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In-vitro Enhancement of Salinity Tolerance in Rice Using Cobalt Sulfate

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Abstract: This investigation was conducted to study the effects of cobalt sulfate on decreasing the negative salt stress effects. Calli of three Egyptian rice cultivars were obtained on LS medium supplemented with cobalt sulfate (5 mg/l), in addition to control treatment. The rate of callus proliferation increased with the presence of cobalt sulfate in the induction medium. Different treatments were made to study the effects of salinity, cobalt sulfate and the combination between them on the studied parameters (callus dry weight, free proline content, macronutrients, micronutrients and plantlets regeneration). Results showed that, under salinity stress, decreasing in dry weight and regeneration capacity, in contrast, an increasing in free proline accumulation was observed. The presence of cobalt sulfate decreased the negative impact of salt stress that observed in these indicators. Also, the levels of macro and micronutrients that affected by salt stress were not occurred in the presence of cobalt sulfate. RAPD studies were conducted using eight primers to find out the differences among them by occurrence of polymorphic bands due to the studied treatments. Distinguished specific bands identified salt stress and cobalt treatments effects on the studied genotypes. It could be concluded that, exposure to cobalt sulfate before exposure to salt stress led to reducing its harmful effects on the studied parameters.

Key words: Cobalt sulfate · Salinity Tolerance · Salt stress

INTRODUCTION

Rice belongs to the genus *Oryza*, which consists of 22 wild species and two cultigens. Although, uncountable numbers of subspecies are available, mainly 3 subspecies of *Oryza sativa* are more important and more common. Rice is one of the most important cereal crops and a major crop consumed by most of human population around the world. Rice was planted on about 11% of the Earth's cultivated land area over a wide number of ecosystems [1]. Rice accounts more than 21% of the basic calories needs of world's population and up to 76% of the calories intake of South East Asia [2]. Globally, rice is the predominant dietary energy source for 17 countries in Asia and the pacific, 9 countries in North and South America and 8 countries in Africa. Rice provides 20% of the world's dietary energy supply, while wheat supplies 19% and maize 5% (FAO).

Salinity is the major environmental stress that adversely affects the plant productivity. Significant reduction of yield in many rice genotypes was observed at a salinity level of 8.5 ds m^{-1} besides the reduction of many yield contributing parameters *viz.,* chlorophyll content, productive tillers plant and fertility percentage [3]. About 6.5% (831 million ha) of the world's total area (12.78 billion ha) is affected by salt in soils (FAO). The area is still increasing due to many factors including climate change, rise in sea levels, excessive irrigation without proper drainage in inlands, underlying rocks rich in harmful salts *etc*. Hence, great efforts are needed to produce varieties with high tolerance level to salinity stress.

Cobalt, a transition element, is an essential component of several enzymes and co-enzymes. It has been shown to affect growth and metabolism of plants, in different degrees, depending on the concentration and status of cobalt in rhizosphere and soil. Nadia [4]

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demonstrated that, cobalt helps tomato plants to resist stresses caused with high salinity. Increasing cobalt applications in growth media leads to increasing cobalt content in shoots and roots of both salt-tolerant and salt-sensitive tomatoes. Cobalt concentrations up to 15 ppm resulted an increase in Ca^{++} and Mg^{++} content of shoots and roots while Na^{++} and Cl^{-} concentrations decreased. Bedoglio [5] pointed that low level of applied cobalt increased the leaf area as well as the size of chloroplasts in potato plants.

Tissue culture techniques are becoming increasingly popular as an alternative means of plant vegetative propagation, mass production of chemicals and genetic engineering [6]. Liu [7] reported that, callus culture is used as an *in-vitro* technique for biochemical and physiological studies in response to stress at the cellular level. At the callus level, two popular markers are used for salt tolerance evaluation; first is the free proline accumulation in tissues, where, the more proline accumulated at callus level, the more salt tolerant cultivar it is [8-11]. Second is the accumulation of Na^{+} and K^{+} in tissues and ratio of them. With salinity increasing in the medium, a less increase in Na^{+}/K^{+} ratio was observed, indicates that the variety is tolerant [10, 12].

RAPD-PCR based technique, using arbitrary primers is used to detect changes in the DNA sequence at sites in the genome, which anneal with the primer. RAPD-PCR also used to identify genes controlling yield and some stress traits [13], it is characterized by its low technical input and small quantity of plant DNA needed for analysis [14]. In rice, RAPD marker is a reliable, efficient and effective technique for the determination of the genetic variation [15, 16]. The objectives of this work were studying the effects of cobalt sulfate on salinity stress tolerance in three Egyptian rice cultivars at cellular and molecular levels.

MATERIALS AND METHODS

Plant Materials: Mature grains of three Egyptian rice varieties (*Oryza sativa* L.) namely Giza 177, Giza178 and Sakha 104, were kindly supplemented from Agriculture Research Center, Egypt. Seeds were manually dehusked and washed with current tap water for one hour, then surface-sterilized with 70 % Ethanol (V/V) for 10 min., 0.1 % Mercuric Chloride for 3 min. and finally by three rinses with sterilized distilled water.

Embryo Culture: Mature embryos were isolated and placed on LS medium [17] supplemented with 2 mg/l 2,4-D., 5 mg/l cobalt sulfate, 1 gm/l casine hydrolysate, 20 gm/l sucrose, 40 mg/l thiamine HCL, 150 mg/l asparagine and gelled with 2.5 gm/l gel-rite after adjusting the pH at 5.7. The medium for control cultures was completely the same excepting the addition of cobalt sulfate. Five embryos cultured in each jar for each treatment, kept in dark at 24-26 ºC for 3 weeks.

A) Effects of cobalt sulfate on callus induction: Percentage of callus induction was measured as the number of calli obtained/total number of seeds cultured x 100, for studying cobalt sulfate effect on callus induction.

The calli with good sizes and appearance were transferred to the studied treatments medium, as shown in the following diagram:

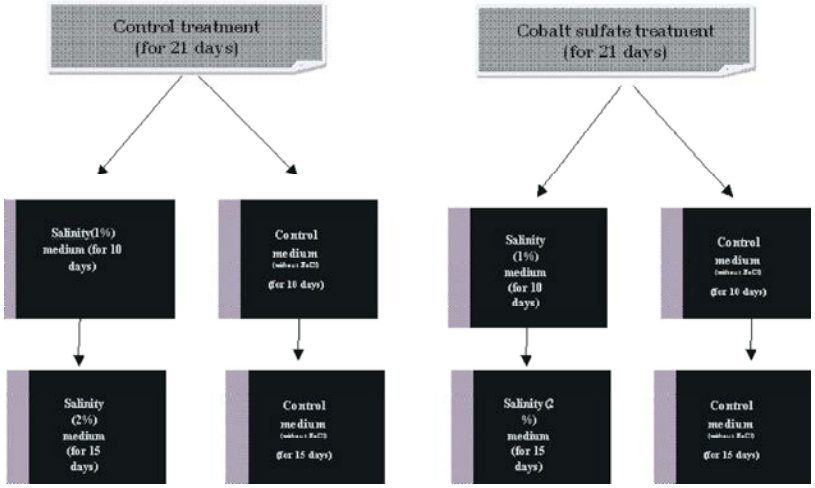

Table 1: List of random primers and their nucleotide sequences used in RAPD analysis.

No.	Primer	Sequence	No.	Primer	Sequence
1	OPX-11	5``-GGAGCCTCAG-3`	5	OPD-13	5`-GGGGTGACGA-3`
2	OPT-08	5`-AACGGCGACA-3`	6	OPW-04	5`-CAGAAGCGGA-3`
3	OPC-19	5`-GTTGCCAGCC-3`	7	OPN-06	5`-GAGACGCACA-3`
4	OPX-17	5`-GACACGGACC-3`	8	OPC-15	5`-GACGGATCAG-3`

The following characteristics were measured as salinity tolerance indicators under all studied combinations; control and the higher salt concentration (2 %).

B) Dry weight: For studying the effect of cobalt, salinity and the combination between them on the dry weight, one gram of fresh callus of each treatment was dried at 60 ^{0}C for three hours; dry weight percentage was calculated as dry weight / fresh weight x 100.
C) Free proline quantification: Estimation of free proline content from calli was done for all studied treatments, according to [18].
D) Measurements of nutritional contents: Macronutrients (N, P, K, Ca, Mg. Na and Cl) and micronutrients (Fe, Mn, Zn and Cu) as well as cobalt were determined from callus tissues of all studied treatments according to [19].

DNA Extraction and RAPD Amplification Conditions: DNA was extracted from rice calli induced in different treatments (control $C^- S^-$, salinity $C^- S^+$, cobalt sulfate $C^+ S^-$ and a combination between them $C^+ S^+$) to be used for RAPD analysis. Freshly collected calli (200 mg) were ground to a fine powder in liquid nitrogen. The genomic DNA was extracted using the Biobasic kit protocol. Isolated DNA was quantified on spectrometer (Bio-mate 3). RAPD analysis was performed using eight random primers (Table 1) produced from Operon Technologies (Metabion International AG). RAPD assay was performed as described [20] with some modifications.

PCR reaction 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 2 min at 72°C. PCR products were analyzed using 1% agarose gel electrophoresis and visualized with ethidium bromide staining. The sizes of the fragments were estimated based on a DNA ladder of 100 bp (Fermentas). The RAPD markers were scored on the presence (1) or absence (0) of amplification products for each treatment and genotype.

Differentiation of calli: Survival rice calli were transferred to regeneration medium (LS), supplemented with 0.1 mg/l 2,4-D., 15 gm/l sucrose and gelled with 2.5 gm/l with gel-rite, pH 5.7, calli with clearly differentiated shoots were scored for all studied treatments as regenerating percentage.

RESULTS AND DISCUSSION

Effect of Cobalt Sulfate on the Rate of Callus Induction: Yellowish and friable embryogenic calli were obtained within 15 days in all studied varieties. In control treatment, Sakha 104 showed the highest callus induction percentage (95%), while, Giza 177 and 178 showed 90 and 92 % respectively, this indicated genotypic differences for their callus induction ability. As shown in Table (2), callus induction percentage was increased in the presence of cobalt sulfate in Giza 177 and 178.

It is noticed that, calli on medium with cobalt sulfate were with bigger sizes and better viability in all studied varieties (Fig. 1). Cobalt is an essential `microelement to the physiological activities in cells. Furthermore, [21] reported that, cobalt is a transition element which is an essential factor in many enzymes and co-enzymes. It affects the growth and metabolism of plants in various degrees depending on the concentration of cobalt in the surrounding medium. Amarasinghe [22] found that the rate of callus proliferation in some rice varieties was significantly higher in the medium supplemented with 5 mg/l copper sulfate and 5-10 mg/l cobalt chloride together. He added that, a high quality granular calli were produced in the medium supplemented with 5 mg/l copper sulfate.

Table 2: Callus induction percentage for the three rice varieties under control and cobalt sulfate treatments.

Varieties	Control	Cobalt sulfate 5 mg/l
Giza 177	90	92
Giza 178	92	95
Sakha 104	95	90

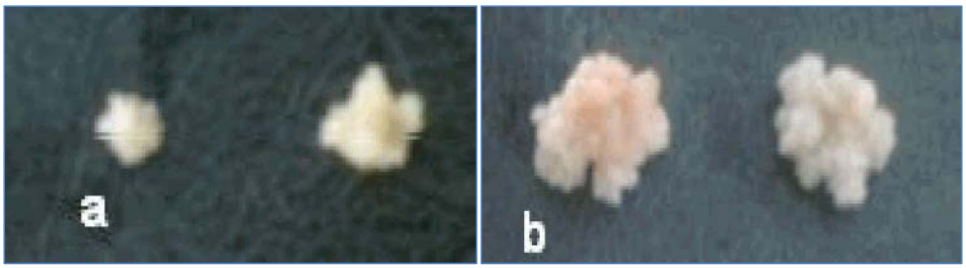

Fig. 1: Effect of cobalt sulfate on callus size, a) callus produced from control treatment, b) callus produced from cobalt sulfate treatment.

Effect of Cobalt Sulfate on Callus Dry Weights: Comparing with control treatment $C^- S^-$, a reduction in callus dry weight due to salt stress $C^- S^+$ was observed in the varieties Giza 177 and 178. In Sakha 104, an increase in callus dry weight percentage (80.9) under salt stress $C^- S^+$ was observed but not enough to reach the increment due to either cobalt treatments $C^+ S^-$ or $C^+ S^+$ that reached to 95.3 and 92,2 respectively.

The percentage of dry weight could be used for the identification of tolerant and sensitive rice varieties. Rahmanzadeh [23] evaluated 4 rice cultivars both in pots and under *in-vitro* conditions, they identified that the Tichung-65 genotype was the most sensitive cultivar based on seedling dry weight, wet weight as well as shoot and root length. Our results indicated that the genotype Sakha 104 showed the highest values for all studied characters (related with salt tolerance) than the other two sensitive genotypes. It means that, cobalt sulfate enhance salt tolerance and it is genotype dependent.

Furthermore, the presence of cobalt sulfate alone $C^+ S^-$ exhibited the highest percentages of callus dry weight in all studied varieties than all other treatments; it reached to 68.3, 90.2 and 95.3 for Giza 177, Giza 178 and Sakha 104, respectively. In addition the presence of cobalt sulfate with salinity $C^+ S^+$ caused an increment in dry weight of all studied genotypes compared with calli grown on control induction medium $C^- S^-$ and $C^- S^+$. Our results were in agreement with the previous studies of [4, 24] who reported that, under salinity conditions, cobalt sulfate increased the dry matter in tomato and wheat plants. The results showed that, not only cobalt sulfate but also cobalt combined with salt stress caused an increase in dry weight that reflects the effect of cobalt in increasing salt tolerance [4, 24].

Effect of Cobalt Sulfate on Proline Accumulation in Callus Tissues: In Table (3), variation in free proline content was observed among the studied genotypes.

Table 3: Effects of cobalt sulfate as single or combined with salinity on calli dry weight percentages and free proline content of rice calli.

Variety	Treatment	D.W %	Proline content (μg/100 mg)
Giza 177	$C^- S^-$	63.8	0.21
	$C^- S^+$	59.6	0.27
	$C^+ S^-$	68.3	0.23
	$C^+ S^+$	60.8	0.38
Giza 178	$C^- S^-$	88.6	0.27
	$C^- S^+$	68.5	0.35
	$C^+ S^-$	90.2	0.26
	$C^+ S^+$	86.7	0.43
Sakha 104	$C^- S^-$	71.9	0.25
	$C^- S^+$	80.9	0.38
	$C^+ S^-$	95.3	0.29
	$C^+ S^+$	92.2	0.45

In control treatment $C^- S^-$, Giza 178 gave the highest proline content (0.27 μg/100 mg), while, Giza 177 exhibited the lowest value. Compared with control treatment, the salinity stress caused an increment in proline accumulation in all studied varieties. This increment reached to 1.29, 1.30 and 1.52 fold times for Giza 177, Giza 178 and Sakha 104, respectively. Our results were in agreement with the earlier findings of [25, 26]. Also, increasing the accumulation of proline due to salinity stress has been reported in many crops such as Lentil [27], Vigna [28], Wheat [29] and soyabeen [30]. Accumulation of organic solutes such as proline under stress conditions are among those non-specific mechanisms [31]. Proline was found to reduce the toxic effects of NaCl for helical destabilization at DNA replication [32].

Also, a higher increment was observed in calli grown on induction medium containing cobalt sulfate $C^+ S^-$ in Giza 177 and Sakha 104 genotypes. Thus, cobalt sulfate enhanced the increment of proline accumulation in these genotypes. Numerous studies emphasized that, proline content in higher plants increases under different environmental stresses, this increment in stressed plants has a protective function.

Table 4: Effect of cobalt on rice cultivars nutrient status under saline condition.

		Macronutrients%			Micronutrients (ppm)					
Variety	Treatment	N	P	K	Mn	Zn	Cu	Fe	Na	Cobalt (ppm)
Giza177	C^-S^-	1.43	0.528	1.29	18.0	15.19	7.89	24.7	2.14	1.66
	C^-S^+	1.38	0.521	1.16	19.1	15.6	7.58	24.0	5.42	0.98
	C^+S^-	1.51	0.541	1.38	18.5	15.41	8.14	25.4	2.03	4.26
	C^+S^+	1.69	0.556	1.46	19.3	15.79	9.06	26.8	3.99	5.59
Giza178	C^-S^-	1.48	0.539	1.24	18.3	16.8	8.02	25.6	2.16	0.37
	C^-S^+	1.42	0.530	1.19	17.5	16.0	7.90	24.8	5.53	0.98
	C^+S^-	1.56	0.546	1.28	18.8	17.6	8.28	26.4	2.10	4.05
	C^+S^+	1.72	0.558	1.35	19.3	18.9	9.17	27.9	4.08	5.30
Sakha 104	C^-S^-	1.49	0.541	1.28	19.3	16.9	8.12	25.2	2.21	1.49
	C^-S^+	1.45	0.535	1.22	18.2	16.5	7.93	24.5	5.61	0.98
	C^+S^-	1.57	0.548	1.33	18.6	17.3	8.31	26.7	2.15	4.18
	C^+S^+	1.73	0.561	1.40	19.9	18.4	9.25	27.5	4.19	5.41

The most important result obtained in the present study is that treated calli with cobalt sulfate in callus induction medium before exposing to salinity $C^+ S^+$, exhibited the highest values in proline content accumulation. Comparing with control treatment, the presence of both cobalt and salinity caused an increase in proline accumulation reached to 1.81, 1.59 and 1.80 fold times, for Giza 177, Giza 178 and Sakha 104, respectively. It could be noticed that, cobalt sulfate alone but more when combined with salinity stress enhanced the accumulation of proline in callus tissues.

Trends of Macro and Micronutrients under Cobalt Sulfate and Salt Stress Macronutrients (N, P and K): Results in Table (4) showed the nutritional status of rice calli under all studied treatments. In comparing with control $C^- S^-$, salt stress alone $C^- S^+$ decreased the content of N, P and K in rice callus of the three varieties (Giza 177, Giza 178 and Sakha 104). While in the presence of cobalt $C^+ S^+$, an increment in the N, P and K content were observed in all studied genotypes. Thus, cobalt addition has significant beneficial effects on the status of macronutrients (N, P and K) and it is genotype independent. These results are in harmony with (33) at the whole plant level. In wheat plants, [24] reported that, cobalt in general increased the content of N, P, K and Ca. The presence or retention of K^+ in rice callus was a key factor for salt tolerance as it was found to be positively correlated with growth while proline was probably the last metabolic device that rice calluses opted for when expose to salt stress [25].

Data presented in Table (4) clearly indicated that under saline condition, sodium content resulted the greatest figures in all rice cultivars. Cobalt addition in plant media reduced Na^{++} content. These results agree with those obtained by [24] who showed that salinity condition increased the content of Ca, Mg, Na and Cl content. All cobalt treatments significantly decreased Na^{++} and Cl contents in wheat shoots. These results are in agreement with [34].

Micronutrients (Mn, Fe, Zn and Cu): In calli that produced on control induction medium, salinity stress $C^- S^+$ affected plant contents of Mn, Fe, Zn and Cu in all rice genotypes. While, the effect of salinity stress was with lesser effect in addition of cobalt sulfate to induction medium $C^+ S^+$ by increasing the levels of micronutrients in all studied genotypes. Thus, Cobalt gave a significant promotive effect on the status of micronutrients (Mn, Fe, Zn and Cu). Our results agree with those obtained at whole plant level by authors [4, 24, 35].

Generally, Sakha 104 (salt – tolerant) gave the highest mineral composition followed by Giza 178, while Giza 177 recorded the lowest one. These data are in harmony with those obtained by authors [4] who demonstrated that, cobalt increased nutritional status in tomato cultivar Edcawy (salt tolerant) compared with Mony Maker (salt sensitive) tomatoes.

Effect of cobalt sulfate and salinity on Na^+ / K^+ ratio: Na^+ / K^+ ratio in leaves of crop plants can be used as an important indicator of salinity tolerance and breeding for low ion accumulation could be a simple way to improve salt tolerance. In general, salt tolerant varieties of rice maintain low concentration of Na^+ in their leaves than those of salt sensitive lines, when exposed to salt stress [36]. Our results showed that, under salt stress, Na^+ / K^+ ratio was decreased in the presence of cobalt to induction medium $C^+ S^+$, it reached to 2.73, 3.02 and 2.99 in

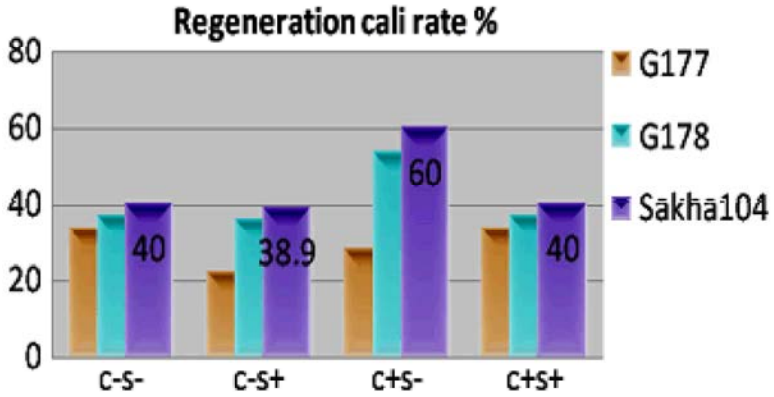

Fig. 2: Plant regeneration percentages under different treatments of cobalt sulfate and salinity for the three rice genotypes.

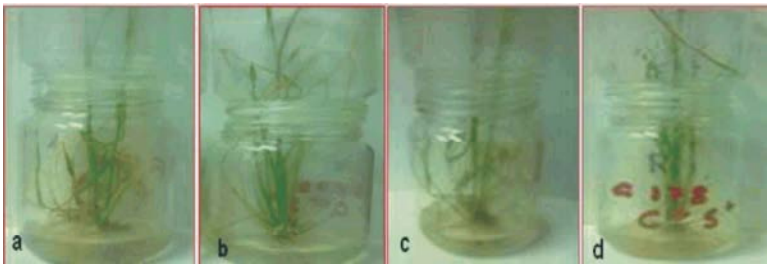

Fig. 3: Plantlets produced calli from the studied treatments; a) control ($C^- S^-$), b) cobalt sulfate ($C^+ S^-$), c) salinity ($C^- S^+$) and d) salinity combined with cobalt sulfate of Giza 178 rice cultivar.

comparing to control treatment $C^- S^+$ which gave a high Na^+ / K^+ ratio in all genotypes (4.67, 4.65 and 4.6) for Giza 178, Giza 177 and Sakha 104. Thus, under salt stress cobalt sulfate caused a significant decrease in Na^+ / K^+ ratio. The genotypes with low Na/ K^+ ratio was highly tolerant and the susceptible one had high Na^+ / K^+ ratio [37].

Plantlet Regeneration of Embryogenic Calli: This experiment was performed to study the effect of cobalt sulfate, salinity and the combination of cobalt with salinity on the regeneration capacity. The survival produced calli from control and cobalt sulfate treatments were transferred to regeneration medium. The plant regeneration capacity was measured on the basis of plant formation.

The salt stress in the culture medium $C^- S^+$ decreased the percentages of plant regeneration in all studied genotypes (22.18, 36.33 and 38.9), when comparing it with control treatment which reached to 33.5, 37.0 and 40% for Giza 177, Giza 178 and Sakha 104, respectively. The effects of salinity on the regeneration capacity have been described by several authors [38, 39, 40]. It could be concluded that, the studied genotypes differed in their ability to tolerate high salinity depending on the genetic performance of the genotype (Fig. 2, 3). The presence of cobalt sulfate in the induction medium $C^+ S^-$ increased the regeneration capacity in two genotypes (Giza 178 and Sakha 104) to 1.45 and 1.5 fold times, respectively, in compared with control treatment. While, the genotype Giza 177 showed a reduction in plant regeneration in the presence of cobalt sulfate in induction medium.

It was observed that, the presence of cobalt in culture medium decreased the negative effect of salinity on regeneration capacity. This effect was observed as a high regeneration capacity under salt stress in all studied genotypes. The increasing in regeneration was reached to 1.19, 1.29 and 1.45 fold times for Giza 177, Giza 178 and Sakha 104, respectively, in compared with $C^- S^+$.

RAPD Analysis: RAPD profiles of the three rice genotypes and their treatments under study were compared to find out the differences among them by occurrence of polymorphic bands due to salinity, cobalt sulfate and salinity with cobalt sulfate. The size of generated bands ranged between 190-2001 bp (Table 5). The eight primers produced multiple band profiles with a number of amplified DNA fragment vary from 3 for OPC-19 to 12 for OPT-08.

Table 5: The number of generated bands per primer, number of polymorphic bands and the percentages of polymorphism among the three rice genotypes and their treatments revealed by RAPD markers.

No	Primers	Band size range (bp)	Generated bands	Polymorphic bands	% polymorphism
1	OPX-11	296-715	4	3	75
2	OPT-08	230-1724	12	10	83.33
3	OPC-19	396-836	3	1	33.33
4	OPX-17	235-973	4	4	100
5	OPD-13	337-818	7	5	71.43
6	OPW-04	190-1590	7	3	42.86
7	OPN-06	340-1736	6	3	50
8	OPC-15	266-2001	10	9	90
Total	-	-	53	38	-
Average	-	-	6.62	4.75	68.24

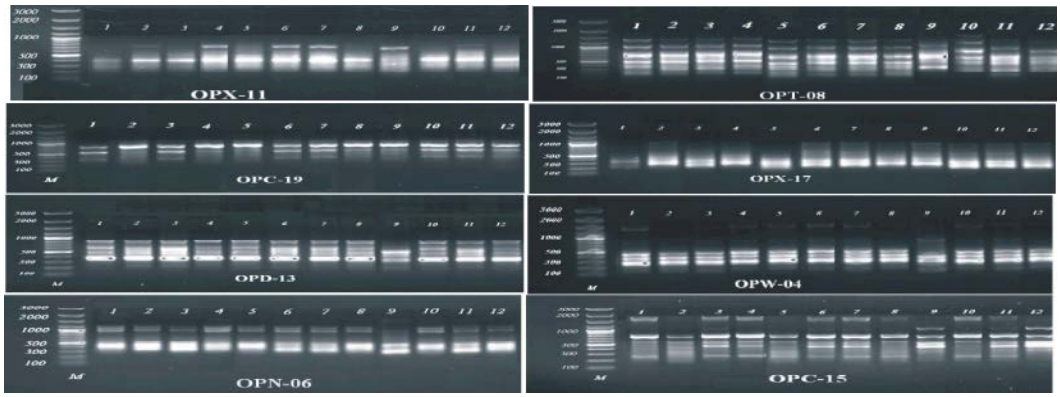

Fig. 4: RAPD banding patterns among the three rice genotypes using eight random primers, M: 100 bp plus DNA ladder (fermentas), 1-4: the rice cultivar Giza 177 with its studied treatment; 1=control ($C^- S^-$), 2=salinity treatment ($C^- S^+$), 3=cobalt sulfate treatment ($C^+ S^-$) and 4=salinity with cobalt sulfate ($C^+ S^+$). From 5-8, 9-12 are the rice genotypes Giza 178 and Sakha 104, respectively, with the same treatments.

Out of 53 total generated bands, 15 were monomorphic and 38 were polymorphic, which revealed 68.24% polymorphism that reflected genetic diversity among the rice genotypes and the differences resulted from the studied treatments (Fig. 4). The level of polymorphism is comparable to that reported by [41] who found 68.94% polymorphism when evaluated 30 rice cultivars differ in their salt tolerance. Choudhury [42] estimated the genetic similarity among 48 cultivars of Indian rice using 58 RAPD primers and found 67.5% polymorphism. However, more polymorphism percentages were reported [43]; [44]; [45] and [15] reached to 80%, 72.9%, 89.4 and 96%, respectively. Differences in polymorphism percentages could be referred to using different rice genotypes in these studies.

Genetic identification by unique bands: Four positive (one with each of primers OPX-11, OPT-08 and two with the primer OPW-04) and two negative (with primers OPD-13 and OPN-06) unique markers were characterized the control treatment $C^- S^-$ of the Sakha 104 genotype. These unique fragments may be contributed to this genotype.

Salinity treatment $C^- S^+$ of the Sakha 104 genotype also produced one specific band with primer OPT-08 with molecular weight of 1724 bp. Two unique bands were produced with the primers OPT-08 and OPC-19 identified the cobalt sulfate $C^+ S^-$ treatment of Sakha 104 genotype with molecular weight of 289 and 1090, respectively. Also, a positive unique band was identified under salinity with cobalt sulfate treatment $C^+ S^+$ of the same genotype with molecular weight of 395 bp. The other two genotypes (Giza 178 and Giza 177) did not exhibit any unique markers.

The band with molecular weight of 1090 bp with primer OPC-15 was found in all cobalt treatment $C^+ S^-$ of all genotypes. Thus, this band could be used to identify effect of cobalt treatment on the studied genotypes. Among the tested primers, four primers OPX-11, OPT-08

(which produced the highest number of unique bands), OPW-04 and OPC-15 exhibited positive unique markers, while two (OPD-13 and OPN-06) revealed negative unique markers. These primers suitable to distinguish rice cultivars under cobalt and salinity as single or combined.

CONCLUSION

Soil salinity is a major problem that limits crop yield and restricts use of land previously uncultivated. It is concluded that using cobalt sulfate decreased the harmful effects caused by salt stress as cobalt is an essential component of several enzymes and co-enzymes. We recommended add cobalt sulfate in rice embryo tissue culture to enhance different characters related to salt stress tolerance.

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