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The Effects of Salinity Conducted on the Main Potato Varieties: Shangi, Tigoni, Chulu, Dutch Robjin, Lenana, and Nyota *in vitro* in Kenya

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ABSTRACT

Salinity stress is a major environmental factor limiting potato (Solanum tuberosum) productivity in Kenya. In this study, we investigate the response of primary in vitro potato varieties to saline conditions, aiming to identify salt-tolerant varieties and explore the underlying physiological mechanisms for arid and semi-arid areas. In vitro, subcultures of different potato varieties were exposed to varying concentrations of sodium chloride (NaCl), 0mm, 10mm, 60mm, and 100mm, simulating saline stress conditions in the Murashige and Skoog (MS) media. Growth parameters, including in vitro height (IH), number of roots (NR) and their density (RD), number of internodes and their length, and number of leaves, were monitored over a set period of 30 days. Results indicated significant varietal differences in response to salinity, with some varieties demonstrating enhanced salt tolerance through better maintenance of cellular integrity and osmotic adjustment. Most varieties proved to be adversely affected by the high levels of NaCl, i.e., 60mm and 100mm. The findings of this study provide valuable insights into the salt tolerance mechanisms of potatoes and highlight the potential of in vitro screening as a tool for breeding salt-resistant cultivars for arid and semi-arid regions under irrigation. These results contribute to developing sustainable potato cultivation practices in saline-affected soils.

Keywords: Solanum tuberosum, MS media, soil salinity, sodium chloride, in vitro, tissue culture, ADC, CIP

INTRODUCTION

In Kenya, potato (*Solanum tuberosum*) is now the second most important food crop after maize. It is grown by an estimated 800,000 small-scale farmers and generates employment for an estimated 2.5 million people along the value chain (CIP, 2019). Thus, improved potato production has the potential to boost farmer incomes and living standards significantly.

Soil salinity refers to the concentration of soluble salts in the soil that can adversely affect plant growth, particularly the development of Irish potatoes (*Solanum tuberosum*). When soil salinity levels are too high, the excess salts interfere with the potato plants' ability to take up water and essential nutrients, reducing crop yields and poor-quality tubers (Shrivastava & Kumar, 2014).

Salinity is one of the most significant abiotic stresses that severely impact agricultural productivity, particularly in regions with arid and semi-arid climates (Wafula, 2021). Potatoes (*Solanum tuberosum*), a major Kenyan food crop, are highly susceptible to salt stress, which adversely affects growth, development, and tuber yield. As the world faces increasing soil salinization due to climate change and poor irrigation practices (JHA, 2017), developing salt-tolerant crop varieties has become more urgent. In Kenya increased population has increased food demand and the young population is consuming more potatoes (Naziri et al., 2024).

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Hence, there is a need to increase the area under potatoes. The majority of potatoes in Kenya are grown in the highlands (NPCKKENYA, 2023).

Region	County				
Central	Nyandarua, Nyeri, Kiambu, Kirinyaga and Muranga				
Eastern	Upper parts of Meru, Machakos, Makueni, Embu and Tharaka Nithi				
Rift valley	Nakuru, Narok, Bomet, Elgeyo Marakwet, Kericho, Uasin Gishu, Nandi, Laikipia, West Pokot, Bering, Trans-Nzoia and Kajiado				
Western	Bungoma and Kakamega				
Coast	Taita-Taveta and Kwale				
Nyanza	Nyamira and Kisii				

Table 1: Potato growing regions in Kenya

These areas are becoming unproductive due to human settlement and increased pest pressure like PCN (Potato Cyst Nematode), Bacterial Wilt, and climate change (Nyang'au et al., 2023). Therefore, more land will be required to be reclaimed in the Kenyan ASAL areas where we have salinity issues (Ng'ang'a, 2023).

Traditional breeding methods to enhance salinity tolerance in potatoes have been slow and resource-intensive, requiring large field trials and long breeding cycles.

In vitro plant tissue culture offers a promising alternative for the rapid screening and selection of salt-tolerant potato varieties (Ahmed et al., 2020). This method allows for the controlled environment necessary to assess plants' physiological and biochemical responses to various stressors, including salinity. Furthermore, *in vitro* systems enable the study of individual plant responses at cellular and molecular levels, providing insights into stress tolerance (Nyang'au et al., 2023).

Several studies have demonstrated the potential of *in vitro* cultures in identifying potato varieties with enhanced resistance to abiotic stresses, including salinity (Rahman, 2009). However, there remains a gap in our understanding of the precise physiological and biochemical adaptations that contribute to potato salt tolerance. This research addresses this gap by investigating the salinity response of main potato varieties, focusing on growth parameters: no. of roots, *in vitro* height, no. of leaves, and the no. of internodes of stress tolerance. The ultimate goal is to identify potato cultivars with superior salt tolerance that can be incorporated into breeding programs, helping to ensure food security in salt-affected regions worldwide.

According to previous studies (Maathuis, 2013), salt is highly toxic to all plants and leads to Na+ and Cl -formation. The accumulation of Na+ and Cl- ions in the cells is very poisonous and can influence all mechanisms and the enzymatic actions of the plants. The main goal of this study was to reveal variability in the salinity stress tolerance of potato varieties using *in vitro* screening. *In vitro*, plantlets consisting of a single node of different varieties were cultured on Murashige and Skoog (MS) media supplemented with varying concentrations of sodium chloride (NaCl) (control, 10mM, 60mM, and 100mM). The Previous studies revealed that leaflet formation of all potato varieties subjected to salt stress was delayed up to 1-2 weeks at higher salt concentrations (100 mM NaCl) (Ahmed et al., 2020).

MATERIALS AND METHODS

Plant Materials and NaCl Treatments

This research was conducted at the Agriculture Development Corporation (ADC) MOLO Potato Complex Tissue Culture Laboratory.

In trial 1, seven potato varieties were investigated for NaCl tolerance at concentrations of 10 mM, 60 mM, and 100mM, and their performance was compared against the control. They included: Shangi, Tigoni, Chulu, Dutch Robjin, Unica, Lenana, and Nyota potato varieties. Disease-free *in vitro* plantlets initially obtained from the International Centre for Potato (CIP) were used. Stem cuttings of about 1cm consisting of a single node were cultured on MS media supplemented by different concentrations of NaCl (10, 60, and 100mM) and 0 mM NaCl as a control.

In trial 2, five major varieties were investigated using the 10mM NaCl concentration and 0 mM as the control. This investigation was to confirm the results observed in trial 1. Here, parameters of growth rate (GR), length of internodes (LI), stem strength (SS), and root system (RS) were observed. The main varieties investigated include Dutch Robjin, Unica, Shangi, Tigoni, and Nyota.

RESULTS AND DISCUSSION

Trial 1

The *in vitro* screening of potatoes for NaCl tolerance was conducted under different concentrations of NaCl: Control, 10 mM, 60 mM, and 100mM. The observed effects on various growth Parameters: *in vitro* height (IH), no. of roots (NR), no of leaves (NL), and no of internodes (NI) at each concentration are as presented below.

Table 2: Average obtained across the seven varieties for concentration of 0 mM	
(control)	

Variety	NL	NI	NR	IH(cm)
Shangi	10.3	8.5	6.5	3.9
Chulu	19.3	16.7	8.3	9.7
Dutch Robjin	16	13.2	9	8.2
Unica	12.7	10.7	10	8.7
Lenana	9.3	6.7	5	4
Nyota	9.5	6.7	7	3.3
Tigoni	10.7	8.3	8.3	4.8

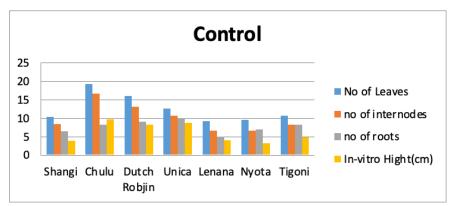


Figure 1: Bar graph illustration

Under control, MS media potato *in vitro* growth habits from best performing were Chulu, Dutch Robjin, Unica, Tigoni, Shangi, Lenana, and Nyota. Chulu displayed good performance across all parameters with a height of 9.7 cm, several leaves at an average of 19.3, roots at 8.3, and internodes at 16.7. Unica proved to have the highest number of roots at 10. Lenana does perform dismally in this control media, with its parameters being the lowest across.

Further investigation of the variance between 0mM of NaCl and 10mM was conducted to establish and ascertain the difference between the concentrations so that we could decide whether the effects of the concentration are viable enough to allow potato production in areas with these salinity levels.

Variety	NL	NI	NR	IH (cm)
Shangi	16.3	13.7	6.3	8.3
Chulu	22	19.7	12	9.7
Dutch Robjin	20.7	19.3	8.3	8.7
Unica	14.3	12.3	9.3	9.7
Lenana	14.3	9.8	7.3	8
Nyota	11.7	10.3	8.3	6.5
Tigoni	13	11.3	9	6.3

 Table 3: Averages obtained across seven varieties for concentration 10mM NaCl

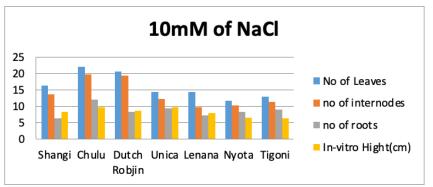


Figure 2: Bar graph illustration

Under slight salinity of NaCl 10mM concentration, most varieties under trial performed better than under control (0Mm), in-vitro growth performance from best: Chulu, Dutch Robjin, Shangi, Unica, Lenana, Tigoni, Nyota. Chulu showed to have the highest no of leaves being 22 compared to control where it had 19.3, Chulu had the highest internodes at 19.7 whereas in control it had 16.7, together with high no of roots at 12 against control which had 8.3. This shows that Chulu does perform best at this level of concentration followed closely by Dutch Robjin.

Variety	NL	NI	NR	IH (cm)
Shangi	8.7	5.7	3	3.2
Chulu	11.7	9.7	11	3.2
Dutch Robjin	17.3	15.3	10.7	6.5
Unica	14.3	9	4	3
Lenana	7.3	4	1.3	1.2
Nyota	5	3.3	5	1.3
Tigoni	8	4	5.3	1.3

Table 4: Averages obtained across the seven varieties for 60 mM concentration

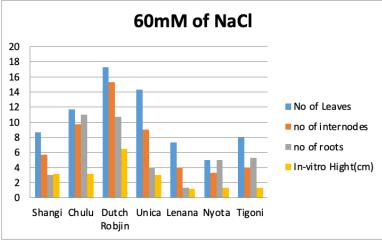


Figure 3: Bar graph illustration

Under 60mM salinity level, crop growth was inhibited and in-vitro performance was poor across all varieties except for Dutch Robjin, which was thriving in all parameters. Indicating that the Dutch Robjin can be cultured onto MS media with a concentration of NaCl 60mM, and its performance would be at best compared to the others, but it's still affected by the high concentration when figures obtained from Table 3 are compared to those in Table 4. It's noted that the number of leaves drops from 20.7 to 17.3, indicating inhibition in performance by high salt concentrations. When compared to the control, Dutch Robjin improves in its parameters except for the height, where there is a slight drop from 8.2 to 6.5.

The best was Dutch Robjin, leading in all parameters, followed by Unica, Chulu, and Shangi are average performers across all parameters, same with Tigoni. Lenana and Nyota performed below average.

Variety	NL	NI	NR	IH (cm)
Shangi	6.3	4	3.3	1.7
Chulu	6.3	2.7	3.2	1
Dutch Robjin	13.3	11	5.7	3.7
Unica	7	2.7	3.3	1.8
Lenana	6.3	4.3	1.3	1.3
Nyota	5.7	3.3	2.7	1.3
Tigoni	4.7	2.7	3.7	0.5

Table 5: Averages obtained across the seven varieties for 100 mM concentration

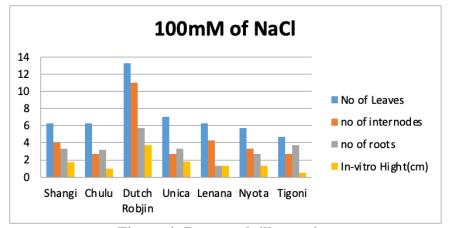


Figure 4: Bar graph illustration

Dutch Robjin was found to be the most tolerant local potato variety at 100mM, and all its traits performed well. It was recorded at a height of 3.7 cm. The number of leaves was 13.3, but there was a significant drop in these figures, indicating low tolerance to any further increase in the saline concentrations. The plant trait most affected by high salinity was height, and Tigoni proved to be the most intolerant, with an average height of 0.5cm recorded.

Dutch Robjin was recorded with an average of 11 internodes. The plant trait that was least affected by high salinity was Dutch Robjin, with several plant leaves with an average of 13.3. Lenana had the least number of roots.

Comparison Made across the Concentrations for Each Variety

Table 6: Comparison of averages for Dutch Robjin across all variables and
concentrations

Variables	NL	NI	NR	IH
0mM	16	13.2	9	8.2
10mM	20.7	19.3	8.3	8.7
60mM	17.3	15.3	10.7	6.3
100mM	13.3	11	5.7	3.7

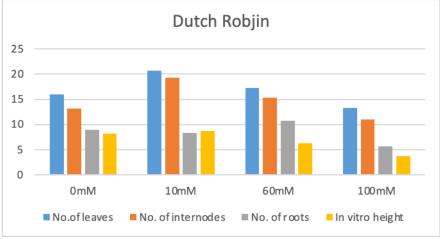


Figure 5: Bar graph illustration

Dutch Robjin showed high tolerance at the 10mM NaCl concentration level, with the number of leaves being 20.7 and 13.3 at 100 mM NaCl, still being the high-performing variable.

At 100mM, the height is recorded at 3.7cm compared to the control at 8.7cm.

The number of roots at 100mm was recorded as 5.7, while the highest was 10.7 at 60 mM

Table 7: Comparison of averages for Unica across all variables and concentrations

Variables	NL	NI	NR	IH
0mM	12.7	10.7	10	8.7
10mM	14.3	12.3	9.3	9.7
60mM	14.3	9	4	3
100mM	7	2.7	3.3	1.8

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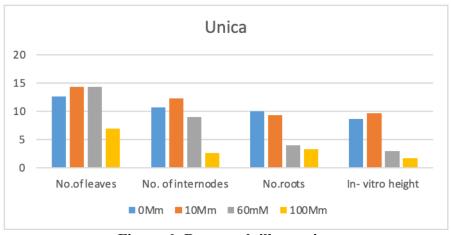


Figure 6: Bar graph illustration

The lowest height recorded was 1.8 in concentration 100mM, while at 10 mM it was 8.7 cm. The lowest number of roots was recorded at 4, and the highest was at 10. The highest number of internodes recorded was at 12.3, and the lowest was 2.7 at concentrations of 100mM. The highest number of leaves was recorded in both concentrations of 60mM and 10 mM; the control was above average in most parameters compared to 100mM, which was the least performing.

 Table 8: Comparison of averages for Shangi across all variables and concentrations

Variables	NL	NI	NR	IH
0mM	10.3	8.5	6.5	3.9
10mM	16.3	13.7	6.3	8.3
60mM	8.7	5.7	3	3.2
100mM	6.3	4	3.3	1.7

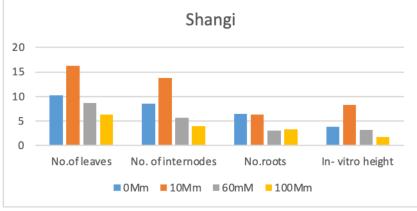


Figure 7: Bar graph illustration

Plant height for change was observed to drastically reduce with an increase in the concentrations except for the concentration of NaCl 10Mm, which was recorded as high. At this point, change seemed to be performing better than the control MS media across all parameters. Thus, Shangi *in vitro* can be cultured at this concentration.

An increase in salt concentration absorbed by the invitros inhibits performance. The inhibition of water absorption by in vitro roots due to the increased osmotic pressure in the 100Mm caused little to no root growth.

An increase in concentration also leads to a decrease in the number of roots.

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Table 9: Comparison of Tigoni across all variables and concentrations

Variables	NL	NI	NR	IH
0mM	10.7	8.3	8.3	4.8
10mM	13	11.3	9	6.3
60mM	8	4	5.3	1.3
100mM	4.7	2.7	3.7	0.5

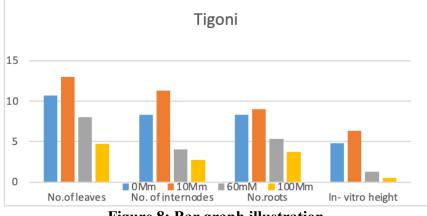


Figure 8: Bar graph illustration

At a 100 mM concentration, *in vitro* performance was affected across all the parameters, with height being the most intolerant, with a height of 0.5 being recorded.

At 10mM, Tigoni has the highest number of leaves recorded across all the concentrations.

Table 10: Comparison of averages for Nyota across all variables and concentrations

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Variables	NL	NI	NR	IH
0mM	9.5	6.7	7	3.3
10mM	11.7	10.3	8.3	6.5
60mM	5	3.3	5	1.3
100mM	5.7	3.3	2.7	1.3

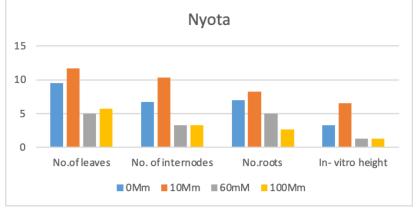


Figure 9: Bar graph illustration

Nyota has tolerance for slight levels of salinity of 10mM, as is shown by the data recorded, all parameters can tolerate these levels of salinity to allow some level of performance. Nyota *in vitro* height is the most intolerant to high levels of 60mM and 100mM, being 1.3 cm in both.

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Table 11: Comparison of averages for Chulu across all variables and concentrations Variables NL NI NR IH 0mM 9.5 6.7 7 3.3 10mM 11.7 10.3 8.3 6.5 60mM 5 3.3 5 1.3 100mM 5.7 2.7 3.3 1.3

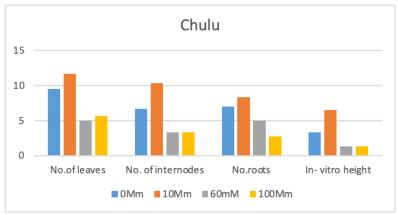


Figure 10: Bar graph illustration

Chulu showed high levels of Inhibition of water absorption in roots due to the increased osmotic pressure in the 100 mM and 60 mM media, causing little growth of roots. With little growth of roots, the other parameters are adversely affected.

In vitro height of 1.3 was recorded in both 60 mM and 100mM, indicating very poor growth, but as observed in 10mM, a record of 6.3 was the highest, showing that it can survive at this level of salinity.

At 10Mm all parameters performed well as compared to the control.

2. CUI	ilparison of averages for Le	nana aci u	55 all v	ariadio	es anu conc	LCIII
	Parameters/	NL	NI	NR	IH (cm)	
	concentrations					
	0mM	9.5	6.7	7	3.3	
	10mM	11.7	10.3	8.3	6.5	
	60mM	5	3.3	5	1.3	
	100mM	5.7	3.3	2.7	1.3	

Table 12: Com	parison of averag	es for Lenan	a across all v	variables and	concentrations
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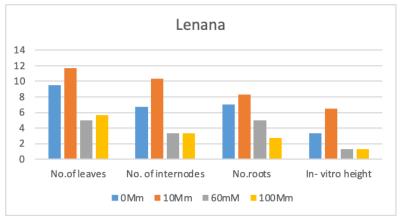


Figure 11: Bar graph illustration

Effects of Different Concentrations of NaCl in the Enhancement of Responses of Potato under Parameters Below, Number of Leaves (NL), Number of Internodes (NN), Number of Roots (NR), In vitro Height (IH)

Trial 1 Conducted in March and April 2024

Table 13: Raw data collected seven days after inoculation	Table 13:	Raw data	collected	seven day	's after	inoculation
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Date	Variety	NL	NI	NR	IH (cm)	Level (mM)
3/15/2024	Shangi	5	3	1.75	1.5	0
	Tigoni	4.8	3.2	4.3	2.5	0
	Chulu	6	6	6	3.9	0
	Unica	4.3	3.2	4.7	2.1	0
	Dutch Robjin	7.3	4.5	8.3	3.2	0
	Lenana	3.8	2.3	2.1	1.5	0
	Nyota	3.3	2.5	5.3	1.3	0
	Shangi	6.1	4.3	2.5	3.4	10
	Tigoni	5.7	3.2	4	1.8	10
	Dutch Robjin	5.6	5	4.3	3.8	10
	Chulu	8.3	5	4.3	4.7	10
	Nyota	4.8	3.3	2.2	2.3	10
	Lenana	5.7	4	2.2	3.3	10
	Unica	6	3.5	4.7	2.2	10
	Shangi	3.3	2.8	1.7	1.2	60
	Chulu	4.2	2.3	2	1	60
	Tigoni	3.8	2	2.7	0.5	60
	Dutch Robjin	6.8	5	3.8	2.8	60
	Lenana	2.2	1.3	0.7	0.4	60
	Nyota	3	1.8	1	1.1	60
	Unica	4.3	2.2	2.3	1.2	60
	Shangi	2	1.3	0	0.5	100
	Chulu	3	1	0	0.3	100
	Tigoni	2.7	0.7	2	0.2	100
	Dutch Robjin	5.7	2.2	0.7	0.8	100
	Lenana	3	0.3	0.3	0.1	100
	Nyota	3.3	2	1	0.4	100
	Unica	3	1.3	0	0.3	100

Table 14: Raw data collected fourteen days after inoculation

Date	Variety	NL	NI	NR	IH (cm)	Level (mM)
3/22/2024	Shangi	5.5	4.5	3	1.8	0
	Tigoni	6	4	5.7	1.7	0
	Chulu	8.8	7.3	7	4.5	0
	Unica	6.8	4.7	6.3	3.3	0
	Dutch Robjin	8.5	7	5.2	4.5	0
	Nyota	4	3.3	6	1.6	0
	Lenana	4.7	2.8	3	1.5	0
	Shangi	7.2	5.8	4.5	4.5	10
	Tigoni	6.2	5	5.3	3	10
	Dutch Robjin	7	6.7	5	4	10
	Unica	6.7	4.7	6	3.3	10
	Lenana	7.3	5.3	4.7	4	10
	Nyota	6.8	5.5	2.7	3	10
	Chulu	12	10	5.3	6.5	10
	Shangi	3.8	3.3	3.3	1.7	60
	Unica	6	3.3	2.7	2	60
	Tigoni	3.7	2	3.2	0.8	60

Nyota	4.3	2.5	2.7	1.2	60
Dutch Robjin	8.5	7.3	4.7	3.3	60
Chulu	6	3.3	3	1.3	60
Lenana	2.7	1.3	1.3	0.8	60
Shangi	3.7	1.7	0.7	0.9	100
Tigoni	3.3	1.3	3.3	0.3	100
Lenana	4	2.3	1	0.5	100
Chulu	4	2	1.7	0.6	100
Nyota	5	3.3	1.7	1	100
Unica	3.7	1.7	0	0.6	100
Dutch Robjin	4.3	2.3	1	1.7	100

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Table 15: Raw data collected twenty days after inoculation

Date	Variety	NL	NI	NR	IH	Level (mM)
3/30/2024	Shangi	8	5	4.5	2.3	0
	Tigoni	6.8	4.2	7.7	2	0
	Chulu	11.7	9	8	6	0
	Unica	7.5	6.3	6.8	3.9	0
	Dutch Robjin	10.2	8.8	8.7	5	0
	Nyota	5.7	4	5	2.4	0
	Lenana	5.5	4.5	3.8	2.4	0
	Shangi	9.3	6.5	4	4.8	10
	Tigoni	7.7	6.2	6.7	3.2	10
	Chulu	13.7	11.3	6	7.2	10
	Dutch Robjin	9	7.3	5.3	6.2	10
	Unica	7	5.5	5.7	4.3	10
	Lenana	7.7	6.5	4.3	5.3	10
	Nyota	8.7	6.5	3.7	4.2	10
	Shangi	4.8	4	4	1.8	60
	Chulu	6.2	3.8	3.3	1.6	60
	Dutch Robjin	9.5	8.2	5.5	4.1	60
	Unica	8.3	4	3.8	2	60
	Lenana	4	2	2.2	0.7	60
	Nyota	4.3	2.7	2.7	1.2	60
	Tigoni	5	1.8	3.5	0.9	60
	Shangi	3.7	2.2	0.7	0.8	100
	Tigoni	3.7	1.3	3.7	0.6	100
	Lenana	4.7	3	1	1.1	100
	Nyota	5	2.7	1.7	0.9	100
	Unica	5	2.2	0	1.2	100
	Chulu	4	1.5	0.7	0.6	100
	Dutch Robjin	3.7	3	2	1.5	100

Table 16: Raw data collected thirty-three days after inoculation

Date	Variety	NL	NI	NR	IH (cm)	Level (mM)
4/11/2024	Shangi	9.5	6	5	2.3	0
	Tigoni	8.7	5.8	8.3	2.5	0
	Chulu	15.3	13	13.3	7.2	0
	Dutch Robjin	12.3	8	12.7	7.5	0
	Unica	9.5	7.7	6.7	5.8	0
	Lenana	7.7	5	5.7	3.1	0
	Nyota	7	5.3	5.7	2.7	0
	Shangi	10.2	7.3	6.7	4.2	10
	Tigoni	8.3	5.8	7	2.6	10
	Chulu	17.3	13.5	11.7	9	10
	Dutch Robjin	14.3	10.3	8	7.7	10
	Unica	10.8	7.8	6.7	6.5	10

Lenana	9.5	7.7	4.7	6.3	10
Nyota	8.5	7	4	5	10
Shangi	7.2	5.2	4.3	2.3	60
Chulu	7	5	3.7	2.2	60
Dutch Robjin	12.5	10.5	6.2	5	60
Unica	11.3	6.3	3.7	2.7	60
Lenana	6.3	3.7	1.3	1.2	60
Nyota	5.3	4.3	3.7	1.5	60
Tigoni	5	2.7	3.7	1.3	60
Shangi	5	3.7	2.7	1.3	100
Chulu	5.3	2.3	2.3	0.7	100
Dutch Robjin	7	5	3	2.5	100
Unica	6	3.3	2.3	1.1	100
Lenana	5	2.7	0.3	0.9	100
Nyota	5.3	3.7	2.7	1	100
Tigoni	3.3	2	2.7	0.4	100

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Table 17: Raw data collected forty-nine days after inoculation

Date	Variety	NL	NI	NR	IH	Level (mM)
4/27/2024	Shangi	10.3	8.5	6.5	3.9	0
	Chulu	19.3	16.7	8.3	9.7	0
	Dutch Robjin	16	13.2	9	8.2	0
	Unica	12.7	10.7	10	8.7	0
	Lenana	9.3	6.7	5	4	0
	Nyota	9.5	6.7	7	3.3	0
	Tigoni	10.7	8.3	8.3	4.8	0
	Shangi	16.3	13.7	6.3	8.3	10
	Chulu	22	19.7	12	9.7	10
	Dutch Robjin	20.7	19.3	8.3	8.7	10
	Unica	14.3	12.3	9.3	9.7	10
	Lenana	14.3	9.8	7.3	8	10
	Nyota	11.7	10.3	8.3	6.5	10
	Tigoni	13	11.3	9	6.3	10
	Shangi	8.7	5.7	3	3.2	60
	Chulu	11.7	9.7	11	3.2	60
	Dutch Robjin	17.3	15.3	10.7	6.5	60
	Unica	14.3	9	4	3	60
	Lenana	7.3	4	1.3	1.2	60
	Nyota	5	3.3	5	1.3	60
	Tigoni	8	4	5.3	1.3	60
	Dutch Robjin	13.3	11	5.7	3.7	100
	Chulu	6.3	2.7	3.2	1	100
	Shangi	6.3	4	3.3	1.7	100
	Unica	7	2.7	3.3	1.8	100
	Lenana	6.3	4.3	1.3	1.3	100
	Nyota	5.7	3.3	2.7	1.3	100
	Tigoni	4.7	2.7	3.7	0.5	100

Trial 2 Conducted in June and July 2024

Observations made between saline MS media composing 10 mM NaCl and control (0 mM NaCl) media

Parameters to be observed include: Growth rate (GR), length of internodes (LI), root system, weak/thin stems, and thick/strong stems.

This trial used control MS potato media and MS media with a concentration of NaCl 10mM at a pH of 5.8 to confirm the results obtained in trial one.

Growth rate (GR) is measured against time. The normal growth time for most varieties is 30 days, at this point, all plantlets are expected to be at container height (7cm). Thus, this growth is termed a normal growth rate.

If the *in vitro* plantlets have not attained the container height at half height, which is 3.5 cm, at 30 days, this growth rate is termed slow.

Internodes (LI) were considered long if the distance between one node and the next was approximately 10mm to 12mm, medium if between 7mm and 10mm, and short if between 3mm and 6mm.

Stems (SS) were observed to determine their characteristics, whether thick and strong or thin and weak.

The root system (RS) was considered to be dense if it covered 100% of the base of the container; roots that spread and covered only 50% of the base of the container are considered to be medium, while roots covering a quarter of the base of the container are 25% and, therefore, considered to be sparse.

Date: 20/6/2024-Variety-Dutch Robjin (CIP)

2 control containers with an estimate of 20 in vitro plantlets inoculated.

2 10Mm MS media containers with an estimated 20 in vitro plantlets inoculated.

Observations were made at 30 days of growth.

Table 10: Dutten Robjin								
Variables	Growth rate	Internodes	Weak /strong stems	Root system (density)				
NaCl(10Mm)	Normal	Medium	Strong/thick	Medium				
Control	Normal	Medium	Strong /thick	Dense				

Table 18: Dutch Robjin

Dutch Robjin exhibited consistency across all parameters in the two Media, with little variation observed in root density in the NaCl 10mM concentration.

Date: 20/6/2024-Variety –Unica (CIP):

2 control Ms Media containers with an estimated 20 in vitro plantlets inoculated.

2 10mM MS media containers with an estimated 20 *in vitro* plantlets inoculated. Observations were made at 30 days of growth.

Table 17. Ulika								
Variables	Growth rate	Internodes	Weak /strong	stems	Root system (density)			
			(thick/thin)					
NaCl(10mM)	Slow	Medium	Weak		Sparse			
Control	Normal	Medium	Weak		Sparse			

Table 19: Unica

At 30 days the *in vitro* sub cultured into the NaCl (10mM) Media was observed to be at half the height of those in the normal media.

At 30 days, the 10mM NaCl Media and control media *in vitro* are at same length, their stems are of similar thinness and seem weak, and at this point they have one or two leaves on them compared to when they were at 30 days; the root hairs have spread from their base in the media to their stems all the way to the tips (network/web of roots).Both 10mM NaCl and control Media exhibited sparse rooting system in the media.

Date: 20/6/2024-Variety – Shangi (CIP):

2 control containers with an estimate of 20 in vitro plantlets inoculated.

2 10mM MS media containers with an estimate of 20 in vitro plantlets inoculated.

Observations were made at 30 days of growth.

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Table 20: Shangi							
Variables	Growth rate	Internodes	Weak /strong stems	Root system			
			(thick/thin)	(density)			
NaCl(10mM)	Normal	Long	Weak /thin	Sparse			
Control	Normal	Long	Strong/thick	Dense			

Both Media were thriving and growing at the same rate. From trial 1 it was observed that Shangi can be cultured on both media without experiencing any effects of the saline concentrations.

Date: 21/6/2024-Variety – Tigoni (CIP)

2 control containers with an estimate of 20 *in vitro* plantlets inoculated 2 10mM MS media containers with an estimate of 20 *in vitro* plantlets inoculated Observations were made starting at 30 days of growth.

Table 21: Tigoni							
Variables	Growth rate	Internodes	Weak /strong stems	Root system			
			(thick/thin)	(density)			
NaCl(10Mm)	Slow	Medium	Strong/thick	Sparse			
Control	Normal	Medium	strong	Medium			

Tigoni showed slow growth in the 10mM concentration with a very sparse root system and was observed to be above average in the control media.

Date: 01/7/2024-Variety-Nyota (CIP)

2 control containers with an estimated 20 in vitro plantlets inoculated and sub-cultured.

2 10mM MS media containers with an estimated 20 *in vitro* plantlets inoculated and sub-cultured.

Observations were made at 30 days of growth.

Tabl	e 22:	Nyota

Variables	Growth rate	Internodes	Weak /strong stems (thick/thin)	Root system (density)
NaCl(10mM)	Slow	Medium	Weak	Medium
Control	Slow	Medium	Weak	Medium

Nyota in general did not respond very well to the 10mM MS media in comparison to the control, the growth rate was the same and very slow. Both Medias exhibited the same characteristics.

In comparison to the other varieties in both the 10mM NaCl and control media, Nyota takes the longest (60days) to grow the height of the container which is about 7cm.

It was observed that Nyota *in vitro* is slow in growth in both control and 10mM NaCl concentration.

CONCLUSIONS

The 10mM concentration of NaCl used in trial 1 was used in trial 2, which was conducted to confirm the findings from trial 1.

Dutch Robjin performed very well in both trial 1 and trial 2 against all concentrations and parameters; Chulu performed best in trial 1.

Lenana, Tigoni is strongly recommended to be done at 0 mM NaCl than any other concentration; hence, it is recommended for MS 0 mM concentration media.

Our findings will contribute to further research on the understanding of stress tolerance in potato *in vitro* production, which will improve varieties for areas with high salinity levels.

The research work noted and recommended that when carrying out *in vitro* propagation of Dutch Robjin,10Mm of NaCl should be added to improve its growth vigor, as it's found to perform better at the starting 10mM NaCl concentration than under normal MS media.

We recommend further in vivo trials to confirm our results. This will improve/enhance breeding programs for saline-tolerant varieties.

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