Journal homepage
www.ajas.uoanbar.edu.iq
Anbar Journal of Agricultural Sciences
(University of Anbar – College of Agriculture)

STUDYING THE EFFECT OF SALT STRESS ON THE GROWTH OF THREE POTATO CULTIVARS (SOLANUM TUBEROSUM L.) GROWN IN VITRO

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Article info

Received: 2022-12-12
Accepted: 2023-02-21
Published: 2025-06-30

DOI-Crossref:
10.32649/ajas.2025.186518

Cite as:
Ali, M. R. K., Mahmooud, S. A., and Mohammed, M. A. (2025). Studying the effect of salt stress on the growth of three potato cultivars (solanum tuberosum l.) grown in vitro. Anbar Journal of Agricultural Sciences, 23(1): 1-11.

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Abstract

Carried out, Elmundo, Arizona) outside the body, the sterilization results showed the superiority of the Mundo variety by giving it the lowest percentage of contamination. The study was conducted in the Plant Tissue Culture Laboratory of the Department of Horticulture and Landscape Engineering - College of Agriculture - Anbar University, for the period from June-2022 to September-2022 with the aim of studying the effect of Salt stress in the growth characteristics of three potato cultivars, namely (Burren), amounted to 13.33%. The Burren cultivar excelled by giving it the highest response percentage of 16.67%. of NaCl salt, which is (0, 8, 10, and 12) ds for a month. The results showed a significant effect of NaCl salt on the vegetative growth characteristics of plants by increasing the salt concentration, as there was a significant reduction in the lengths and number of vegetative branches, their fresh and dry weights, and the number of nodes and leaves. It was also observed that there was a significant effect of the cultivars, as the Arizona cultivar recorded the highest average number and length of branches with a value of (2.800 cm and 3.275 branches explants^{-1}), respectively, while the Elmundo cultivar recorded the highest average number of nodes and leaves with a value of (7.12

nodes explants^{-1} and 10.83 leaves explants^{-1}), while the Burren variety gave the highest mean of fresh and dry weight with a value of (0.0947 and 0.00975 gm), respectively.

Keywords: Salt stress, Growth, Potato, In vitro.

دراسة تأثير الاجهاد الملحي على نمو ثلاثة أصناف من البطاطا Solanum tuberosum L. المزروعة خارج الجسم الحي

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الخلاصة

نفذت الدراسة في مختبر زراعة الأنسجة النباتية التابع لقسم البستنة وهندسة الحدائق – كلية الزراعة – جامعة الانبار، للفترة من حزيران– 2022 الى أيلول– 2022 بهدف دراسة تأثير الاجهاد الملحي في صفات النمو لثلاثة اصناف من البطاطا وهي (Burren، Elmundo، Arizona) خارج الجسم الحي، أظهرت نتائج التعقيم تفوق الصنف الموندو بإعطائه اقل نسبة للتلوث بلغت 13.33%. وتفوق الصنف بورين بإعطائه اعلى نسبة مئوية للاستجابة بلغت 16.67%. اما بالنسبة لتجربة الملوحة استعمل وسط MS مزود بتراكيز مختلفة من ملح NaCl وهي (0، 8، 10 و12) ديسي سيمينز تم اضافتها الى الوسط الغذائي MS لمدة شهر، أظهرت النتائج وجود تأثير معنوي لملح NaCl في صفات النمو الخضري للنبيتات بزيادة تركيز الملح إذ حصل اختزال معنوي في اطوال الافرع الخضرية وعددها واوزانها الطرية والجافة وعدد العقد والأوراق، كما لوحظ وجود تأثير معنوي للأصناف إذ سجل الصنف Arizona اعلى متوسط لطول الافرع واعدادها بقيمة بلغت (2.800 سم و3.275 فرع نبات$^{-1}$) بالتتابع بينما سجل الصنف Elmundo اعلى متوسط لعدد العقد والأوراق بقيمة بلغت (7.12 عقدة نبات$^{-1}$ و10.83 ورقة نبات$^{-1}$) في حين اعطى الصنف Burren اعلى متوسط للوزن الطري والجاف بقيمة بلغت (0.0947 و0.00975 غم) بالتتابع.

كلمات مفتاحية: الاجهاد الملحي، نمو، البطاطا، خارج الجسم الحي.

Introduction

The potato (*Solanum tuberosum L.*), which belongs to the Solanaceae family, is one of the most economically important vegetable crops worldwide. It ranks fourth globally after wheat, rice, and maize as a major food crop (12). In recent years, potato productivity has declined due to soil salinity, as salt stress is one of the primary factors

that reduce the growth of agricultural crops and limit their productivity. High salinity affects the normal growth of plants by altering the plant cell membrane, proteins, and lipid structure (8). Salinity is one of the most significant problems facing Iraqi soils, particularly in the central regions, where 75% of these soils are affected by salinity, especially in potato cultivation areas (7).

Salinity is a major issue that reduces the cultivation of many crops by affecting the morphological traits of crops grown in the field and in vitro. It impacts roots, stems, leaves, and other plant parts. Previous studies on potatoes have shown that increasing the concentration of sodium chloride in the growth medium causes a reduction in the growth of vegetative parts in tissue culture, including plant height, leaf number, and fresh and dry weights. Additionally, there is variation in the growth rate of tissue-cultured plantlets depending on the plant variety and the salt concentrations used in the growth medium (6 and 14). Increasing salinity in the plant growth medium causes a reduction in cell, leaf, and stem size, as well as thickening and hardening of plant cell walls, making them less flexible and more rigid due to salt accumulation in the cells (4). Salinity also stunts main stems, reduces the number of lateral branches, causes the death of newly formed branches, and inhibits cambium activity (5).

A significant effect of sodium chloride at concentrations (0, 50, 100, and 150) mM was observed in the MS growth medium supplemented with the growth regulator IAA at a concentration of 1 mg L^{-1} on the vegetative growth traits of tissue-cultured *Eigenheimer* and *Bintje* varieties grown in vitro. The results showed that increasing salt concentration in the growth medium caused a significant reduction in shoot length, leaf number, and fresh and dry weights. The interaction between 0 mM sodium chloride and the *Bintje* variety recorded the highest shoot length and leaf number, while the interaction between 50 mM and the *Eigenheimer* variety gave the highest fresh and dry weights of the vegetative parts. However, increasing sodium chloride concentration in the growth medium had a negative effect on vegetative growth traits (11). Therefore, the current study aimed to utilize plant tissue culture technology to identify the best-performing potato variety under salt stress conditions, with the goal of cultivating it in salt-affected soils.

Materials and Methods

Sterilization Experiments and Cultivation of Plant Parts: To achieve the optimal concentration of sodium hypochlorite (NaOCl) for obtaining contamination-free cultures, which is a critical step in establishing and initiating tissue cultures, different concentrations of sodium hypochlorite (commercial "Vas" at 6%) were used at 25%, 50%, and 75%. Green vegetative buds, approximately 1 cm in length, were excised from the tubers of the studied potato varieties. These buds were washed under running water for half an hour to remove any adhering dirt or debris. The green buds were then immersed in sodium hypochlorite solution for each potato variety according to the aforementioned concentrations for ten minutes, with continuous agitation (2).

Afterward, the buds were rinsed three times with sterile distilled water for three minutes each time to ensure the removal of any residual sterilizing agent. This process was carried out in a laminar airflow cabinet. The sterilized green buds were then transferred to sterile Petri dishes inside the cabinet. The lower part of the bud, coated

with paraffin, was removed, and the upper part of the bud, approximately 0.5 cm in length, was cultured on a regeneration medium. The explants were placed in test tubes containing 15 mL of the vegetative propagation medium, which consisted of MS salts.

Germination Experiment: For the germination stage, the MS (Murashige and Skoog) medium (13) of American origin was used, supplemented with the growth regulator NAA at a concentration of 0.2 mg L^{-1}. The green buds excised from the tubers were used after sterilization using the method described in the previous section, with one bud per vial. The cultures were incubated in a growth room at a temperature of 25 ± 2°C with a constant light cycle of 16 hours of light and 8 hours of darkness. Measurements were taken after one month of cultivation for the following traits:

1. Contamination Percentage: Calculated using the following formula: Contamination Percentage (%) = (Number of Contaminated Explants / Total Number of Explants) × 100
2. Germination Percentage: Calculated using the following formula: Germination Percentage (%) = (Number of Germinated Buds / Total Number of Buds) × 100 (1).

Cultivation of Explants on Saline Media: Four salinity levels were prepared by adding laboratory-grade (pure) sodium chloride to the MS medium. The salt was dissolved in water dropwise, in a manner similar to adjusting pH, and the salinity levels were calibrated using a salinity meter. The levels were as follows: 0, 8, 10, and 12 dS/m. The stem nodes obtained from the cultures during the germination stage were then transferred to the saline media. The cultures were incubated under the same conditions mentioned earlier (25 ± 2°C, 16 hours light/8 hours dark). After one month of cultivation, the required measurements were taken.

Traits Studied in the Salinity Experiment:

1. Plant Height (cm)
2. Number of Branches (branches per plant)
3. Number of Nodes (nodes per plant)
4. Number of Leaves (leaves per plant)
5. Fresh Weight (g)
6. Dry Weight (g)

Experimental Design: The experiment was conducted as a factorial experiment using a Completely Randomized Design (CRD) with ten replications for each treatment (3). Statistical analysis was performed using the Genstat software.

Results and Discussion

Sterilization of Plant Parts: The results of the statistical analysis, presented in Table 1, show significant differences in the percentage of contamination when using sodium hypochlorite. The 75% concentration yielded the lowest contamination rate of 12.78%, followed by the 50% concentration with a contamination rate of 24.44%, while the 25% concentration resulted in the highest contamination rate of 32.22%. The same table also indicates significant differences among the varieties in terms of contamination percentage. The *Mondo* variety showed the lowest contamination rate at 13.33%, followed by the *Burren* variety with a rate of 18.34%, while the *Arizona* variety had the highest contamination rate at 37.78%.

Regarding the interaction between sodium hypochlorite concentration and the varieties, the combination of the 75% concentration with the *Mondo* variety resulted in the lowest contamination percentage of 5.00%. In contrast, the interaction between the 25% concentration and the *Arizona* variety yielded the highest contamination percentage of 53.33%.

Table 1: Effect of different concentrations of sodium hypochlorite on the contamination percentage of three potato varieties (%).

Mean of Concentration	Arizona Variety	Burren Variety	AL Mondo Variety	Sodium Hypochlorite Concentration
32.22	53.33	21.67	21.67	25%
24.44	33.33	26.67	13.33	50%
12.78	26.67	6.67	5.00	75%
	37.78	18.34	13.33	Mean of Variety
Variety| × Concentration	Variety		Concentration	L.S.D 0.05
4.67**	**2.70****		**2.70****	

Response Percentage of Excised Buds to Tissue Culture for the Studied Potato Varieties (%): The results of the statistical analysis in Table 2 show significant differences among the different concentrations of sodium hypochlorite in the percentage response of excised buds from potato varieties cultured on MS medium. The 25% concentration recorded the highest response percentage at 25.55%, followed by the 50% concentration with a response percentage of 7.22%, while the 75% concentration showed no significant response.

Additionally, the same table indicates significant differences among the varieties in the response of buds to tissue culture. The *Burren* variety recorded the highest response percentage at 16.67%, followed by the *Arizona* variety with a response percentage of 8.33%, while the *Mondo* variety showed the lowest response percentage at 7.78%.

Regarding the interaction between sodium hypochlorite concentrations and the varieties in the response percentage of buds, the combination of the 25% concentration with the *Burren* variety yielded the highest response percentage at 28.33%, followed by the 25% concentration with the *Arizona* variety at 25.00%. In contrast, the 50% concentration with the *Burren* variety showed the lowest response percentage at 21.67%, while no significant response was observed for the 75% concentration across all three varieties.

Table 2: The percentage of response of the three potato varieties to tissue culture one month after planting (%).

Mean of Concentration	Arizona Variety	Burren Variety	Mondo AL Variety	Sodium Hypochlorite Concentration
25.55	25.00	28.33	23.33	25%
7.22	0.00	21.67	0.00	50%
0.00	0.00	0.00	0.00	75%
	8.33	16.67	7.78	Mean of **Variety**
Concentration × Variety	Variety		Concentration	L.S.D 0.05
2.86**	1.65**		1.65**	

Effect of Different Concentrations of NaCl on Plant Height for Three Potato Varieties (cm): The results of the statistical analysis in Table 3 show significant differences among the different NaCl treatments. The T0 treatment (control, 0 dS/m) recorded the highest average plant height of 7.147 cm, while the T3 treatment (highest salinity level) recorded the lowest average plant height of 4.833 cm.

Regarding the effect of varieties, the table indicates significant differences among the varieties. The Arizona variety recorded an average plant height of 5.765 cm, followed by the Burren variety with an average height of 5.528 cm, while the Mondo variety showed the lowest average plant height of 5.138 cm.

Concerning the interaction between NaCl treatments and varieties, the table shows significant differences in plant height. The interaction between the T0 treatment and the Burren variety yielded the highest average plant height of 7.940 cm, while the interaction between the T3 treatment and the Burren variety recorded the lowest average plant height of 3.940 cm.

Table 3: Effect of Different Concentrations of NaCl on Plant Height for Three Potato Varieties (cm).

Mean of treatments	Arizona Variety	Burren Variety	Mondo AL Variety	treatment
7.147	7.060	7.940	6.440	T0
5.037	5.110	5.060	4.940	T1
4.890	4.890	5.170	4.610	T2
4.833	6.000	3.940	4.560	T3
	5.765	5.528	5.138	Mean of **Variety**
T × Variety	Variety		T	L.S.D 0.05
1.634	0.817**		0.944	
T0= 0 d.s, T1= 8 d.s, T2= 10 d.s, T3= 12 d.s.				

Effect of Different Concentrations of NaCl on the Number of Branches for Three Potato Varieties (branches per plant): The results of the statistical analysis in Table 4 indicate significant differences among the different NaCl concentrations in the number of branches for potato plantlets grown in vitro. The control treatment (T0) recorded the highest average number of branches at 3.633 branches per plant, while the T3 treatment recorded the lowest average number of branches at 2.400 branches per plant.

Regarding the effect of varieties on the number of branches, the table shows significant differences among the varieties. The Arizona variety recorded the highest average number of branches at 3.275 branches per plant, while the Burren variety showed the lowest average number of branches at 2.350 branches per plant.

Concerning the interaction between NaCl treatments and varieties, the statistical analysis indicates significant differences in the number of branches. The interaction between the T0 treatment and the Arizona variety yielded the highest average number of branches at 4.200 branches per plant, while the interaction between the T1 treatment and the Burren variety recorded the lowest average number of branches at 1.800 branches per plant.

Table 4: Effect of different concentrations of NaCl on the number of branches of three potato varieties one month after planting (plant branch^{-1}).

Mean of treatments	Arizona Variety	Burren Variety	Mondo AL Variety	treatment
3.633	4.200	3.000	3.700	**T0**
2.567	3.500	1.800	2.400	**T1**
2.567	3.300	1.900	2.500	**T2**
2.400	2.100	2.700	2.400	**T3**
	3.275	**2.350**	**2.725**	Mean of Variety
T × Variety	Variety		**T**	**L.S.D 0.05**
0.7776	**0.3888**		**0.4489**	

Effect of different concentrations of NaCl on the number of nodes for three potato varieties (node plant^{-1}): Table 5 shows significant differences between different concentrations of NaCl in the number of nodes trait for plants growing outside the living body, as treatment T2 outperformed by giving the highest average number of nodes, reaching 6.83 nodes plant^{-1}, while treatment T1 recorded the lowest average number of nodes, reaching 6.10 nodes plant^{-1}.

The same table also shows significant differences between varieties in the number of nodes trait, as the Mondo variety recorded the highest average number of nodes, reaching 7.12 nodes plant^{-1}, followed by the Borin variety with an average of 6.28 nodes plant^{-1}, which did not differ significantly from the Arizona variety, which recorded an average of 5.98 nodes plant^{-1}.

As for the interaction between NaCl salt and varieties, the results of the statistical analysis in the table below indicate significant differences in the number of nodes of plants growing outside the living body, as the interaction between treatment T2 and the Mondo variety recorded the highest average of 8.40 nodes plant^{-1}, while the interaction between treatment T1 and the Borin variety recorded the lowest average of 5.20 nodes plant^{-1}.

Table 5: Effect of different concentrations of NaCl on the number of nodes of three potato varieties one month after planting (node plant^{-1}).

Mean of treatments	Arizona Variety	Burren Variety	Mondo AL Variety	treatment
6.27	6.40	6.30	6.10	**T0**
6.10	5.70	5.20	7.40	**T1**
6.83	6.00	6.10	8.40	**T2**
6.63	5.80	7.50	6.60	**T3**
	5.98	**6.28**	**7.12**	Mean of Variety
T × Variety	Variety		**T**	**L.S.D 0.05**
1.129	**0.565**		**0.652**	

Effect of different concentrations of NaCl salt on the number of leaves for three potato varieties (leaf per plant^{-1}): The results of the statistical analysis in Table 6 indicate that there are significant differences between the different concentrations of NaCl salt in the number of leaves trait for potato plants grown outside the living body, as the comparison treatment recorded the highest average number of leaves, reaching 11.53 leaves per plant^{-1}, while the T1 treatment gave the lowest average number of leaves, reaching 8.50 leaves per plant^{-1}.

The results of the same table indicate that there are significant differences between the varieties in the number of leaves trait, as the Mondo variety recorded the highest average, reaching 10.83 leaves per plant^{-1}, followed by the Borin variety with an average of 9.35 leaves per plant^{-1}, which did not differ significantly from the Arizona variety, which gave the lowest average, reaching 8.85 leaves per plant^{-1}.

As for the effect of the interaction between NaCl salt and the varieties, the same table shows significant differences in the number of leaves trait, as the interaction between treatment T0 and the Mondo variety recorded the highest average number of leaves, reaching 13.00 leaves per plant^{-1}, while the interaction between treatment T1 and the Borin variety gave the lowest average number of leaves, reaching 7.00 leaves per plant^{-1}.

Table 6: Effect of different concentrations of NaCl on the number of leaves of three potato varieties one month after planting (leaf of plant^{-1}).

Mean of treatments	Arizona Variety	Burren Variety	Mondo AL Variety	treatment
11.53	10.90	10.70	13.00	**T0**
8.50	8.20	7.00	10.30	**T1**
9.37	8.60	7.80	11.70	**T2**
9.30	7.70	11.90	8.30	**T3**
	8.85	**9.35**	**10.83**	Mean of Variety
T × Variety	Variety		**T**	**L.S.D 0.05**
2.345	**1.173**		**1.354**	

Effect of different concentrations of NaCl on fresh weight of three potato varieties (g): The data in Table 7 indicate that there are significant differences between different concentrations of NaCl in the fresh weight of potato plants grown outside the living body, as the comparison treatment outperformed by giving the highest average fresh weight of 0.1131 g, while the T3 treatment gave the lowest average fresh weight of 0.0697 g.

The same table also indicates that there are significant differences between the varieties in the fresh weight of plants grown outside the living body, as the Borin variety recorded the highest average fresh weight of 0.0947 g, while the Arizona variety gave the lowest average fresh weight of 0.0728 g.

As for the interaction between NaCl salt and varieties, the interaction between T0 and the Borin variety gave the highest average fresh weight of 0.1398 g, while the interaction between T3 and the Arizona variety gave the lowest average fresh weight of 0.0510 g.

Table 7: Effect of different concentrations of NaCl on the fresh weight of three potato varieties one month after planting (g).

Mean of treatments	Arizona Variety	Burren Variety	Mondo AL Variety	treatment
0.1131	0.1018	0.1398	0.0977	**T0**
0.0793	0.0712	0.0826	0.0840	**T1**
0.0703	0.0674	0.0712	0.0722	**T2**
0.0697	0.0510	0.0851	0.0731	**T3**
	0.0728	**0.0947**	**0.0818**	Mean of Variety
T × Variety	Variety		**T**	**L.S.D 0.05**
0.02296	**0.01148**		**0.01326**	

Effect of different concentrations of NaCl on the dry weight of three potato varieties (g): The results of the statistical analysis in Table 8 show significant differences between the different concentrations of NaCl in the dry weight trait, as the comparison treatment T0 outperformed by giving the highest average dry weight of 0.01009 g for potato plants grown outside the living body. It was followed by treatment T1 with an average dry weight of 0.00939 g, while treatment T2 recorded the lowest average dry weight of 0.00741 g.

As for the effect of varieties on the dry weight trait, the same table shows significant differences between the different varieties, as the Borin variety outperformed by giving the highest average dry weight of 0.00975 g, followed by the Mondo variety by giving an average dry weight of 0.00922 g, while the Arizona variety gave the lowest average dry weight of 0.00681 g.

Regarding the interaction between NaCl salt and varieties, the results of the statistical analysis in the table below show that there were significant differences in the dry weight trait, as the interaction between T1 and the Mondo variety recorded the highest average dry weight of 0.01297 g. While the interaction between T1 and the Arizona variety gave the lowest average dry weight of 0.00568 g.

Table 8: Effect of different concentrations of NaCl on the dry weight of three potato varieties one month after planting (g).

Mean of treatments	Arizona Variety	Burren Variety	Mondo AL Variety	treatment
0.01009	0.00891	0.01222	0.00913	**T0**
0.00939	0.00568	0.00953	0.01297	**T1**
0.00741	0.00654	0.00817	0.00751	**T2**
0.00750	0.00612	0.00909	0.00728	**T3**
	0.00681	**0.00975**	**0.00922**	Mean of Variety
T × Variety	Variety		**T**	**L.S.D 0.05**
0.00288*	**0.00144****		**0.00167****	

We conclude from this study that the effect of different salt concentrations on plant growth varies according to the genetic differences of the varieties, and the reason for the negative effect of salinity on plant length, number of branches, number of nodes and leaves, and decrease in fresh and dry weights may be attributed to the fact that increasing salt concentrations in the nutrient medium for plant growth reduces the ability of plant tissues to absorb water and thus reduces the absorption of nutrients and minerals necessary for plant growth due to osmotic stress and thus a decrease in metabolic processes (9). As for the length and number of branches, their decrease is due to the increase in NaCl concentrations in the nutrient medium, which causes direct and indirect effects on their growth and development through its effect on the hormonal and ionic balance and vital activities within the plant, especially on the processes of cell division and elongation, which is reflected in the length and number of branches (10). The same is the case with the number of leaves, which decreased due to the increase in osmotic pressure in the growth medium, which causes the majority of the energy available for the metabolic processes of the cultivated tissue to be converted towards building osmotic potential inside the cell to confront the high osmotic pressure in the nutrient medium at the expense of the building processes necessary for the

growth of plant parts, including leaves (15). The decrease in the fresh and dry weight of the green branches is due to the same reasons mentioned previously due to salinity. These results agree with (6 and 14) in terms of the decrease in the length of the branches, their number, and the fresh and dry weight.

Conclusions

Al Mondo variety excelled in providing the lowest contamination rate, while the Bourne variety excelled in providing the highest percentage of response. The vegetative growth characteristics of the seedlings were affected by the addition of sodium chloride to the growing medium. The Arizona variety recorded the highest average for branch length and number, while Al Mondo variety recorded the highest average for the number of nodes. On the other hand, the Bourine variety gave the highest average for fresh and dry weight.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Mohammed Rajab Kamel Ali; methodology, writing—original draft preparation, Saad A. Mahmooud and Mohammed A. Mohammed —review and editing. All authors have read and agreed to the published version of the manuscript.

Funding:

This research received no external funding.

Institutional Review Board Statement:

The study was conducted following the protocol authorized by the Head of the Ethics Committee, University of Anbar, Iraq Republic.

Informed Consent Statement:

No Informed Consent Statement.

Data Availability Statement:

No Data Availability Statement.

Conflicts of Interest:

The authors declare no conflict of interest.

Acknowledgments:

The authors are thankful for the help of The College Dean, and the Head of the Horticulture and Landscape Architecture Dept. The College of Agriculture, University of Anbar, Iraq.

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