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Role of Salicylic Acid in Alleviation of Aluminum Effects on Growth and Biochemical Processes in Lupin (*Lupinus termis* L.) Plant

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HEAVY metal toxicity is one of the major abiotic stresses leading to inhibition of growth and yield of plants. Aluminum (Al^{3+}) treatment resulted in a significant decrease in the fresh and dry biomasses of shoots and roots as well as leaf relative water content (RWC) of lupin plants. This reduction was associated with a significant decrease in IAA and GA_3 contents and increase in ABA level. Furthermore, increasing Al^{3+} levels induced generation and accumulation of H_2O_2 and MDA in shoots and roots of lupin plants. The enhancement of oxidative stress was associated with biosynthesis of non-enzymatic antioxidants including proline, phenolics and trigonilline components together with an increase of G6PDH activity and $NADP^+$/NADPH ratio. In addition, Al^{3+} stress triggered an increase in antioxidant enzymes SOD and CAT, whereas GPx, PPO, GR, and APx activities were markedly suppressed. Priming treatment of lupin seeds with salicylic acid could considerably reverse the Al^{3+}-induced inhibitory effects *via* induction of defense mechanisms and increase growth regulator contents.

Keywords: Aluminum, Antioxidant enzymes, Hormones, Malondialdehyde, Phenolic compounds, Salicylic acid, Trigonilline.

Introduction

Aluminum (Al^{3+}) is the most abundant metal in acid soil, in which it becomes soluble at acidic solution (pH<5.0) and available to plants in the forms of $Al(OH)^{3+}$ (Abdul Ghani, 2010) causing various cytotoxic inhibition and damages to cell structures and finally suppression of growth and crop yield (Tahara et al., 2008). The earliest symptoms of Al^{3+} toxicity is the reduction of root growth (Xiao et al., 2004). In addition, Al^{3+} inhibition includes disturbance of plasma membrane properties (Ruan et al., 2011), decreases water uptake and causes nutrient imbalance (Kochian et al., 2007), damages ultra-structure of chloroplasts, decreases stomatal conductance and CO_2 assimilation (Yang, 2002) and induces the generation of reactive oxygen species (Smirnov et al., 2014). Furthermore, Gururani et al. (2015) and Bücker-Neto et al. (2017) concluded that phytohormones levels were markedly decreased in response to Al^{3+} toxicity leading to plant growth inhibition.

There are several reports that discuss the defense mechanisms of Al^{3+} tolerance in plants. Organic acids, such as citrate, malate and oxalate that secreted by plants, can form chelate components with Al^{3+} in order to protect these plants (Ma et al., 1998). Kawano (2003) and Maron et al. (2008) mentioned that due to the great affinity of Al^{3+} to oxygen ligand components, e.g. ATP and PO_4^{3+}, plants possess another mechanism for detoxification of Al^{3+} in cytosol. Moreover, the induction of non-enzymatic and enzymatic antioxidant systems have been reported for shift off the inhibitory effects of Al^{3+} (Chen et al., 2000; Xiao et al., 2003; Upali etal., 2013). Among non-enzymatic antioxidants, a marked increase of phenolic compounds under Al^{3+} stress was shown in several plants (Malusà et al., 2006; Smirnov et al., 2015). The role of phenolics was related to their ability to donate H^+ for reducing generated ROS and/or bind the metals with their functional groups (OH, COOH) to shift off their harmful effects on a plant (Sasi et al., 2019; Selem &

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Naguib, 2018). Trigonilline (alkaline compound) might play an important role as antioxidant and osmoregulatory agent under various stresses (Minorsky, 2002). Naik et al. (2009) reported that, there was a marked increase of CAT, APx and GPx activities in leaves of two *Populous* species under Al^{3+} treatment. Birben et al. (2012)stated that APx and SOD activities were markedly increased in roots of Al-tolerant and Al-sensitive triticale cultivars, while Singh et al. (2011) found that, under Al^{3+} treatment, there was a marked increase of APx and SOD and GR activities in greengram seedlings, whereas a decline in CAT activity and AsA and GSH contents. Sharma & Dubey (2007) reported that increasing Al^{3+} levels in rooting media of rice plants resulted in an increase the activity of cytosolic SOD, APx and GPx in shoots and that was associated with a decrease of CAT and chloroplast APx as well as AsA, but had no effect on total glutathione content.

Salicylic acid (SA), an active molecule and considered as phytohormone with phenolic nature (Surapu et al., 2014; Khan et al., 2015), plays an important role in induction of acclamatory effects against various environmental stress. Krantev et al. (2008) and Pandey et al. (2013) reported that exogenous application of SA may improve the growth of plants under various stresses via increase stomatal openings and rate of photosynthesis, water and mineral uptake, protection of plasma membranes integrity and induce antioxidant system. Metwally et al. (2003) reported that SA pretreatment markedly increased antioxidant enzymes activity such as CAT, AP_X in barley plants exposed to Cd stress. Similar findings were reported in several plants exposed to various heavy metal stresses, (Pandey et al., 2013; Khan et al., 2015; Liu et al. 2017). The aim of present study is to evaluate the alleviating efficiency of salicylic acid (10µM) against the toxic effects of aluminum stress (0 and 100ppm Al^{3+}) on physiological and biochemical changes in the lupine plant.

Materials and Methods

Lupin seeds (*Lupinus termis* L.) were kindly supplied by the Agricultural Research Center (GARC), Giza, Egypt. The seeds were selected uniformity of size, shape and color. Prior to germination, seeds were surface sterilized by soaking for two minutes in 4% (v/v) sodium hypochlorite, then washed several times with distilled water. The sterilized seeds were soaked in 10µM salicylic acid for 8hrs or distilled water, then transferred to plastic pots (20cm in diameter, 20cm length with a hole at the bottom) filled with fixed amount of previously acid-washed quartz sand. Ten seeds were germinated in each pot and the pots were placed under natural environmental conditions (photoperiod, 16hrs/ 8hrs ligh/ dark, temperature, 27$\pm$ 2^{o}C light/ 23$\pm$2^{0}C dark; light intensity, PPFD 23µ mol m^{-2} $s.^{-1}$) with 80% water holding capacity. The pots were irrigated with 1/10 strength modified Hoagland solution alone as control (Epstein, 1972) or supplemented with 100ppm Al^{3+} as ($AlCl_3$). The pots were irrigated every two days with growth media interval with distilled water, to prevent $AlCl_3$ accumulation, keeping the water holding capacity at 80% along the experimental period. Each treatment was represented by three replica. At 21-days, homologous plants were harvested, washed thoroughly from adhering soil particles, gently plotted, dissected to shoots and roots and quickly saved at -80^{o}C in a cooling room for estimation of various growth parameters and chemical analyses. Determinationof fresh and dry biomass, shoot height and root length.

The shoots and roots of homologous plants (three replicates) were taken and weighed for fresh biomass. The oven dry biomass was determined after drying the samples in an oven at 50^{o}C till constant weight. Shoot height and root length were determined to nearest centimeter.

Determination of relative water content (RWC)

Relative water content (RWC) was determined after Silveira et al. (2003) based on the following equation:

$$RWC = [\ (f.m. - d.m.)/ (t.m. - d.m.)\ ] \ . \ 100$$

Where f.m. is the fresh biomass, d.m. the dry biomass after drying at 50^{o}C for 48hrs and t.m. is the turgid mass (after soaking in water for 4hrs at room temperature).

Determination of proline content

Determination of free proline was carried out using the acid ninhydrin method described by Bates el al. (1973).

Determination of hydrogen peroxide (H_2O_2) and malondialdhyde contents (MDA)

H_2O_2 content in shoots and roots of lupine plants was determined according to Velikova et al. (2000). The level of lipid peroxidation was measured

according to the thiobarbituric acid (TBA) test and the MDA concentration was calculated using its extinction coefficient $155mM^{-1}\ cm^{-1}$ (Heath & Packer, 1968).

Determination of total phenolic contents

Extraction and estimation of the phenolic compounds were carried out according to Malusà el al. (2006). Phenolic concentration was calculated according to a calibration curve using gallic acid as a standard.

Determination of trigonilline content

Trigonilline was extracted and estimated according to the method of Zheng & Ashihara (2004). An aliquot of extract was filtered through a 30µm syringe filter and 50µ1 of the filtrate were used for determination of trigonilline by High-performance liquid chromatographic using a HPLC system (Perkin Elmer series 200 LC and UV/VIS detector 200 LC, USA).

Determination of NADP and NADPH content

High-performance liquid chromatographic (HPLC) method was used for determination of $NADP^+$ and NADPH according to Caruso et al. (2004). The plant samples (0.3gm) were subjected to either acid extraction using 0.6M perchloric acid (for measurement of $NADP^+$ and NAD or alkaline extraction using 0.5M potassium hydroxide (for measurement of NADPH and NADH). The extracts were centrifuged at 14.000g at 4°C for 10min followed by neutralization with either 0.5M KOH or 1M KH_2PO_4, respectively, and re-centrifugation at 14.000 gat 4°C for 10min to remove the precipitate. The supernatants were used for the assay after filtration through 0.22µm-syringe filters.

Determination of plant growth regulators

The method of extraction of plant growth regulators was adopted by Shindy & Smith (1975) and described by Hashem (2006). To estimate the amounts of hormones IAA, ABA and GA_3, the plant hormone fractions and standard ones were methylated according to Vogel (1975) to be ready for GC analysis. Flame ionization detector was used for identification and determination of hormones using Gas Chromatography (Helwett Packered 5890, USA).

Assays of antioxidant enzymes

Extraction

Assays of some antioxidant enzymes, an aliquot of fresh shoot and root (0.2g) was homogenized in a mortar and pestle with 4ml of ice-cold extraction buffer (100mM potassium phosphate buffer, pH 7, 0.1mM EDTA). The homogenate was filtered through muslin cloth and centrifuged at 16.000g for 15min. The supernatant fraction was used as crude extract for enzyme activity. All operations were carried out at 4°C (Azevedo-Neto el al., 2006).

Superoxide dismutase (SOD) activity

Superoxide dismutase activity was determined by measuring its ability to inhibit the photochemical reduction of nitrobluetetrazolium chloride (NBT) as described by Giannopolitis & Ries (1977). One unit of SOD activity (U) was defined as the amount of enzyme required to cause 50% inhibition of the NBT photo-reduction rate and the result expressed as $U\ g^{-1}$f.m.

Catalase (CAT) activity

Catalase activity was measured according to the method of Beers & Sizer (1952), with minor modifications as described by Azevedo-Neto et al. (2006).

Ascorbate peroxidase (APx) activity

Ascorbate peroxidase activity was assayed according to Nakano & Asada (1981), taking into consideration that 2mole ascorbate are required for reduction of 1mole H_2O_2 (Mckersie & Leshem, 1994).

Guaiacol peroxidase (GPx) activity

Guaiacol peroxidase activity was determined as described by Urbanek et al. (1991). Taking into consideration that 4mole H_2O_2 are reduced to produce 1 mole tetraguaiacol (Plewa et al., 1991).

Polyphenol oxidase (PPO) activity

Polyphenol oxidase activity was assayed as the method described by Kumar & Khan (1982).

Glutathione reductase (GR) activity

Glutathione reductase activity was assayed according to Foyer & Halliwell (1976),with minor modifications as described by Azevedo-Neto et al. (2006).

Glucose-6-phosphate dehydrogenase (G6PDH) activity

Glucose-6-phosphate dehydrogenase activity was extracted according to the method described by Conkle et al. (1979). Fresh plant material

(0.5g) was homogenized extract in 0.05M Tris-HCl buffer pH 7.0. The homogenate was filtered through four layers of cheesecloth and centrifuged at 15.000g for 20min at 4°C. Activity of G-6-PDH was assayed according to the method of Ashihara & Komamine (1976).

Statistical analysis

Statistical analysis of the results was done using SPSS software package version 20.0 to obtain the mean and for comparison between the different groups involved in this study, ANOVA was used for comparison between independent samples, LSD was estimated $P \leq 0.05$.

Results

Changes in fresh and dry biomasses

Supplementation the nutrient media of lupine plants with 100ppm Al^{3+} levels resulted in a significant decrease of fresh and dry matter of shoots and roots compared to controls. The decrease in fresh mass of shoots and roots of 100ppm Al^{3+}-treated plants was 79% and 94% respectively, compared to untreated controls. With SA-pretreatment the respective suppression was reached to 67% and 85% (Table 1) compared to control (SA-pretreated in absence of Al^{3+}). The decline in dry mass of shoots and roots was 55% and 61%, respectively, under 100ppm Al level. Whereas, the corresponding values were 41% and 51%, respectively, in plants generated from SA pretreated seeds (Table 1).

There was a marked decrease of RWC of leaves of Al^{3+}-imposed lupin plants in comparison to controls. The RWC of leaves of control plants raised from water-soaked seeds was 89%, this value decreased to 41% in 100ppm Al^{3+}-stressed ones. The plants from SA-pretreated seeds showed a higher RWC compared to those untreated in presence of Al^{3+}. The RWC in leaves of SA-pretreated control was 91%, whereas under 100ppm Al^{3+} was 64%.

The shoot height and root length of lupine plants raised from water-imbibed and SA-pretreated seeds were significantly decreased in response to Al^{3+} treatment compared to controls, but the suppression in the former was markedly higher than those of the latter (Table 1).

Changes in H_2O_2 and MDA contents

Contamination of the nutrient medium with 100ppm Al^{3+} concentration resulted in a significant increase of H_2O_2 and MDA contents in shoot and root of SA-untreated or pretreated lupine plants, compared to Al^{3+}-untreated controls. At 100ppm Al^{3+} level, the increment in H_2O_2 and MDA in shoots of control plants raised from water-soaked seeds was 7.8 and 10.4 fold, respectively. The corresponding values for roots were 6.1 and 10.8 fold, respectively (Table 2). However, on SA-pretreated, the increment of H_2O_2 and MDA in shoots of 100ppm Al^{3+}-treated was 6.1 and 7.6 times, respectively, over control. In the roots, this increment was 4.7 and 7.9 fold, respectively. These observations indicate a marked decline of oxidative damage under SA pretreatment.

TABLE 1. Effect of salicylic acid (10µM) pretreatment of lupin seeds on fresh and dry biomasses of shoots and roots, shoot height, root length and leaf relative water content (RWC) under imposing Al^{3+}- stress for 21-d. The plants raised from non-primed or SA-primed seeds were saved as controls.

Treatment		Shoots		Roots		Shoot	Root	Leaf
Al ppm	SA µ	f.m gm plant $^{-1}$	d.m. gm plant $^{-1}$	f.m. gm plant $^{-1}$	d.m. gm plant $^{-1}$	Height cm	Length cm	RWC %
0	0	4.486±0.280	0.218±0.018	2.226±0.186	0.099±0.008	13.3±0.831	7.7±0.481	89±8.091
100	0	0.961±0.060	0.109±0.008	0.141±0.009	0.039±0.003	3.8±0.317	1.9±0.127	41±2.929
0	10	4.591±0.353	0.219±0.014	2.797±0.215	0.132±0.008	12.1±0.931	8.2±0.586	91±7.000
100	10	1.523±0.138	0.129±0.010	0.411±0.029	0.064±0.004	5.6±0.509	2.3±0.164	64±5.333
P value		0.001*	0.041*	0.036*	0.042*	0.005*	0.015*	0.003*
LSD≤0.05		2.1	0.100	1.00	0.05	2.6	2.00	15.2

- Data are the mean values of three replicates (n= 3).
- * : Statistically significant at $P \leq 0.05$.

TABLE 2 . Effect of salicylic acid (10µM) pretreatment of lupin seeds on hydrogen peroxide (H_2O_2), molandialdehyde (MDA), proline total phenolic compounds (TPC) and trigonilline contents in shoots and roots under imposing Al^{3+}- stress for 21-d.

Treatment Al^{3+} ppm	SA µM	H_2O_2 µmol H_2O_2 g^{-1}f.m.		MDA µmol g^{-1}f.m.		Proline contents mg g^{-1}d.m.		Phenolic compounds (TPC) µg eggallic acid g^{-1}d.m.		Trigonilline µmol g^{-1}f.m.	
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0	0	13.77±0.98	22.10±1.84	8.51±0.61	8.7±0.73	66.84±4.18	53.14±4.09	173±11.53	204±18.55	48.45±4.40	23.9±1.99
100	0	107.99±0.81	135.61±11.30	88.09±5.51	93.63±7.80	10.95±0.68	9.28±0.84	507±36.21	791±60.85	16.11±1.07	12.96±1.00
0	10	11.87±0.85	19.35±1.21	8.53±0.71	6.5±0.41	39.37±3.03	24.85±2.26	215±17.92	274±19.57	43.74±3.36	24.01±1.72
100	10	72.26±4.82	90.54±6.96	64.48±5.37	51.75±3.23	8.26±0.64	6.02±0.43	413±37.55	592±49.33	82.18±6.85	34.82±2.18
P value		0.005*	0.001*	0.001*	0.001*	0.002*	0.002*	0.001*	0.0023*	0.005*	0.003*
LSD≤ 0.05		20.2	15.5	20.00	10.8	10.2	10.0	105.0	100.0	20.1	8.5

- The plants raised from non -primed or SA-primed seeds were saved as controls.
- Data are the mean values of three replicates (n= 3).
- *: Statistically significant at $P \leq 0.05$.

Changes in proline content

There was a significant increase of proline content in shoots and roots of Al^{3+}-stressed lupine plants comparing to unstressed controls. At 100ppm Al^{3+}, the proline content in shoots and roots were 5.5 and 6.9 fold of untreated controls, respectively. Whereas, the corresponding values were 3.6 and 2.7 times over control raised from SA-soaked seeds (Table2).

Changes in total phenolics content

The total phenolics contentin shoots and roots of 21-d old lupin plants raised from unprimed or SA-primed seeds were significantly increased in response to Al^{3+} stress comparing to controls (Table 2). The total phenolics in shoots and roots of 100ppm Al^{3+}- stressed lupin plants were 2.9 and 3.9 fold of Al^{3+}-untreated control, respectively. Salicylic acid pretreatment significantly enhanced the accumulation of total phenolics in shoots and roots of Al^{3+} unstressed plants. The increase of total phenolics in shoots and roots in SA-pretreated plants was 24% and 34%, respectively compared to untreated controls. However, there was a significant increase of total phenolics in Al^{3+}-stressed plants raised from SA-pretreated seeds; the attained values were markedly lower than those SA-untreated ones. The increment of total phenolics in shoots and roots of SA-pretreated plants imposed to 100ppm Al^{3+} was 1.9- and 2.2-fold of SA-pretreated control, respectively.

Changes in trigonilline content

There was a significant suppression of trigonilline content in shoots and roots of Al^{3+}-stressed lupin plants.(Table 2). The decrease of trigonilline of shoots and roots of 100ppm Al^{3+}-treated plants was 67% and 46%, respectively compared to Al^{3+}-untreated control. On the other hand, SA- pretreatment resulted in a significant increase of trigonilline concentration in shoots and roots of Al^{3+}-stressed plants, in comparison to those untreated. The trigonilline content in 100ppm Al^{3+}- treated shoots and roots oflupin plants raised from primed- SA seeds were 5.1 and 2.7 fold of those SA-non primed ones. Priming treatment of lupin seeds with 10µM SA insignificantly changed the trigonilline content compared to control plants.

Changes in $NADP^+$ and NADPH content

Under Al- stress in plants raised from non-primed and primed-SA seeds, $NADP^+$ content and $NADP^+/NADPH^+$ ratio in shoots and roots of

lupin plants were significantly increased compared to control plants (Table 3). The increase of NADP in shoots and roots under 100ppm Al was 58% and 36%, respectively compared to controls, the corresponding values for primed –SA shoots and roots were 41% and 12%, respectively. There was a significantly decreased in NADPH content in shoots and roots raised from primed-SA seeds in responsible to Al^{3+} treatments, these values were higher than those of unprimed plants.

Changes in hormonal contents

The contents of IAA and GA_3 in shoots and roots of SA-untreated or treatedlupin plants were significantly decreased in response to Al^{3+} levels (Table 4). The decline in IAA and GA_3 in shoots of 100ppm Al^{3+}-treated plants was 50% and 43%, respectively compared to untreated control. The corresponding values for roots were 41% and 51%, respectively. With SA pretreatment, the reduction in IAA and GA_3 was 37% and 28% in shoots and 35% and 42% in roots, respectively, compared to control plants raised from SA-soaked seeds. On the other hand, Al^{+3} stress significantly increased ABA content in lupin plants. The level of ABA in shoots and roots increased by 2.2 and 2.8 times over control under 100ppm Al^{3+}, respectively, whereas the corresponding values were 1.5 and 1.8 times over SA-pretreatment in absence of Al^{3+}, respectively.

TABLE 3. Effect of salicylic acid (10µM) pretreatment of lupin seeds on NADP and $NADPH^+$ (µg g^{-1}f.m.) content in shoots and roots under imposing Al^{3+}-stress for 21-d.

Treatment		NADPH/NADP ratio					
		Shoots			Roots		
Al ppm	SA µM	$NADP^+$	$NADPH^+$	$NADP^+$/ $NADPH^+$	$NADP^+$	$NADPH^+$	$NADP^+$/ $NADPH^+$
0	0	7.81±0.56	47.52±2.97	0.16±0.014	4.12±0.37	31.72±2.27	0.10±0.012
100	0	12.37±0.88	18.49±1.68	0.67±0.04	5.59±0.43	10.37±0.65	0.54±0.04
0	10	6.36±0.42	44.74±4.07	0.11±0.016	5.66±0.51	31.83±2.45	0.18±0.013
100	10	8.95±0.64	24.89±2.07	0.36±0.021	6.35±0.58	16.71±1.52	0.39±0.021
P value		0.0321*	0.005*	0.025*	0.042*	0.003*	0.025*
LSD≤ 0.05		2.9	7.5	0.20	1.00	8.25	0.22

- *: Statistically significant at $P \leq 0.05$.
- The plants raised from non-primed or SA-primed seeds were saved as controls.
- Data are the mean values of three replicates (n= 3).

TABLE 4. Effect of salicylic acid (10µM) pretreatment of lupin seeds on hormonal contents in shoots and roots under imposing Al^{3+}-stress for 21-d.

Treatment		Hormone contents µg^{-1} 100g f.m					
		Shoot			Root		
Al ppm	SA µM	IAA	GA_3	ABA	IAA	GA_3	ABA
0	0	62.11±5.18	1028.26±93.48	36.9±3.08	30.9±2.81	341.42±28.45	18.54±1.55
100	0	31.08±2.22	581.72±44.75	81.08±5.79	18.38±1.53	168.12±15.28	51.24±3.66
0	10	74.01±5.69	1172.77±78.18	39.36±2.81	55.42±3.46	422.85±35.24	22.63±1.89
100	10	46.57±3.10	844.26±70.36	60.84±4.68	36.13±2.41	245.44±20.45	41.51±3.46
P value		0.0013*	0.001*	0.021*	0.0041*	0.0032*	0.0025*
LSD≤0.05		8.5	105.0	5.60	7.0	81.0	6.2

- *: Statistically significant at $P \leq 0.05$.
- The plants raised from non-primed or SA-primed seeds were saved as controls.
- Data are the mean values of three replicates (n= 3).

Changes in enzymatic activities

Under 100ppm Al^{3+} level, the SOD activity in the shoots and roots was triggered by 4.2 and 5.6 times over the control raised from water-imbibed seeds, while SA- pretreatment seeds enhanced SOD activity to reach 5.3 and 6.1 times over control to the 100ppm Al^{3+}-stressed seeds (Table 5). The CAT activity in shoots and roots was 4.4 and 6.3 fold under 100ppm Al^{3+} compared to Al^{3+}-untreated control. Priming treatment with SA resulted in an induction of CAT activity was 6.3 and 7.9 fold under Al^{3+}- stress relative to SA-pretreated control. On the other hand, the decrease of APx activity in shoots and roots under 100ppm Al^{3+} was 74% and 83% in response to control raised from water-soaked seeds during 100ppm Al toxicity. SA- pretreatment could restore back the APx activity in shoots and roots under Al^{3+} stress, so that the decline was amounted to 34% and 53%, respectively compared to Al^{3+}- unstressed control raised from SA pretreated seeds.

However, GPx activity in shoots and roots was significantly decreased under Al^{3+} treatment; SA pretreatment significantly restore GPx activity (Table 5). The suppression of GPx in shoots and roots of 100ppm Al^{3+}-stressed plant was 45% and 39%, respectively compared to unstressed control, whereas the GPx in SA-treated shoots and roots under Al^{3+} treatments was 2.1 and 1.7 fold of control plants in absence of Al^{3+}. The PPO activity in shoots and roots also showed a significant decline under Al stress without SA-pretreatment, conversely the PPO activity in shoots and roots was 1.6 and 1.9 fold, respectively in the plants from SA- pretreated seeds (Table 5).

Application of Al^{3+} with nutrient media significantly suppressed GR activity of lupin plants compared to untreated control (Table 6). However the GR activity in shoots and roots raised from SA-pretreated lupine seeds was markedly suppressed in response to Al^{3+} stress, but the attained values were greatly higher than those of Al^{3+} stressed in absence of SA. The decrease of GR activity in shoots and roots under Al^{3+}-stress was 68% and 73%, respectively compared to control in absence of SA. Whereas with SA-pretreatment, the respective suppression was 38% and 51%, respectively. During Al stress, the increment in G6PDH activity in shoots and roots was 7.6 and 5.7 fold of plants raised from water-soaked seeds; this increment was 10.7 and 8.7 fold on seeds pre-soaked with SA, respectively (Table 6).

TABLE 5. Effect of salicylic acid (10µM) pretreatment of lupin seeds on superoxidedismutase (SOD), catalase (CAT), ascorbate peroxidase (APx), gauaicol peroxidase (GPx) and polyphenol oxidase (PPO) activities in shoots and roots under imposing Al^{3+}-stress for 21-d.

Treatment Al ppm	Treatment SA µM	Enzymatic antioxidants activities									
		SOD $Ug^{-1}f.m.$		CAT µmol H_2O_2, $g^{-1}f.m.$ min^{-1}		APx µmol$H_2O_2g^{-1}f.m.min^{-1}$		GPx µmolgauaicol$g^{-1}f.m.min^{-1}$		PPO $Ug^{-1}f.m.$ min^{-1}	
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0	0	4.83±0.32	6.31±0.45	9.18±0.83	7.94±0.53	37.4±2.34	26.59±1.90	15.85±1.32	16.06±1.15	1.96±0.12	1.01±0.06
100	0	20.45±1.70	35.62±2.74	40.84±2.92	49.95±3.33	9.91±0.66	4.6±0.29	8.78±0.63	9.72±0.75	0.43±0.03	0.56±0.05
0	10	5.98±0.54	8.49±0.61	9.58±0.64	8.1±0.62	35.63±3.24	28.09±2.01	25.63±1.71	20.85±1.60	2.47±0.16	2.13±0.19
100	10	31.51±2.25	52.54±4.04	59.99±5.45	63.76±4.25	23.51±1.47	13.34±1.21	52.88±4.81	36.35±2.27	3.92±0.36	4.11±0.37
P value		0.003*	0.0052*	0.0012*	0.005*	0.006*	0.005*	0.002*	0.004*	0.036*	0.025*
LSD≤ 0.05		5.00	4.80	5.01	7.01	5.2	4.5	6.00	4.20	0.50	0.70

- *: Statistically significant at $P \leq 0.05$.
- The plants raised from non-prime or SA-primed seeds were saved as controls.
- Data are the mean values of three replicates (n=3).

TABLE 6. Effect of salicylic acid (10µM)pretreatment of lupin seeds onglutathione reductase (GR) and glucose-6-phospate dehydrogenase (G6PDH) activities in shoots and roots under imposing Al^{3+}-stress for 21-d.

Treatment Al ppm	Treatment SA µM	GR µmolNADPH$_2$g^{-1}f.m. min^{-1}		G6PDH µmol g^{-1}f.m min^{-1}	
		Shoot	Root	Shoot	Root
0	0	32.96±2.20	23.79±2.16	0.014±0.0012	0.012±0.001
10	0	10.46±0.80	6.31±0.39	0.107±0.01	0.068±0.0012
0	10	33.79±2.82	20.83±1.39	0.026±0.0013	0.019±0.0016
100	10	20.95±1.50	10.18±0.73	0.278±0.02	0.166±0.02
P value		0.0025*	0.0028*	0.011*	0.025*
LSD ≤0.05		4.55	4.9	0.07	0.050

- *: Statistically significant at P≤ 0.05.
- The plants raised from non-prime or SA-primed seeds were saved as controls.
- Data are the mean values of three replicates (n= 3).

Discussion

Exposure of lupin plants to 100ppm Al^{3+} resulted in a significant suppression in vegetative growth. Similarly, many authors have been reported that Al^{3+} stress markedly inhibit the growth biomarkers of several plants such as *Plantago* plant (Neusa et al., 2012), rice (Marciano et al., 2010) and tea plants (Mukhopadyay et al., 2012). Kuo & Kao (2003) mentioned that Al stress has detrimental effect on plasma membranes. Tahara et al. (2008) revealed that the suppression in growth of Al-treated plants could be attributed to the inhibitory effect of ROS on tip growth and cell division. Thus, the decrease of growth biomarkers in Al-stressed lupin plants, in the present study, might be related to disturbance of water and growth regulators balance, manifested by the decrease of RWC and plant hormones (IAA, GA3). Moreover, there was a significant increase of H_2O_2 and MDA contents in shoots and roots of Al^{3+} -stressed lupin plants indicating the impairment of plasma structure and water uptake, as well as oxidation of macromolecules, DNA and RNA in tips, hence inhibition of cells division and growth.

Roychoudhury et al. (2016) suggested that pretreating mung bean seeds with SA could help in Cd stress adaptability and improve their vigor stress. The observed increase of growth biomarkers and RWC of Al^{3+}-treated lupine plants toward the control treatments might indicate partial recovery from Al^{3+} stress with SA pre-treatment. These results coincides with the findings by Kabiri et al. (2014). Tonel el al. (2013) stated that improve the growth of plants raised from primed-SA seeds under various stresses, was mainly related to reduce the generation of ROS and lipid peroxidation. In accordance with these views, there was a significant decrease of H_2O_2 and MDA contents in shoots and roots of Al^{3+}-stressed lupine plants raised from primed-SA seeds and that associated with increase of fresh matter and leaf RWC. These observations might indicate the role of SA in improving the plasma membranes structure of lupin plants under Al^{3+} stress and resulting in an increase of water and nutrients absorption and therefore enhancing the growth.

The preservation of heavy metals-induced oxidative stress necessitates the biosynthesis of osmolytes and enhances of non-enzymatic and enzymatic antioxidant (Surapu et al., 2014). In this study, increased proline and phenolic contents in shoots and roots of lupin plants with respect to control was noteworthy following Al^{3+} treatment. These observations in agreement with those shown in several plants grown under various stresses (Matsui et al., 2007; Ezzat et al., 2015). Proline accumulation in Al^{3+}-stressed lupin plants, in this study, might impose in cellular osmotic adjustment protecting the enzyme structure and stabilizing the cell organelles, to some extent, from oxidative stress. In addition, the increment of phenolics content in Al^{3+}-treated lupin plants was accompanied with a significant increase of H_2O_2 and decrease of GPX activity, revealing the lower

involving of phenolics as H-donors for scavenging generated ROS. Also, it seen that, the accumulation of phenolics was associated with a significant decline of IAA. Taffouo et al. (2009) suggested that increased phenolics content might stimulate IAA-oxidase and hence decrease IAA content and growth. Thus, one feature of suppression the growth of Al^{3+}-stressed lupin plants, in this study, could be explained by decrease of growth regulators content due to enhancement of phenolics accumulation which induce IAA-oxidase activity. Moreover, the increase of phenolics with enhancement of GPx and PPO activities in plants raised from SA-primed seeds might reveal the role of SA pretreatment in improvement the lupin growth *via* raising the level of non-enzymatic antioxidative mechanism under Al^{3+}stress. These observations are well supported by earlier reports of Senaratna et al. (2003) and Sgherri et al. (2004).

Rajasekaran et al. (2001) postulated that accumulation of trigonilline, under stress, might play a role as osmo-regulatory agent,while Zheng et al. (2005) suggested that the increase of trigonilline content could be attributed to detoxification of nicotinic acid and nicotinamide released from pyridine nucleotide cycle. In this study, the suppression of trigonilline content in Al^{3+}-stressed lupin plants might be attributed to enhancement the nicotinic acid and nicotinamide biosynthesis during pyridine nucleotide cycle as indicated by decrease of NADPH. Conversely, trigonilline content in shoots and roots of lupin plants raise from SA-primed seeds was significantly increased in respect to Al^{3+} stress, and that was accompanied with a marked increase of growth. Therefore, it is possible to suggest that under pretreatment with SA, accumulated trigonilline might maintain the osmotic pressure of cells and decline the toxicity of released nicotinic acid in lupin plants imposed to Al^{3+}-stress, and hence sustain the growth. Minorsky (2002) and Zheng et al. (2004) postulated that accumulation of trigonilline acts as similar manner of cytokinins promoting cell cycle and growth.

Foyer & Noctor (2005) have reported that $NADPH^{+}$ is a key cofactor in cellular redox states and a branch between ROS-enhancing and ROS-eleminating. Results in Table 3 showed a marked decrease of $NADPH^{+}$ content and increase of $NADP/NADPH^{+}$ ratio in shoots and roots of lupin plants raised from unprimed or SA-primed seeds imposed to Al^{3+}-stress in comparison to controls; the NADP/NADPH ratio in SA-primed lupin plants was markedly lower than those in unprimed ones. On the other hand, there was a significant increase of G6PDH (as NADPH-producing) and decrease of GR (as NADPH consuming) activities. Thus, it is possible to suggest that the $NADPH_2$ in Al^{3+}-treated lupin plants might be induced in the generation H_2O_2 *via* enhancement of $NADPH_2$-oxidase (Seong et al., 2007) and hence increase of ABA content and/or releasing the toxic nicotinic acid. Singh (2017) and Bücker-Neto (2017) reported that environmental stress signals resulted in $NADPH_2$ oxidase expression and that account for H_2O_2 generation and ABA production leading to stomatal closure and causing suppression of growth. In addition, the marker decrease of $NADPH_2$ in SA-primed plants could be induced the biosynthesis of osmo-regulatory agent such as proline and trigonilline, activated the enzymatic antioxidants as glutathione peroxidase and glutathione-S- transferase (Birben et al., 2012) as well as and/or enhanced ascorbate peroxidase *via* ASA-GSH cycle for defense mechanisms, and that might improve the lupin growth. In this study, there was a significant increase of GR and APx activity in lupin plants raised from SA-primed seeds compared to unprimed ones under Al^{3+}-treatments, these observations might indicate the induction of AsA-GSH cycle for scavenging generated H_2O_2. Farhud & Yazdanpanah (2008) have concluded that $NADPH_2$ acts as a cofactor of GR by transferring its proton for reducing the oxidized glutathione (GSSG) to reduce from (GSH) and hence can scavenge the generated H_2O_2 directly *via* glutathione peroxidase and/or indirectly by APx; through AsA-GSH cycle. Therefore, the switch off NADP/NADPH could mark a detectable sense in particular situation during imposing unprimed or SA-primed lupin seeds to Al^{3+}-stress.

The balance between enzymatic antioxidants, SOD, APx, GPx and PPO activities is important factor for controlling the level of generated ROS. In this study, contamination the growth media of lupin plants with Al^{3+} significantly activated SOD and CAT. These results in agreement with earlier observation of Xu et al. (2012) in wheat, Ramírez-Benítez et al. (2008) in coffee plant and Seong et al. (2007) in tomato plant. The enhancement of SOD and CAT activity might be related to H_2O_2 acts as signal transduction for enzymes (Foyer & Noctor, 2005) or increases of enzyme isoforms (Roychoudhury et al., 2016). On the other hand, APx, GPx and PPO activities in Al^{3+}-stressed lupin plants were significantly more or less suppressed,

comparing to untreated controls. These observations were associated with a significant increase of H_2O_2 and MDA content, revealing that the harmony balance between these studied enzymes seems to be unable to scavenging ROS at the Al^{3+} concentration used and hence impaired the lupin growth.

SA-pretreatment declined the inhibitory effect of Al^{3+} on lupin growth, in this study, was associated with the enhancing the activity of antioxidant enzymes *via*, SOD, CAT, APx, GPx and PPO. Similarly, Liu et al. (2017) have suggested that alleviation the inhibitory effect of various environmental stresses by application of SA was might be attributed to the regulation of enzymatic antioxidant activities.

Conclusion

It is concluded that priming treatment of lupin seeds with SA might shift off-to some extent- the inhibitory action of Al^{3+} on the growth, through enhancement of enzymatic and non-enzymatic antioxidants and that was associated with a marked decrease of ROS generation. In addition, the change in oxido-reduction homeostasis as indicated by $NADP/NADPH_2$ could play an important role between the enhancement of oxidative stress and induction of defense mechanism.

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دور حمض الساليسيليك في التخفيف من آثار الألمونيوم على النمو والعمليات البيوكيميائية في نبات الترمس

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تعتبرسمية المعادن الثقيلة واحدة من الضغوط الرئيسية التي تؤدي إلى التثبيط والآثر السلبي في حياة النباتات. وفي الدراسة الحالية ادت المعالجة بعنصر الألمونيوم إلى الأنخفاض المعنوي في النمووالمحتوى المائي لكل من المجموع الحضري والجذري لنبات الترمس. وقد صاحب هذا الانخفاض انخفاضا كبيرا في محتوى هرموناندول حمض الخليك وحمض الجبريلليك مع زيادة في حمض الابسيسك. وعلاوة على ذلك فإن زيادة مستويات الألومنيوم في المحلول المائي قد ادت إلى توليد وتراكم فوق اكسيد الهيدروجين ومالونالدهيد (الدال على اكسدة الأغشية البلازمية) في نبات الترمس. ارتبطت زيادة الإجهاد التأكسدي نتيجة تصاعد شوادر الأوكسجين الحرة (ROS) إلى التخليق الحيوي لمضادات الأكسدة غير الأنزيمية (البرولين والفينولات والتريجونيلين) ونسبة إنزيمات مضادات الأكسدة (كاتاليز ,سوبر اوكسيد ديسميتيز)، مع انحفاض نشاط كل من GR، PPO، GPx و APx بشكل ملحوظ. ولقد ادت المعالجة بتركيزات الألمونيوم إلى نشاط انزيم جلوكوز6-- فوسفات ديهيدروجينيز والتغير في الجهد التأكسيدي للسيتوبلازم بدلالة نقص كل من نسبةNADP+/ NADPH وGSH/AsA فكان هذا مصاحبا بالنقص في نمو نبات الترمس.

اشارت الدراسة ايضا أن المعالجة الأولية لبذور الترمس ب10 ميكرومول من حمض الساليسليك قد ادت إلى تخفيف ملحوظ من التأثير المثبط لعنصر الألمونيوم على نمو نبات الترمس. وأظهرت النتائج أن المعالجة بحمض الساليسيلك قد ادت الزيادة تحفيزمكونات مضادات الأكسدة غير الإنزيمية ونشاط انزيمات مضادات الأكسدة مثل سوبراوكسيد ديسميتيز و كاتاليزواسكوبريك اوكسيديز و فينول بيراوكسيديز وفينول اوكسيديز في النباتات المعالجة مقارنة بالنباتات غير المعالجة. وكذلك زيادة معنوية في هرمونات النمو (الأندول حمض الخليك، حمض جبريلليك) ونقص في انتاج هرمون الاجهاد (حمض الابسيسيك). وبالإضافة إلى توازن الأكسدة الخلوية من خلال زيادةGSH/AsA، NADP+/ NADPH+، مع الملاحظة أن الزيادة في النمو لنبات الترمس بالمعالجة بحمض الساليسليك كان مرتبطا بنقص تراكم تولد اوكسجين الحرة مما قد يوحي إلى نشاط انزيمات المضادة للأكسدة مع زيادة نشاط دورة GSH/AsA.