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In vitro Response of Some Banana Cultivars to Salicylic Acid Treatment Under Salinity Stress

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Abstract:

The effect of Salicylic acid (SA) on three Cavendish banana cultivars (i.e. Zeef, imported and local Grand Naine) subjected to *in vitro* salt stress was evaluated. Five concentrations of NaCl (0,30,60,120 and 200 mM) were tested, among which the optimum concentration for screening was determined at 120 mM. Different concentrations of SA (0, 0.5 and 1 mM) were assessed for several vegetative and physiological traits of the three cultivars cultured upon medium containing 120 mMNaCl. Analysis of variance showed highly significant differences among cultivars, SA concentrations and salinity levels, as well as their interactions in most of the studied traits. NaCl treatment caused severe loss in vegetative and physiological traits in all tested cultivars and activated several protein bands compared with non-saline conditions. Moreover, addition of SA exhibited a beneficial effect in reducingdamage caused by salinity in several traits i.e. survival percentage, plantlet length, fresh weight, total chlorophyll, carotenoids and proline content and Potassiumuptake. In addition, SA at 0.5 mM was more effective in enhancement salinity tolerance, while high concentration inhibited most of protein bands enhanced under salinity. The results showed that among the evaluated cultivars, both Grand Naine cultivars were more tolerantthanZeef cultivar. The information resulted herein is valuable and could be help inimprovement of salt tolerance in banana.

Keywords: *Salicylic acid, Musa, Salinity, Protein, abiotic stress*

Introduction:

Banana (*Musa* L.) belongs to family Musaceaeis considered one of the most important fruit crops worldwide due to its nutritional facts. Bananaiscontinuously facing a various array of environmental biotic and abioticstresses which affect its growth and yield. Among numerous abiotic stresses, salinity is one of themajor factors that limits crop production (Misra *et al.,* 1990). Salinity ruins the metabolism in plant tissue that is exhibited in terms of modified growth performance and physiological process to provide tolerance against salinity induced stress (Sairam and Tyagi, 2004 and Mahajan and Tuteja, 2005). Approximately 7% of the world's land area is affected by salinity which resulted in more than 35% reduction in agricultural production worldwide (Tanji, 2002). Accordingly, attempts are being made to evaluate crop cultivars for their salt tolerance. Several organized systems of biochemical and physiological processes have been developed by plants to defend themselves from salinity-induced damages. These include ionic homoeostasis, antioxidant responses, and/or os-

moregulation (Hasegawa *et al.,* 2000; Parida and Das, 2005).

Salicylic acid (SA) has been reported to enhance plant tolerance abiotic stresses including salinity by increasing biomass, chlorophyll content and activation of antioxidant enzymes that are responsible to activate the photosynthetic process and improved useful tool for evaluation of environmental stress tolerance which could be oxidative stress (Li *et al.,* 2014) as well as it repairs membrane potential and prevents salt-induced potassium loss via GORK channel (gated outwardly-rectifying K^+ channel) (Jayakannan *et al.,* 2013). The effect of SAon decreasing salinity oxidative stress has been reported in several species including *A. thaliana* (Jayakannan *et al.,* 2013), *Hordeumvulgare* (Fayez and Bazaid, 2014 and Khan *et al.,* 2014) and *Torreyagrandis* (Li *et al.,* 2014).

Tissue culture technique is providing a easily controlled (Errabii *et al.,* 2006). Likewise, *In vitro* culture systems decrease environmental differences due to defined nutrient media, controlled conditions and symmetry of stress application. Also, the simplicity of manipulation enables to investigate large number of plants and stress treatments in a limited space and a short period. (Das *et al.,* 1990; Misra *et al.,* 1990;Misra *et al.,* 2002; Abouzaid *et al.,* 2016). The effect of saline conditions on *in vitro* banana micropropagation has been reported with various effects on several characteristics. NaCl caused decreasing in total proteins, K^+ uptake, explant proliferation, fresh and dry weight, number of plantlets per explant, psuedostem diameter, root proliferation and growth rate. While, salinity causes increasing in proline, glycinebetain and carotenoids content (De Macêdo *et al.,* 2005; Ikram-ul-Haq *et al.,* 2007; Ikram-ul-Haq *et al.,* 2011; Chhatoi *et al.,* 2016).

The objectives of this work were to: 1) determine the effect of different SA concentrations on *in vitro* salinity tolerance in some banana cultivars and 2) detect the variability among banana cultivars in response to salinity and SA treatment.

Materials and Methods:

Plant materials:

Three Cavendish banana cultivars (*Musa acuminate* Colla, AAA) were used in this study, namely Zeef (a local grown cultivar in Egypt with superior characterization for fruit quality) and two Grand Naine cultivars (Local (LGN) and Imported (IGN)). The meristem was obtained from developing suckers of the three cultivars grown under field conditions. The suckers were washed thoroughly under running tap water, roots and outer tissues were removed with a sharp knife until reach a size of 10×2.5 cm, then they were soaked in commercial sodium hypochlorite for 25 minutes followed by washing three times with sterilized distilled water.

The explant size was reduced under laminar flow hood condition through dissection and removal of leaf sheath until a size of 4×1 cm. Explants sterilized again with 10% sodium hypochlorite for ten minutes followed by washing three times with sterilized distilled water. The initial explant was then prepared by removal of outer tissue with sterile scalpel to reach 1x0.5cm size, then explant cul-

tured upon the establishment medium vertically. Cultures were transferred to growth room at 25±1°C in dark for one week then transferred to a 16h light system (2000 lux) for growth and development. After two months, cultures were transferred into the growth medium.

Culture media:

The establishment medium was composed of MS (Murashige and Skoog 1962) full-strength supplemented with 9µM 6-Benzylaminopurine (BAP), 1µMIndole-3-acetic acid (IAA), 3% sucrose and 1.25 mM KH_2Po_4 with 2 g/l gelrit. The growth medium consists as establishment medium with 1µM BAP. The pH was adjusted at 5.8 and the media were then autoclaved at 1.2 kg/cm2 pressure at 121°C for 20 minutes. The growth regulators were added by filtration and the media poured to jars under laminar flow hood aseptic conditions.

Salinity tolerance evaluation and effect of salicylic acid:

A preliminary experiment was performed to determine the optimal concentration of Nacl to be used for salinity evaluation. Growth medium was supplemented with five concentrations of NaCl (0,30,60,120 and 200 mM) and those were used on Zeef cultivar. After 45 days, Survival percentage, some vegetative characteristics and chlorophyll content were recorded. The main experiment was performed on the three cultivars using zero (as control) and the optimum concentration of NaCl. Non-saline and saline media were supplemented with different concentrations of SA (0, 0.5 and 1 mM). Salicylic acid was used in a sodium salicylate formula (160 g/mol) and added by filtration.

The experiment was conducted in a complete randomized design (CRD) with three replicates with five explants for each replicate, by a total of 90 explants for each cultivar (2 NaCl concentrations ×3 SA concentrations×3 replicates× 5 explants). The whole experiment was repeated twice for result validation. After 45 days, some vegetative characteristics were recorded, including the percentage of survival (as the number of survive explants divided by the total number of explants), plantlet length (cm), the average number of shoots per explant (NSE), fresh and dry weight. Some other physiological characteristics were also recorded, includingphotosynthetic pigments *via*, chlorophyll-a, chlorophyll-b and carotenoids according to Lichtenthaler (1987), proline content following the method of Bates *et al.,*(1973) and Potassium uptake using Jackson, (1973) method.

Statistical analysis

MSTAT-C statistical program (Nissen 1984) was used to perform analysis of variance (ANOVA). Means were separated by revised least significant difference (LSD`) test at levels of 5 and 1 % probability. Percentage of reduction (%RED) due to NaCl was estimated for each cultivar in each characteristic as $(Y_1-Y_2)/Y_1\times 100$,where Y_1 the average of trait under the control (0 mMNaCl) and Y_2 is the average of trait under the treatment (120mMNaCl).The effect of salicylic acid (SA) on salinity tolerance was estimated for each characteristic as $(T_1-T_2) / C \times 100$, where T_1 the average of trait under saline medium containing SA, T_2 is

the average of trait under saline medium without SA and C is the average of trait under non-saline medium without SA (control).

Total protein profile analysis:

Total protein was extracted from *in vitro* plantlets of the three cultivars cultured upon different combinations of SA on saline and non-saline media after 2 months Each sample was represented by different tissues including leaves, psuedostem and roots. The protocol of Laemmli, (1970) was used with some modifications. Eco-Mini vertical electrophoresis unit (Biometra ®, Germany) was used for SDS-PAGE, then gels were stained with Commassie brilliant blue staining solution and captured using UVP-Gel Doc-II documentation system. For analysis, 1D scan software (scanalytics) was used for band detection, molecular weight estimation and retention factor (RF) determination.

Results:

Five concentrations of NaCl (0, 30, 60, 120 and 200 mM) were used to determine the optimum concentration for salinity evaluation, among which the preliminary result on Zeef cultivar showed that NaCl at 30 and 60 mM has nosignificant effect on plantlet growth compared with control, while NaCl at 120 mM gave 50% survival of plantlets and affected most of the vegetative parameters, and the treatment with 200 mM was enough to inhibit the growth completely and caused total death of the plantlets (Fig 1). The main experiment was achieved using the three cultivars (Zeef, imported Grand Naine (IGN) and local Grand Naine (LGN)) which were evaluated under saline (120 mMNaCl) and non-salinemedia, both supplemented with different concentrations of salicylic acid (SA). Analysis of variance showed highly significant effects of cultivars, NaCl and SA treatments and their interactions on most of the evaluated traits (Table 1).

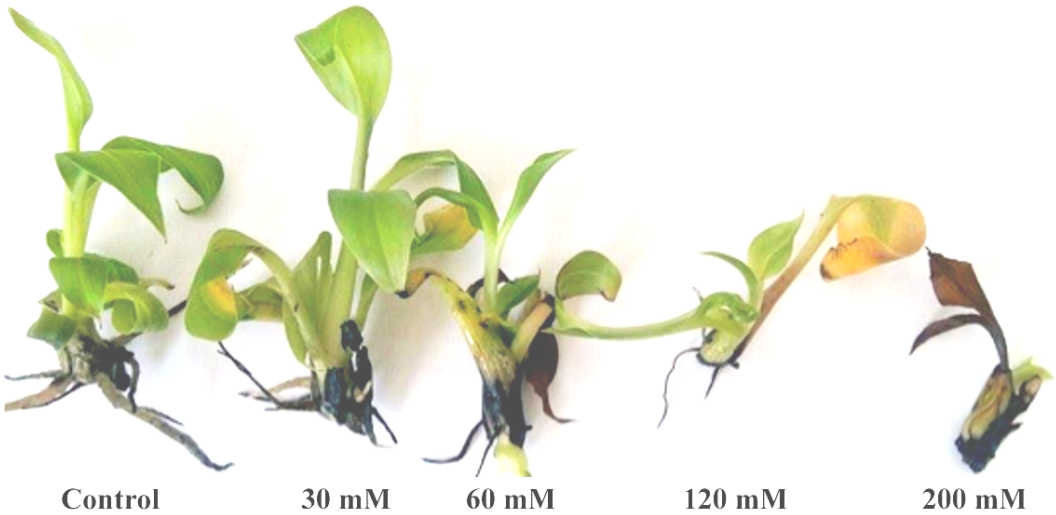

Figure (1): Effect of *in vitro* treatment with different concentration of NaCl on Zeef banana cultivar.

Effect of SA under non-saline conditions:

Under normal conditions (non-saline medium), SA at both concentrations didn't affect the survival percentage comparing with control. However, it has various effects on the other vegetative and physiological traits. In this regard, overall the three cultivars, 0.5 mM SA caused increasing in number of shoots per explant (NSE), plantlet length (PL), proline content, while it decreased fresh and dry weights, chlorophyll a and b, carotenoid content and potassium uptake (Table 2). The effect of the higher concentration of SA (1 mM) was slightly different which increased PL, carotenoid content, proline content and K^+ uptake and decreased NSE, FW, DW, chlorophyll a and b. Moreover, the response of the three cultivars to SA treatment was inconstant (Fig 2, Table 2).

Table 1. Analysis of variance of the tested cultivars for some vegetative and physiological traits under different combinations of NaCl and salicylic acid treatments.

Trait	Source of variance	df	MS	Trait	Source of variance	df	MS
Number of shoots per explant	Cultivars (CV)	2	25.461**	Chlorophyll-b	Cultivars (CV)	2	0.004**
	NaCl	1	84.125**		NaCl	1	0.196**
	SA	2	0.932*		SA	2	0.002*
	CV × NaCl	2	8.25**		CV × NaCl	2	0.001NS
	CV × SA	4	5.099**		CV × SA	4	0.000NS
	NaCl × SA	2	0.774NS		NaCl × SA	2	0.002NS
	CV × NaCl × SA	4	7.221**		CV × NaCl × SA	4	0.001NS
	Error	36	0.253		Error	36	0.000
Plantlet length	Cultivars (CV)	2	5.586**	Total chlorophyll	Cultivars (CV)	2	0.071**
	NaCl	1	46.296**		NaCl	1	1.581**
	SA	2	16.568**		SA	2	0.005NS
	CV × NaCl	2	4.725**		CV × NaCl	2	0.025**
	CV × SA	4	6.465**		CV × SA	4	0.020**
	NaCl × SA	2	2.597*		NaCl × SA	2	0.049**
	CV × NaCl × SA	4	10.604**		CV × NaCl × SA	4	0.018**
	Error	36	0.718		Error	36	0.003
Fresh weight	Cultivars (CV)	2	1.167NS	Carotenoids	Cultivars (CV)	2	0.002*
	NaCl	1	96.133**		NaCl	1	0.043**
	SA	2	3.090**		SA	2	0.001NS
	CV × NaCl	2	0.502NS		CV × NaCl	2	0.002*
	CV × SA	4	0.597NS		CV × SA	4	0.003**
	NaCl × SA	2	6.395**		NaCl × SA	2	0.001NS
	CV × NaCl × SA	4	1.358*		CV × NaCl × SA	4	0.002*
	Error	36	0.402		Error	36	0.001
Dry weight	Cultivars (CV)	2	0.004*	Proline	Cultivars (CV)	2	0.052*
	NaCl	1	0.088**		NaCl	1	0.068*
	SA	2	0.019**		SA	2	0.200**
	CV × NaCl	2	0.001NS		CV × NaCl	2	0.213**
	CV × SA	4	0.002NS		CV × SA	4	0.156**
	NaCl × SA	2	0.031**		NaCl × SA	2	0.162**
	CV × NaCl × SA	4	0.002NS		CV × NaCl × SA	4	0.107**
	Error	36	0.001		Error	36	0.011
Chlorophyll-a	Cultivars (CV)	2	0.050**	Potassium uptake	Cultivars (CV)	2	1.216**
	NaCl	1	0.664**		NaCl	1	5.907**
	SA	2	0.008*		SA	2	0.256**
	CV × NaCl	2	0.016**		CV × NaCl	2	0.371**
	CV × SA	4	0.021**		CV × SA	4	3.147**
	NaCl × SA	2	0.035**		NaCl × SA	2	1.437**
	CV × NaCl × SA	4	0.012**		CV × NaCl × SA	4	0.803**
	Error	36	0.002		Error	36	0.024

NS: non-significant, * significant at $P< 0.05$ and ** significant at $P<0.01$.

Table 2. Average values of different vegetative and physiological traits evaluated under different combinations of NaCl and Salicylic acid in three Cavendish banana cultivars.

Media	Cultivar	SA (mM)	Vegetative and physiological traits (Average ± SE)									
			NSE	**PL**	**FW**	**DW**	**ChA**	**ChB**	**ChT**	**Car**	**Pro**	**K**
Non-saline (zero NaCl)	Zeef	Zero	3.03±0.38	3.80±0.12	5.29±0.25	0.30±0.05	0.45±0.02	0.16±0.01	0.61±0.02	0.17±0.00	0.64±0.01	5.45±0.03
		½	2.57±0.07	9.67±1.13	5.00±0.40	0.25±0.03	0.27±0.01	0.12±0.00	0.38±0.01	0.09±0.01	0.60±0.07	5.47±0.07
		1	2.47±0.23	6.73±0.90	2.49±0.22	0.12±0.01	0.17±0.02	0.13±0.02	0.30±0.04	0.09±0.03	0.78±0.05	5.01±0.18
	IGN	Zero	2.73±0.15	9.67±0.58	5.77±0.35	0.24±0.02	0.43±0.01	0.19±0.01	0.62±0.00	0.11±0.02	0.84±0.11	5.94±0.03
		½	6.13±0.27	7.33±075	4.27±0.81	0.25±0.02	0.39±0.00	0.14±0.01	0.53±0.01	0.13±0.02	0.81±0.02	5.01±0.06
		1	5.57±0.64	6.40±0.21	3.83±0.27	0.17±0.01	0.42±0.01	0.15±0.01	0.57±0.01	0.16±0.01	0.73±0.00	6.43±0.12
	LGN	Zero	8.77±0.39	5.70±0.46	5.17±0.18	0.27±0.01	0.36±0.01	0.19±0.01	0.54±0.01	0.11±0.02	0.58±0.02	5.07±0.12
		½	6.00±0.00	8.50±0.17	5.70±0.38	0.31±0.02	0.44±0.04	0.17±0.03	0.61±0.06	0.16±0.03	0.74±0.08	5.13±0.06
		1	4.37±0.63	8.10±0.40	4.17±0.94	0.16±0.01	0.47±0.03	0.19±0.00	0.66±0.03	0.16±0.01	1.15±0.12	5.24±0.14
Saline (120 mMNaCl)	Zeef	Zero	2.03±0.03	4.70±0.55	1.90±0.15	0.14±0.02	0.08±0.01	0.03±0.01	0.11±0.02	0.06±0.01	0.60±0.03	4.68±0.04
		½	1.17±0.09	5.60±0.42	1.93±0.24	0.16±0.01	0.16±0.01	0.04±0.00	0.20±0.01	0.07±0.01	1.41±0.04	4.92±0.05
		1	1.23±0.15	6.90±0.25	2.10±0.06	0.17±0.01	0.13±0.02	0.03±0.00	0.16±0.02	0.07±0.00	0.95±0.11	3.84±0.07
	IGN	Zero	2.57±0.27	4.83±0.20	1.33±0.09	0.09±0.01	0.15±0.02	0.03±0.01	0.18±0.03	0.08±0.02	0.76±0.02	4.22±0.02
		½	2.20±0.10	7.70±0.15	2.27±0.07	0.16±0.01	0.29±0.07	0.05±0.01	0.34±0.08	0.12±0.02	0.65±0.01	4.56±0.06
		1	2.57±0.18	6.17±0.12	1.63±0.03	0.14±0.01	0.19±0.01	0.03±0.00	0.22±0.01	0.09±0.00	0.63±0.02	6.13±0.09
	LGN	Zero	2.07±0.07	3.47±0.12	1.97±0.03	0.15±0.01	0.04±0.00	0.07±0.01	0.12±0.01	0.03±0.01	0.65±0.05	4.39±0.11
		½	2.83±0.17	4.73±0.46	1.83±0.19	0.16±0.01	0.19±0.04	0.04±0.01	0.23±0.05	0.08±0.01	0.97±0.08	6.08±0.10
		1	2.50±0.29	5.13±0.13	2.70±0.31	0.17±0.01	0.16±0.01	0.03±0.00	0.19±0.01	0.08±0.01	0.92±0.03	3.98±0.08
LSD` 0.05			0.74	1.29	1.14	0.06	0.07	0.001	0.09	0.06	0.16	0.23

IGN: imported Grand Naine, LGN: local Grand Naine, SA: salicylic acid, NSE: number of shoots per explant, PL: plantlet length, FW: fresh weight, DW: dry weight, ChA: chlorophyll-a, ChB: chlorophyll-b, ChT: total chlorophyll, Car: carotenoid, Pro: proline, K: Potassium uptake.

Table 3. Percentage of increasing or decreasing due to SA treatment in vegetative and physiological traits under saline conditions, as an average of the three banana cultivars.

Medium	**Cv.**	**SA (mM)**	**NSE**	**PL**	**FW**	**DW**	**ChA**	**ChB**	**ChT**	**Car**	**Pro**	**K**
Non-saline	Zeef	½	-15.38	154.39	-5.54	-16.48	-40.30	-28.57	-37.16	-44.00	-5.94	0.31
		1	-18.68	77.19	-52.96	-61.54	-61.19	-22.45	-50.82	-44.00	22.57	-8.19
	IGN	½	124.39	-24.14	-26.01	4.17	-8.53	-25.00	-13.51	14.71	-3.42	-15.66
		1	103.66	-33.79	-33.53	-30.56	-2.33	-17.86	-7.03	41.18	-12.79	8.31
	LGN	½	-31.56	49.12	10.32	14.81	22.43	-7.14	12.27	46.88	26.94	1.12
		1	-50.19	42.11	-19.35	-41.98	31.78	0.00	20.86	46.88	96.89	3.42
Saline	Zeef	½	-28.57	23.68	0.63	6.59	18.66	3.67	14.64	6.60	127.08	4.28
		1	-26.37	57.89	3.78	10.99	12.09	-3.27	7.98	8.00	55.58	-15.46
	IGN	½	-13.41	29.66	16.18	27.78	32.56	10.71	25.95	35.29	-13.51	5.78
		1	0.00	13.79	5.20	19.44	10.08	2.50	7.78	12.35	-15.50	32.10
	LGN	½	8.75	22.22	-2.58	4.94	41.12	-16.07	21.47	40.63	54.66	33.46
		1	-2.52	27.13	7.60	12.30	17.62	-8.39	9.74	18.28	23.72	3.99

Effect of SA under salinity:

NaClat 120 mM significantly affected all evaluated traits. The survival percentage was decreased dramatically when they cultured on saline medium with 60, 70 and 60% survival percentage for Zeef, IGN and LGN, respectively. Results showed that NSE was severely affected by NaCl, especially in LGN cultivar. On the other hand, NaCl didn't affect PL in Zeef cultivar in contrary to the other two cultivars, which were significantly affected by stress. The percentage of reduction (%RED) due to salinity as an average of all cultivars ranged from 2.8 to 78.1% for proline content and chlorophyll-a respectively. However, the three cultivars varied in their response to saline stress, for instance, in Zeef cultivar the highest %RED was 82.8% for chlorophyll-b and the lowest %RED was 6.7% for proline, while PL was increased under salinity by 23.7% compared to control. The %RED due to salinity in IGN cultivar ranged from 6.1% for NSE to 85.7% for chlorophyll-b. While, the highest %RED in LGN cultivar was 87.9% for chlorophyll-a and the lowest was 13.5% for K^+ uptake and unlikely it was an 11.1% increase in proline content due to NaCl treatment (Table 2, Fig 3). NaCl stress decreased chlorophyll a, chlorophyll b and subsequently total chlorophyll content and K^+ uptake as compared to the non-saline medium.

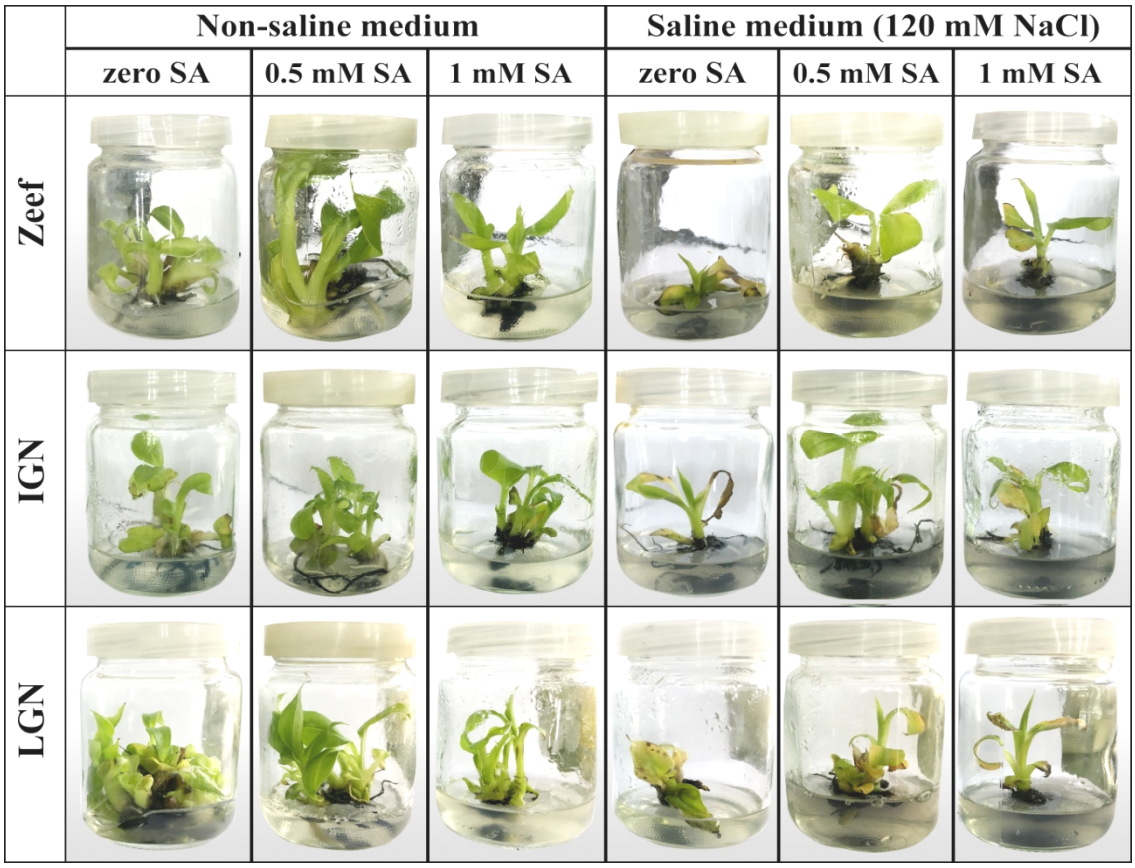

Figure (2): *In vitro* response of three Cavendish banana cultivars to different combinations of NaCl and salicylic acid treatments.

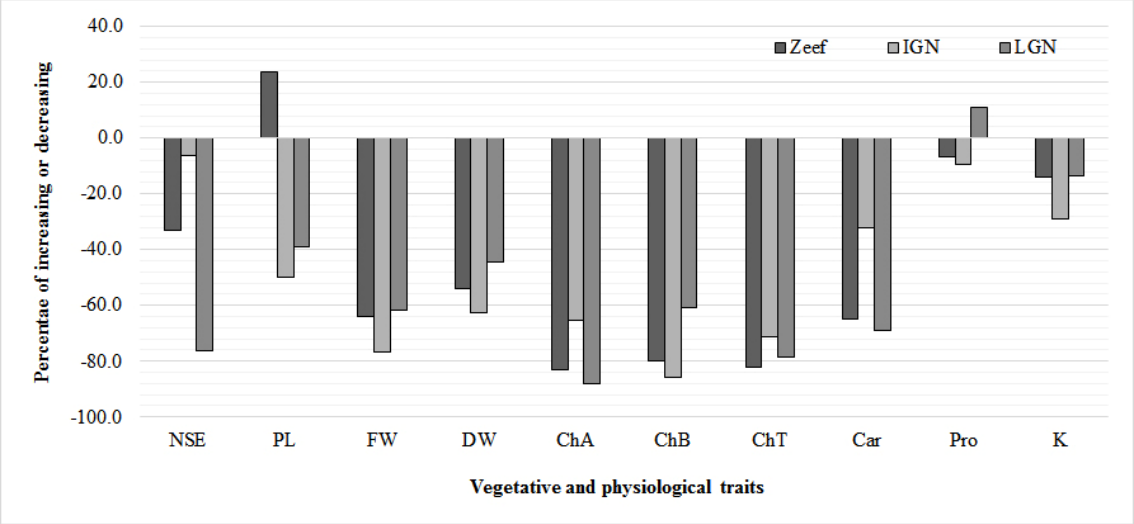

Figure (3): Effect of salinity stress (120 mMNaCl) on some vegetative and physiological traits in three Cavendish banana cultivars.

The treatment of SA under saline medium enhanced all the vegetative and physiological traits evaluated in this study compared with saline stress except NSE and chlorophyll-b (Fig. 4). In this regard, results indicate that SA at 0.5 mM raised the survival rate up to 80, 80 and 90% for Zeef, IGN and LGN, respectively. Meanwhile, the addition of 1 mM of SA resulted in a slight decrease in survival rates (50, 75 and 70% for the three cultivars, respectively). In addition, results showed that SA at 0.5 mM was more effective in increasing most of the studied traits compared with the other concentration (Fig. 4). The percentage of increasing due to SA treatment as an average of all cultivars ranged from 5.14 to 49.27% for FW and prolinecontent, respectively (Fig. 4). However, the response of each cultivar to SA treatment was different (Table 3). SA at 0.5 mM significantly ameliorated the total chlorophyll content, carotenoid in the three cultivars compared with saline stress. The proline accumulation in LGN was noticeable significantly in treatments containing NaCl and/or SA. On the other hand, in Zeef cultivar, the proline content highly increased when the saline medium was supplemented with SA, where the proline content increased from 0.60 mg/g DW on saline medium to 1.4 and 0.95 mg/g DW on saline medium supplemented by 0.5 and 1 mM SA, respectively. On the contrary, the proline content in IGN was decreased when culture media were supplemented with NaCl and/or SA at both concentrations (Table 3).Addition of SA at 0.5 mM enhanced Potassium uptake under salinity in Zeef and LGN cultivars while SA at 1 mM enhanced K^+ uptake in IGN (Table 3).

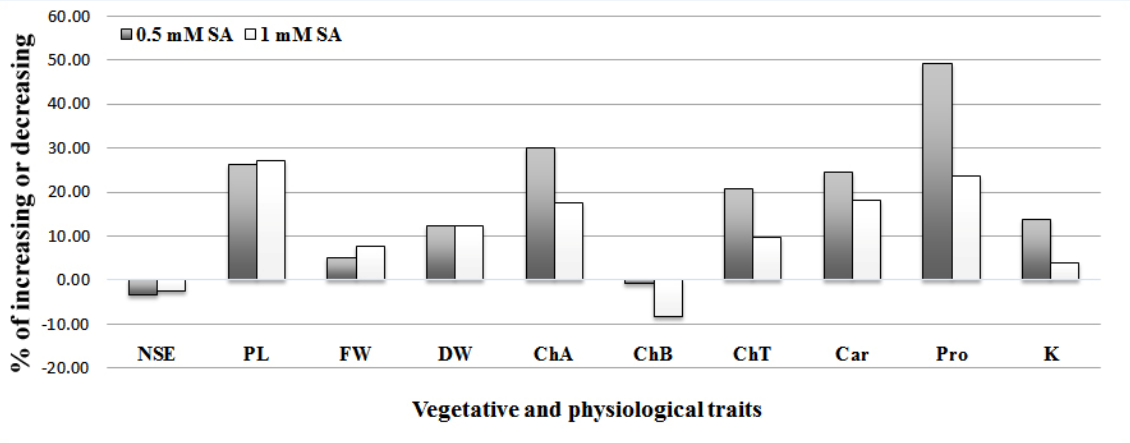

Figure (4): Effect of salicylic acid treatment on some vegetative and physiological traits under salinity compared with NaCl treatment, as an average of three Cavendish banana cultivars.

Effect of salt stress and SA on total protein profile:

SDS-PAGE protein profiles were performed to the *in vitro* plantlets of the three banana cultivars evaluated under different combinations of NaCl and SA. Results showed that, addition of SA with both concentrations didn't induce or reduce any band of the protein pattern compared with control in all cultivars. However, salinity stress induced several bands of proteins in the evaluated cultivars compared with control. In this regard, three bands (44, 30 and 28KD) were newly expressed in all cultivars, two bands (26 and 17 KD) were induced only with Zeef cultivar, and one band (19 KD) was newly expressed in both IGN (Table 4 and Fig. 5). Addition of SA to saline media showed inhibition of induction in some bands expressed due to NaCl stress. Incidentally, SA at 0.5 mM inhibited one bands (44 KD) in Zeef and LGN cultivars and another band (17 KD) in Zeef cultivar, while it didn't affect the profile of IGN compared with saline stress. Unlike low concentration of SA, higher concentration (1 mM) successfully inhibited all bands induced by NaCl stress with exceptionof three bands (30, 28 and 26 KD) in Zeef cultivar (Table 4 and Fig. 5).

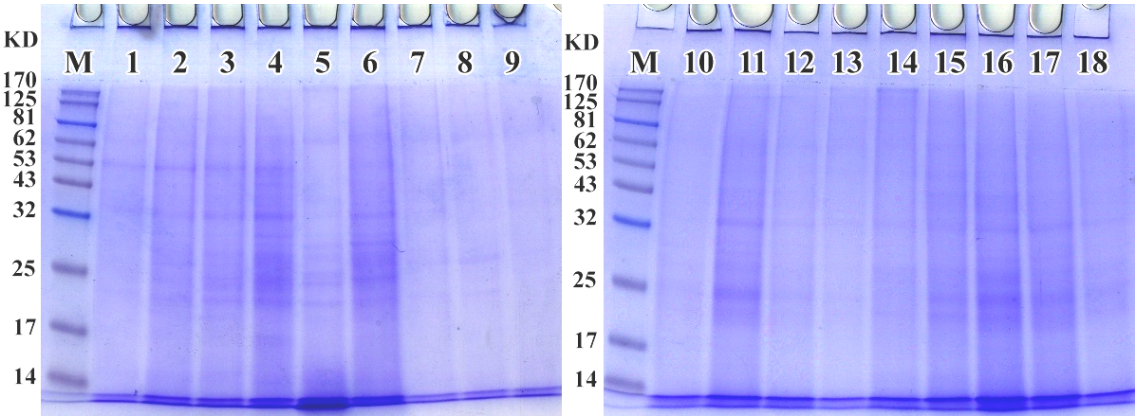

Figure (5): SDS-PAGE of total protein in three Cavendish banana cultivars under different combinations of NaCl and salicylic acid treatments.

Table 4. Presence and absence matrix of SDS-PAGE total protein in three Cavendish banana cultivars under different combinations of NaCl and salicylic acid.

MW	RF	Zeef						IGN						LGN					
		Non-saline			Saline			Non-saline			Saline			Non-saline			Saline		
		Zero SA	0.5 mM SA	1 mM SA	Zero SA	0.5 mM SA	1 mM SA	Zero SA	0.5 mM SA	1 mM SA	Zero SA	0.5 mM SA	1 mM SA	Zero SA	0.5 mM SA	1 mM SA	Zero SA	0.5 mM SA	1 mM SA
66	19.9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
53	28.3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
44	35.2	–	–	–	+	–	–	–	–	–	+	+	–	–	–	–	+	–	–
33	46.3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30	53.6	–	–	–	+	+	+	–	–	–	+	+	–	–	–	–	+	+	–
28	57.0	–	–	–	+	+	+	–	–	–	+	+	–	–	–	–	+	+	–
27	60.6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
26	46.4	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
24	68.4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
21	74.6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19	79.9	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	+	+	–
17	86.9	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	+	+	–

Disscution:

Tissue culture techniques have solved many of the restrictions which block abiotic stress evaluation in several species, providing place, technique, time saving, accuracy, etc. (Sakthivelu *et al.,* 2008). In this study, *in vitro* response to salicylic acid (SA) under normal and saline conditions was evaluated in three Cavendish banana cultivars. Significant variations among cultivars showed in this study indicate their

suitability for stress screening. In addition, the significance of interactions between cultivars and both NaCl and SA levels clearly exhibited that the response to the different treatments was due to genotype and concentrations.

Some previous studies have investigated the effect of NaCl on *in vitro* growth and related parameters in banana, in which it was reported that salinity caused decreasing in some traits and increasing in other. In this regard, some studies reported decreasing inmicropropagation rate, fresh weight, dry weight, number of plantlets per explant, psuedostem diameter and Potassium uptake (Ikram-ul-Haq *et al.,* 2007), chlorophyll a, b and total (Ikram-ul-Haq *et al.,* 2011), root length and root proliferation (Chhatoi *et al.,* 2016). These results are matched with our findings since NaCl caused decreasing in all evaluated traits except plantlet length and proline in some cultivars. However, other traits have been reported that were increased due to NaCl treatment, this includes reducing sugar content (Ikram-ul-Haq *et al.,* 2007), proline, glycinebetain and carotenoids content (Ikram-ul-Haq *et al.,* 2011).

Salicylic acid known as a substance which acts as hormone and has been reported as an elicitor for abiotic and biotic stressesat *in vitro* or *in vivo* levels in different plant species, including *Coffeaarabica* (Quiroz-Figueroa *et al.,* 2001), *Astragulusadsurgens* (Luo *et al.,* 2001), and *Avenanuda* (Hao *et al.,* 2006). In the present study, the application of SA on non-saline medium decreased some traits and increased other depending on genotype and SA concentration which is agree with Arfan *et al.,*(2007) who stated the impact of SA in inducing stress tolerance depends on type of species or concentration of SA. On the other hand, under saline condition SA increased most of the evaluated traits compared with NaCl treatment. These findings indicate that SA can acts as abiotic stress when it applied solely but it could reduce the effect of other abiotic stress such salinity. The results in this study agree with Shakirova *et al.,*(2003), Arfan *et al.,*(2007) and Sakhanokho and Kelley, (2009) who reported that SA application improves growth and opposed the growth inhibition induced by abiotic stresses.

Chlorophylls have focal part in photosynthesis; therefore, any changes in their level can affect plant growth. In our study, salinity stress decreased total chlorophyll content in leaves. Similar results have been reported in radish (Chaparzadeh and Behboud 2015), *Thellungiellahalophila* (Vera-Estrella *et al.,* 2005) and *Brassica juncea* (Yusuf *et al.,* 2008). Additionally, carotenoids have a critical role as antioxidant molecules for free radical scavenging, thus, increasing of carotenoids in plants under salinity stress and SA treatment could boost their ability to minimize the damage caused by ROS (Azooz, 2009).

When exposed to high salt conditions, plants maintain their water content by accumulation of compatible organic solutes, like proline, in their cytoplasms (Harinasut *et al.,* 2000). These organic compounds act as osmoprotectants as a response to

abiotic stresses, such as increased salinity. Proline accumulation under salinity has been reported and suggested to be a biochemical marker for increased salt tolerance in several plant species e.g. mulberry (Harinasut *et al.,* 2000), acacia (Yokota, 2003), and sugarcane (Gandonou *et al.,* 2006).In our study, we found that the accumulation of proline content was higher in local Grand Naine (LGN) followed by Imported Grand Naine (IGN) then Zeef cultivar, which reflect their degree of tolerance or sensitivity. In this regard, Igarashi *et al.* (1997), found that the level of proline accumulation in a salt-tolerant rice species was higher than in a salt-sensitive species under high salinity conditions. In contrary with Yokota, (2003) who reported a larger accumulation of proline in salt-hypersensitive plants than in salt-tolerant ones.

Our results showed that, all the three cultivars exhibited significant reduction in K^+ due to salinity stress. Sarwarand Ashraf, (2003)suggested that the decrease in K^+ uptake might be because the excessive presence of Na in the medium, as high Na content is known to have an antagonistic effect on K^+ uptake in plants. It is also known that salt tolerance is associated with K^+ contents, because of its part in osmotic regulation and competition with Na (Ashraf *et al.,* 2005). SA increased the uptake of K^+ as compared to non-SA treatment (El-Tayeb 2005 and Abdi *et al.,* 2011). These results are consistent with our results. Also, Szepsi *et al.,* 2005 found out that exogenous application of SA stimulated K^+ uptake.

Analysis of total protein pattern performed in the present study clearly confirmed the variability in performance showed among the three cultivars of Cavendish banana in affected traits due to salinity and application of SA. Although all cultivars represented the same profile of protein under normal conditions, they varied in the number of bands induced due to salinity and SA. These results confirmed that banana cultivars used in this study varied in their reaction and response to NaCl and SA which reflects their tolerance and susceptibility. In this regard, LGN was the most responsive cultivar to SA treatment while Zeef was less responsive. In addition, high concentration of SA was enough to inhibit the induction of salinity newly proteins unlike low concentration which remained the induced proteins under saline condition. This result matched with that of Conrath *et al.,* (1995) who found that SA at 1 mM was enough to inhibit catalase activity in tobacco. Induction of some proteins showed in this study agreed with previous finding, e.g. in Soybean, salt stress activated mitogen-activated protein kinase (MAPK) (Im *et al.,* 2012) and a 38-kD MAPK is activated by salt stress in alfalfa (Munnik *et al.,* 1999).

In conclusion,*in vitro* response to treatments of SA under saline conditions was mainly due to genotype and concentration of SA. Both Grand Naine cultivars showed their tolerance by exhibiting less reduction under salinity and high response to SA-compared with Zeefcultivar. Additionally, SA application reduces effectively the loss caused by NaCl however high concentration of SA reduced most of the protein bands induced under stress, while 0.5 mM SA

was more effective in enhancing performance of cultivars under saline condition and protein synthesis. These results supported the valuable application of SA to plants under salinity to enhance their performance and tolerance which could be used in improvement programs.

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إستجابة بض أصناف الموز للمعاملة بحض لسلسيلك تحت تأثير الملوحة معمليا

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الملخص:

تم دراسة تأثير حمض السالسيلك معمليا على ثلاثة أصناف من الموز (زيف و جراندنان مستورد ومحلى). فى التجربة المبدئية تم إختبار خمس تركيزات من كلوريد الصوديوم (صفر،٣٠،٦٠،١٢٠،٢٠٠ مليمول) وتم إختيار تركيز ١٢٠ مليمول من كلوريد الصوديوم لإجراء تجربة السالسيلك حيث أنه التركيز الذى أعطى ٥٠% نقص فى الصفات الخضرية.

تم إستخدام حمض السالسيلك بتركيزين ٠،٥ و ١ مليمول لإختبار تأثيره على الصفات الخضرية للأصناف الثلاثة المزروعة معمليا على بيئة تحتوى على ١٢٠ مليمول من كلوريد الصوديوم وقد أظهرت النتائج اختلافات معنوية واضحة بين الأصناف من جهة وتأثير حمض السالسيلك من جهة أخرى. المعاملة بكلوريد الصوديوم ادى الى تدهور فى معظم الصفات الخضرية والفسيولوجية بالنسبة للثلاث أصناف كما أدى لتنشيط بعض البروتينات بالمقارنة بالنباتات المزروعة على بيئة خالية من الملح. بالإضافة الى ذلك فإن اضافة حمض السالسيلك أظهر أثر ملحوظ فى تقليل ضرر الملوحة فى معظم الصفات.

إضافة الى ذلك فان حمض السالسيلك بتركيز ٠،٥ مليمول كان أكثر فاعلية فى تحسين المقاومة للملوحة فى حين أن التركيز الأعلى (١ مليمول) سبب تثبيط فى معظم البروتينات التى نشطت بالمعاملة بالملوحة. أيضا أظهرت النتائج أن صنفى الجراندنان المحلى والمستورد :انا أكثر مقاومة يليهما الصنف زيف.