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SPERMIDINE AS AN ELEVATOR OF SALINITY INDUCED STRESS ON TWO VARIETIES OF *TRITICUM DURUM* DESF. (KARIM AND RAZZEK)

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Abstract

Salinity is one of the main abiotic constraints affecting crop productivity. Recently, it is reported that exogenous application of spermidine (Spd) could significantly alleviate the effect of salt stress. In this study, we aim to evaluate the effects of Spd application on salt stress resistance in two durum wheat (*Triticum durum*) varieties (Karim and Razzek) in Tunisia. Seedlings of both varieties were subjected to different NaCl concentrations (0, 20, 50, 100, 150 and 200 mM) in the presence and absence of 1 mM of Spd for 10 days. Results showed that Spd significantly increased the weight of both fresh and dry matter of NaCl-stressed plants. Similarly, Spd increased soluble sugars, and chlorophylls a and b contents particularly under 150 and 200 mMNaCl. However, exogenous Spd application decreased the content of proline and the activity of aminating glutamate dehydrogenase (GDH) enzyme. Our analysis, suggested that the application of Spd could be used as an effective approach in alleviating the NaCl-induced toxic effect in durum wheat.

Key words: Exogenous spermidine, GDH enzyme, Salinity tolerance, *Triticum durum*.

Introduction

Cereal grains are considered among the main component in human diet where more than 50 % of the daily caloric intake comes directly from the consumption of the cereals (Awika, 2011). They are also considered as one of the major sources of nutrients, such as carbohydrate, protein, vitamins and minerals for livestock that form an important part of the food chain (Mckevith, 2004). However, salinization poses a real threat to global food security by lowering crop yields and irreparably damaging the agriculture lands, crop productivity and quality. Salt-affected lands are increasing at a rate of 10% each year mainly due to irrigation with NaCl-rich water, elevated evaporation especially in arid environment, weathering of native rocks, low precipitations, and poor cultural practices. By 2050, it is estimated that, more than 50% of the arable land, will be affected by salinization (Shrivastava & Kumar, 2015).

In semi-arid regions, the salt content in soil can reach up to 100 mM, which is beyond the tolerance limit of most cultivated crops and inhibits their growth and productivity especially in sensitive species such as wheat (Gentili *et al*., 2018).

For these reasons, salinity is considered as the major abiotic constraint limiting plant growth and yield. Salt-induced plant growth inhibition is generally associated with reduced water availability and toxic ions (Na^+, Cl^-) accumulation, causing mineral imbalance responsible for morphological, physiological and metabolic disturbances (Ventura *et al*., 2014). From a physiological standpoint, the harmful impact of salinity is reflected by three basic effects (i) osmotic effects through NaCl impact on water potential resulting in a reduction of the water availability for the plant, (ii) mineral effects by limiting the absorption of essential nutrients specially potassium (K^+) and nitrogen (NO_3^-) and (iii) toxic effects following the excessive accumulation of toxic ions in tissues that negatively affect metabolic processes (Almeida-Machado & Serralheiro, 2017). In addition, elevated concentration of NaCl in the culture medium is able to reduce the chlorophyll content (Shah *et al*., 2017) and limit the plant's access to essentials nutriments, such as nitrogen (N) which is implicated in the composition of many compounds including amides, proteins amino acids and polyamines (Assaha *et al*., 2017). The disruption of N assimilation by NaCl generally results in an increase in ammonium (NH_4^+) content and activation of aminating glutamate dehydrogenase (GDH) pathway. This pathway could be involved in the detoxification of NH_4^+ and the replacement of glutamate pool, which is largely involved in the synthesis of protective metabolites including proline and soluble sugar (Ashraf *et al*., 2018).

Plants have developed three main resistant strategies against salt constraint: detoxification, restoration of homeostasis and growth regulation (Hanin *et al*., 2016). Pertinent tolerance mechanism involves the accumulation of compatible solutes such as; proline, glycine, and betaine in the cytoplasm to equilibrate the decrease in water potential occurring in the vacuole due to the accumulation of salt ions (Negrao *et al*., 2017). Polyamines (PAs), including spermidine (Spd), spermine (Spm) and their precursor, putrescine (Put), are involved in plant salt tolerance through numerous mechanisms (Ghabriche *et al*., 2017). The abundance of these metabolites, mainly Spd, was connected with improved salinity tolerance suggesting that Spd is involved in the sequestration of excessive Na^+ in the vacuole and maintaining the pH balance (Todorova *et al*., 2013).

In this study, we evaluated the effect of exogenous Spd in enhancing salinity tolerance in durum wheat (*Triticum durum*). We measured some physiological and

biochemical indicators such as plant fresh and dry weight, chlorophyll content, proline, total soluble sugars as well as the key enzymes involved in the plant metabolism (NADH-GDH and NAD^+- GDH) in roots and leaves of two varieties of *T. durum*.

Material and Methods

Plant material and growth conditions: Seeds of two durum wheat (*Triticum durum*Desf.) varieties, Karim (Jori"S"/Anhinga"S"//Flamingo"S", CIMMYT-Mexico, 1980) and Razzek (Dmx69-331/Karim, INRAT-Tunisie, 1987), were provided by the Ministry of Agriculture of Tunisia. The seeds were sterilized with sodium hypochlorite solution (10%) for 20 min and washed 3 times with distilled water. After that, they were germinated in Petri dishes on wet filter paper at 25°C in the dark for 5 days. Healthy seedlings of the same size were transferred to buckets (1.3L) of continuously aerated nutrient Hoagland solution. The nutrient solution was constantly aerated using an electric pump and renewed every 4 days. Plants were grown in a growth chamber under 26°C and 40% relative humidity during the light period and 20°C and 50% relative humidity during the dark period (16 h photoperiod with a light illumination of 150 µmol m^{-2} s^{-1} at the canopy level and 8 h dark period). Salt stress treatment was applied by adding different NaCl concentrations (0, 20, 50, 100, 150 and 200 mM) to the nutrient solution. At the same time, another group of treated plants with NaCl (20, 50, 100, 150 and 200 mM) had received Spd at 1 mM concentration to the nutrient solution. 12 plants per treatment were harvested 10 days after salinity treatment.

Determination of water content: The water content shoot and root was determined as described by Molassiotis *et al*., (2010).

Tissue ion content: Mineral elements in dried leaf and roots materials were extracted with 0.5% HNO_3 and were assayed with flame photometry as previously described (M'rah *et al*., 2006).

Glutamate Dehydrogenase assay: The extraction of GDH was performed according to the method of Dguimi *et al*., (2019). Briefly, frozen samples were homogenized in a cold mortar and pestle with Tris-HCl (100 mM, pH 7.5), 2-mercaptoethanol (14 mM), and PVP (1% (w/v)). The extract was centrifuged (5804R, Eppendorf, USA) at 4°C for 20 min at 10,000. NADH-GDH and NAD^+-GDH activities were determined at 340 nm, using UV/Vis spectrophotometer (UV-1600PC, VWR, USA).

Determination of chlorophylls content: Chlorophyll was extracted in 80% acetone solution and determined according to Lichtenthaler & Wellburn (1983). The fresh leaves (100 mg) were transferred into 2 ml of 80% acetone, vortexed and kept at 4°C overnight. The obtained suspension was centrifuged at 10,000*g for 15 min and the supernatant absorbance was measured at 3 wavelengths (645, 652 and 663 nm).

Analysis of proline content: Proline content in tissues was determined according to Bates method as described by Ben Fattoum *et al*., (2016). Samples (0.5 g) were homogenized with 5 mL of sulphosalycylic acid (3% (W/V)); then were filtered through filter paper. After the addition of 2 mL of a mixture containing; (acidic acid, distilled water and orthophosphoric acid (85%) at a ratio of 6:3:1) and 1 mL of glacial acetic acid, the resulting solution was incubated in a water bath at 100°C for 1 h. The reaction was then stopped using an ice bath. The mixture was extracted with toluene, and the absorbance was measured at 520 nm. The concentrations of proline were expressed on µg per g fresh weight (µmol g^{-1} FW).

Determination of soluble sugars content: Soluble sugars were measured according to Dguimi *et al*., (2019). Soluble sugars were extracted from fresh leaves and roots samples (0.5 g), that were crushed in a mortar and homogenized with 5 mL of hot alcohol (80%). The homogenates were centrifuged at 9000 g for 15 min and the supernatant was used for the analysis. The concentrations of total soluble sugar were calculated on a fresh weight basis (µmol g^{-1} FW).

Statistical analysis

Analysis of variance (ANOVA) was used to compare parameters significance in each variety. ANOVA was also applied on variety, NaCl concentrations, Spd and theirs interaction to test the significance of parameters. Analyses of variance based on Duncan's multiple range test using Xlstat (www.xlstat.com). The difference was considered statistically significant at $p<0.05$.

Results

Growth status and water content: Under different NaCl concentrations, significant decreases were observed in FW of leaf and root of Karim and Razzek varieties. This decrease was remarkable at the highest dose of NaCl (200 mM), where the FW of leaves were decreased from 0.96 to 0.13 mg and from 1.14 to 0.31 mg for Karim and Razzek varieties, respectively (Table 1). Root FW also were decreased from 0.85 to 0.12 mg (Karim) and from 0.95 to 0.41 mg (Razzek). Likewise, the DW of leaves and roots was significantly influenced by high NaCl concentrations (Fig. 2A, B, C and D). Despite the decrease in FW and DW, Razzek variety remained more resistant than Karim. The addition of Spd (1 mM) showed an increase in the FW of both leaves and roots. This improvement was more remarkable in Razzek variety. Addition of Spd reduced the effect of salt stress at different NaCl concentrations for both Karim and Razzek varieties.

Salinity increased WC in the roots treated with 100 and 150 mM NaCl. However, this effect is reduced by the presence of Spd (1mM) for both Karim and Razzek varieties. Similarly, it can be noted that in the presence of Spd, WC appears almost to the same level as the control treatment especially at 50 mM and 100 mM treatments for Karim and Razzek varieties, respectively.

Table 1. Growth status and water content of durum wheat (*Triticum durum* Desf.) varieties (Karim and Razzek) under salt stress and of spermidine application.

	FWL (g)	FWR (g)	DWL (g)	DWR (g)	WCL (%)	WCR (%)
	NaCl (mM)			Karim		
0 mM	0,960 ± 0,019^{A}	0,846 ± 0,025^{AB}	0,230 ± 0,022^{A}	0,105 ± 0,009^{A}	320,505 ± 41,175^{E}	708,274 ± 64,064^{E}
20 mM	0,790 ± 0,017^{B}	0,736 ± 0,028^{BC}	0,164 ± 0,005^{B}	0,069 ± 0,002^{B}	381,438 ± 15,989^{DE}	946,912 ± 57,563^{CD}
50 mM	0,598 ± 0,011^{C}	0,590 ± 0,032^{D}	0,116 ± 0,005^{C}	0,042 ± 0,002^{C}	417,355 ± 27,345^{DE}	1291,791 ± 61,498^{A}
100 mM	0,478 ± 0,036^{D}	0,206 ± 0,009^{F}	0,062 ± 0,003^{D}	0,025 ± 0,004^{D}	670,588 ± 30,638^{C}	740,431 ± 169,252^{DE}
150 mM	0,190 ± 0,016^{E}	0,097 ± 0,004^{G}	0,012 ± 0,001^{E}	0,009 ± 0,000^{E}	1512,238 ± 116,953^{A}	1023,870 ± 44,265^{BC}
200 mM	0,133 ± 0,008^{F}	0,073 ± 0,006^{G}	0,010 ± 0,000^{F}	0,006 ± 0,000^{E}	1250,208 ± 72,791^{B}	1096,394 ± 123,944^{ABC}
20 mM+Spd	0,828 ± 0,023^{B}	0,788 ± 0,030^{A}	0,172 ± 0,008^{B}	0,072 ± 0,002^{B}	380,808 ± 17,946^{DE}	998,968 ± 68,714^{BC}
50 mM+Spd	0,656 ± 0,023^{C}	0,604 ± 0,030^{C}	0,146 ± 0,005^{C}	0,046 ± 0,003^{C}	349,956 ± 5,296^{DE}	1209,135 ± 143,208^{AB}
100 mM+Spd	0,538 ± 0,020^{D}	0,338 ± 0,020^{D}	0,088 ± 0,003^{D}	0,033 ± 0,003^{D}	511,577 ± 22,469^{CD}	928,571 ± 63,888^{CDE}
150 mM+Spd	0,362 ± 0,025^{E}	0,180 ± 0,042^{E}	0,051 ± 0,004^{E}	0,018 ± 0,004^{E}	609,296 ± 58,663^{C}	900,000 ± 0,000^{CDE}
200 mM+Spd	0,306 ± 0,009^{F}	0,124 ± 0,018^{E}	0,026 ± 0,004^{F}	0,011±0,002^{E}	1118,379 ± 206,544^{B}	1048,134 ± 205,193^{BC}
				Razzek		
0 mM	1,140 ± 0,134^{A}	0,952 ± 0,016^{A}	0,230 ± 0,013^{A}	0,234 ± 0,023^{A}	139,055 ± 49,616^{C}	254,685 ± 146,443^{B}
20 mM	0,822 ± 0,025^{BC}	0,808 ± 0,023^{C}	0,164 ± 0,018^{B}	0,192 ± 0,004^{B}	127,197 ± 40,277^{C}	252,107 ± 141,179^{C}
50 mM	0,716 ± 0,021^{BC}	0,724 ± 0,042^{D}	0,116 ± 0,004^{E}	0,118 ± 0,008^{C}	392,275 ± 173,963^{BC}	427,980 ± 246,008^{B}
100 mM	0,640 ± 0,019^{CD}	0,458 ± 0,033^{F}	0,062 ± 0,005^{EF}	0,064 ± 0,002^{E}	518,714 ± 245,664^{B}	481,169 ± 271,108^{B}
150 mM	0,432 ± 0,026^{DE}	0,230 ± 0,025^{G}	0,012 ± 0,005^{FG}	0,022 ± 0,003^{F}	534,573 ± 257,910^{A}	767,089 ± 458,461^{B}
200 mM	0,314 ± 0,013^{E}	0,140 ± 0,012^{H}	0,010 ± 0,004^{G}	0,012 ± 0,002^{F}	1121,196 ± 598,172^{A}	956,380 ± 580,305^{A}
20 mM+Spd	0,884 ± 0,080^{B}	0,852 ± 0,046^{B}	0,172 ± 0,020^{A}	0,204 ± 0,011^{AB}	103,041 ± 36,542^{C}	257,485 ± 149,573^{B}
50 mM+Spd	0,888 ± 0,113^{B}	0,764 ± 0,015^{CD}	0,146 ± 0,006^{A}	0,174 ± 0,011^{B}	109,711 ± 40,141^{C}	274,736 ± 155,240^{B}
100 mM+Spd	0,848 ± 0,105^{BC}	0,604 ± 0,027^{E}	0,088 ± 0,011^{B}	0,110 ± 0,007^{CD}	140,449 ± 55,576^{BC}	350,313 ± 196,488^{B}
150 mM+Spd	0,764 ± 0,159^{BC}	0,436 ± 0,015^{F}	0,051 ± 0,012^{C}	0,099 ± 0,007^{DE}	190,939 ± 82,474^{C}	268,223 ± 152,687^{B}
200 mM+Spd	0,640 ± 0,177^{CD}	0,408 ± 0,015^{F}	0,026 ± 0,011^{D}	0,095 ± 0,004^{CDE}	214,376 ± 109,226^{C}	254,853 ± 143,377^{B}

The values are mean (n = 5) ± SD. Means with the different letters within same column were significantly different from each other at P = 0.05; FWL: fresh weight of leaves, FWR: fresh weight of roots, DWL: dried weight of leaves; DWR: dried weight of roots; WCL: water content of leaves; WCR: water content of roots

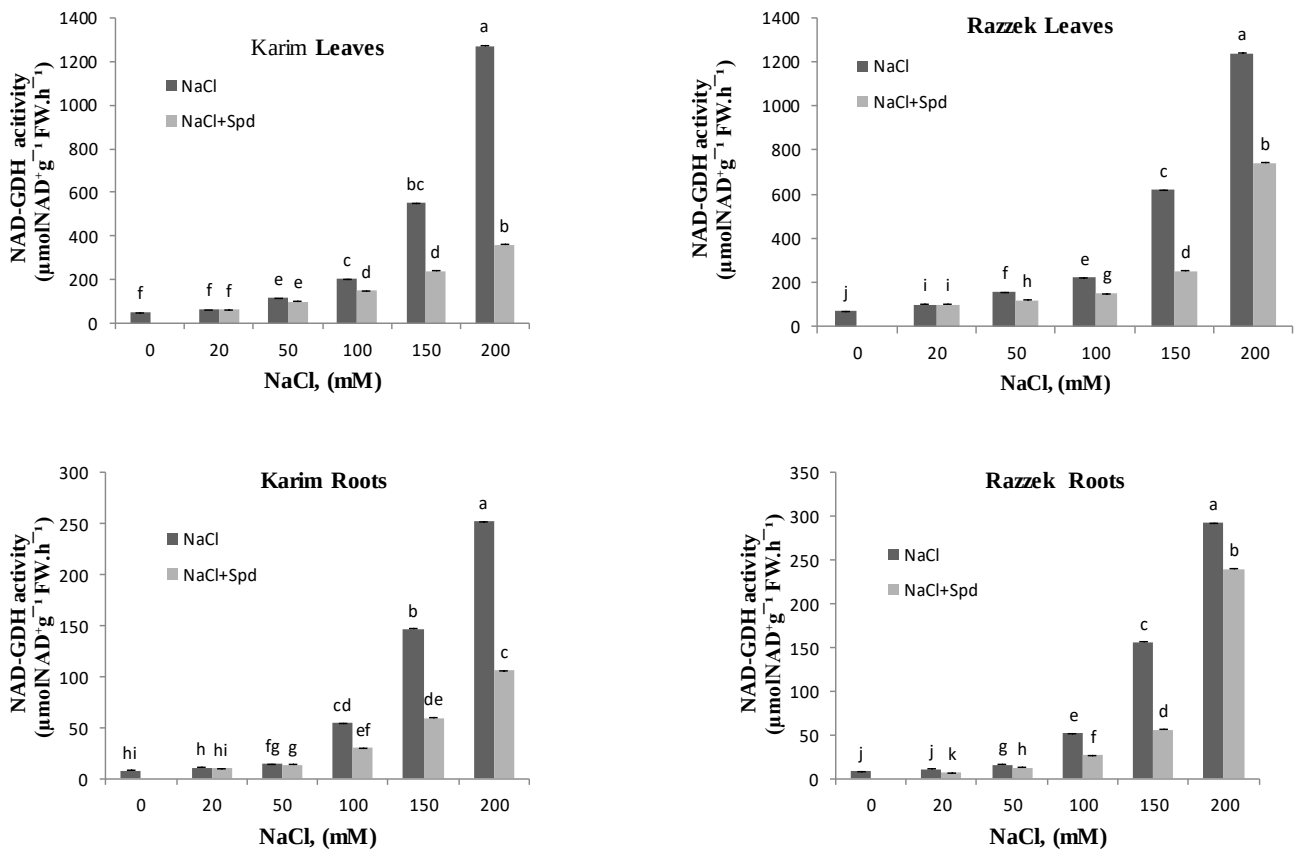

Fig. 1. Activity of (NAD^+-GDH) in leaves and roots of Karim and Razzek varieties cultivated under salt stress and exogenous spermidine application. Means followed by different letters are significantly different ($p \leq 0.05$) as determined by analysis of variance (ANOVA).

Table 2. Ions content of durum wheat (*Triticum durum* Desf.) varieties (Karim and Razzek) under salt stress and of spermidine application.

(µg/gDW)	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-
NaCl	**Karim variety**			**Razzek variety**		
	Shoot					
0 mM	172.1 ± 17.3^{F}	1228.3± 29.2^{A}	260.8±25.6^{E}	203.0±5.5^{G}	1723.0±54.3^{A}	310.7±10.5^{G}
20 mM	296.6 ± 13.4^{E}	1178.3±8.5^{A}	292.3±5.4^{E}	298.7±7.5^{F}	1248.7±61.9^{B}	405.3±5.7^{F}
50 mM	411.0 ± 9.5^{D}	985.3±14.4^{B}	354.3±6.7^{DE}	554.3±10.6^{E}	1002.9±5.4^{D}	533.0±10.6^{E}
100 mM	882.7 ± 24.8^{C}	767.7±25.2^{D}	544.0±34.5^{C}	1349.0±37.6^{C}	818.7±12.8^{E}	848.5±38.7^{C}
150 mM	1486.3 244.8^{B}	577.4±27.2^{D}	708.0±15.2^{B}	1974.3±6.5^{B}	518.0±16.1^{G}	1013.0±17.5^{B}
200 mM	2267.7 ± 51.4^{A}	419.0±12.4^{F}	884.7±30.5^{A}	4003.7±5.3^{A}	296.9±14.7^{H}	1606.6±10.9^{A}
20 mM+Spd	197.4 ± 1.4^{F}	1203.3±5.1^{A}	203.9±12.2^{G}	212.7±9.7^{G}	1243.7±37.7^{B}	292.3±5.8^{G}
50 mM+Spd	197.3 ± 12.4^{F}	996.4±7.1^{B}	248.0±31.0^{F}	325.7±13.3^{F}	1110.7±10.2^{C}	410.3±10.1^{F}
100 mM+Spd	400.3 ± 8.9^{D}	876.0±29.^{1C}	402.0±5.3^{D}	653.3±7.0^{DE}	935.0±11.0^{D}	694.3±5.9^{D}
150 mM+Spd	343.6 ± 41.5^{DE}	811.3±11.4^{CD}	575.7±22.4^{C}	707.0±9.1^{D}	769.3±37.3^{E}	907.0±10.7^{C}
200 mM+Spd	420.8 ± 17.1^{D}	738.7±23.7^{D}	689.3±15.5^{B}	765.0±16.4^{D}	707.0±64.3^{FG}	995.6±4.5^{B}
	Root					
0 mM	253.8 ±18.4^{G}	741.0±34.4^{A}	306.7±6.6^{G}	269.7±11.4^{F}	818.7±34.8^{A}	453.7±34.7^{F}
20 mM	372.7±26.0^{F}	637.2±29.0^{B}	399.6±1.1^{F}	421.5±13.2^{E}	711.3±10.5^{B}	533.3±25.9E
50 mM	605.3±6.3^{D}	581.7±11.3^{C}	523.0±22.0^{E}	756.6±12.7^{D}	608.0±11.5^{C}	770.0±30.7^{D}
100 mM	2000.0±9.2^{C}	419.020.4±^{E}	800.7±16.3D	2937.3±47.7^{C}	394.5±6.6^{D}	995.7±6.0^{C}
150 mM	2993.0±79.0^{B}	377.3±9.6^{E}	996.3±7.3^{B}	4217.7±14.6^{B}	299.3±1.0^{E}	1482.3±47.7^{B}
200 mM	3410.0±89.0^{A}	300.4±12.1^{F}	1292.0±19.6^{A}	5043.4±52.2^{A}	204.0±6.4^{F}	1925.7±20.9^{A}
20 mM+Spd	343.9±25.7^{FG}	720.3±18.4^{A}	286.3±14.2^{G}	287.5±8.4^{F}	788.7±21.5^{A}	413.3±10.9^{F}
50 mM+Spd	480.3±26.8^{E}	695.3±6.3^{A}	339.0±30.3^{G}	431.3±38.1^{E}	714.7±6.5^{B}	533.7±40.6^{E}
100 mM+Spd	641.9±25.9^{D}	603.3±14.3^{C}	556.0±35.9^{E}	811.6±8.3^{D}	694.0±9.2^{B}	801.0±10.1^{D}
150 mM+Spd	606.5±9.1^{D}	605.7±11.6^{C}	907.0±14.3^{E}	878.0±25.5^{D}	607.3±10.6^{C}	1033.0±49.3^{C}
200 mM+Spd	710.7±8.8^{D}	506.6±19.1^{D}	926.3±20.6C	900.0±8.8^{D}	561.7±32.0^{C}	1607.7±14.0^{B}

The values are mean (n = 5) ± SD. Means with the different letters within same column were significantly different from each other at P = 0.05

Ions accumulation: In Table 2, we summarized the variation in cations and chloride concentrations in leaves and root tissues of plants subjected to different treatments. In control treatment (no salt), Razzek variety accumulated more amounts of Na^+ compared to Karim, in both leaves and roots (Table 2). Under 200 mM NaCl, Karim variety accumulated 2267 µeqg^{-1}DW and 3410 µeqg^{-1}DW of Na^+ in the shoots and in the roots, respectively. However, for the same treatment, Na^+ accumulations in Razzek plants were 4003 µeqg^{-1}DW and 5043 µeqg^{-1} DW respectively, in shoots and roots. The amendment of the NaCl-containing medium (200 mM) with 1 mM spermidine reduced significantly the accumulation of Na in both shoot and root tissues. In Karim, 1 mM spermidine was able to decrease the accumulation of sodium by 5-fold in both tissues under 200 mM NaCl, while in Razzek, it was 5- and 5.6-fold for shoot and root respectively under the same conditions. These results strongly suggest a more limited absorption of Na^+ in both varieties in the presence of spermidine. Similarly, salinity resulted an increase in Cl^- accumulation in shoots and roots of both varieties (Table 2). Under salt treatment, the addition of spermidine decreased the Cl^- accumulation in both tissues. Under control conditions, both tissues of Razzek had slightly more K^+ than Karim. The presence of NaCl decreased K^+ levels in leaves and roots of both varieties, especially under 200 mM NaCl. The addition of spermidine partially restored the accumulation of K^+ in leaves and roots of NaCl-treated plants.

Glutamate Dehydrogenase assay: The GDH amination (NADH-GDH) (Fig. 1) and deamination (NAD^+-GDH) (Fig. 2) activities are more predominant in leaves than roots. In the absence of salt stress, GDH has a dominant catabolic function in leaves reflecting that the NAD^+-GDH activity is more important than NADH-GDH. Contrarily, in roots, the amino activity (NADH-GDH) is predominant. The addition of NaCl in the medium stimulates NADH-GDH activity while inhibits the NAD^+-GDH in leaves. There is trend in the increase in NADH-GDH activity and the reduction in NAD^+-GDH activity according to the salt treatment. Therefore, the NADH-GDH activity is positively correlated and the NAD^+-GDH activity is negatively correlated with NaCl concentration. Similarly, in the roots, the amino function of GDH is stimulated by NaCl treatment, which is opposite to that of the NAD^+-GDH activity which is reduced at higher NaCl levels. Spd addition to the culture medium Spd addition minimizes the NADH-GDH and NAD^+-GDH fluctuations. It is noted that after treatment with NaCl and Spd, there was a slight increase in NADH-GDH at 50 mM treatment, in both leaves and roots, however under 150 mM NaCl treatment, the NAD^+-GDH activity was decreased.

Chlorophyll content: Total chlorophyll content in leaves were decreased in salt treated samples and was negatively correlated with NaCl concentration. However, the use of Spd resulted in an increase in Chlorophyll content in all NaCl concentration (Fig. 3). At 200 mM of NaCl, the content of chl *a* (Fig. 4) and chl b (Fig. 5) severely decreased compared to the control plants in both varieties. Similar to total chlorophyll, the presence of Spd improved the content of chl *a* and Chl b of stressed plant, and it was able to recover up to 80% of the reduction caused by NaCl.

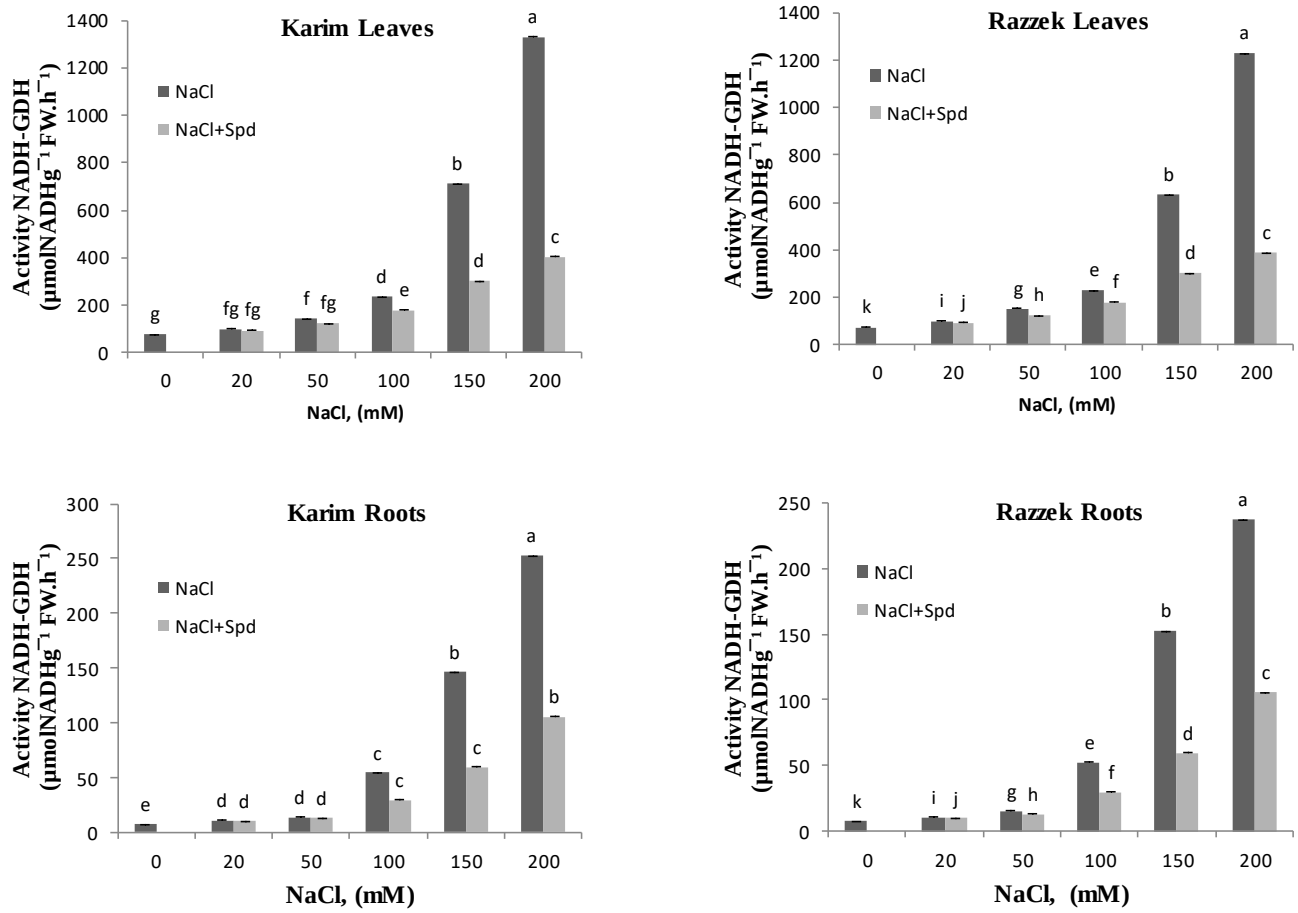

Fig. 2. Activity of (NADH-GDH) in leaves and roots of Karim and Razzek varieties cultivated under salt stress and exogenous spermidine application. Means followed by different letters are significantly different ($p \leq 0.05$) as determined by analysis of variance (ANOVA).

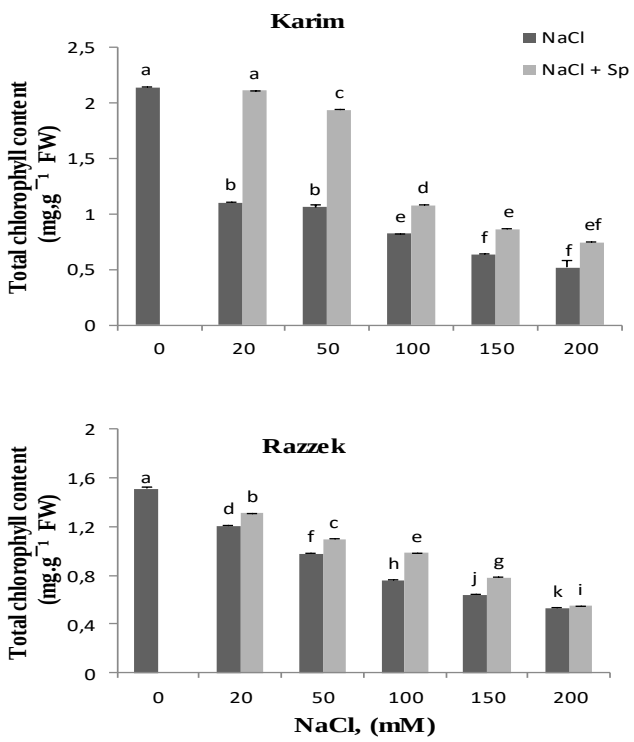

Fig. 3. Total chlorophyll content in leaves of Karim and Razzek varieties cultivated under salt stress and exogenous spermidine application. Means followed by different letters are significantly different ($p \leq 0.05$) as determined by analysis of variance (ANOVA).

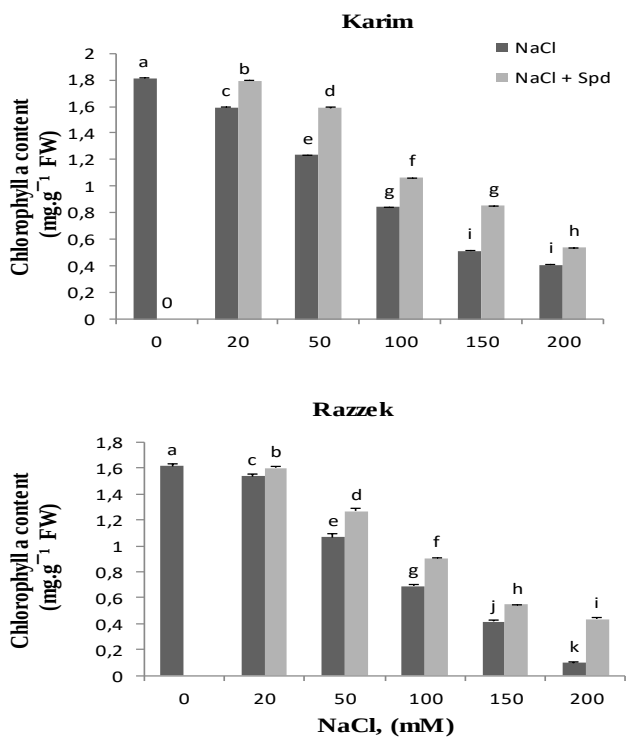

Fig. 4. Chlorophyll a content in leaves of Karim and Razzek varieties cultivated under salt stress and exogenous spermidine application. Means followed by different letters are significantly different ($p \leq 0.05$) as determined by analysis of variance (ANOVA).

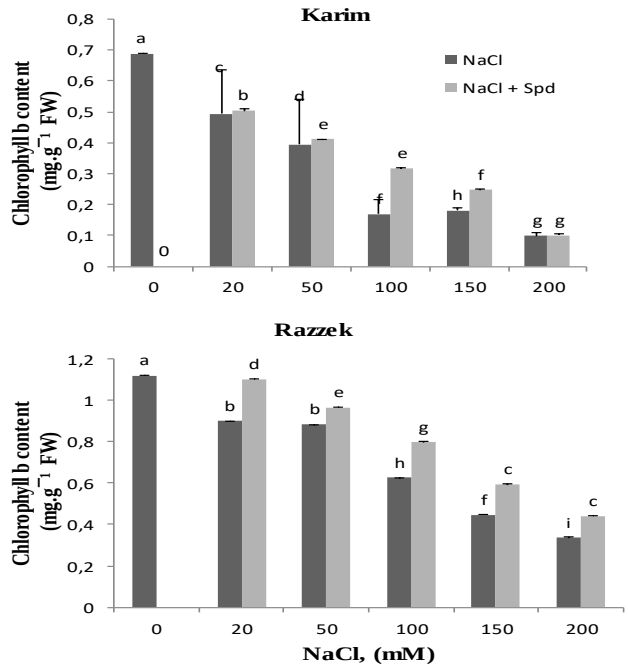

Fig. 5. chlorophyll b content in leaves of Karim and Razzek varieties cultivated under salt stress and exogenous spermidine application. Means followed by different letters are significantly different ($p \leq 0.05$) as determined by analysis of variance (ANOVA).

Proline content: In response to saline stress, both varieties accumulate proline at the leaf and root level. In leaf tissues, there was a significant increase in proline synthesis under 100 mM and 200 mM in both varieties (Fig. 6). In root samples, the increase in proline is less than the foliar level. However, the proline content increased under 100 mM NaCl (8.27 and 7.96 µmol.g^{-1} FW for Karim and Razzek, respectively) and reach the highest values under 200 mM NaCl (32.37 and 32.02 µmol.g^{-1} FW for Karim and Razzek, respectively). Foliar and root accumulation of proline seems to be a common response to saline stress, but we did not observe this behavior for plants grown in medium containing Spd. Proline content showed a slight increase under salt stress in the presence of Spd (Fig. 6).

Soluble sugars (SS) content: In control plants, the soluble sugars concentration was greater in the roots for both varieties. Salt induced an increase in SS concentration in the shoots and roots of both varieties (Fig. 7). Exogenous application of Spd increased soluble sugar content in both root and leaf tissue at low NaCl concentrations (20 and 50 mM) however it reduced the SS in plants subjected to higher NaCl concentrations (>100 mM).

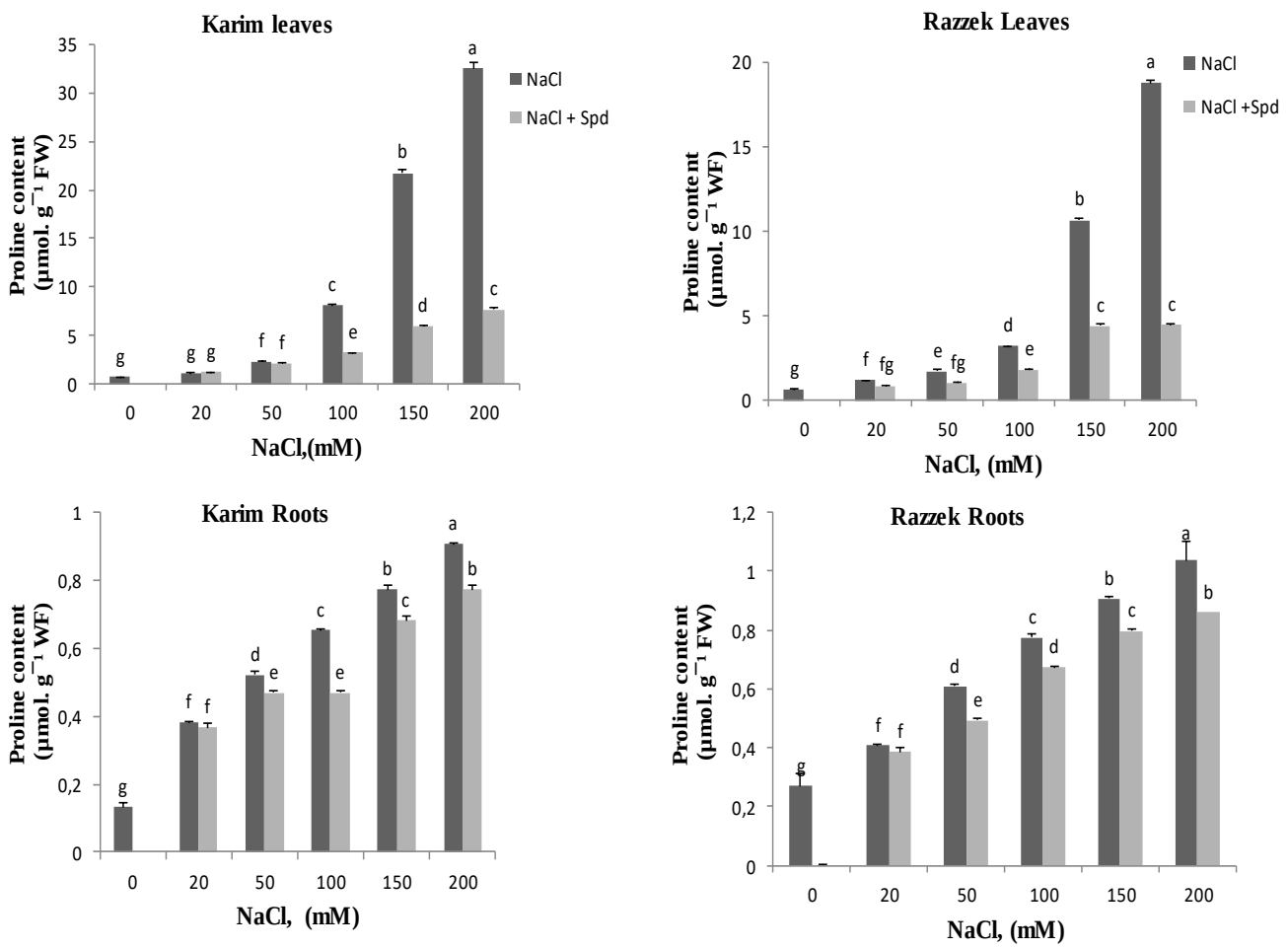

Fig. 6. Proline content in leaves and roots of Karim and Razzek varieties cultivated under salt stress and exogenous spermidine application. Means followed by different letters are significantly different ($p \leq 0.05$) as determined by analysis of variance (ANOVA).

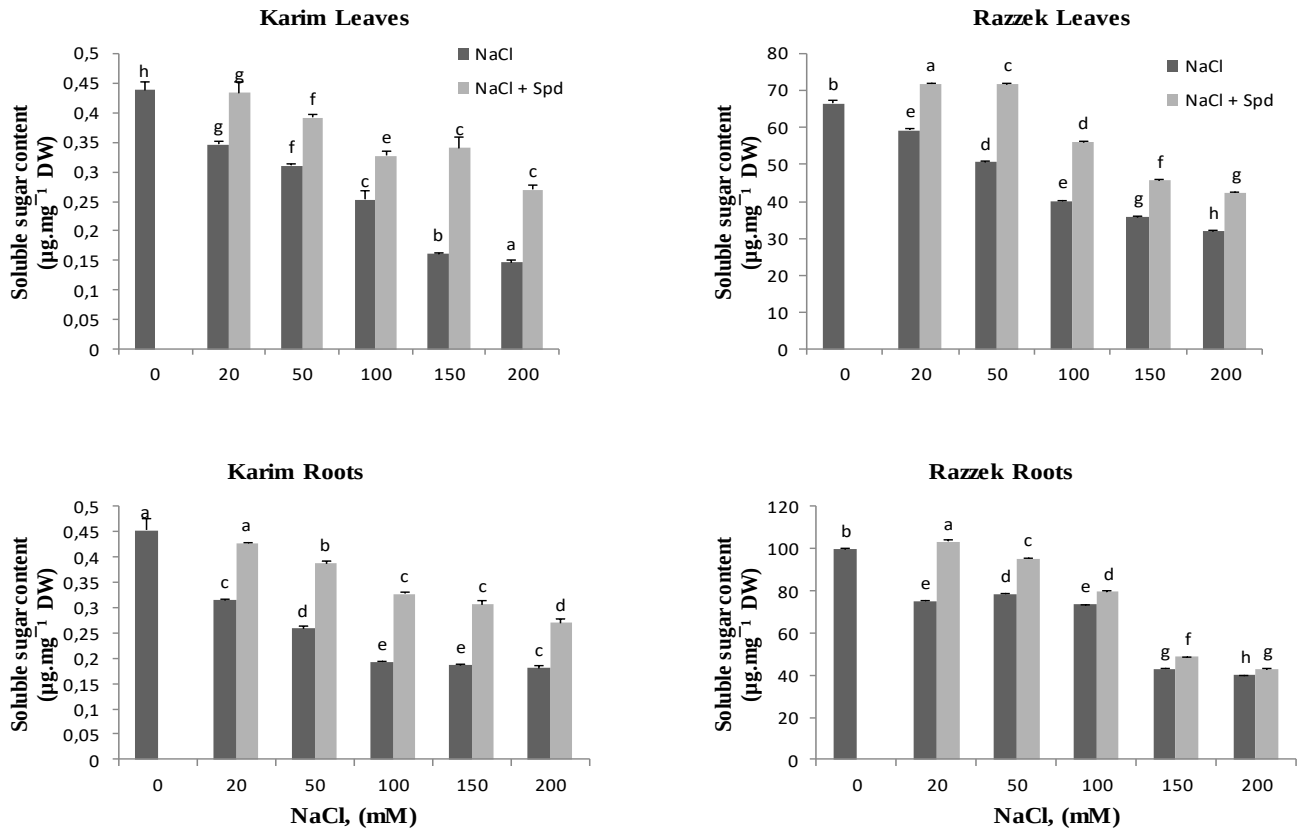

Fig. 7. Soluble sugar content in leaves and roots of Karim and Razzek varieties cultivated under salt stress and exogenous spermidine application Means followed by different letters are significantly different ($p \leq 0.05$) as determined by analysis of variance (ANOVA).

Discussion

Salinity is considered as one of the most serious environmental constraints in plant growth and productivity. The selection of NaCl-resistant plants could be considered as important alternative way to increase food security in many parts of the world suffering from soil salinization (Dwivedi *et al*., 2016). Durum wheat is one of the most important cereal crops in the world and there is a need to improve its productivity under salt stress conditions (Shahrayini *et al*., 2018). To control salinity stress, plants develop several adaptive strategies varying according to the species and environmental conditions. A plant's tolerance to saline stress can be defined, from a physiological stand point, by its ability to survive and grow at higher salinity levels than sensitive plants. A plant's tolerance to salt stress appears to be the result of many morphological, physiological and biochemical changes that interact to maintain the plant's growth and development. Plant tolerance could be improved by an external factor such as foliar application of polyamines that play a key role in regulating fundamental processes.

Spd is one of the polyamines that has been used to reduce the negative effects of stress. Spd regulates plant growth and developmental process such as germination, cell proliferation, enzyme activities, nutrient acquisition and membrane stability (Khare *et al*., 2018). In this study, we demonstrated that Spd application has resulted in an improved plant performance against salt stress in two durum wheat varieties. The addition of Spd (1 mM) alleviated NaCl-induced phytotoxicity in *T. durum* growth. We showed the positive impact of Spd on plant development and the biomass production in durum wheat.

The reduction of plant growth by salt stress could be due to a restriction in the water up-take, inhibition of leaf expansion and/or a decrease of chlorophylls content. In this study, salinity treatments significantly reduced chlorophylls content; however, exogenous application of Spd increased the chlorophyll content in leaves. However, the effect of Spd on Chl was more pronounced in Razzek than in Karim. We demonstrated here the intraspecific action of Spd in the alleviation of NaCl-induced chlorophyll inhibition. The positive correlation between reduction of Chl content under salt stress and the sensitivity to salt was demonstrated (Sayyad-Amin *et al*., 2016). Previous studies showed that, chlorophyll was more degraded under NaCl constraint in sensitive wheat cultivars than in NaCl-tolerant cultivars (Maghsoudi *et al*., 2015), which is consistent with our findings.

To develop resistance against high level of NaCl, plants must maintain adequate hydration of tissues which is related to the capability of root system to absorb water and the osmotic adjustment at the cellular level. Plants can accumulate compatible solutes inside their cells to compensate the osmotic imbalance caused by accumulation of Na^+ in their vacuole under salt stress. In this study, our results showed that the higher levels of proline accumulation in Razzek, suggested that this cultivar is able to cope better with salinity. It has been reported that free proline content increases when plants grow under various stress conditions, including salinity, and it is suggested that proline accumulation can act as an

adaptive mechanism in higher plants (Kumar *et al*., 2017). The physiological effects of proline accumulation can be observed in photosynthetic maintenance, self-regulation, macromolecular protection against damage and reduction of cellular acidity under saline stress conditions (Ben Rejeb *et al*., 2012). In our experiments, proline content is significantly higher in durum wheat leaves and roots subjected to NaCl stress. These results are consistent with previous studies on durum wheat (Yousfi *et al*., 2012) and wheat (*Triticum aestivum*) seedlings growing under high salinity (Ouhaddach *et al*., 2018). In addition, our results showed that Razzek variety has a considerable ability to accumulate proline in both organs, which could explain its relatively higher tolerance to salinity as compared to Karim. This behavior (proline accumulation) has also been described in other salt-tolerant plants that accumulate more proline than salt-sensitive plants (Ashraf & Foolad, 2007). Therefore, proline accumulation was qualified as indicator of salt tolerance in most plant species (Ashraf *et al.,* 2018).

The accumulation of soluble sugars is necessary to stabilize sub-cellular structures (membrane and proteins), neutralize the osmotic imbalance imposed by saline stress and thus improve carbon metabolism (Hayat *et al*., 2012). It has been shown that the accumulation of soluble sugars is a common adaptive response to drought and salinity (Ying *et al*., 2010). These metabolites provide energy for plants to resume growth and relieve the inhibitory effects of Na^+ and Cl^- ions on enzyme activities (Wang & Song, 2012). Our results suggest that the treatment with Spd induced defense responses against saline condition as confirmed on rice under stressed conditions (Chattopadhaya *et al*., 2002). Under salt stress, Na^+ and Cl^- can be accumulated in the vacuole of tolerant plants or in the cytoplasm of sensitive cultivars. Therefore, salinity causes ionic disparity and water stress causing membrane destruction. However, it has been demonstrated that polyamines are able to inhibit the uptake of Na^+, prevent the loss of K^+, and reduce the leakage of amino acids and can associate with anionic membrane component, thus reducing cell membrane damage under stress conditions (Chattopadhaya *et al.*, 2002; Shi & Chan, 2014). Spermidine acts on the stressed plant by closing the Na^+ and Cl^- dependent channels that block the entry and accumulation of these ions.

Our results also showed that under the same growth conditions, the two studied varieties (Karim and Razzek) respond differently to salinity stress. The second interaction (variety-[NaCl], variety-spermidine and [NaCl]-spermidine) and the third interaction (variety-[NaCl]-spermedine) showed significant effects on all studied parameters. In this sense, intraspecific variability offers a valuable tool to determine tolerant genotype and for studying mechanisms of salt tolerance (Gregorio *et al*., 2002).

Conclusion

Our results showed that salt stress applied on two durum wheat varieties significantly affects growth parameters, water content, chlorophylls content and NADH-GDH and NAD^+- GDH activities of leaves and roots. Exogenous application of Spd at 1 mM significantly reduced the damage induced by NaCl in both durum wheat varieties. It can be concluded that despite the presence of salt at 200 mM, wheat plants are able to survive and tolerate saline stress when treated with Spd. Thus, the exogenous application of spermidine could be considered as an effective approach in alleviating the damage of durum wheat varieties under salinity stress. The significant effect of variety, [NaCl] and spermidine and their interaction offers a valuable tool to determine tolerant genotype for studying the mechanisms of exogenous application of spermidine under salt stress to determine the mechanisms underlying the alleviation effects.

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