Opti-Sciences, Inc*., Hudson, NH, USA). Photosynthetic gas-exchange rates were measured with a *Li-6400* portable photosynthesis system (*LICOR Inc.,* Lincoln, NE, USA) at the flowering stage between 10:00 and 13:00 h. The P_N, g_s, C_i, and transpiration rate (*E*) were measured by maintaining air temperature at 25°C, the relative humidity at 50−60%, and at light intensity of 1,200 µmol(photon) m^{-2} s^{-1}. These measurements were made on four fully expanded leaves per plants and were taken, when a steady state was obtained.

WUE and WUE_i: WUE was calculated as a ratio of P_N to *E* (Bertolde *et al.* 2012) and as an estimate of WUE_i the ratio of P_N to g_s was taken (Rouhi *et al.* 2007).

Yield and yield components: At harvest, the number of pods per plant, number of grains per pod, 100-grain mass, grain yield, and harvest index of bean genotypes were determined. Harvest index (HI) was calculated as: (grain yield/biological yield) × 100.

Statistical analysis: All measurements were done in triplicate, and the results were averaged and processed by one-way variance analysis test (*ANOVA*) using a *Statistical Analysis Software Version 9.1* (*SAS Institute Inc*., Cary, North Carolina, USA). For the comparison of data means, least significant difference (LSD) test was used at the $P \leq 0.05$ level of significance.

Results

Leaf RWC, contents of Chl and Car: The statistical analysis of the data revealed that RWC affected by water deficit and spermidine treatments. Foliar application of spermidine increased leaf RWC under both MWD and SWD significantly, but the extent of the enhancement was greater under SWD than that of MWD. Contrary, leaf RWC of unstressed plants was not affected by spermidine spray (Fig. 1*A*).

In order to better understand the roles of water stress and spermidine for pigment contents in bean, the contents of Chl *a*, *b*, and Car were determined. Water deficit and spermidine strongly affected leaf Chl and Car contents. There are no 2- or 3-way interactive effects of year, water deficit, genotype, and spermidine on Chl and Car contents (Table 1). Under MWD and SWD, leaf Chl was reduced on average by 14 and 34%, respectively, compared to well-watered plants. However, the leaf Car content increased under MWD and SWD by 1.3 and 2.6 folds, respectively (Table 1). In this study, exposure of bean plants to spermidine considerably elevated Chl and Car contents. According to our results presented in Table 1, Chl *a*, *b*, total and Car contents were improved by 15, 10, 14, and 18% by spermidine relative to control plants. Meanwhile, year, water stress, genotype, and spermidine had no effects on Chl *a*/*b* ratio.

Proline and MDA contents: Consistent with the results, year, water stress levels, genotypes, spermidine, and interaction of water deficit × genotype had a significant influence on the content of proline in leaves. As shown in Fig. 1*B*, the proline content was positively correlated with water scarcity. With increasing water deficit intensity,

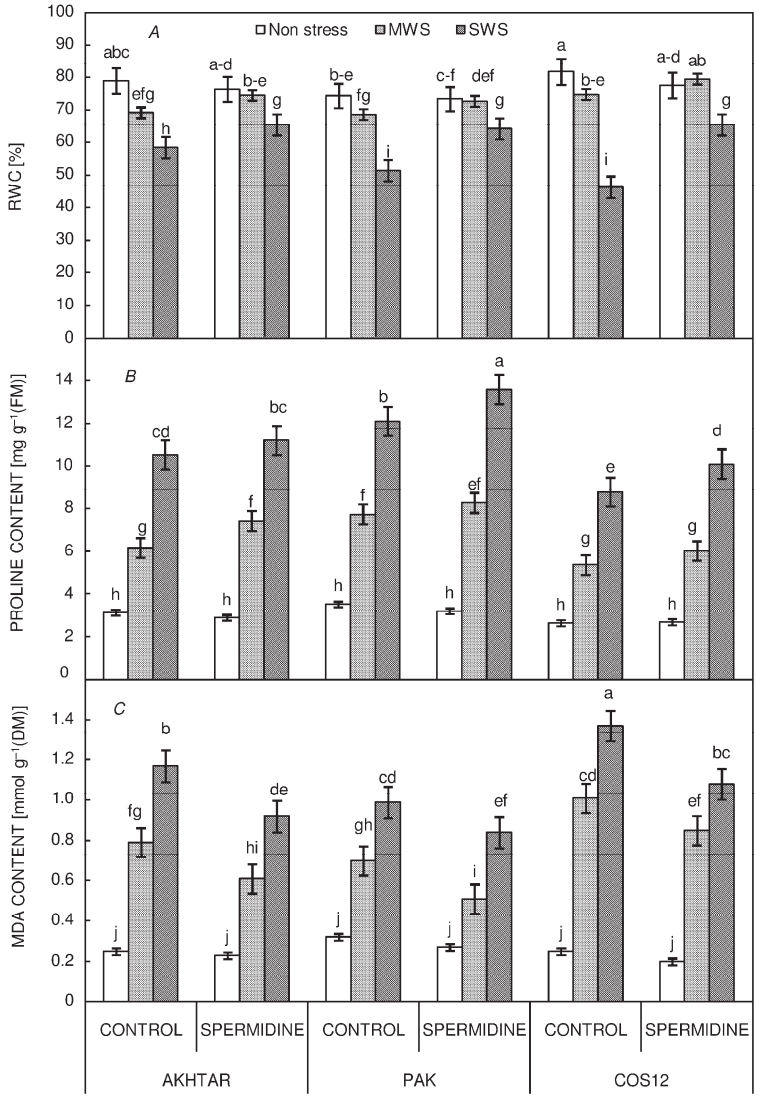

Fig. 1. Effect of spermidine application on relative water content (RWC) (*A*), proline (*B*) and malondialdehyde (MDA) (*C*) contents of common bean genotypes under water stress levels. Columns with *the same letter(s)* are not significantly different at $P \leq 0.05$ probability. Bars on the columns are means ± standard error. MWS – moderate water stress, SWS – severe water stress.

the proline content increased; whereas, the value of increment varied among genotypes. Under SWD, the highest proline content belonged to Pak. Additionally, no conspicuous difference in the proline contents was observed under non-stressed condition. Compared with control, the spermidine-sprayed plants showed slightly higher proline contents.

Variations in the MDA content, which indicated lipid peroxidation, are presented in Fig. 1*C*. Substantial effects of water stress levels, genotypes, spermidine, and water stress × genotypes on leaf MDA content were found. The MDA content under MWD and SWD strongly increased. Nonetheless, the extent of MDA increment was different between bean genotypes under water deficit. The maximum amount of MDA was recorded in COS12 under SWD. Exogenous application of spermidine appreciably diminished the leaf MDA content by 19% compared with control, irrespective of water stress level and genotype.

F_v/F_m and gas-exchange parameters: Results of *ANOVA* for quantum yield showed that F_v/F_m was notably affected by water stress and spermidine. There was no significant difference in F_v/F_m between genotypes as indicated in Table 1. Water stress treatments caused a remarkable decrease of F_v/F_m, which was 9% under MWD and was 22% under SWD compared with no stress treatment. Averaged across water stress and genotypes, F_v/F_m was improved by spermidine application by 6% compared with control. There were no 2- or 3-way interactive effects of year, water deficit, genotype, and spermidine on F_v/F_m (Table 1).

In order to determine the water stress and spermidine effects over the gas-exchange parameters of three common bean genotypes, P_N, g_s, C_i, and E were measured. Water deficit, genotype, and spermidine considerably affected P_N, g_s, C_i, and E. The interactive effects of water deficit × genotype on P_N, g_s, and E, and water deficit × spermidine

Table 1. Effects of spermidine on chlorophyll (Chl) *a*, *b* and total contents, Chl *a*/*b* ratio, carotenoid (Car) content, and maximal quantum yield of PSII (F_v/F_m) of common bean genotypes under water deficit. Values are means ± standard errors. Means followed by *the same letter* in the same column are not significantly different at the 5% probability level by LSD test. Analysis of variance (*ANOVA*) *P* values were shown ($P<0.05$ – significant; $P<0.01$ – markedly significant; $P>0.05$ – not significant). MWS – moderate water stress, SWS – severe water stress; FM – fresh mass.

Treatment	df	Chl *a* [mg g^{-1}(FM)]	Chl *b* [mg g^{-1}(FM)]	Total Chl [mg g^{-1}(FM)]	Chl *a*/*b*	Car [mg g^{-1}(FM)]	F_v/F_m
Water deficit							
No stress		4.76 ± 0.14^{a}	2.29 ± 0.06^{a}	7.05 ± 0.06^{a}	2.09 ± 0.08^{a}	0.23 ± 0.03^{c}	0.77 ± 0.01^{a}
MWS		4.05 ± 0.15^{b}	1.96 ± 0.05^{b}	6.01 ± 0.05^{b}	2.03 ± 0.08^{a}	0.55 ± 0.03^{b}	0.70 ± 0.02^{b}
SWS		3.07 ± 0.14^{c}	1.35 ± 0.05^{c}	4.42 ± 0.05^{c}	2.36 ± 0.07^{a}	0.83 ± 0.03^{a}	0.60 ± 0.01^{c}
Spermidine							
No spray		3.68 ± 0.10^{b}	1.77 ± 0.07^{b}	5.45 ± 0.04^{b}	2.14 ± 0.06^{a}	0.49 ± 0.04^{b}	0.67 ± 0.01^{b}
1 mM		4.24 ± 0.11^{a}	1.96 ± 0.04^{a}	6.21 ± 0.05^{a}	2.23 ± 0.08^{a}	0.58 ± 0.03^{a}	0.71 ± 0.01^{a}
Genotype							
Akhtar		3.98 ± 0.11a	1.91 ± 0.04^{a}	5.59 ± 0.06^{a}	2.13 ± 0.07^{a}	0.51 ± 0.04^{b}	0.69 ± 0.02^{a}
Pak		4.06 ± 0.12^{a}	1.9 ± 0.05^{a}	5.97 ± 0.05^{a}	2.2 ± 0.06^{a}	0.59 ± 0.03^{a}	0.70 ± 0.01^{a}
COS12		3.83 ± 0.15^{a}	1.79 ± 0.06^{a}	5.62 ± 0.06^{a}	2.23 ± 0.07^{a}	0.53 ± 0.04^{ab}	0.68 ± 0.01^{a}
Significance test of variations source (*P* values)							
Year (Y)	1	0.42	0.35	0.28	0.32	0.59	0.44
Water deficit (W)	2	<0.01	<0.01	<0.01	0.15	<0.01	<0.01
Genotype (G)	2	0.49	0.08	0.19	0.84	0.08	0.54
Spernidine (S)	1	<0.01	<0.01	<0.01	0.47	<0.01	<0.01
Y×W	2	0.36	0.17	0.26	0.25	0.21	0.48
Y×G	2	0.49	0.49	0.58	0.45	0.03	0.42
Y×S	1	0.03	0.95	0.06	0.07	0.34	0.34
W×G	4	0.72	0.38	0.41	0.98	0.77	0.86
W×S	2	0.88	0.38	0.97	0.84	0.51	0.60
G×S	2	0.97	0.71	0.97	0.74	0.62	0.93
Y×W×S	2	0.96	0.57	0.90	0.98	0.33	0.71
Y×S×G	2	0.73	0.79	0.64	0.87	0.99	0.88
Y×W×G	4	0.96	0.77	0.97	0.59	0.57	0.95
W×G×S	4	0.99	0.73	0.99	0.78	0.53	0.92
Y×W×S×G	4	0.97	0.78	0.99	0.92	0.87	0.91

on *E* were also detected (Table 2). Regardless of genotype and spermidine, P_N, g_s, C_i, and *E* noticeably declined in the plants exposed to water stress conditions. Averaged across water deficit and genotype, gas-exchange parameters were elevated when plants were exposed to spermidine. According to Table 2, the highest value of photosynthetic parameters was observed in Pak with spermidine under well-watered plants, while the lowest value belonged to COS12 without spermidine under SWD. Averaged over the genotypes, usage of spermidine increased *E* differently, when plants were subjected to water deficit. Application of spermidine enhanced *E* by 8, 22, and 32% under no stress, MWD, and SWD, respectively (Table 2).

WUE and WUE_i: Water deficit and genotype significantly affected WUE; however, spermidine and interactive effects of water deficit × genotype also influenced WUE_i. As an average, severe water deficit increased WUE by 13% in comparison with well-watered beans, while there was no considerable difference between well-watered and MWD treatments. The maximum WUE was recorded in COS12 under SWD (Table 2). Irrespective of spermidine, WUE_i varied markedly among the bean genotypes when exposed to three water stress treatments. Under MWD, WUE_i of all genotypes was reduced; however, under SWD, WUE_i of Akhtar and Pak genotypes increased compared with non-stressed plants (Table 2). Averaged across water stress and genotypes, foliar application of spermidine increased the WUE_i by 7%; however, spermidine usage had no significant effects on the WUE of bean genotypes.

Yield and yield components: Fig. 2 illustrates the changes in the yield and yield components of bean genotypes at the harvest stage. Water deficit, genotype, spermidine, and interactive effects of water deficit × genotype were noticeable on the number of pods per plant, number of grains per pod, 100-grain mass, and grain yield. The interactive effect of water deficit × spermidine on 100-grain mass was also detected. Water deficit conditions disrupted yield-related traits in terms of the number of pods and grains per plant, 100-grain mass, and grain yield in comparison to well-watered treatment. The extents of reductions under MWD in Akhtar, Pak, and COS12 were

Table 2. Effects of spermidine on stomatal conductance (g_s), intercellular CO_2 concentration (C_i), net photosynthetic rate (P_N), transpiration rate (E), water-use efficiency (WUE) and intrinsic water-use efficiency (WUE_i) of common bean genotypes under water deficit. Means ± standard errors followed by *the same letter* in the same column are not significantly different at the 5% probability level by LSD test. Analysis of variance (*ANOVA*) *P* values were shown ($P<0.05$ – significant; $P<0.01$ – markedly significant; $P>0.05$ – not significant). MWS – moderate water stress, SWS – severe water stress.

Genotype	Treatment	g_s [mmol(H_2O) m^{-2} s^{-1}]	C_i [µmol(CO_2) mol^{-1}]	P_N [µmol(CO_2) m^{-2} s^{-1}]	E [mmol(H_2O) m^{-2} s^{-1}]	WUE [mmol(CO_2) mol^{-1}(H_2O)]	WUE_i [mmol(CO_2) mol^{-1}(H_2O)]
Akhtar	No stress	502 ± 8.5^{c}	325 ± 5.5^{abc}	22.7 ± 0.55^{bc}	12.7 ± 0.33^{b}	1.78 ± 0.04^{efg}	$0.044 \pm 0.0007^{c–g}$
	No stress+spermidine	517 ± 9.7^{bc}	327 ± 5.8^{ab}	23.5 ± 0.56^{ab}	11.3 ± 0.34^{d}	2.07 ± 0.04^{cd}	$0.044 \pm 0.0007^{c–g}$
	MWS	408 ± 9.3^{g}	273 ± 5.8^{fgh}	18.7 ± 0.50^{efg}	10 ± 0.32^{e}	$1.86 \pm 0.04^{d–g}$	0.04 ± 0.0007^{gh}
	MWS+spermidine	435 ± 11^{f}	296 ± 6.5^{def}	20.1 ± 0.54^{de}	11.7 ± 0.35^{cd}	1.74 ± 0.04^{fg}	$0.045 \pm 0.0006^{b–f}$
	SWS	322 ± 8.5^{jk}	221 ± 5.5^{k}	12.2 ± 0.55^{j}	6.32 ± 0.33^{h}	1.93 ± 0.04^{def}	0.041 ± 0.0007^{fg}
	SWS+spermidine	343 ± 8.3^{ij}	242 ± 5.1^{ijk}	15.9 ± 0.52^{h}	8.47 ± 0.34^{f}	$1.90 \pm 0.03^{d–g}$	0.053 ± 0.0006^{a}
Pak	No stress	516 ± 9.1^{bc}	341 ± 5.3^{a}	23.7 ± 0.53^{ab}	14.4 ± 0.31^{a}	1.67 ± 0.03^{g}	$0.045 \pm 0.0007^{b–f}$
	No stress+spermidine	548 ± 8.4^{a}	344 ± 5.2^{a}	24.6 ± 0.55^{a}	14.3 ± 0.32^{a}	1.73 ± 0.04^{fg}	$0.044 \pm 0.0007^{c–g}$
	MWS	461 ± 10.3^{de}	$298 \pm 6.2^{c–f}$	19 ± 0.55^{ef}	11.3 ± 0.35^{d}	1.69 ± 0.03^{fg}	0.041 ± 0.0007^{fg}
	MWS+spermidine	478 ± 10.3^{d}	$320 \pm 5.2^{a–d}$	21.4 ± 0.56^{cd}	12 ± 0.35^{c}	1.78 ± 0.04^{efg}	$0.045 \pm 0.0007^{b–f}$
	SWS	345 ± 9.1^{i}	260 ± 5.3^{hij}	16.4 ± 0.53^{h}	7.96 ± 0.31^{fg}	2.08 ± 0.03^{cd}	0.048 ± 0.0007^{bc}
	SWS+spermidine	378 ± 8.4^{h}	$286 \pm 5.7^{e–h}$	18.5 ± 0.55^{fg}	9.67 ± 0.32^{e}	$1.92 \pm 0.04^{d–g}$	0.049 ± 0.0007^{ab}
COS12	No stress	507 ± 9.4^{c}	$312 \pm 5.1^{b–e}$	23.2 ± 0.53^{ab}	12.1 ± 0.30^{bc}	$1.90 \pm 0.04^{d–g}$	$0.046 \pm 0.0007^{b–e}$
	No stress+spermidine	531 ± 10.4^{ab}	324 ± 5.2^{abc}	24.1 ± 0.49^{ab}	12.2 ± 0.33^{bc}	2.01 ± 0.04^{cde}	0.047 ± 0.0007^{bcd}
	MWS	460 ± 10.3^{de}	267 ± 5.8^{ghi}	17.2 ± 0.55^{gh}	7.77 ± 0.35^{g}	2.25 ± 0.04^{bc}	0.042 ± 0.0007^{efg}
	MWS+spermidine	449 ± 9.5^{ef}	291 ± 5.3^{efg}	18.9 ± 0.53^{ef}	10 ± 0.36^{e}	$1.90 \pm 0.03^{d–g}$	$0.043 \pm 0.0007^{d–g}$
	SWS	291 ± 8.3^{l}	216 ± 5.2^{k}	11.8 ± 0.51^{j}	4.75 ± 0.32^{i}	2.51 ± 0.04^{a}	0.036 ± 0.0007^{h}
	SWS+spermidine	301 ± 10.5^{kl}	235 ± 5.2^{jk}	13.8 ± 0.55^{i}	6.22 ± 0.35^{h}	2.35 ± 0.04^{ab}	0.042 ± 0.0007^{efg}
Significance test of variations source (*P* values)							
Year (Y)	1	0.77	0.19	0.90	0.63	0.80	0.59
Water deficit (W)	2	<0.01	<0.01	<0.01	<0.01	<0.05	0.27
Genotype (G)	2	<0.01	<0.01	<0.01	<0.01	<0.01	0.28
Spermidine (S)	1	<0.01	<0.05	<0.01	<0.01	0.66	<0.05
Y×W	2	0.31	0.98	0.13	0.96	0.44	0.08
Y×G	2	0.18	0.34	0.36	0.08	0.83	0.70
Y×S	1	0.61	0.94	0.09	0.63	0.28	0.18
W×G	4	<0.01	0.83	<0.05	<0.01	0.33	<0.05
W×S	2	0.71	0.59	0.31	<0.01	0.24	0.20
G×S	2	0.43	0.98	0.96	0.46	0.61	0.53
Y×W×S	2	0.64	0.99	0.15	0.66	0.53	0.47
Y×S×G	2	0.22	0.93	0.53	0.40	0.42	0.25
Y×W×G	4	0.55	0.77	0.54	0.12	0.72	0.47
W×G×S	4	0.79	0.99	0.85	0.11	0.78	0.65
Y×W×S×G	4	0.71	0.99	0.99	0.70	0.98	0.98

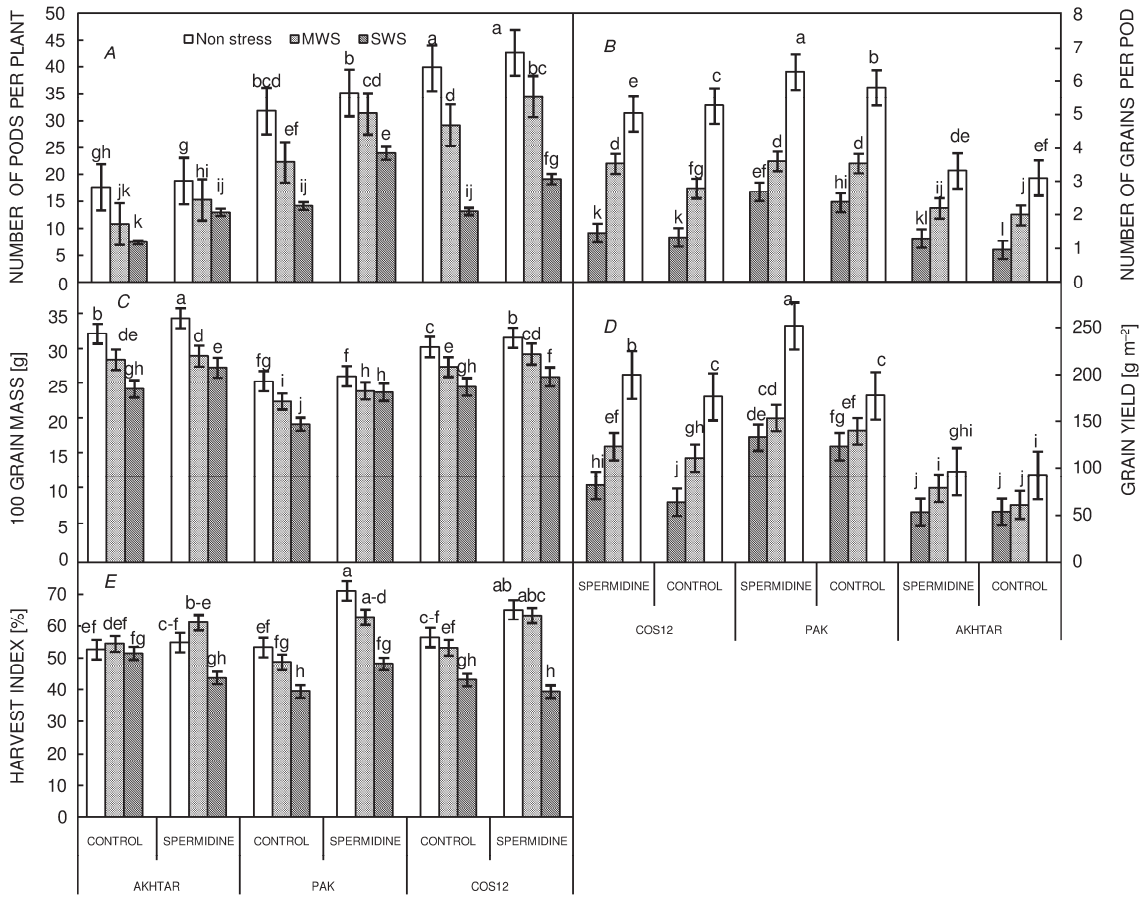

Fig. 2. Effect of spermidine application on number of pod per plant (*A*), number of grain per pod (*B*), 100-grain mass (*C*), grain yield (*D*), and harvest index (HI) (*E*) of common bean genotypes under water stress levels. Columns with *the same letter(s)* are not significantly different at $P \leq 0.05$ probability. Bars on the columns are means ± standard error. MWS – moderate water stress, SWS – severe water stress.

28, 20, and 24% and 43, 43, and 69%, respectively, under SWD for the number of pods per plant (Fig. 2*A*). For number of grains per pod, the values declined in Akhtar, Pak and COS12 by 35, 41, and 38% under MWD; while they were 64, 58, and 72%, respectively, under SWD (Fig. 2*B*). The reductions in the extent of the 100-grain mass in Akhtar, Pak, and COS12 under MWD were 13, 9, and 8%, and under SWD, the reductions were 22, 17, and 18%, respectively, compared with non-stressed plants (Fig. 2*C*). Finally, the reductions in grain yield in Akhtar, Pak, and COS12 were 24, 31, and 36% under MWD, and 42, 41, and 60%, respectively, under SWD (Fig. 2*D*).

Averaged over water stress and genotypes, when bean plants were sprayed with spermidine, the number of pods per plant, number of grains per pod, 100-grain mass, and grain yield improved by 25, 8, 7, and 31%, respectively, compared with control. Although exogenous application of spermidine increased 100-grain mass in plants under well-watered and water-deficit conditions, the extents of increment varied. A higher (14%) 100-grain mass was observed in spermidine-treated plants compared with control in response to SWD; this was the greatest enhancement (Fig. 2*C*).

Water deficit and spermidine considerably affected HI of bean plants. There were no 2- or 3-way interactive effects of year, water deficit, genotype, and spermidine on HI. Averaged across genotype and spermidine, HI noticeably declined in the plants subjected to water stress conditions, especially under SWD. Moreover, HI was enhanced approximately by 12% when plants were exposed to spermidine, irrespective of water stress and genotype (Fig. 2*E*).

Discussion

Water deficit is a well-known environmental stress factor that restricts the growth and yield of crop plants worldwide and can trigger a series of physiological and biochemical responses in plants (Yue *et al.* 2012). The main physiological and biochemical characteristics are not limited to stomatal closure, decrease in tha Chl content,

reduction in transpiration, and inhibition of photosynthesis (Nezhadahmadi *et al.* 2013). Under water deficit, decreases in leaf RWC and water potential induce stomatal closure, leading to a reduction in CO_2 availability and consequently to a decline in P_N and WUE (Bota *et al.* 2004). The first response of plants to soil moisture reduction is stomatal closure (Chaves *et al.* 2003). Stomatal closure and down-regulation of photosynthesis are both common responses of plants to drought (Dietz and Pfannschmidt 2011). For common bean, the values of RWC, g_s, C_i, E, and P_N were significantly diminished by water-stress treatments in the present study. These results suggest that the diminution of RWC in concert with the decline of g_s, E, and C_i contributed to the significant decrease in P_N under water scarcity. Chl fluorescence reflects the maximal efficiency of excitation energy captured by open PSII reaction center. Fluorescence determination has become a widely used method to study the functioning of the photosynthetic apparatus and is a powerful tool to study the responses of plants to environmental stress (Massacci *et al.* 2008). Decrease in PSII quantum yield during leaf drying occurs at lower water potentials (Brodribb and Holbrook 2003). Generally, water stress may damage oxygen-evolving complex and PSII reaction centers (Subrahmanyam *et al.* 2006). In our study, F_v/F_m decreased by 9 and 22% under MWD and SWD, respectively.

The results presented in Table 1 showed that the Chl content of bean plants declined by 14 and 34%; while Car improved 1.3 and 2.6 folds under MWD and SWD, respectively, relative to well-watered plants. The reduced or unchanged Chl content depended on the duration and severity of drought stress (Anjum *et al.* 2011). Drought or heat stresses increase ROS productions which can lead to lipid peroxidation and consequently to Chl destruction (Foyer *et al.* 1994). In fact, ROS can cause membrane damage and MDA accumulation and finally cell death. MDA has been considered an indicator for the degree of oxidative stress (Dionisio-Sese and Tobita 1998). In our study, leaf MDA content increased when plants were exposed to water stress. Osmotic adjustment is also an important mechanism to resist drought stress in plants. The osmoregulation substances, such as proline, soluble sugars, and soluble proteins, play vital roles in maintaining osmotic equilibrium and the integrity of membranes under water deficit (Mahajan and Tuteja 2005). Proline accumulation may occur due to an increased activity of pyroline-5-carboxylase reductase and reduced activitys of proline oxidase under stress conditions (Nounjan *et al.* 2012). As an average, leaf proline content in bean plants increased by water deficit almost 1.8 folds in comparison with well-watered plants. Car as accessory pigments play a critical role in the assembly of the light-harvesting complex and in thermal dissipation of excess light energy (Demmig-Adams and Adams 1992). We observed that the increase in the Car content was closely associated with the water stress level (Table 1). In drought conditions, plants usually increase endogenous Car contents to cope with oxidative stress (Eskling *et al.* 1997).

According to our results presented in Table 2, WUE increased only under SWD. Zhang *et al.* (2006) reported that deficit of irrigation could result in higher WUE in spring wheat under an arid environment. Declining of g_s under drought treatment improves WUE when g_s is the dominant factor controlling gas exchange (Li *et al.* 2011). Water deficiency considerably impaired the yield and yield component-related traits, such as the number of pods per plant, number of grains per pod, 100-grain mass, grain yield per plant and harvest index. Decreasing the yield under water deficit could be a result of a reduction in the Chl content as our data indicated, and consequently, photosynthesis efficiency. Martínez *et al.* (2007) demonstrated that water stress reduced grain yield up to 70% in common bean under field conditions. Bean genotypes showed different responses to water deficit in this experiment. The results show that the changes in grain production under water stress and their relationship with the water deficit resistance of bean genotypes varied greatly with the severity of the stress. Among bean genotypes, the lowest decrease of gas-exchange parameters belonged to Pak; in contrast, the highest was recorded in COS12 under water stress conditions, which led to similar trends in yield and yield components. In agreement with this observation, the lowest value of MDA content and the highest increment in proline under water scarcity belonged to Pak.

PAs are ubiquitous aliphatic amines associated with regulation of plant stress tolerance due to their roles in free radical scavenging, osmotic adjustment, and maintaining a cation–anion balance under abiotic stress (Roychoudhury *et al.* 2011). Exogenously applied PAs could improve drought tolerance of plants by inhibiting lipid peroxidation, increasing WUE and modulating plant metabolism (Sagor *et al.* 2013). In the current study, spermidine application led to increase in leaf RWC, Chl, Car, and proline contents, F_v/F_m, C_i, g_s, E, P_N, and WUE_i in three bean genotypes compared with control. Enhanced RWC, g_s, E, and Chl content in spermidine- treated plants resulted in higher P_N and improved WUE_i. It suggests that spermidine is able to influence P_N through increasing RWC, g_s, and Chl, Car, and proline contents under water stress. When RWC falls below 70%, a cascade of physiological processes is initiated with negative impacts for the plant. These changes include turgor loss, decrease in leaf water potential, stomatal closure, decrease in internal CO_2 concentration, all of which can lead to impairment of photosynthetic activity (Singh and Reddy 2011). The rises of P_N in concert with the enhanced g_s have contributed to the significant increase in the WUE_i. PAs compounds effectively enhance photosynthesis since they are capable to reverse the stress-induced damage in the photosynthetic apparatus (Sfakianaki *et al.* 2006). Zhang *et al.* (2009) reported that exogenous application of PA increased photosynthetic rates in cucumber cultivars.

Besford *et al.* (1993) stated that exogenous spermine decreased Chl degradation and protected leaf Rubisco under water stress, while increased contents of spermine were associated with enhanced photosynthesis (Islam *et al.* 2003). Li *et al.* (2015) concluded that exogenous spermidine significantly improved leaf RWC and maintained the Chl content better, which demonstrate that spermidine pretreatments delayed leaf senescence in creeping bentgrass under drought stress. Our data clearly showed the direct correlation between spermidine use and Car, proline and Chl contents in response to water stress. It means that keeping the balance between Chl and Car biosynthesis and their catabolism were important for plants to deal with water stress. Moreover, accumulated organic solute and enhanced Car metabolism caused by spermidine may be involved in osmotic adjustment and osmoprotection, which explains the higher Chl and F_v/F_m and better photosynthesis regarding the response of water stress. These data suggested that the better stress tolerance of treated plants by spermidine could be associated with increased RWC, Car, proline, and Chl pigment contents, g_s and E, which strongly improved P_N. Enhanced proline and Car contents in spermidine-treated leaves resulted in lower lipid peroxidation and improved cell membrane stability, as demonstrated by lower MDA concentrations.

In addition, the data in this study also showed that spermidine usage could increase the yield and yield components of bean plants. Increasing grain yield is primarily the result of increased number of pods per plant and number of grains per pod rather than 100-grain mass in spermidine-treated plants. Ndayiragije and Lutts (2007) demonstrated that exogenous putrescine, and to a lesser extent spermidine, improved salt-damaged yield-related characters in rice including grain yield per plant, panicle number, spikelets per panicle, and 1,000-grain mass. Saleethong *et al.* (2013) stated that the exogenous spermidine application alleviated the adverse effects of NaCl stress on the reproductive processes leading to an improvement in rice yield. Stimulation of reproductive development by PAs has also been reported in apricot (Alburquerque *et al.* 2006) and cotton (Bibi *et al.* 2010) but the mechanisms of PAs action on promoting reproductive development are still largely unknown. We think the increase in g_s, E, photosynthetic pigment contents, and P_N by spermidine can lead to the yield and yield components improvements in common bean plants. Our results suggested that spermidine is directly involved in mitigation of water stress in bean plants.

Conclusion: Water stress inhibits growth and reduces grain yield of common bean plants through Chl diminution and photosynthesis inhibition. We conducted experiments with three conventional common bean genotypes. Based on our results, Pak and COS12 appeared to be water stress-tolerant and water stress-sensitive, respectively, because of the reduction in some gas-exchange parameters, grain yield, and yield components affected by diminished water availability. The present study also confirmed that spermidine as a free radical scavenger counteracted impressively and alleviated the negative effects of water deficit stress in three bean genotypes, as demonstrated by higher RWC, Chl content, g_s, C_i, E, P_N, and increasing grain yield in comparison with control bean. This study also suggests that spermidine induced accumulation of Car and proline, which play a principle role in improving drought tolerance associated with osmotic adjustment and osmoprotection. Likewise, spermidine usage reduced the MDA content, which lowered lipid peroxidation and cell damage, and caused a higher membrane stability. It is proposed that spermidine as a polyamine would help plants resist stress and prevent them from being seriously damaged. Therefore, we can speculate that application of PAs may be useful in common bean for coping with water-stress conditions.

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