Morphological and Physiological Response of Potato for Radiation and Salt Stress *in vitro*

الاستجابة المظهرية والفسيولوجية للبطاطا للتشعيع والشد الملحي خارج الجسم الحي

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Abstract

The experiments were undertaken to study the response and behavior of two mutant clones of Potato genotype for salt stress by exposing it to different salt levels of sodium chloride (with electrical conductivity of 8, 10, 12 dS m-1) and compare with those in the control treatment 6 dS m-1. The morphological response was determined by measuring the morphological characteristics of vegetation, root growth, tuber formation. While the physiological response was determined by estimating some ions in the vegetative and root growth. The results showed a significant decrease in the morphological characteristics of the vegetative growth (number of shoots, plant height and dry weight) and tuber formation by increasing salt levels, while the characteristics of the root growth (number of roots, lengths and dry weight) were not affected. There is no significant difference in the behavior of the two clones under saline levels, except for the superiority of vegetative clone (C_2) at comparison treatment in the number of shoots (2.00 shoot / plant), and vegetative clone (C_1) at comparison treatment and 12 ds m-1 in the shoot length and the percentage of tuber formation (13.40 cm and 100% Respectively). The results also recorded that the root growth of vegetative clone (C_2) was a significant accumulation of Na^+ and Ca^{++} at 12 dS m-1 which reached 8.33 and 23.38 mg-1 gm dry weight, respectively, while the accumulation of K+ in vegetative clone (C_1) was increased by the root growth in the control treatment which reached 45.03 mg -1 gm dry weight.

Key words: Potato, Na^+, K^+, Ca^{++}, Microtubers, *In vitro*.

الملخص

درست استجابة وسلوك سلالتين طافرتين من البطاطا المتحملة للملوحة للشد الملحي بتعريض النباتات الى مستويات ملحية مختلفة لملح كلوريد الصوديوم (8 ،10 و12 ديسي سيمنز. م$^{-1}$) ومقارنتها مع تلك النامية في معاملة السيطرة (ذات الايصالية الكهربائية 6 ديسي سيمنز. م$^{-1}$). حددت الاستجابة المظهرية بدراسة الصفات المظهرية للنمو الخضري والجذري وتكوين الدرينات الدقيقة وحددت الاستجابة الفسيولوجية بتقدير تراكيز بعض الايونات في الجزء الخضري والجذري لتلك السلالتين. أظهرت النتائج انخفاض معنوي في الصفات المظهرية للجزء الخضري عدد الفروع وارتفاع النبات والوزن الجاف) والدرينات الدقيقة بزيادة المستويات الملحية في حين لم تتأثر صفات الجزء الجذري (عدد الجذور وأطوالها والوزن الجاف بتلك المستويات. لم يختلف سلوك السلالتين معنويا في المستويات الملحية المختلفة ماعدا تفوق السلالة الخضرية (C_2) في معاملة المقارنة في عدد الأفرع (2.00 فرع / نبات) والسلالة الخضرية (C_1) في معاملة المقارنة والمستوى الملحي 12 ديس سيمنز م$^{-1}$ في طول الأفرع والنسبة المئوية لتكوين الدرينات الدقيقة (13.40 سم و 100 % على التوالي). كذلك سجلت النتائج تفوق المجموع الجذري للسلالة الخضرية (C_2) في تراكم ايونات الصوديوم والكالسيوم في المستوى الملحي 12 ديسي سيمنز م$^{-1}$ (8.33 و 23.38 ملغم غم$^{-1}$ وزن جاف على التوالي) في حين ازداد تراكم ايونات البوتاسيوم في السلالة الخضرية (C_1) بواسطة المجموع الجذري في معاملة السيطرة اذ بلغ (45.03 ملغم غم$^{-1}$ وزن جاف).

الكلمات الدالة: البطاطا، K^+ ، Na^+، Ca^{++} ، الدرينات الدقيقة، خارج الجسم الحي.

Introduction

Biotic and Abiotic environmental stresses, particularly drought and salinity, are the main threat to agricultural activity . Given the magnitude of the losses caused by these stresses on plant growth and reduction of yield [1,2]. Crops vary in their ability to tolerate saline levels, sensitive to tolerate. Potato classified as moderately sensitive to salinity, with a threshold of 1.7 ds^{-1}. It was noted that plant growth was reduced and tuber reduced to 50% at 50 m mol NaCl while completely inhibiting plant growth when treated with 150 mM NaCl [3].

The importance of laboratory experiments at their three levels tissue, cellular and molecular were highlighted by their role in the understanding tolerant mechanism, As differences in tissue composition, ion distribution and physiological

changes induced by the salinity effect should help identify salinity tolerance mechanisms [4]. Plant tissue culture technique was an opportunity offered as a source of access to clones or cultivars with desirable agricultural traits [5], or selection for environmental stresses such as salinity and drought [6]. Which allows for the screening and isolation of cells with desired traits from thousands of cells in a short period of time, limited space, and the possibility of evaluating the selected traits and study the physiological basis for stress tolerance with a greater chance for the getting genetic

variation and opportunity *in vitro* pre-selected to evaluate the behavior of any tolerant mutant clone to salt stress compared with those planted in the control treatment (with electrical conductivity 6 dS m^{-1}) [7].

The genotypes and clones of potato were different in their response to the sodium chloride,were represented as morphological and physiological, which gives an indication to tolerate clones [8, 9]. Zaman et al [10] found a correlation between the increase in salt levels and plant height. Studies have also indicated the salinity effect on accumulation of ions in the plant parts of potato, whether leaves or roots, whose varied in those parts with varying salinity levels. Jaarsma et al [10] mentioned to the effect of salt levels of sodium chloride (0, 60 and 180 mM) on decrease K^+ in the leaves and increase its concentration in the roots, specifically at the salt level 60 mM , while there were no differences between the stem and roots in the accumulation of Na^+. While Gao [12] found a high accumulation of Na^+ and Cl^- and a reduction in K^+ in the leaf with inhibition of growth at 200 mM NaCl when potato cultivar "Longshu no.3" planted at different salinity levels (0, 25, 50, 100 and 200 m mol NaCl). The study of the behavior and response of any genotypes or mutant clone when exposed to any environmental stress is an essential requirement for studying that reflects their ability to grow, develop and understand the mechanism.Therefore, this study was carried out to achieve this for two mutant clones of Riviera genotype induced from tolerant calli (planted at salt levels 8, and 12 dS m^{-1}) after exposing to 18 Gy.

Materials and Methods

The experiments were conducted at the Genetic Engineering Department in Agricultural Research Directorate in Ministry of Science and Technology/Iraq. Potato variety (Riviera) was established on semi-solid medium MS [Murashige and Skoog 13]. pH was adjusted to 5.7 prior to autoclaving at 1.2 kg cm^{-2} and 121ºC for 20 minutes. Plantelets were exposed to Gamma rays (source Co ^{60}) at dose 18 Gy. the internode cuttings (stem segments approximately 1- 1.5 cm length, without node) from *in vitro* irradiated plantlets were used for callus induction, which cultured on petri dish (9cm) containing MS with 3% sucrose, 7% agar and 0.1, 100, 0.5, 0.5,0.5, 2, 2 mg L^{-1} of Thiamine –HCL, Inositol, Glycin, Nicotinic Acid, Pyridoxine-HCL, Benzyl adenine, 2,4-D, respectively. All cultures were incubated in the growth room chamber at 25°C±2 under a 16 h light and 8 h dark for months. Calli were transferred to regeneration media containing MS salt supplemented with mg L^{-1} of 0.4 Thiamine HCL, 100 Inositol, 0.5 Glycine, 0.5 Nicotinic Acid, 0.5 Pyridoxine-HCL, 3 BA, 0.5 GA3, 0.03 NAA and 30 gm L^{-1} sucrose [14] with different levels of NaCl to generate EC at 8, 12 dS m^{-1}. Plants were formed after 70 days as a mutant clone: Vegetative clone (C_1): [plants induced from salt tolerant Calli (at 8 dS m^{-1} NaCl), Vegetative clone C_2): [plants induced from salt tolerant Calli (at 12 dS m^{-1} NaCl) Figure (1).

A. Clone $_1$ **B. Clone $_2$**

Fig. (1): (A and B) are the formation of vegetative growths from callus which inducted from Riviera irradiated plantlets (18 Gy) After 70 days. from Left-to-Right : 8 ds m^{-1} 12 ds m^{-1}.

Effect of salinity on morphological traits and tuber formation of mutant clones

Plantlets of two clones were micropropagated and cultured on semi-solid propagation medium (MS salt supplemented with mg L^{-1} of 0.4 Thiamine HCL, 100 Inositol, 2 Glycine, 2 Nicotinic Acid and 1 Indole Acetic Acid and 30 gm L^{-1} sucrose for subsequent experiments. Plantlets were divided into two parts: 1) Nodal cuttings (with length 1-2 cm in with one node) were collected from regenerated plantlets and planted on previous propagation semi– solid medium supplemented with different levels of NaCl to generate EC at 8, 10, 12 dS/m, the EC of the control treatment (MS basal medium, without adding NaCl) was 6 dS m^{-1}. All cultures were incubated at 25±2 °C under 16 h light 1000 lux and 8h. dark photoperiod. Data of the number of shoots, nodes/plant, plant height, and fresh, dry weight of vegetative growth

and the number of roots/plant, root height and fresh, dry for root growth were taken After 30 days. 2) For tuber formation, Nodal cuttings (with length 1-2 cm in with two nodes) were planted in 25 ml of semi- solid MS salt supplemented with mg L^{-1} of 0.4 Thiamine HCL, 100 Inositol, 2 Glycin, 2 Nicotinic Acid, 1 Indole Acetic Acid, 4 Kintein and 80 gm L^{-1} sucrose, supplemented with the same levels of NaCl. incubated for 10 days, photoperiod 16 h light and 8 h dark before placed in darkness . After that all the plantlets were placed in a growth room chamber at 18±2 °C with darkness until microtubers harvest. After the tuberization period was completed (90 days) data were recorded for % tuberization, number of microtubers/plantlet, microtuber weight (gm), microtuber diameter (cm) [15].

Determination of ions content

150 mg dry weight of vegetative and root growth were taken and placed in beaker containing 9 ml digesting mixture (10 Nitric acid: 4 Perchloric acids: 1 sulfuric acid). The beakers were heated up to 60 ºC until the solution became colorless, then the digestion diluted with distilled water. Concentrations of Ca^{++}, Na^{+} and K+ were measured using Atomic Absorption Spectrophotometer (Shimadzo AA-670) according to the manufacturer's recommendation [15] , Results were statistically analyzed using GenStat program and means were separated using Duncan's test at a probability level of 5% [17].

Results and Discussion

Effect of salinity on morphological traits of vegetative growth

Results in Table (1) showed that decrease in these characterizes by increasing salinity as compared with the control treatment (6 ds m^{-1}). The plants of vegetative clone (C_2, plants induced from salt tolerant calli which planted at salt levels 12 ds m^{-1}) at control treatment significantly surpassed in the number of shoots, fresh and dry weight reached 2.0 shoot/ plant, 160.0 mg and 16.36 mg respectively, while plants of vegetative clone (C1, plants induced from salt tolerant calli which planted at salt levels 8 ds m^{-1}) at the same treatment showed significantly higher average reached was 13.40 cm/plant and 15.00 node/ plant, respectively, and differed signifecantly from others.

Table (1): Effect of Salt levels of NaCl on morphological characterize of vegetative growth for two vgetative clones after 30 days.

Ve getative clones	Salt levels ds m^{-1} 6	8	10	12
	Number of shoots / plant			
C_1	1.40 b	1.00 b	0.80 b	0.80 b
C_2	2.00 a	1.20 b	1.00 b	0.80 b
	Plant height cm / plant			
C_1	13.40 a	8.60 ab	7.60 b	4.80 b
C_2	8.20 b	5.10 b	4.90 b	4.70 b
	Number of nodes / plant			
C_1	15.00 a	8.60 bc	8.40 bc	6.80 bc
C_2	11.20 ab	5.20 c	5.20 c	5.00 c
	Fresh weight mg			
C_1	154.0 ab	110.0 ab	108.0 ab	104.0 ab
C_2	160.0 a	142.0 ab	68.0 b	64.0 b
	Dry weight			
C_1	13.20 abc	10.50 bcd	8.30 d	8.30 d
C_2	16.40 a	14.20 ab	9.40 cd	6.70 d

Means followed by the same letters are not significantly different (P≤0.05) according to Duncan's test

Effect of salinity on morphological traits of root growth

The results in table 2 revealed that two clones of potato were no significant differences in the number and length of roots at all salt levels. On the other hand, there were significant difference in fresh and dry weight, vegetative clone (C_1)and (C_2) surpassed in fresh weight trait at salt levels 6 and 8 ds m^{-1} while they superior in dry weight at control treatment reached (13.34 and 23.06 mg respectively) and differed from others.

Table (2): Effect of Salt levels of NaCl on morphological characterizes of root growth vegetative clones after 30 days.

Vegetative clones	Salt levels ds m^{-1}			
	6	8	10	12
	Number of roots			
C_1	15.00 a	11.40 a	10.20 a	7.60 a
C_2	16.40 a	11.00 a	8.00 a	7.60 a
	Root length cm			
C_1	5.70 a	5.00 a	4.40 a	4.30 a
C_2	5.80 a	5.70 a	4.60 a	2.88 a
	Fresh weight mg			
C_1	86.0 a	72.0 a	40.0 b	18.0 bc
C_2	68.0 a	66.0 a	28.0 b	14.0 c
	Dry weight mg			
C_1	13..34 a	11.44 b	2.66 b	2.20 b
C_2	23.06 a	6.84 b	6.46. b	2.02 b

Means followed by the same letters are not significantly different (P≤0.05) according to Duncan's test

Table (3): Effect of Salt levels on vegetative characterize of Microtubers vegetative clones after 90 days

Vegetative clones	Salt levels ds m^{-1}			
	6	8	10	12
	% micrtuber formation			
C_1	100 a	100 a	100 a	100 a
C_2	100 a	80 ab	80 ab	70 b
	Number of tubers			
C_1	1.20 a	1.10 ab	1.10 ab	1.00 abc
C_2	1.20 a	0.80 bc	0.80 bc	0.70 c
	Diameter of tubers cm			
C_1	0.37 a	0.32 ab	0.30 ab	0.29 ab
C_2	0.39 a	0.22 b	0.21 b	0.19 b
	Weight of tubers mg			
C_1	70.00 a	56.00 ab	53.00 ab	52.00 ab
C_2	67.00 a	30.00 ab	25.00 b	23.00 b

Means followed by the same letters are not significantly different (P≤0.05) according to Duncan's test

Effect of salinity on microtuber formation

Plants of vegetative clone (C_1 and C_2) were observed significant differences in all traits at all salt levels (6, 8, 10 and 12 ds m^{-1}) Table (3). While vegetative clone (C_2) was superior in the number, diameter and weight of microtubers at control treatment reached (1.20 tuber $plant^{-1}$, 0.38 cm and 67.00 mg, respectively, that differ from others levels exception 8 ds m^{-1} for the weight of microtuber trait. The lowest percentage of microtuber formation, number, diameter, and weight of tuber was 70%, 0.70 tuber $plant^{-1}$, 0.19 cm and 23.00 mg respectively at 12 ds m^{-1}.

In general, it seems that decrease in all morphological characteristics of the vegetative and root growth and microtuber formation by increasing salt levels compared with the cotrol treatment (with the electrical conductivity 6 ds m^{-1}), these results were agreed with many studies [10, 18]. These results may be explained by the role of salts in their negative effects in the readiness of water and food soluble in the saline medium for most of the cell's processes, and thus reflected negatively on the division and growth of cells [19]. Or due to effect of radiation [20]. The difference in the behavior of the two mutant clones may be due to genetic variations, either by irradiation or by somaclonal variation [21, 22] as a result from induced irradiated plantlets.

Effect of salinity on ion accumulations

The results in Table 4 showed that Na^+ significant differences in both parts of plantlets of two vegetative clones at all salt levels., where the root parts of the vegetative clone (C_1) accumulated highest Na^+ at salt level 12 ds m^{-1} reached 8.33 mg gm^{-1} dry weight while the vegetative parts accumulated the lowest ion content at level 8 ds m^{-1} of C_2 was 1.08 mg gm^{-1} dry weight. Thr result presented in Table 4 also showed that Salinity levels affecte in accumulation of K+ in the plantlets parts of the two mutant clones. In clone (C_1) the vegetative parts not significantly differs from K^+ content at 8, 10 and 12 ds m^{-1} of NaCl and differed from the control treatment, which gave 7.16 mg gm^{-1} dry weight, while root part differed at all levels of salinity and control treatment superioty in the content of K^+ (45.03 mg gm^{-1} dry weight). As for the C_2, the vegetative parts did not differ in K^+ content at control treatment and 8 ds m^{-1} that differed significantly from the 10 and 12 ds m^{-1}, which were not significantly different from each other. Root part differed at levels 8, 10 and 12 ds m^{-1}, which differed from the control treatment. Results in Table 4 showed in C_1, there was a significant difference in Ca^{++} accumulation at salt levels 8,10 and 12 ds m^{-1} in both two parts. While, no differences appeared in a control treatment. Root part surpassed at12 ds m^{-1} was reached 23.38 mg gm^{-1} dry weight that differs from others treatments.

It seems that both clones were no significant differences in accumulation of Na^+, while C_1 was accumulated K^+ and C_2 accumukated Ca^{++}. It may be explained to clones dependent and the source of variations in their tissues , either due to variability or irradiation, that may be the effect on the mechanism of ions absorption by causing damage to the membranes and thus their permeability or transport system ions. [8]. While, increase in Na^+ and decrease in K^+ at 12 ds m^{-1} was a result of the negative effect of Na^+ ions (as they are dominant in the medium) in the absorption and entry of K^+ ions into cells due to the competition between these two ions [22].

Table (4): Effect of salt levels on Sodium (Na^+), potassium (K^+) and Calicium Ca^{++} ions (mg gm^{-1} dry weight) for both of vegetative and root growth of vegetative clones after 30 days.

		Na^+ (mg gm^{-1} dry weight)			
Vegetative clones	Parts	Salt levels ds m^{-1}			
		6	8	10	12
C_1	Vegetative	3.15 f	6.95 b	5.85 de	5.44 e
	Root	1.75 ghi	2.28 g	1.68 ghi	6.16 cd
C_2	Vegetative	2.94 f	1.08 i	6.49 bcd	1.88 gh
	Root	6.64 bc	3.20 f	1.55 hi	8.33 a
		K^+ (mg gm^{-1} dry weight)			
Vegetative clones	Parts	Salt levels ds m^{-1}			
		6	8	10	12
C_1	Vegetative	7.16 g	14.17 ef	11.53 f	13.00 f
	Root	45.03 a	17.72 e	37.35 b	28.32 c
C_2	Vegetative	12.46 f	12.09 f	1.11 h	1.09 h
	Root	24.16 d	1.30 h	2.79 h	2.51 h
		Ca^{++} (mg gm^{-1} dry weight)			
Vegetative clones	Parts	Salt levels ds m^{-1}			
		6	8	10	12
C_1	Vegetative	5.05 i	5.35 hi	4.03 j	5.77 gh
	Root	6.52 e	6.46 ef	8.49 d	14.63 b
C_2	Vegetative	4.46 ef	5.64 gh	6.54 e	8.91 d
	Root	6.06 fg	4.18 j	10.51 c	23.38 a

Means followed by the same letters are not significantly different ($P \leq 0.05$) according to Duncan's test

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