

BIOCHEMICAL AND PHENOTYPIC CHARACTERIZATION OF SALINITY POTATO TOLERANCE IN TISSUE CULTURE

Fatma A. Elatar, I.A. Ibrahim, Awatef M. Badr Elden, K.F. Abdellatif and Amal M. Zweil

Plant Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Egypt



Scientific J. of
Horticultural Research,
1(4):1-14 (2023).

Received:

15/7/2022

Accepted:

8/8/2022

Corresponding author:

Awatef M. Badr Elden

awatef.badrelden@gbri.usc.edu.eg

ABSTRACT: One of the main abiotic factors that significantly reduces the production of agricultural crops in arid and semiarid areas is salinity. The current study aims to create an effective *in vitro* screening method for four potato genotypes (Lady Rosetta, Hermes, Cara, and Spunta) to test their salt tolerance at concentrations of 0.0, 250, 500, 1000, 2000, and 4000 mg/l NaCl supplemented with MS media. Buds on Murashige and Skoog medium fortified with varying doses of BAP and KN (0.0, 0.5, 1.0, 1.5, and 2.0 mg/l) were inoculated in order to initiate shoot growth. In the MS medium containing 1.0 and 1.5 mg/l KN, the most multiple shoots were produced. However, Lady Rosetta also set the record for the most shoots per jar (13.70l). On the other side, Lady Rosetta's concentration of 2.00 mg/l KN produced the most leaves (39.44/jar). At 0.50 mg/l KN, Hermes and Spunta produced shoots with the same maximum length (10.00 cm). Different NaCl concentrations in MS media had a negative impact on the quantity of shoots. When grown on MS medium enriched with 4000 mg/ NaCl, Lady Rosetta is more resilient to salt stress. The Lady Rosetta experiment with a 1000 mg/l NaCl concentration yielded the most leaves (94.00 leaves/jar). A decrease in fresh weight was caused by higher salt concentrations. In comparison to other genotypes, Hermes' dry weight (0.67g/jar) with treatment with 250 mg/l NaCl was the greatest. Four potato genotypes had considerably lower levels of photosynthetic pigments, such as chlorophyll a, chlorophyll b, and total carotenoid, which was associated with a drop in osmotic pressure in the culture medium. It will make it easier to conduct research on the growth of potato plants that can withstand salt from *in vitro*-selected cells. In dry and semi-arid regions of the world, as well as in the creation of novel, unconventional potato breeding projects, it might be economically significant.

Keywords: Micropropagation, potato genotypes, photosynthetic pigments and salinity tolerance

INTRODUCTION

Salinity, one of the most important and pervasive agricultural issues that contributes to decreased crop production and arable land, is an abiotic factor that combines components of water shortage and sodium (Na) toxicity (FAO, 2019). Salt-affected soil, particularly

in semi-arid and arid regions, covers at least 25% of the world's agricultural land area (Flowers *et al.*, 2015). The potato (*Solanum tuberosum* L.), one of the most important vegetable crops in the world, is widely recognized. It ranks as the fourth-most significant food crop in the world, behind rice, wheat, and corn, and is used for human

consumption, animal feed, and as a source of starch for the brewing of alcoholic beverages (Gowayed *et al.*, 2017). It is becoming more crucial for industrial purposes in temperate and tropical regions as a source of carbohydrates, vitamins, and minerals. The wide range of biotic and abiotic stresses that can affect potatoes is concerning (Adolf *et al.*, 2020). Within potato cultivars, a genetic bottleneck has resulted from a historically limited inflow of variety. Therefore, it is anticipated that creating potato cultivars with new genetic diversity will increase their tolerance to biotic and abiotic restrictions (Munoz *et al.*, 2019). With approximately 759,200 tonnes exported to the European Union and Russia in 2018, Egypt surpassed China to rank fifth in potato exports. Egyptian exports of potatoes in 2019 totalled 259.6 million dollars, accounting for 5% of the global market (FAO, 2019). While the potato plant is thought to be fairly sensitive to salinity, Van Hoorn *et al.*, (1993) found that irrigated conditions containing 5.9 ds m⁻¹ of salt resulted in a 37 percent reduction in potato output. According to Katerji *et al.* (2000) the plant's leaves have the highest salt sensitivity. Potato production is reported to be reduced by salt concentrations above 50 mM NaCl (Rahman *et al.*, 2019).

It is challenging to create a variety that can withstand these abiotic challenges due to the autotetraploidy and complicated genetic makeup of potatoes. Evaluation of potato cultivars for salt stress can be done quickly and effectively using *in vitro* methods and micropropagation (Byun *et al.*, 2007). To make crop plants more resilient to environmental challenges, particularly salt stress, plant tissue culture techniques have joined forces with traditional breeding and biotechnology (Rahman *et al.*, 2008). A quick and effective method appears to be the research of plant salt tolerance to determine crop vulnerability (Zhu, 2007).

It is understood that the negative consequences of salinity result from osmotic stress, stoppage of metabolic activity by ionic excess and imbalance, and interference of salt

ions with the intake of crucial macro- and micronutrients (Gowayed *et al.*, 2017). Plants produce antioxidants in response to osmotic stress, but they also produce compatible solutes like proline, which were formerly believed to serve as osmotic buffers, and accumulate these solutes in the cytosol (El-Sayed, 2021). A critical component of potato production is the identification and testing of commercial cultivars for salt stress tolerance using an *in vitro* system since different potato cultivars respond differently to salinity stress (El-Sayed *et al.*, 2021).

The objectives of this study were to assess the impact of NaCl salinity levels on the vegetative growth of Lady Rosetta, Hermes, Cara, and Spunta parameters (shoot number, number of leaves, shoot length, fresh weight, and dry weight), as well as some chemical components of potato genotypes (chlorophyll a, chlorophyll b, and carotenoid) under *in vitro* growing conditions.

MATERIALS AND METHODS

Explant preparation and sterilization:

Four potato genotypes, Lady Rosetta, Hermes, Cara and Spunta, were collected from the greenhouse of Tissue Culture and Genetic Engineering Centre, Genetic Engineering and Biotechnology Research Institute University of Sadat City, Egypt, to obtain shoots as explants after 4-5 weeks, Explants taken from the mother plant them washed by tap water and double distilled water. After primary sterilization, the explants were treated with 70% ethanol for 90 seconds and washed with distilled water. Next, the explants were dipped in 1% HgCl₂ (mercuric chloride) for 4 to 8 minutes (Zaman, *et al.*, 2015).

Shoot Multiplications:

After 30 days of growth on the culture initiation, Murashige and Skoog (1962) medium MS supplemented with 2 mg/l KN, young and healthy shoots with nodes cuttings were cultured on a shoot multiplication, MS medium containing different concentrations of BAP or KN (0.0,0.5,1.0, 1.5, 2, mg/l). Five

stem cuttings were inoculated per jar with ten replications for each treatment. The culture jars were placed in the growth room chambers with 16 hours photoperiod (8 hours dark) under light intensity 2000 lux and at 25 ± 2 °C. The developed shoots at different multiplication were observed and recorded after 30 days, shoot number, leave number and shoot length (cm).

***In vitro* screening of potato for salt tolerance:**

The studied four potato cultivars were screened for salt tolerance using MS at various concentrations (0, 250, 500, 1000, 2000 and 4000 mg/l NaCl). The stem cuttings were used as explants. Each experiment consisted of 4 explants with ten replications. Data were taken after five weeks as, number of shoots, number of leaves, shoot length (cm), fresh and dry weights (g), and photosynthetic pigments (chlorophyll a, b and carotenoids).

Determination of photosynthetic pigments:

The quantity of photosynthetic pigments (chlorophyll a, b, total and carotenoids) was determined according to the method of Lichtenthaler (1987). Shoot samples (0.25 g) were homogenized in acetone (80%). The extract was centrifuged at 3000 xg and absorbance was recorded at wavelengths of 646.8 and 663.2 nm for chlorophyll assay and 470 nm for carotenoids assay by a UV-Vis spectrophotometer (Cary50, Germany). Chla, Chlb, ChlT and carotenoids were calculated using the following formulas:

$$\text{Chla} = (12.25 A_{663.2} - 2.79 A_{646.8})$$

$$\text{Chlb} = (21.21 A_{646.8} - 5.1 A_{663.2})$$

$$\text{ChlT} = \text{Chla} + \text{Chlb}$$

$$\text{Car} = (1000 A_{470} - 1.8 \text{Chla} - 85.02 \text{Chlb}) / 198$$

Statistical analysis:

Data collected in this study were statistically analysed using the generalized linear models (GLM) of SAS (2003). Separation among means was achieved using the least significant difference (LSD).

RESULTS AND DISCUSSION

Effect of different concentrations of BAP and KN on *in vitro* regeneration of four potato genotypes:

Explants implanted on MS media enriched with plant growth regulators during the initial stage of *in vitro* proliferation responded favourably, producing excellent outcomes on regeneration. Following inoculation according to specifications, shoot explants of four distinct potato genotypes were grown on MS media supplemented with various concentrations of BAP and KN (0, 0.5, 1.0, 1.50, and 2.0 mg/l). The results of the statistical analysis were then compared. Tables (1, 2 and 3) and Figs. (1 and 2) present the findings. The number of shoots per plantlet was considerably impacted by various cytokinin supplement amounts (BAP and KN). The highest and lowest shoot counts (16.08 and 16.41, respectively) were recorded at (1.0 and 1.5 mg/l KN) and (6.91, respectively), respectively, at 2.0 mg/l BAP (Table, 1). However, the Cara genotype produced the fewest shoots (8.96), while Lady Rosetta genotype had the maximum number of shoots (13.70). (Fig., 2). Due to the interaction of variety and cytokinin, significant differences in the number of shoots were discovered (BAP and KN). Lady Rosetta, which performed better than the other three genotypes, had the most shoots (24.00) when KN was presented at 2.0 mg/l. The Lady Rosetta has the most number of leaves (30.18). The Spunta genotype, in contrast to the other three genotypes, had the fewest leaves (19.03). The number of leaves was considerably impacted by various cytokinin (BAP and KN) supplement doses. The greatest number of leaves (38.33) were found at 2.0 mg/l KN, and the fewest number (13.83) were found at 2.00 mg/l BAP (Table, 2). However, Lady Rosetta's genotype at 2.00 mg/l KN level had the largest number of leaves (39.44). Due to the interaction between variety and cytokinin, significant changes in terms of the number of leaves were discovered (BAP and KN). Significant differences in shoot length were seen between

Table 1. Effect of different concentrations of BAP and KN on shoots number of four potato genotypes.

Genotypes	Shoots number									Mean (A)
	Cytokinin conc. (mg/l)									
	BAP			KN						
	0.00	0.50	1.00	1.50	2.00	0.50	1.00	1.50	2.00	
Lady Rosetta	12.33	7.00	12.66	11.00	9.66	9.00	17.33	20.33	24.00	13.70
Hermes	9.33	8.33	13.00	11.33	5.00	12.00	16.00	13.66	13.66	11.37
Cara	11.66	13.66	5.66	7.33	6.333	10.66	10.00	8.00	7.33	8.96
Spunta	7.33	5.33	11.66	7.33	6.66	15.66	21.00	23.66	13.66	12.48
Mean (B)	10.16	8.58	10.75	9.25	6.91	11.83	16.08	16.41	14.66	
LSD at 0.5										
A					0.3765					
B					0.6547					
AxB					1.1294					

Table 2. Effect of different concentrations of BAP and KN on leaves number of four potato genotypes.

Genotypes	Leaves number									Mean (A)
	Cytokinin conc. (mg/l)									
	BAP			KN						
	0.00	0.50	1.00	1.50	2.00	0.50	1.00	1.50	2.00	
Lady Rosetta	41.66	16.33	17.33	12.66	16.33	37.33	40.00	40.66	49.33	30.18
Hermes	27.00	21.00	18.33	16.66	15.33	33.00	35.33	29.66	42.66	26.55
Cara	25.66	28.33	14.00	16.66	13.33	20.00	21.33	24.66	37.66	22.40
Spunta	26.33	10.66	12.33	10.33	10.33	23.00	26.33	28.33	23.66	19.03
Mean (B)	30.16	19.08	15.50	14.08	13.83	28.33	30.75	30.83	38.33	
LSD at 0.5										
A					1.1954					
B					0.7969					
AxB					2.3907					

Table 3. Effect of different concentrations of BAP and KN on shoots length (cm) of four potato genotypes.

Genotypes	Shoots length (cm)									Mean (A)
	Cytokinin conc. (mg/l)									
	BAP			KN						
	0.00	0.50	1.00	1.50	2.00	0.50	1.00	1.50	2.00	
Lady Rosetta	8.83	5.66	4.33	3.66	3.50	8.66	9.33	9.66	9.83	7.05
Hermes	9.50	5.50	3.83	2.33	2.00	10.00	8.66	8.00	4.66	6.05
Cara	9.50	8.16	3.33	3.00	3.00	9.83	9.50	6.66	7.33	6.70
Spunta	10.00	4.50	3.50	3.00	2.66	10.00	9.16	8.16	7.00	6.44
Mean (B)	9.45	5.95	3.75	3.00	2.79	9.62	9.16	8.12	7.20	
LSD at 0.5										
A					0.1846					
B					0.2769					
AxB					0.5537					

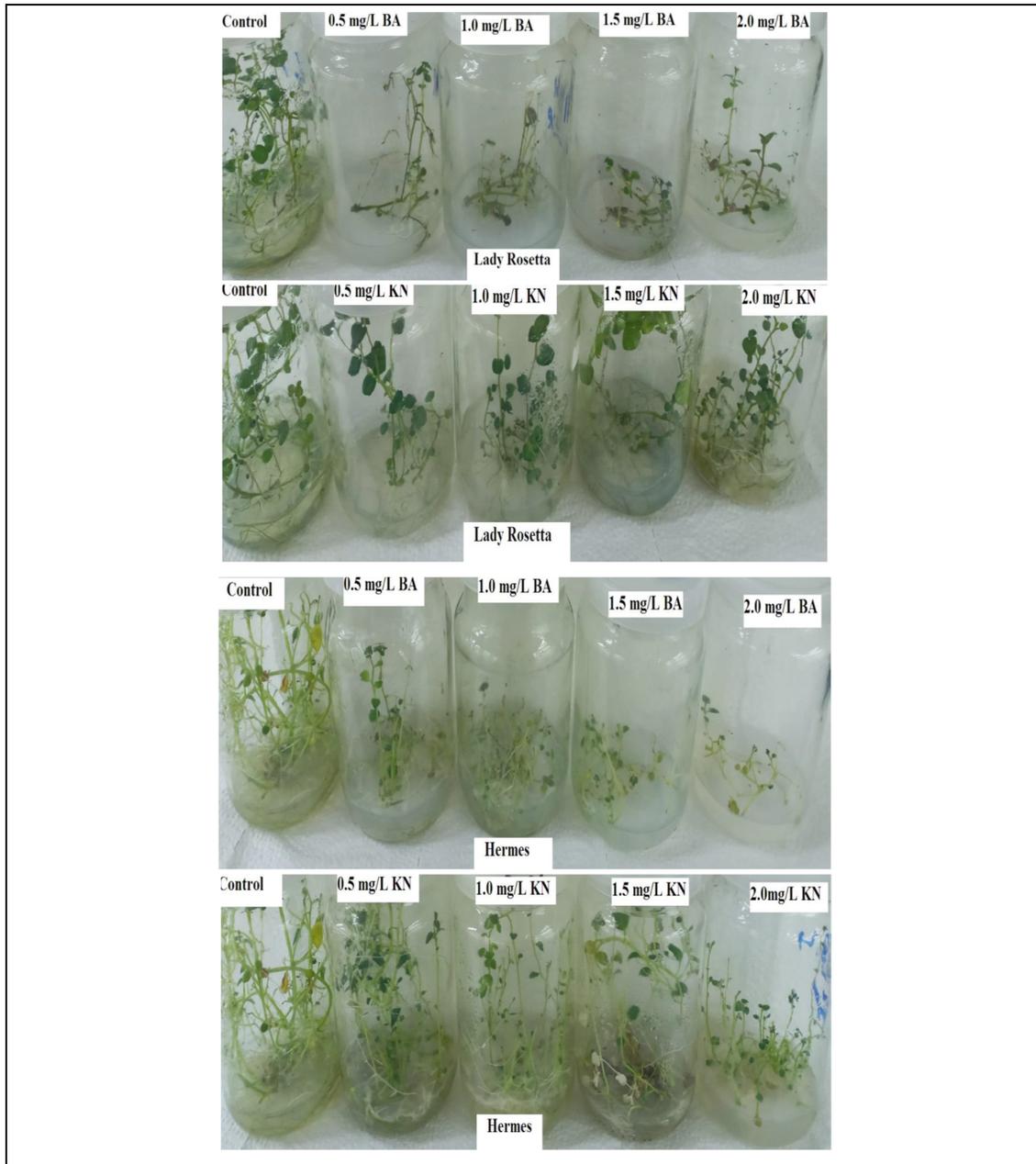


Fig. 1. Effect of cytokinin concentrations on shooting of Lady Rosetta and Hermes.

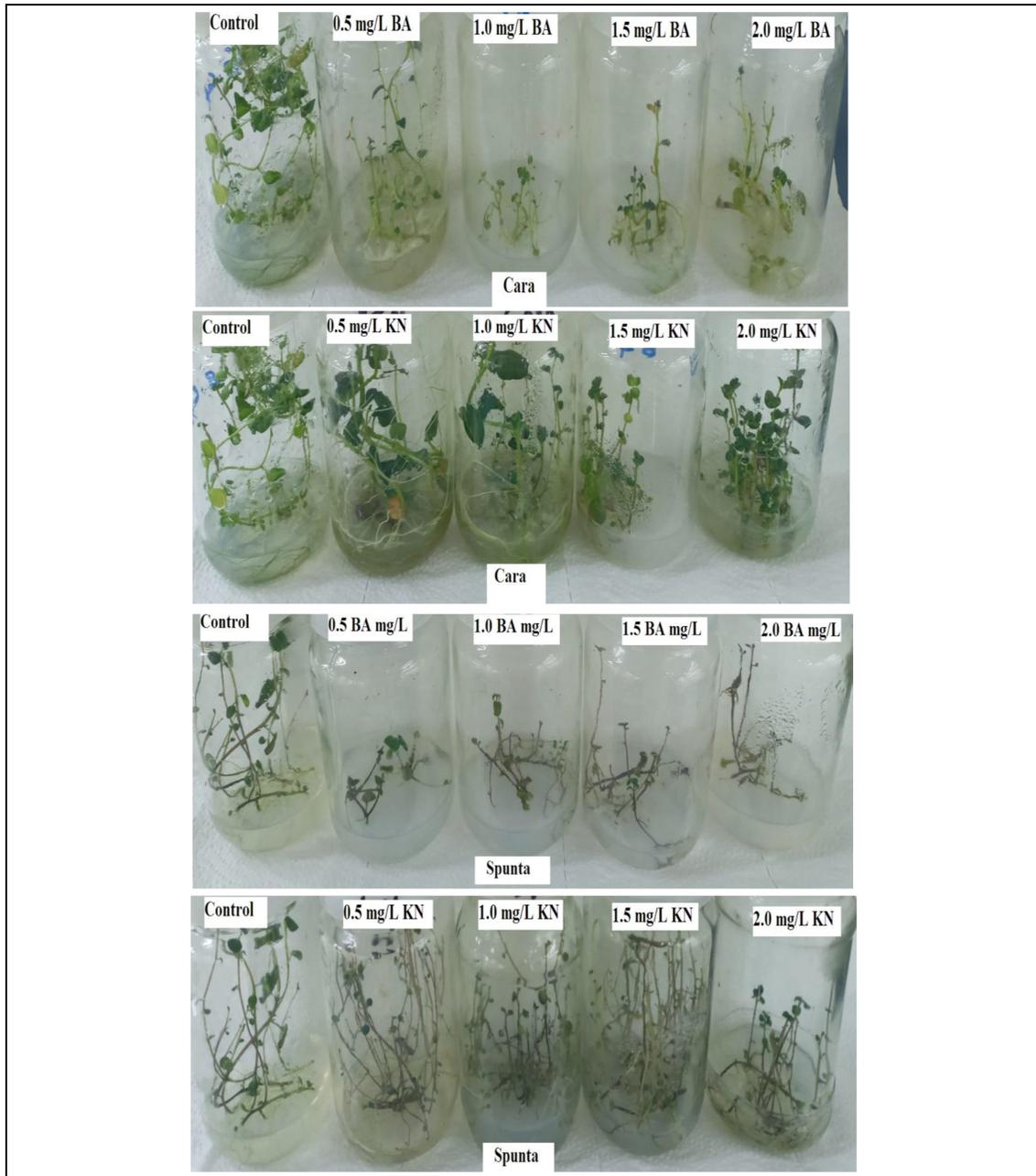


Fig. 2. Effect of cytokinin concentrations on shooting of Lady Rosetta and Hermes.

genotypes (Table, 3). Lady Rosetta's genotype had the longest shoots (7.05 cm), followed by Cara (6.70 cm) (Figs., 1 and 2). Additionally, KN treatments at 0.50 mg/l outperformed all other treatments, and their

maximum shoot length was estimated to be (9.62 cm). When the genotypes Hermes and Spunta interacted, the highest length of shoots was obtained at 0.50 mg/l KN (10.00 cm). The genotypic effect may be a contributing

factor in the various ways that shoots multiply.

Like the findings of the present study, Sarker and Mustafa (2002) discovered that as BAP or KN concentration increased, the number of numerous shoots per explant (internode and leaf) increased. In semi-solid medium, Hossain *et al.*, (2005) obtained fresh shoots in 3–5 days. According to Dessoky *et al.* (2016), shoot initiation occurred between 2.75 and 6.25 days for BAP treatments and 4.25 and 7.25 days for KN treatments. The lowest proportion (15%) was on MS medium without growth regulators, which produced 14 shoots of magenta from 6 nodes using 3 mg/l GA₃ and 0.1 mg/l KN. In the MS medium containing 2.5 mg/l KN, most potato multiple shoots were produced (Hajare *et al.*, 2021).

Effect of various salt stress treatments on different phenotypic parameters and photosynthetic pigments of four potato genotypes:

The presence of sodium chloride in the growth medium leads to often induces secondary stresses. *In vitro* screening of salt stress tolerance was implemented to obtain four potato genotypes that tolerate NaCl. The plantlets grown on MS media containing different concentrations of NaCl (Table, 4 and Figs, 3-5) exhibited significant differences in Lady Rosetta and Spunta. Results showed that plant growth was not influenced by all concentrations of salinity and generally it was almost similar to the control treatment, while high levels of salinity at 4000 mg/l NaCl with Lady Rosetta increased plantlets development and produced (45.66 plantlets/jars) (Table, 4 and Fig., 3). Shoots number were negatively affected by different levels of NaCl in MS media. The highest number of leaves without significant reduction was given by Lady Rosetta under all tested levels of salinity compared to the other tested genotypes. There was a significant difference in the interaction treatments of leaves number. The highest value (94.00 leaves/jars) was obtained from Lady Rosetta with 1000 mg/l NaCl concentration. On the other hand, the lowest

one was obtained from Spunta with 500 mg/l NaCl (28.00 leaves/jars) (Table, 5). The highest NaCl levels prevented the growth of new roots. These results are similar to previous studies (Ahmed *et al.*, 2020). However, the tallest plants were obtained with Lady Rosetta and Cara (6.83 and 6.72 cm, respectively) than other genotypes. Plantlets length was greatly affected by NaCl at different concentrations (Table, 6), shoot length of potato plantlets was significantly decreased due to increasing salinity level from 250 to 4000 mg/l NaCl as compared to control plants. Moreover, the decrease in shoot length was gradual parallel to the increase in the salinity level from 250 to 4000 mg/l NaCl. In contrast, the shortest plants (3.0 cm) were recorded by 4000 mg/l NaCl with Spunta. It can be noticed that potato genotypes differ in their tolerance to salinity concentrations of the plantlets' length. Spunta is the most sensitive one for the effect of salinity on shoot length. This effect may be attributed to NaCl, which inhibits the absorption of water and mineral elements by the roots, resulting in the lack of growth requirements of the plant and the appearance of symptoms based on the resistance of each genotype. In our study, fresh weight was reduced severely by concentrations of NaCl stress in MS media, due to the effect of salt highly significant results were recorded (Table, 7). The maximum fresh weight (8.35 g/jars) was recorded in Hermes than other genotypes. Increased salt levels resulted in a reduction in fresh weight. The maximum fresh weight (8.35 g/shoots) was recorded in Hermes. The highest fresh weight (7.52 g/shoots), was produced at control treatment than other treatments. The lowest value of fresh weight was obtained from Cara with 4000 mg/l NaCl compared to other genotypes. Interaction between four potato genotypes and NaCl at different concentrations, showed the highest value of fresh weight with Hermes at 250 mg/l NaCl. The dry matter of the potato genotypes was marginally impacted by salinity. Maximum dry weight was observed at 250 mg/l NaCl with Hermes than other genotypes as shown in Table (8).

Table 4. Effect of sodium chloride (NaCl) on *in vitro* shoots number of four potato genotypes.

Genotypes	Shoots number						Mean (A)
	NaCl conc. mg/l						
	0	250	500	1000	2000	4000	
Lady Rosetta	27.00	28.66	38.66	35.33	37.00	45.66	35.38
Hermes	31.33	34.33	34.33	37.66	36.00	26.66	33.38
Cara	15.00	23.00	21.33	22.00	23.66	25.00	21.66
Spunta	43.00	43.66	37.00	36.00	34.00	31.33	37.50
Mean (B)	29.08	32.41	32.83	32.75	32.66	32.16	
LSD at 0.5							
A			4.0981				
B			5.0191				
AxB			10.038				

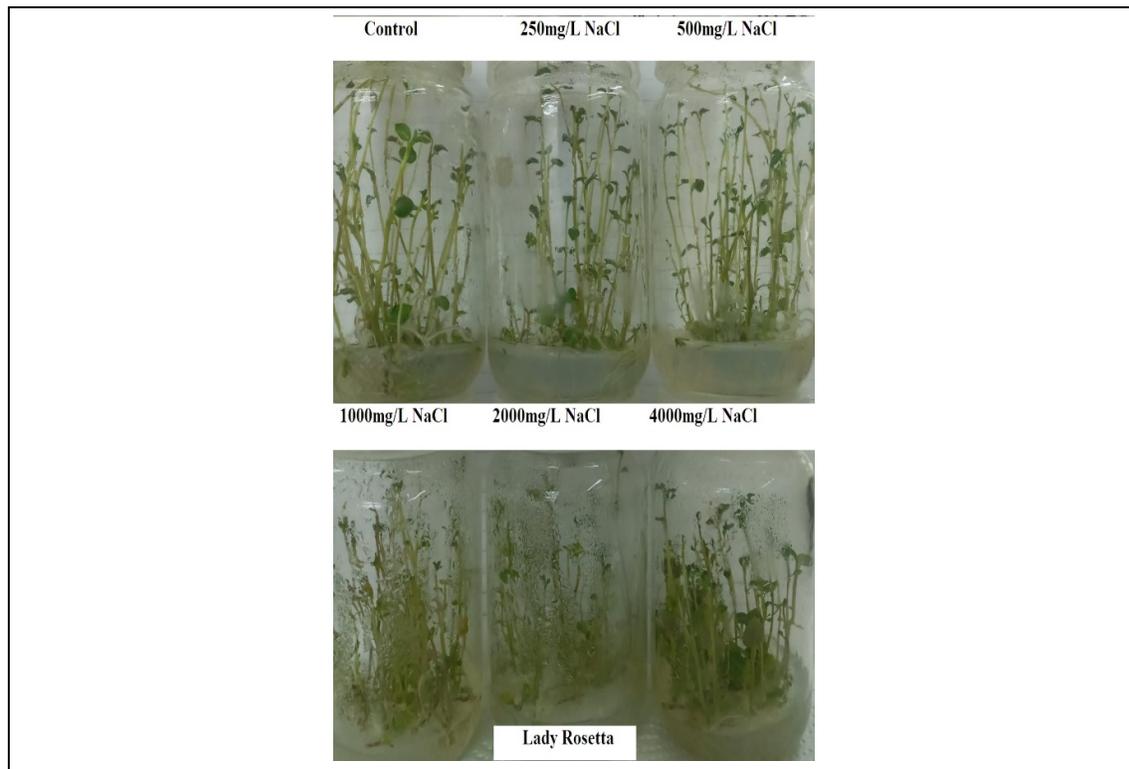


Fig. 3. Effect of different NaCl concentrations on Lady Rosetta genotype.



Fig. 4. Effect of different NaCl concentrations on Hermes genotype.

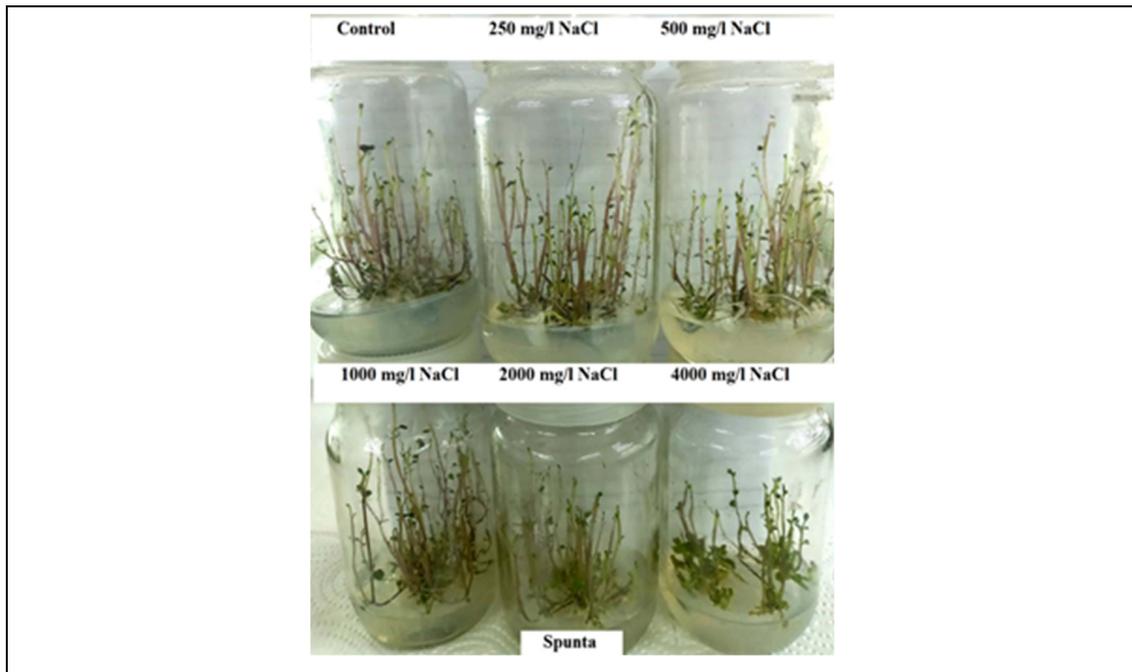


Fig. 5. Effect of different NaCl concentrations on Spunta genotype.

Table 5. Effect of sodium chloride (NaCl) on *in vitro* leaves number of four potato genotypes.

Genotypes	Leaves number						Mean (A)
	NaCl conc. mg/l						
	0.000	250	500	1000	2000	4000	
Lady Rosetta	75.00	87.66	91.00	94.00	84.66	53.33	80.94
Hermes	32.66	48.33	47.00	47.66	47.00	58.33	46.83
Cara	67.33	75.00	66.66	66.66	45.00	40.33	60.16
Spunta	32.66	31.00	28.00	50.00	50.00	56.33	41.38
Mean (B)	51.91	60.50	58.25	64.58	56.66	52.08	
LSD at 0.5							
A				2.5288			
B				3.0971			
AxB				6.1942			

Table 6. Effect of sodium chloride (NaCl) on *in vitro* shoot length of four potato genotypes.

Genotypes	Shoot length (cm)						Mean (A)
	NaCl conc. mg/l						
	0	250	500	1000	2000	4000	
Lady Rosetta	9.66	9.00	7.33	5.66	5.33	4.00	6.83
Hermes	9.00	6.33	5.00	4.66	5.00	4.66	5.77
Cara	5.66	6.66	7.67	8.00	7.00	5.00	6.72
Spunta	5.33	5.33	4.00	4.00	3.66	3.00	4.22
Mean (B)	7.41	6.83	6.00	5.58	5.25	4.25	
LSD at 0.5							
A				0.4673			
B				0.5723			
AxB				1.1446			

Table 7. Effect of sodium chloride (NaCl) on *in vitro* fresh weight of four potato genotypes.

Genotypes	Fresh weight (g)						Mean (A)
	NaCl conc. mg/l						
	0	250	500	1000	2000	4000	
Lady Rosetta	7.20	6.13	7.13	6.33	5.40	9.03	6.80
Hermes	9.80	10.00	7.60	9.06	6.23	7.43	8.35
Cara	5.10	4.76	3.67	5.60	3.76	2.66	4.26
Spunta	8.00	5.43	8.80	5.70	8.66	6.63	7.20
Mean (B)	7.52	6.58	6.80	6.67	6.01	6.43	
LSD at 0.5							
A				0.1373			
B				0.1681			
AxB				0.3362			

Table 8. Effect of sodium chloride (NaCl) on *in vitro* dry weight of four potato genotypes.

Genotypes	Dry weight (g)						Mean (A)
	NaCl conc. mg/l						
	0	250	500	1000	2000	4000	
Lady Rosetta	0.48	0.38	0.51	0.40	0.32	0.56	0.44
Hermes	0.61	0.674	0.44	0.58	0.38	0.51	0.53
Cara	0.28	0.27	0.20	0.35	0.21	0.17	0.25
Spunta	0.40	0.27	0.45	0.34	0.52	0.37	0.39
Mean (B)	0.44	0.40	0.40	0.42	0.36	0.40	
LSD at 0.5							
A			0.0079				
B			0.0097				
AxB			0.0195				

Dry weight was severely reduced by increasing NaCl stress level in MS media and produced the highest dry weight was observed at control (0.44 g/jars). The highest dry weight (0.67 g/shoots) was obtained from Hermes with treatment 250 mg/l NaCl, compared to other treatments. Similar results were furnished by (Pour *et al.*, 2010 and Askari *et al.*, 2012). The *in vitro* selection pattern for salt tolerant cultivars based on NaCl is less time consuming and allows quick identification of tolerant cultivars (Mousavi *et al.*, 2020).

Abdelsalam *et al.* (2021) noticed that there were significant differences in response for NaCl concentrations among cultivars. Data in Tables (9, 10 and 11) revealed that photosynthetic pigments, including chlorophyll a, chlorophyll b, and total carotenoids of four potato genotypes were significantly reduced, related to the decrease in osmotic pressure in the culture media. The effect of potato genotypes and salinity stress on photosynthetic pigments chlorophyll a, chlorophyll b and carotenoids were different. The higher NaCl senility levels decreased chlorophyll a content in all potato genotypes except Hermes was increased in chlorophyll a content. In addition, maximum chlorophyll a content with Lady Rosetta at 2000 mg/l NaCl and Cara at 500 mg/l NaCl (13.60 and 13.22, respectively). Concerning the chlorophyll b results, increasing level of NaCl reduced chlorophyll b except Hermes at 2000 mg/l

NaCl as shown in Table (10). Thus, potato cultivars respond differently under salinity growing conditions and decreasing trend was observed in chlorophyll a and chlorophyll b at higher NaCl level. Salinity stress reduced the carotenoids content in all tested genotypes at the higher levels except Hermes and Spunta. The decrease was more pronounced in Lady Rosetta than in the other tested genotypes. In the medium with 500 mg/l NaCl, plantlets of Cara genotype had higher carotenoids content than those of the other studied genotypes Table (11). Abdullah *et al.* (2018) showed that the addition of NaCl to the MS growing medium induced salt stress that adversely affected shoot, root growth and development of the plantlets of the eighth studied cultivars. This decrease could be as a result of inducing modifications of balance, water status, mineral nutrition as well as efficiency of photosynthesis as reported by. In the medium with 120 Mm NaCl, plantlets of Diamant and Burren cultivars had higher carotenoids content than those of the other studied cultivars (Abdelsalam *et.al.*, 2021).

CONCLUSION

Finally, we have created a system for the regeneration of four potato genotypes from explants of shoots grown in a dish under salt stress. Instead of using BAP for shoot induction, the approach was applied to *in vitro* regeneration using KN. The tolerance to salt stress of the four genotypes of potatoes varied

Table 9. Effect of sodium chloride (NaCl) on chlorophyll a content of *in vitro* four potato genotypes.

Genotypes	Chlorophyll a content mg/kg					
	NaCl conc. mg/l					
	0	250	500	1000	2000	4000
Lady Rosetta	3.54	2.58	2.79	1.86	13.60	11.16
Hermes	2.12	1.97	4.30	2.44	3.94	6.40
Cara	10.75	12.56	13.22	7.82	10.01	5.56
Spunta	3.44	3.75	4.22	4.42	6.04	5.66

Table 10. Effect of sodium chloride (NaCl) on chlorophyll b content of *in vitro* four potato genotypes.

Genotypes	Chlorophyll b content mg/kg					
	NaCl conc. mg/l					
	0	250	500	1000	2000	4000
Lady Rosetta	7.01	5.56	6.07	3.86	26.55	21.92
Hermes	4.53	4.10	8.28	4.67	7.54	12.16
Cara	20.01	23.93	25.15	14.85	19.07	10.62
Spunta	6.60	7.04	8.07	8.40	11.45	10.72

Table 11. Effect of sodium chloride (NaCl) on carotenoids content of *in vitro* four potato genotypes.

Genotypes	Carotenoids content mg/kg					
	NaCl conc. mg/l					
	0	250	500	1000	2000	4000
Lady Rosetta	0.19	0.78	0.20	0.64	2.68	0.77
Hermes	0.51	0.35	0.39	0.16	0.02	1.27
Cara	1.39	1.20	3.46	1.34	0.34	0.72
Spunta	1.05	0.87	0.01	1.78	1.37	1.91

greatly. When cultivated on MS medium with 4000 mg/l NaCl supplement, Lady Rosetta is more resistant to salt stress than Hermes, Cara, and Spunta. The development of stable, salt-tolerant potato plants from carefully chosen *in vitro* cells will be made easier by this research. The creation of innovative non-traditional programmes for potato breeding and its potential economic significance in arid and semi-arid regions of the world. In addition, the researched cultivars might be appropriate for cultivation on recently reclaimed land.

REFERENCES

- Abdelsalam, Z.K.; Ezzat, A.; Tantawy, I.A.A.; Youssef, N. and Gad EL-Hak, S.H. (2021). Effect of NaCl salinity stress on potato (*Solanum tuberosum* L.) plantlets grown and development under *in vitro* conditions. Scientific Journal of Agricultural Sciences, 3(2):1-12.
- Abdullah, A.; Hossain, M. and Akhter, S. (2018). Screening of CIP potato clones for salinity tolerance in pot and field condition. Adv. Plants Agric. Res., 8(6):573-580.

- Adolf, B.; Andrade-Piedra, J.; Bittara, Molina, F.; Przetakiewicz, J.; Hausladen, H. and Kromann, P. (2020). Fungal, Oomycete, and Plasmodiophorid Diseases of Potato. In: Campos H., Ortiz O. (eds) The Potato Crop. Springer, Cham, Switzerland, pp. 307-350.
- Ahmed, H.A.A.; Şahin, N.K.; Akdoğan, G.; Yaman, C.; Köm, D. and Uranbey, S. (2020). Viability in salinity stress tolerance of potato (*Solanum tuberosum* L.) varieties using in-vitro screening. *Ciência e Agrotecnologia*, 44:1-14. <https://doi.org/10.1590/1413-7054202044004220>
- Askari, A.; Pepoyan, A. and Parsaeimehr, A. (2012). Salt tolerance of genetic modified potato (*Solanum tuberosum*) cv. Agria by expression of a bacterial mtID gene. *Adv. Agri. Botanic.*, 4(1):10-16.
- Byun, M.; Won, H.B.K. and Park, S.C. (2007). Recent advances in genetic engineering of potato crops for drought and saline stress tolerance. In: Jenks, M.A.; Hasegawa, P.M. and Jain, S.M. (eds.), *Advances in Molecular Breeding towards Drought and Salt Tolerant Crops*, Springer, Dordrecht, Netherlands, pp. 713-737.
- Dessoky, E.S., Attia, A.O.; Ismail, I.A. and El-Hallous, E.I. (2016). *In vitro* propagation of potato under different hormonal combinations. *International Journal of Advanced Research*, 4(1):684-689.
- El-Sayed, S.F.; Taha, S.S.; Darwish, O.; Sand, M. and Wesongo, S.Z. (2021). Effect of silver thiosulfate and photoperiod in the *in vitro* tuberization of three potato (*Solanum tuberosum* L.) cultivars. *Plant Archives*, 21: 308-317.
- FAO (2019). FAOSTAT: FAO Statistical Database. Food and Agriculture Organization, New York, USA. <https://www.fao.org/faostat>
- Flowers, T.J.; Munns, R., Colmer, T.D., (2015). Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. *Ann. Bot.*, 115:419–431.
- Gowayed, M.H.; Al-Zahrani, H.S., and Metwali, E.M. (2017). Improving the salinity tolerance in potato (*Solanum tuberosum*) by exogenous application of silicon dioxide nanoparticles. *International Journal of Agriculture and Biology*, 19:183-192.
- Hajare, S.T.; Chauhan, N.M.; and Kassa, G. (2021). Effect of growth regulators on *in vitro* micropropagation of potato (*Solanum tuberosum* L.) Gudiene and Belete Varieties from Ethiopia. *The Scientific World Journal*, 1:1-8.
- Hussain, I.; Muhammad, A.; Chaudhury, Z.; Asghar, R.; Naqvi, S.M.S. and Rashid, H. (2005). Morphogenic potential of three potato (*Solanum tuberosum* L.) cultivars from diverse explants, a prerequisite in genetic manipulation. *Pakistan J. Bot.*, 37(4):889-898.
- Katerji, N.; Van Hoorn, J.W.; Hamdy, A. and Mastrorilli, M. (2000). Salt tolerance classification of crops according to soil salinity and to water stress day index. *Agric. Water Manage.*, 43:99-109.
- Lichtenthaler, H.K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods Enzymol.*, 148:350-382.
- Mousavi, S.N.; Ebadi, M.; Khorshidi, M.; Hokmabadi, H. and Masoudian, N. (2020). Effect of salinity on photosynthetic and enzymatic activities and tuberization yield in the genotype of potato cultivar Agria under *in vitro* conditions. *J. Neotropical Agric.*, 7:8-19.
- Munoz, M.; Díaz, O.; Reinún, W.; Winkler, A. and QuevedoMunoz, R. (2019). Slow growth *in vitro* culture for conservation of Chilotanum potato germplasm. *Chilean Journal of Agricultural Research*, 79(1):26-35.
- Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and

- bioassays with tobacco tissue cultures. *Physiologiae Plantarum*, 15(3):473-497.
- Pour, M.S.; Omid, M.; Majidi, I.; Davoodi, D. and Tehrani, P.A. (2010). *In vitro* plantlet propagation and microtuberization of meristem culture in some of wild and commercial potato cultivars as affected by NaCl. *African Journal of Agricultural Research*, 5(4):268-274.
- Rahman, M.H.; Islam, R.; Hossain, M. and Haider, S.A. (2008). Differential response of potato under sodium chloride conditions *in vitro*. *J. Bio-Sci.*, 16:79-83.
- Rahman, M.H.; Islam, R.; Hossain, M. and Haider, S.A. (2019). Differential response of potato under sodium chloride stress conditions *in vitro*. *J. BioSci.*, 16:79-83.
- Sarker, R.H. and Mustafa, B.M. (2002). Regeneration and agrobacterium-mediated genetic transformation of two indigenous potato varieties of Bangladesh. *Plant Tissue Culture*, 12(1):69-77.
- SAS (2003). Statistical Analysis System. SAS Release 9.1 for windows, SAS Institute Inc. Cary, NC, USA.
- Van Hoorn, J.W.; Katerji, N.; Hamdy, A. and Mastrorilli, M. (1993). Effect of saline water on soil salinity and on water stress, growth, and yield of wheat and potatoes. *Agric. Water Manage*, 23:247-265.
- Zaman, M.S.; Ali, G.M.; Muhammad, A.; Farooq, K. and Hussain, I. (2015). *In vitro* screening of salt tolerance in potato (*Solanum tuberosum* L.) varieties. *Sarhad J. Agric.*, 31:106-113.
- Zhu, J.K. (2007). Plant salt stress. *Encyclopedia of life sciences*, John Wiley & Sons, Ltd., <https://doi.org/10.1002/9780470015902.a0001300.pub2>

التوصيف الكيمياء الحيوي والمظاهر لتحمل البطاطس الملوحة في زراعة الأنسجة

فاطمة أمير العطار، إبراهيم عبد المقصود إبراهيم، عواطف محمود بدر الدين، كمال عبد اللطيف، أمال محمد زويل
قسم البيوتكنولوجيا النباتية، معهد بحوث الهندسة الوراثية والتكنولوجيا الحيوية، جامعة مدينة السادات، مصر

تعتبر الملوحة من أهم المعوقات اللاأحيائية التي تؤثر بشدة على إنتاجية المحاصيل الزراعية في المناطق الجافة وشبه الجافة. الهدف من هذا البحث هو تطوير نظام فعال في زراعة الأنسجة لأربعة طرز وراثية من البطاطس (ليدي روزيتا، هيرميس، كارا وسبونتانا) لتحمل الملوحة عند ٠،٠، ٢٥٠، ٥٠٠، ١٠٠٠، ٢٠٠٠ و ٤٠٠٠ مجم / لتر كلوريد الصوديوم في بيئة موراشيغ وسكوج. تم زراعة النباتات على بيئة موراشيغ وسكوج بتركيزات مختلفة من البينزيل امينو بيورين والكاينيتين بتركيزات ٠،٠، ٥٠، ١٠٠، ١،٥، ٢،٠ و ٢،٠ مجم / لتر). تم الحصول على أكبر عدد من النباتات في بيئة موراشيغ وسكوج التي تحتوي على ١،٠ و ١،٥ ملجم / لتر كاينيتين. ومن ناحية أخرى، تم تسجيل أكبر عدد من النباتات (١٣،٧٠) مع الصنف ليدي روزيتا. ومن ناحية أخرى، تم تسجيل أعلى عدد من الأوراق (٣٩،٤٤) عند ٢،٠٠ ملجم / لتر كاينيتين مع ليدي روزيتا. بينما سجل كلا من الصنفين هيرميس وسبونتانا عند ٠،٥٠ ملجم / لتر كاينيتين نفس الطول للنباتات (١٠،٠٠ سم). تأثر عدد النباتات سلباً بالمستويات المختلفة من كلوريد الصوديوم في بيئة موراشيغ وسكوج وتعتبر ليدي روزيتا أكثر تحملاً للإجهاد الملحي عند زراعتها على بيئة موراشيغ وسكوج المحتوية على ٤٠٠٠ ملجم / لتر من كلوريد الصوديوم. تم الحصول على أعلى عدد من الأوراق (٩٤،٠٠) للصنف ليدي روزيتا مع تركيز ١٠٠٠ ملجم / لتر من كلوريد الصوديوم. أدت زيادة تركيزات الملح إلى انخفاض الوزن الطازج. و تم الحصول على أعلى وزن جاف (٠،٦٧ جم) من الصنف هيرميس مع معاملة ٢٥٠ ملجم / لتر كلوريد الصوديوم، مقارنة بالطرز الجينية الأخرى. تم تقليل أصباغ التمثيل الضوئي، بما في ذلك الكلوروفيل أ، والكلوروفيل ب والكاروتين الكلي لأربعة طرز وراثية من البطاطس بشكل كبير، بسبب انخفاض الضغط الأسموزي في وسط الزراعة. سيسهل البحث لإنتاج نباتات البطاطس المستقرة التي تتحمل الملوحة من نباتات ناتجة في المعمل. قد تكون ذات أهمية اقتصادية في الأراضي القاحلة وشبه القاحلة في العالم وتطوير برامج جديدة غير تقليدية لتربية البطاطس.