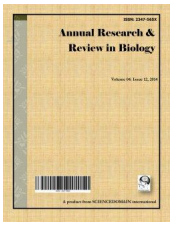

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Improving Salt Stress Tolerance of Pineapple cv. Queen Using Cobalt *In vitro*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors HEE and EAMA performed the tissue culture part, wrote the protocol and wrote the first draft of the manuscript. Authors AMFAA and AAR performed the practical part, analysis data of DNA-RAPD and performed the statistical analysis. Author NG did the chemical analysis of the study. Author MAH manage the literature searches and designed the study. All authors read and approved the final manuscript.

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ABSTRACT

This investigation aimed to focus on how cobalt can avoid the damage caused by salinity stress (NaCl) on Pineapple cv. Queen *in vitro*. Multiplicated pineapple explants (10 – 12 mm) were subjected for eight weeks to different NaCl conc. (0, 65, 135 or 200 mM) half of them were treated firstly with 5 mg/L Cobalt sulphate. Vegetative growth parameters (no.of shoots, no. of leaves, and shoot length/explant), mineral composition (N, P, K, Na, Cl, Fe, Mn, Zn, Cu and cobalt), proline and protein content were determined. Molecular characterization using PCR based RAPD was carried out to describe the genetic differences resulted from the studied treatments, (salinity and salinity combined with cobalt sulfate). Results show that, pineapple explants growth under salt stress wasn't prohibited completely specially below 135 mM of NaCl, but it affected negatively with the highest salt stress 200 mM of NaCl. Explants treated with cobalt before subjected to salinity scored the highest significant percentage of vegetative growth characteristics compared with those

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untreated. Explants treated firstly with cobalt resulted in a significantly decrease of Na^+ and Cl^-. Cobalt has a positive effect on Macro and Micronutrients, proline and protein content. A total of 34 DNA fragments varying from 186-1456 (bp) were amplified, of which 16 were polymorphic and seven observed as a unique markers that revealed 64.03% polymorphism.

Keywords: Pineapple; salt stress; cobalt; macro and micronutrients content; RAPD analysis; in vitro.

1. INTRODUCTION

The pineapple plant (*Ananas comosus Merr.* L.*)* belongs to bromeliad family (*Bromeliaceae)*, which contains 50 genera and about 2500 known species [1]. It is a tropical plant , South America is the original home of this plant, which has spread from it through immigration to Central and North America and grows best in a moderately warm climate (16-33°C) with low, but regular rainfall [2]. The pineapple fruit is important due to its high sugar content and attractive flavor; additionally it contains vitamins A and C [3]. Many studies showed millions of pineapple plants can be produced by tissue culture of the crown or shoot tip per year.

Salinity stress is the most significant problem facing plant agriculture in many regions of the world, which adversely affect the productivity of agriculture crops. The issue of salt tolerance is expected to become more serious as human population growth in the tropics begins to compete for finite water resources [4]. The increasing number of salt-affected hectares of valuable arable land, combined with the increasing demand for food to feed the ever-rising world population, makes mediation of salinity stress in plants greatly important.

Traditional breeding technologies and proper management strategies continue to play a vital role in crop improvement. However they have little success and have failed to provide desirable results [5]. Therefore, it is needed to deploy the biotechnological tools for addressing the critical problems of crop improvement for sustainable agriculture. Tissue culture techniques are becoming increasingly popular as an alternative means of plant vegetative propagation, mass production of chemicals and genetic engineering. Many authors have reported successful production of pineapple *via* micro-propagation system during the last few years [6,7].

Cobalt is considered to be a beneficial element for higher plants. It is an essential element for the synthesis of vitamin B12 which is required for human and animal nutrition [8]. Unlike other heavy metals, cobalt is saver for human consumption up to 8 ppm can be consumed on a daily basis without health hazard; it does not accumulate in human body as other heavy metals with the increase in age. Human body could get rid of cobalt through urination, [9] who added that, cobalt-dense compounds found in the pigments are necessary for plant to resist fungal and insect attack consumed by animals and humans, these compounds act as antioxidants. [10] reported that, cobalt is required in low levels for maintaining high yields of squash [11], groundnut [12] and [13] on rice.

Under salinity conditions, [14] and [15] found that cobalt reduced the salinity and injury effect on tomato [16] and [17] pointed out that, cobalt was used to reduce the harmful effect of salinity on tomato plants. [13] cleared that, the presence of cobalt sulfate decreased the negative impact of salt stress that observed in *in vitro* rice vegetative growth. Therefore, a suggestion being introduced for possible use of cobalt to irrigate transplants with saline water to overcome the salinity hazard. The ability of plants to tolerate excess salts in the rhizosphere is of considerable importance in the arid and semi –arid regions where salinization of soils usually prevails.

Randomly amplified polymorphic DNA (RAPD) was introduced by [18] to measure genetic diversity and genetic relationship among individuals and populations. Also, RAPD based fingerprinting was successfully used for the identification of markers linked to salinity in wheat [19], sorghum [20], barley [21], cotton [22] and other crops.

The aim of this study was a trail for enhancing pineapple explants to overcome salt stress damage using cobalt sulphate. This can be done by studying the effects of salinity combined with cobalt sulfate compared with salt stress alone on some *in-vitro* culture parameters and nutritional status, as well as check the differences resulted from the studied treatments at molecular level.

2. MATERIALS AND METHODS

The present study was carried out during (2015-2016) seasons at plant tissue culture Laboratory,

Genetics and Cytology Department and Plant Nutrition Lab., Plant Nutrition Department, National Research Centre, Dokki, Giza, Egypt.

2.1 Preparation of Tissue Culture Stock

Sterilized axillary buds excised from shoots of pineapple cv. Queen were cultured on sterilized MS medium [23] supplemented with 2.0 mg/l (BA), 0.2 mg/l IBA +30 g/l sucrose+7 g/l agar and incubated at 27 ± 2°C under 3000 Lux light intensity for 16 hrs. The explants were re-cultured to a fresh medium every four weeks intervals.

2.2 Effect of Cobalt on Salinity Treatments

Three months old multiplicated pineapple explants were divided into two main groups, the first one was cultured on the same starting medium free from cobalt (Co^-) while the other group was cultured on the same starting medium supplemented with 5 mg/L cobalt sulphate (Co^+) as recommended by [13] for 4 weeks.

2.3 Effect of NaCl Treatment on Vegetative Growth

Multiplicated pineapple explants of(10 – 12 mm) that without cobalt (Co^-) or with cobalt sulphate (Co^+) were subjected to four different treatments of salt stress which were done by adding 0 (control), 65, 135 or 200 mM NaCl [24] to starting medium in order to investigate the effect of different salt stress individually or under cobalt sulphate treatments to focus on its effect on tolerating salt stress of vegetative growth in pineapple explants *in vitro*, eight treatments are demonstrated in Table 1. Different vegetative characteristics (avg. no. of shoots, avg. no of leaves, and shoot length/explant in cm) were recorded after eight weeks.

Table 1. Salt stress treatments without or with cobalt

NaCl Treatments	Cobalt treatments	
	Without cobalt Co^-	**With cobalt Co^+**
Control. (cont.)	Co^-	Co^+
65 mMNaCl (S_1)	Co^-	Co^+
135 mMNaCl (S_2)	Co^-	Co^+
200 mMNaCl (S_3)	Co^-	Co^+

2.4 Chemical Determination

Plant samples were dried then grinded and digested for assayment of cobalt as well as P, K, Fe, Mn, Zn, Cu, Na^+, and Cl^- according to [25]. Nitrogen (N) % was estimated using the Microkjeldahal and colorimetrically according to the stannous molybdat [26]. Percentage of N was multiplied by a factor of 6.25 for conversion of total N to protein percent [27]. Leaf fresh samples (0.5 g) were taken for proline content determination according to [28].

3. DNA EXTRACTION AND RAPD AMPLIFICATION CONDITIONS

3.1 RAPD Analysis

DNA was extracted from in-*vitro* grown pineapple leaves after the ending of studied treatments (control and three salinity levels with or without cobalt). Freshly collected young tissues (200 mg) were ground to a fine powder in liquid nitrogen. The genomic DNA was extracted using the Biobasic kit protocol. The concentration and purity of each DNA sample were estimated by 0.8% agarose gel electrophoresis in comparison to HindIII digested λ DNA marker.

3.2 DNA Amplification

Ten-mer primers (Operon Technologies, Alameda, 4USA) were used for polymerase chain reaction (PCR) amplification. RAPD assay was performed as described [29] with some modifications as follows:

3.3 PCR Reaction

It was used in a final volume of 20 µl containing 1X PCR buffer, 2 mM $MgCl_2$, 200 mM dNTPs, 0.25 mM of each primer, 1 unit of Taq DNA polymerase (Promega Inc., USA) and 2 µl (50 ng) template DNA. PCR amplification was performed in PTC-100 PCR version 9.0 from M J Research- USA, programmed for 95°C for 5 min (denaturation), 36 cycles of {94°C for 1 min, 36°C for 1 min and 72°C for 1 min (annealing) and a final extension of 5 min at 72°C. PCR products were analyzed using 1% agarose gel electrophoresis. Gels were stained with 0.5 µg/ml ethidium bromide solution and visualized on a UV transilluminator. The fragments were estimated based on a DNA ladder of 100 bp (Fermentas). The RAPD bands were recorded according to the presence (1) or absence (0) of a

DNA band at the same location on the gel. Data were statistically analyzed by the software Gel Analyzer 3.

3.4 Statistical Analysis

Data were subjected to the analysis of variance (ANOVA) allocate to the factorial in a randomized complete block design according to the procedure out- lined by [30] where the salinity is the main factor and cobalt is the second factor with four replicates (each replicate contains 3 explants/jars). The significant differences between treatments were compared after Duncan [31].

4. RESULTS AND DISCUSSION

4.1 Vegetative Characteristics

The effect of treating pineapple explants cv. Queen with cobalt sulphate to overcome the damage effect of different salinity stress conc.(NaCl) on some vegetative characteristics during two successive seasons *in vitro* are presented in Figs.1, 2 and 3.

4.1.1 Shoots number

Data in Fig. 1 showed a significant effect of exposuring pineapple explants to different levels of salinity as well as the presence of cobalt sulphate. Concerning effect of different NaCl conc., data showed that, increasing salinity from S_1 to S_3 associated with a noticeable increase in shoot number till S_3 which decreased dramatically during both seasons of study. It was clear that, cobalt encouraged shoot multiplication as it recorded the highest significant percentage of shoot number/explant compared with control (cobalt absence) during both seasons regardless the effect of salinity presence. Results in Fig. 1 also revealed a significant interaction between salt stress and cobalt, where cobalt plays an important helping role in pineapple explants to avoid the damage caused by NaCl treatments, whereas explants exposured to S_2 treatment gave the greatest significant number of shoots for both seasons of study with contrary to those received the highest salinity dose (200 mM). Meanwhile, the same conc. missing cobalt scored only (one shoot/explant&2.0) for two successive seasons respectively.

4.1.2 Leaves number

Results in Fig. 2 exhibited the effect of different salinity treatments (NaCl) and cobalt sulphate on the average number of leaves of pineapple explants *in vitro*. As for the effect of salinity stress one can notice that, during both seasons of study, pineapple explants exposured to135 mM NaCl (S_2) resulted in highest significant leaves number /explant whereas increasing salinity level up to 200 mM NaCl, showed the lowest multiplication leaves value during both seasons of study compared with other treatments. Data also indicated that, cobalt presence affected significantly avg. number of leaves, where pineapple explants cultured on MS medium and supplemented with 5mg/l cobalt sulphate scored the greatest number of leaves/explant compared with those cultured on MS medium free from cobalt, although there was no significant differences between presence or absence of cobalt during second season only. Regarding the interaction, it was clear that, cobalt sulphate minimizes the effect of salinity stress in terms of number of leaves as salinity increased the highest number of leaves was observed in explants treated with S_2(which scored three fold no. of leaves than control) . On the contrary, pineapple explant received the same treatment (S_2) but without cobalt resulted in almost half the number of leaves /explant.

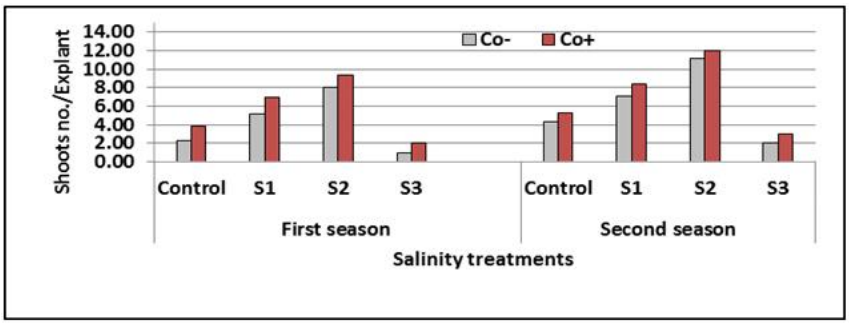

Fig. 1. Effect of different salinity levels of (NaCl) and cobalt on the average shoots number/explant during two successive seasons of pineapple cv .Queen *in vitro*

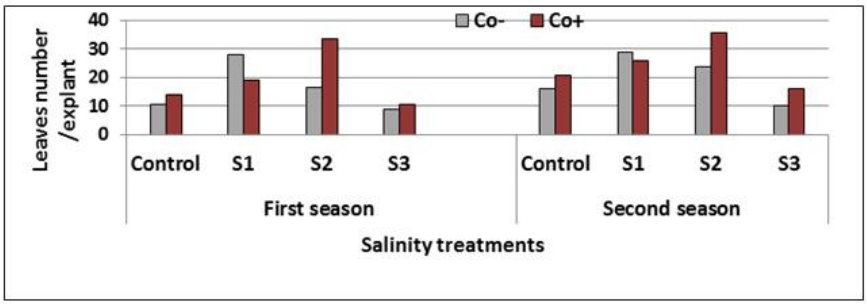

Fig. 2. Effect of different salinity levels of (NaCl) and cobalt on the average leaves number/explant during two successive seasons of pineapple cv. Queen *in vitro*

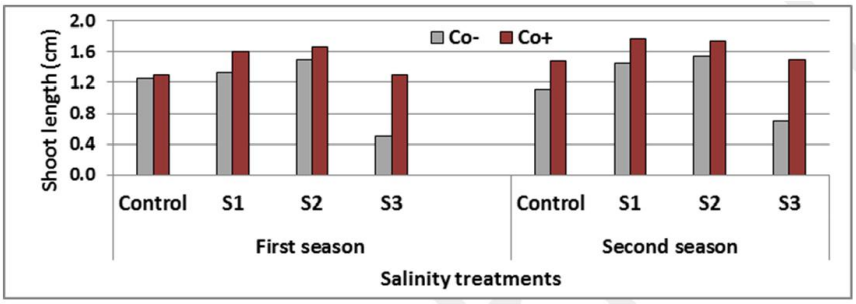

Fig. 3. Effect of different salinity levels of (NaCl) and cobalt on the average shoot length in cm /explant during two successive seasons of pineapple cv. Queen *in vitro*

4.1.3 Shoot length

The effect of exposuring pineapple explants to different NaCl conc. without or after cobalt sulphate treatments on average of shoot length in cm are presented in Fig. 3. Results indicated that, the tallest explants were achieved *in vitro* when pineapple explants were treated with 135 mM NaCl(S_2), while there was no significant differences between $S_1$and S_2 during the second season.Concerning the presence of cobalt sulphate, data cleared that, cobalt sulphate improves explant height as it scored the highest significant shoot length during both seasons.Data also showed a significant interaction between different NaCl conc. and cobalt sulphate .It seems that ,the presence of cobalt could minimize harmful effect of salt stress on the avg. shoot length *in vitro* .Although pineapple explants were under stress of NaCl at 135mM (S_2)but it resulted in the tallest explants for both seasons respectively. On the other hand, the role of cobalt became very clear specially when explants treated with the highest salt stress conc. (S3) after cobalt, they scored (2-3 fold) of explants height compared to those without cobalt.

It is obvious that ,all growth parameters of pineapple explants cv. queen were not affected by increasing NaCl concentrations up to 135 mM, followed by a clearly decrease by 200 mM. This result is supported by the other reports mentioning that photosynthesis process is not always slowed down by salinity treatments but in some cases can be stimulated by low level of salt concentration Therefore, enhanced photosynthesis rate at this level leads to enhancement of water absorption from medium which reflected positively on explant growth parameters as avg.shoot number, leaves number and shoot length [24].

Explants treated with 65 and 135 mM NaCl and responded better may be due to the ability to absorb Na^+ and Cl^-ions into their vacuoles (compartmentalized) to lower the vacuoles water potential, and hence the cytosol can continue absorbing water from the medium [32]. Our results are in agreements with [24], who reported that, the growth of pineapple under tissue culture conditions was not inhibited by salt concentration below than 135 mM NaCl. Salt concentrations at 200 - 250 mM reduced plantlets growth significantly, but did not completely inhibit. Moreover, [33] who found that, increasing seawater levels in proliferation medium increased callus fresh weight as well as shoot multiplication of Jojoba Plants *in vitro*.

[34] Indicated that, the *in vitro* performance in Sri Lankan traditional indica rice varieties can be improved by using the media containing both copper sulphate and cobalt chloride. Similar results have been observed by [13] who cleared that, the presence of cobalt sulfate decreased the negative impact of salt stress as the rate of callus proliferation increased with the presence of cobalt sulfate in the induction medium of rice. [35] demonstrated that, addition of copper sulphate and Cobalt chloride to the medium was most effective for shoots regeneration from callus and enhanced regeneration frequency as well as number of shoots obtained per explant, the best result (7.12 shoot/explant) was obtained by using copper sulphate and cobalt chloride at 2.0 µM. [36] demonstrated that, the plants treated with saline irrigation water containing cobalt resulted in higher plant growth characters and chemical constituents values than those treated with saline irrigation water alone. [37] stated that, all cobalt treatments significantly increased growth and yield parameters, minerals composition and chemical constituents compared with untreated plants.

4.2 Minerals Content

4.2.1 Macronutrients content

The effect of different salinity treatments and cobalt on macronutrients (N, P and K) content of pineapple explants cv. Queen *in vitro*.is presented in Table 2. Results in Table 2 show that, all salinity levels increased N contents compared with control without any significant differences in between .As for P and K contents it was clear that, the greatest content was obtained with explants treated with both S_2 and S_3 (without any significant differences) followed by S_1.On the other hand, pineapple explants pretreated 4 weeks with cobalt before NaCl had the promotive effect on N, P, K, content compared with control (cobalt absence). Concerning the interaction, it was cleared that, cobalt presence scored the highest significant macronutrients content specially after they were subjected to different salinity levels (without any significant differences in-between).

4.2.2 Micronutrients content

Results on Table 3 represented the micronutrient contents as affected by salinity and cobalt treatment. It was cleared that, the same trend of macronutrients content was obtained in micronutrients as all salinity treatments increased micronutrient contents especially (S_3) compared with control. Data also indicated that, the presence of cobalt affected significantly micronutrients content whereas, explants pretreated with cobalt scored the greatest amount of micronutrients. As for the interaction, it was noticed that, the greatest micronutrient content was observed when pineapple explants were treated with cobalt then subjected to the highest level of salinity (S_3).

These results are in good agreement with those obtained by [38] who showed that cobalt significantly increased macro and micronutrients content in groundnut seeds compared with untreated plants. [39] added that cobalt gave a beneficial effect on nutrients status in tomato shoots under different levels of salinity. Moreover, [40,41] reported that, cobalt helped wheat and barley plants to tolerate high salinity and increase plant growth and yield under saline conditions. [13] demonstrated that, cobalt gave a significant promotive effect on the status of macro and micronutrients of rice *in vitro*. [36] reported that, the black seed, *Nigella sativa* L., plants treated with saline irrigation water containing cobalt resulted in higher plant growth characters and chemical constituent's values than those treated with saline irrigation water alone.

Table 2. Effect of salinity treatments (NaCl) and cobalt on the macronutrient contents of pineapple explants cv. Queen *in vitro*

	Macronutrients (%)								
	N			P			K		
	Co-	Co+	Mean	Co-	Co+	Mean	Co-	Co+	Mean
Cont.	0.63e	0.76d	0.70b	0.27c	0.29c	0.28c	1.08c	1.13c	1.11c
S1	0.96c	1.23a	1.10a	0.29c	0.40a	0.35b	1.39b	1.78a	1.59b
S2	1.12b	1.32a	1.22a	0.33c	0.42a	0.37a	1.48b	1.86a	1.67a
S3	1.18b	1.38a	1.28a	0.34b	0.44a	0.39a	1.57b	1.97a	1.77a
Mean	0.97b	1.17a		0.31b	0.39a		1.38b	1.69a	

Means having the same letter/s within each column aren't significantly different at 5% level

4.2.3 Na^+ and Cl^- contents

Data in Fig. 4 indicated that, there was a direct relationship between salinity treatments and pineapple tissue contents of Na^+ and Cl^- ,as pineapple explants treated with different saline levels resulted in high concentrations of potentiality toxic ions such Na^+ and Cl^-. On the other hand, pineapple explants pretreated 4 weeks with cobalt before different NaCl treatments exhibited significantly decrease in Na^+ and Cl^- compared with control. Regarding the interaction it was clear that, as soon as salt stress increased Cl^-% and Na^+% increased until it reached its highest level at S_3 that scored the greatest Cl^-% and Na^+% contents either with Co^+ or Co^-.

These results are in harmony with those obtained by [42] who stated that, irrigation of faba bean with seawater levels of 3.13 and 6.25 dsm^{-1} led to induced higher content of Na^+ and Cl^-. [13] observed that, all cobalt treatments significantly decreased Na^+ and Cl^- contents in rice.

4.3 Protein Content

Results in Fig. 5 revealed direct relationship between salinity treatments and protein content as the highest salinity level(S_3) increased protein content followed by (S_2 and S_1) with no significant differences in between. Moreover, cobalt has a significant positive effect on protein content in pineapple explants. As for interaction, it was noticed that, pretreatment with cobalt increased protein % for all salinity levels with no significant differences in between compared with control.

[24,43] found that, cobalt had beneficial values of both pineapple and groundnut seeds like total proteins, total carbohydrates, starch, soluble sugars and amino acids compared with control. Moreover, [44] found that, cobalt enhanced the content of chemical parameters of tomato fruits such as total soluble solids, total soluble sugar, total protein and vitamin “C”.

4.4 Proline Content

Results in Fig. 6 demonstrated that, proline content significantly increased when it was treated with the highest level of salinity. Moreover, pineapple explants treated with cobalt scored the highest proline content. Concerning the interaction it can be concluded that, cobalt pretreatment increased proline % for all salinity levels with no significant differences in between.

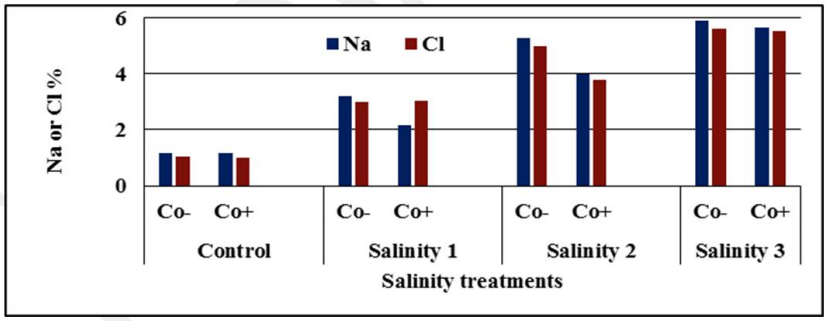

Fig. 4. Effect of salinity levels and cobalt on Na^+ and Cl^- Contents of pineapple cv. Queen *in vitro*

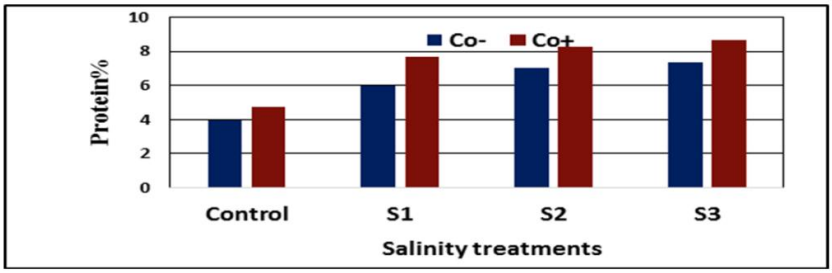

Fig. 5. Effect of different salinity levels and cobalt sulphate on accumulation of protein of pineapple cv. Queen *in vitro*

Our results agreed with those obtained by [45] who showed that leaves proline concentration markedly increased on guava under salt stress. [24] demonstrated that, salinity stress caused an increment in proline accumulation of pineapple *in vitro*. Moreover, cobalt increased proline content at all salinity levels, the obtained result confirms those previously discussed by [40] who cleared that, increasing cobalt concentration, led to an increase in proline content in wheat leaves. In addition [13] and [33] cleared that, salinity stress caused an increment in proline accumulation of rice and Jojoba *in vitro*.

4.5 Molecular Study

Molecular analysis was conducted in order to obtain molecular markers related to salinity stress and salinity combined with cobalt sulfate. Out of ten RAPD primers, five primers (Table 4 and Fig. 7) produced polymorphic bands in all samples. From eight pineapple DNA samples differ only in its applied treatments, 34 RAPD fragments have been generated with molecular weight ranging from 186-1456 bp. The studied primers produced multiple band profiles with a number of amplified fragments vary from 5 for OPD-11 and OPN-16 to 9 with primer OPB-02. From the generated fragments, 12 were monomorphic and 16 were polymorphic, which revealed 64.03% polymorphism that reflected the differences resulted from the culture treatments. The genetic characterization by unique bands was recorded in three primers, one with primer OPD-11, one with primer OPB-02 and five with primer OPD-02. The highest molecular weight of these markers was observed at 1456 bp and could be as a result of using the highest level of salt stress. Three positive unique markers appeared at low molecular weight of 186, 290 and 442 bp (one with primer OPD-11 and two with primer OPD-02). These unique fragments may be refers to the general control of this genotype. The other three unique markers were identified under the second level of salt stress combined with cobalt sulfate (S_2 Co^+) with primer OPD-02 at molecular weight of 334, 730 and 1015 bp.

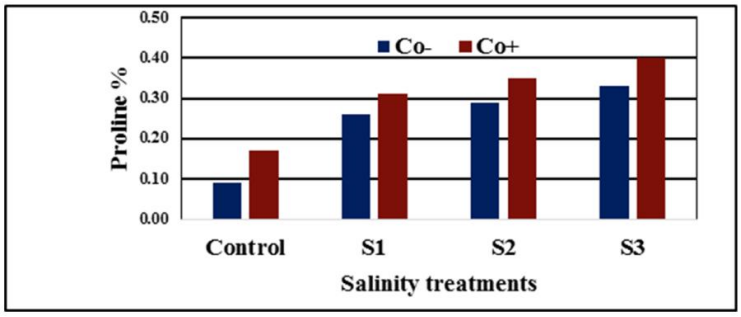

Fig. 6. Effect of different salinity levels and cobalt sulphate on accumulation of proline of pineapple cv. Queen *in vitro*

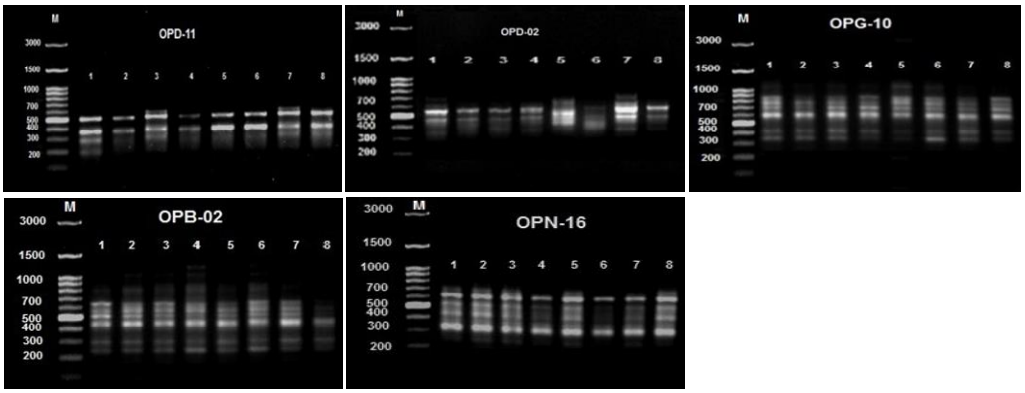

Fig. 7. PCR based RAPD band profiles among different samples of pinapple cv. Queen, M: 100 bp plus DNA ladder (fermentas),1= general control; 2, 3 and 4= samples treated with salt (S1, S2 and S3, respectively); 5= Cobalt sulfate; 6, 7 and 8= samples treated with salt S1, S2, S3 combined with the same concentration of cobalt sulfate respectively)

Table 3. Effect of salinity treatments (NaCl) and cobalt on the micronutrients content and cobalt of pineapple explants cv. Queen *in vitro*

	Micronutrients (ppm)														
	Mn			Zn			Cu			Fe			Co		
	Co-	Co+	Mean	Co-	Co+	Mean	Co-	Co+	Mean	Co-	Co+	Mean	Co-	Co+	Mean
Cont.	15.00e	15.80e	15.40b	19.00d	19.30d	19.15c	13.00d	13.10d	13.05d	23.30e	23.30e	23.30d	0.36d	0.68d	0.52c
S1	17.60c	18.50b	18.50a	21.00c	23.10b	22.05b	14.40c	15.10b	14.75c	24.60d	26.40b	25.50c	0.97d	2.63b	1.80b
S2	17.10c	18.90b	18.00a	22.70b	24.90a	23.80a	14.90b	15.80b	15.35b	25.30c	27.80a	26.55b	1.08c	3.14b	2.11b
S3	16.70d	19.60a	18.15a	23.40b	25.20a	24.30a	15.60a	16.40a	16.00a	26.50b	28.60a	27.55a	1.14c	4.18a	2.66a
mean	16.60b	18.20a		21.53b	23.13a		14.48b	15.10a		24.93b	26.53a		0.89b	2.66a	

Means having the same letter/s within each column aren't significantly different at 5% level

Table 4. The number of amplified bands per primer, number of polymorphic bands and the percentages of polymorphism among the studied treatments of pineapple cv. Queen revealed by RAPD markers

No	Primer name	Sequence 5′----3′	Number of Amplified bands	Band size range (bp)	Number of Monomorphic bands	Number of polymorphic bands	Percentage of polymorphism %
1	OPD-11	AGCGCCATTG	5	186-759	2	2	60.00
2	OPD-02	GGACCCAACC	8	290-1015	1	2	87.50
3	OPG-10	AGGGCCGTCT	7	330-1094	3	4	57.143
4	OPB-02	TGATCCCTGG	9	296-1456	4	5	55.56
5	OPN-16	AAGCGACCTG	5	326-606	2	3	60.00
Total			34		12	16	
Average			6.8		2.4	3.2	64.03%

The observed polymorphism percentage was less than the previous findings of [46,47] and [48] who found 72.0, 70.4 and 75.0 % polymorphism, respectively. This lower percent may be due to the use of one genotype subjected to different treatments. The found unique bands could be used to identify the effects of salt stress with cobalt sulfate. Among the studied primers, OPD-02 that produced the highest number of unique bands is suitable to distinguish the studied genotype under cobalt and salinity. The observed alteration in the DNA fragments of this pineapple genotype exposed to salinity combined with cobalt sulfate may be attributed to the activation of new genes whose transcripts and expression are controlled under salinity stress and cobalt sulfate treatment. DNA markers are innumerable, highly polymorphic and at the same time reliable, not influenced by the environment, lack pleiotropic or epistatic effects [49].

5. CONCLUSION

Cobalt can minimize the harmful effects of salinity stress of pineapple explants that reflected positively on vegetative growth characteristics(shoots number, leaves number and shoot length/expant), minimized Na^+ and Cl^- contents and had a promotive effect on macronutrient (N,P and K) and micronutrients (Mn, Zn, Fe and Cu), proline and protein content. Therefore, it is recommended to treat explants with cobalt sulfate in order to overcome the damage caused by salinity stress. Molecular study of DNA recommends 2 primers suitable to detect positive unique bands, the first primer is OPD-11 and the other is OPD-02.They could be used to identify the effect of salt stress with cobalt sulphate. Some of them related to salt stress and others referred to the pine apple variety itself in this research.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Duval MF, Buso GC, Ferreira FR, Noyer JL, Coppens EG, Hamon P, Ferreira ME. Relationships in Ananas and other related genera using chloroplast DNA restriction site variation. Genome. 2003;46:990-1004.
2. Py C, Lacoeuilhe J, Teisson C. The pineapple: Cultivation and uses. Techniques Agricoles et Productions Tropicales, Maisonneuve and Larose, Paris. 1987;570.
3. Escalona M, Lorenzo JC, Gonzalez B, Daquinta M, Gonzalez JL, Desjardins Y, Borroto CG. Pineapple, *Ananas comosus* (L.) Merr., micropropagation in temporary immersion systems. Plant Cell Rep. 1999; 18:743-748.
4. Shannon MC. The effects of salinity on cellular and biochemical processes associated with salt tolerance in tropical plants. Proc. Plant Stress in Trop. Environ. 1992;56-63.
5. Purohit M, Srivastava S, Srivastava PS. In: Srivastava, P.S. (Ed.), Plant tissue culture and molecular biology: Application and prospects. New Delhi; 1998.
6. Be LV, Debergh PC. Potential low-cost micropropagation of pineapple (*Ananas comosus*). South Africa Journal Botany. 2006;72:191-194.
7. Danso KE, Ayeh KO, Oduro V, Amiteye S, Amoatey HM. Effect of 6-benzylaminopurine and naphthalene acetic acid on *in vitro* production of MD2 pineapple planting materials Ghana. World Applied Sciences Journal. 2008;3(4):614-619.
8. Smith RM. Trace elements in human and animal nutrition. Micronut. News. Info. 1991;119.
9. Young SR. Recent advances of cobalt inhuman nutrition. Victoria M.C. Canada. Micronutrients News. 1983;313.
10. Atta-Aly MA, Shehata NG, El-Kobbia TM. Effect of cobalt on tomato plant growth and mineral content. Annals Agric. Sci. 1991; 36:617-624.
11. Helmy Laila M, Nadia Gad. Effect of cobalt fertilization on the yield, quality the essential oil composition of parsley leaves. Arab Univ. J. Agric. Sci. 2002;10(3):779-802.
12. Nadia Gad. Response of groundnut *(Arachis hypogaea)* plants to cobalt and molybdenum mixture. Middle East Journal of Applied Sciences. 2012;1:19-25.
13. Attia SAA, Nadia Gad, Abdel-Rahman HM, Shenoda JE, Rizkalla Aida A. *In-vitro* enhancement of salinity tolerance in rice

using cobalt sulfate. World Applied Sciences Journal. 2014;31(7):1311-1320.
14. Angelov M, Tsonev T, Dobrinov K, Velikova V, Stoynaova T. Changes in some photosynthetic parameters in pea plants after treatment with cobalt. Hytosynthetica. 1993;8:289-295.
15. Stewart, S. E. Effect of cobalt on tomato plants under saline irrigation. Hort. Sci. 2001;6:82-85.
16. Shanon MC. Effect of cobalt on tomato plants grown under salinity conditions. J. Amer. Soc. Hort. Sci. 2002;12:644.
17. Nadia Gad. Interactive effect of cobalt and salinity on tomato plants. 1-some physiological parameters as affected by cobalt and salinity. Research Journal of Agriculture and Biological Sciences. 2005a;1(3):270-276.
18. Welsh J, Mcclell M. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 1990;18:7213-7218.
19. Rahman M Ur, Malik TA, Chowdhary MA, Iqbal MJ, Zafar Y. Application of random amplified polymorphic DNA (RAPD) technique for the identification of markers linked to salinity tolerance in wheat *(Triticum aestivum* L.*)*. Pak. J. Botany. 2004;36:595-602.
20. Younis RAA, Ahmed MF, EL-Menshawi MM. Molecular genetic markers associated with salt tolerance in grain sorghum. Arab J. Biotech. 2007;10:249-258.
21. Abdel-Hamid AME. Physiological and molecular markers for salt tolerance in four barley cultivars. European Scientific Journal. 2014;3:252-272.
22. Saleh B. Salt tolerance evaluation in cotton (*Gossypium hirsutum* L.) using RAPD marker. Journal of Stress Physiology & Biochemistry. 2015;11:11-20.
23. Murashige T, Skoog FAA. Revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant. 1962;15:473-497.
24. Hasan SMZ, Abdallah NS. Effect of salinity on growth, Proline accumulation and Malate content of pineapple (*Ananas comosus* L. *Merill*.) Under Tissue Culture Condition. Malays. Appl. Biol. 2007;36:57-63.
25. Cottenie A, Verloo M, Kiekens L, Velgh G, Camerlynck R. Chemical Analysis of Plant and Soil. State Univ. Ghent. 1982; 44-45.
26. A.O.A.C. Association of Official Agricultural Chemists. In Hortwitz, W., Latimer, G.W. (ed.): Official Methods of Analysis. 18th. Gaithersburg; 2005.
27. Kelly JD, Bliss FA. Habitability estimated of percentage seed protein and available methionine and correlations with yield in dry bean. Crop Science. 1975;15:753-757.
28. Bates LS, Walden RP, Teare ID. Rapid determination of free proline for water stress studies. Plant and Soil. 1973;39: 205-208.
29. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 1990;18:6531-6535.
30. Dospekhov BA. Field experimentation. Statistical procedures. Mir Publishers, Moscow.
31. Dunn OJ. Multiple comparisons using rank sums. Technometrics. 1964;6:241-252.
32. Parida AK, Das AB. Salt tolerance and salinity effects on plants: A review. Ecotoxicology and Environmental Safety. 2005;60:324-349.
33. Taha RA. Effect of growth regulators and salinity levels on *in vitro* cultures of jojoba. World Applied Sciences Journal. 2014;31: 751-758.
34. Amarasinghe AAY. Effect of copper sulphate and cobalt chloride on *in vitro* performance of traditional Indica rice (*Oryza sativa* L.). The Journal of Agricultural Sciences. 2009;4:132-141.
35. AL-Mayahi AMW. Effect of copper sulphate and cobalt chloride on growth of the *in vitro* culture for date palm (*Phoenix dactylifera* L.) cv. Ashgar. American Journal of Agricultural and Biological Sciences. 2014; 9:6-18.
36. Khalid KA, Sheddeed MR. The effects of saline irrigation water and cobalt on growth and chemical composition in *Nigella sativa*. Nusantra Bioscience. 2014;6:146-151.
37. Nadia Gad, Bekbayeva L, Surif M. Effect of cobalt on *Moringa olifera* growth yield quantity and quality. International J. of Sci. Res. 2015;4:82-85.
38. Jana PK, Karmakar S, Ghatak S, Barik A, Naybari A, Souda G, Mukher AK, Saren K. Effect of cobalt and rhizobium on yield, oil content and nutrient concentration in irrigated summer groundnut. Indian J. Agric. Sci. 1994;630-632.
39. Nadia Gad. Interactive effect of cobalt and salinity on tomato plants. I-growth and Mineral composition as affected by Cobalt

and salinity. Research Journal of Agriculture and Biological Sciences. 2005b;1:261-269.
40. Nadia Gad, Abd El-Moez MR, Hala Kandil. Barley response to salt stress at varied levels of cobalt II. Some physiological and chemical characteristics. Journal of Applied Sciences Research. 2011a;7: 1447-1453.
41. Nadia Gad, Hala Kandil. Maximizing the tolerance of wheat plants to soil salinity using cobalt. π some physiological and chemical characteristics. Journal of Applied Sciences Research. 2011b;7: 1551-1557.
42. Sadak MSH, Abdelhamid MT, Schmidhalter U. Effect of foliar application of amino acid on plant yield and some physiological parameters in Beans plants irrigated with seawater. Acta Biologica Colombiana. 2015;20(1):141-152.
43. Vijayarengan P, Abdul- Jaleel C, Chang-Xing Z, Jayakumar K, Azooz MM. Biochemical variation in Groundnut under application. Global. Journal of Molecular Science. 2009;4:19-22.
44. Nadia Gad, Hala Kandil. Influence of cobalt on phosphorus uptake, growth and yield of tomato. Agriculture and Biological J. of North Am. 2010;1(5):1069-1075.
45. El-Hefnawy SM. Physiological studies on guava. PhD. Thesis. Fac. Agric. Zagazig Univ., Egypt; 1986.
46. Sripaoraya S, Blackhall NW, Marchant R, Power JB, Lowe KC, Davey MR. Relationships in pineapple by random amplified polymorphic DNA (RAPD) analysis. Plant Breeding. 2001;120:265-267.
47. Popluechai S, Ont S, Eungwanichayapant PD. Relationships between some Thai cultivars of pineapple *(Ananas comosus)* revealed by RAPD analysis. Songklanakarin. J. Sci. Technol. 2007; 29(6):1491-1497.
48. Roostika I, Khumaida N, Ardie SW. RAPD analysis to detect somaclonal variation of Pineapple *in-vitro* culturers during micropropagation. Biotropia. 2015;22:109-119.
49. Grover S Y Singh, Pareek N, Malik CP. DNA fingerprinting: Approaches and applications with emphasis on random amplified polymorphic DNA (RAPD). *The* Journal of Plant Science Research. 2012; 28(1):1-14.

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