

Effect of Salt Stress on *in Vitro* Propagation of Three Potato Cultivars

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ABSTRACT

The research was carried out at Biotechnology Laboratory, Horticulture Department, Patuakhali Science and Technology University, Dumki, Patuakhali, Bangladesh from 16 March to October 2016 to study the effects of different amount of NaCl (0, 1, 1.5, 2, 2.5 and 3%) on propagation of three potato cultivars. Diamant, Cardinal and Granula under *in vitro* conditions. Observations were made on the shoot and root generation. Significant differences were observed in respects of all the parameters in all cultivars used in this study. Among the cultivars, Granula performed best than others in respects to days (17.32) needed for shoot creation, amount of shoot (50.04%) initiation, shoot size (3.91 cm), number of leaves (3.34) per plant let, amount of nodes (3.26) per shoot. In case of different treatments of NaCl concentrations, control showed the best performance in respects of days (16.76) needed for shoot creation, percentage of shoot (61.18%) initiation, shoot length (4.90 cm), number of leaves (4.12) per plantlet, number of nodes (3.83) per shoot. Among the interaction effect the best performance showed “Granula” with control in respects to days (15.96) needed for shoot initiation, percentage of shoot (69.27%) initiation, shoot size (5.47 cm), number of nodes (4.35) per shoot. In contrast, Cardinal with MS medium with 3% NaCl showed the lowest performance over others in respects to the lowest percentage shoot (35.32%) creation, and lowest amount of leaves (2.05) per plantlet, and lowest amount of nodes (2.00) per shoot.

Keywords: Potato, *In vitro*, salt stress, Cultivars, propagation.

INTRODUCTION

Potato (*Solanum tuberosum*L.) is a tuberous crop and it is regard as important edible starch-rich, popular and nutritious vegetables in the world. Potato contributed alone as much as 50 percent of the total annual vegetable production in Bangladesh (Anonymous, 1998). Potato is the 4th most famous vegetable crop globally after rice, wheat and corn and the 8th most vegetable crop (FAO, 2008). It is a cheap source of vitamins, proteins, carbohydrates and minerals (Anonymous, 2014). Due to the large scale production of potatoes, it has become a necessary crop to lessen food shortages.

In Bangladesh, potato is mainly used as a vegetable, although in many countries, it constitutes the staple food and supply more than 90% of the carbohydrates. In Bangladesh, about 9.2 million tons of potatoes were produced from yearly 0.45 million hectares with an average yield of 20 tons/ha during 2015-2016 (BBS, 2016). Salinity is one of the large nonliving constraints that extremely affect productivity of agricultural products especially in arid and semiarid regions. The rate of photosynthesis and respiration in crop plants is severely interfered causing reduced plant growth and low productivity at high salts (Silva *et al.*, 2001, Zhang *et al.*, 2005, Fidago, 2004). Higher level of salinity disrupts plant roots causing water deficiency, nutrients imbalance by altering uptake and transport, ionic stress by higher Na^+ and Cl^- accumulation, cell membrane ineffectiveness and interfering cellular processes like cell division and geno-toxicity as well resulting in reduced plant growth, development and yield (Munns, 2002). Salinity and dearth/aridity are interlinked factors occurring simultaneously in the green sector that's why the problem of salinity exists in the high land regions of the world. There are two types of salinity, one is coastal salinity and the other one is induced by irrigation, in Bangladesh coastal salinity is the most common. Coastal area covers about 2.5 million hectare of land which is about 25% of the net cultivable land. About one million hectares of coastal area covering 64 upazilas of 12 districts are affected by different kinds of salinity (Yasmen, 2006). Cropping intensity in the saline area of Bangladesh is comparatively low, ranging from 76% in the Chittagong costal region to 132% in the Patuakhali coastal region compared to 179%, the national average (Karim *et al.*, 2012). However, when salinity is nonlethal, other soil factors may also become a major growth-limiting factor. In case of the coastal saline soils studied, the direct sources of soluble salts were saline tidal and underground waters, the ultimate salt source being seawater. The predominance of cations in saline soil is in the order, $\text{Na}^+ > \text{Mg}^{2+} > \text{Ca}^{2+} > \text{K}^+$ (Panauallah, 1995). The predominance of Na^+ might cause an antagonistic effect on nutrient uptake, resulting in nutrient deficiencies, particularly of K^+ and Ca^{2+} in the field. In such a situation, nutritional imbalance might become a major limiting factor for crop production.

Potato growth under different salt stress helps to develop an efficient screening technique of salinity-resistant potato lines and to get somaclonal variants under different salt stress. *In vitro* culture and micropropagation are very rapid and modern ways for the estimation of potato cultivars for salt stress (Byun *et al.*, 2007). Plant tissue culture techniques in association with conventional breeding and biotechnology have become popular approaches for developing crop plants tolerant to environmental stresses especially salt stress (Rahman *et al.*, 2008). *In vitro* determination of salinity tolerance, utilizing nodal cuttings of plants kept ranking of potato cultivars and wild species (Pour *et al.*, 2009; Silva *et al.*, 2001; Morpurgo and Silva, rodriguez, 1987). A highly significant correlation was found between *in vitro* growth parameters and the field results of ten potato clones exposed to a high level of NaCl (Morpurgo, 1991). So needs salt stress tolerant variety developed. The works done in this respect are not available. So, therefore the research was conducted with the following objectives:

- i. To introduce an efficient *in vitro* propagation protocol applying salt stress for different potato cultivars;
- ii. To identify salt-tolerant potato varieties and
- iii. To determine the tolerance level of potato cultivars against salt stress.

MATERIALS AND METHODS

Time and location of the experiment

The present observation was accomplished in the Department of Horticulture, Patuakhali Science and Technology University from march to October 2016.

Experimental materials

Three Potato (*Solanum tuberosum* L.) cultivars namely Diamant, Cardinal and Granula were used as experimental materials in the present investigation.



Plate1. Experimental materials (Diamant(A), Cardinal(B) and Granula(C) cultivar).

Three Potato (*Solanum tuberosum* L.) cultivars namely Diamant, Cardinal and Granula were taken experimental materials in the present research.

Layout and design of the experiment

The research was completed in a Completely Randomized Design (CRD) with three replications. The analysis of variance was presented and means were distinguished by Duncan's Multiple Range Test (DMRT) for interpretation of results.

METHODS

The following methods were taken for the present investigation, each for special purposes. Those were:

- 1) For subculture of plantlets: Salt free MS medium
- 2) For shoot starting: MS medium complemented with NaCl
- and 3) For root starting: MS medium complemented with NaCl.

Culture media

Murashige and Skoog (1962) medium was taken with one salt complements as a culture system for shoot improvement, maintenance and improvement of roots from excised shoots for the production of plants.

Macronutrients

The Stock solution of macronutrients was making up to 20 times the concentration of the final medium in 1 liter of distilled water. Twenty times the weight of the salts required per liter of the culture were weighed properly and dissolved by using a magnetic stirrer in about 750 ml of distilled water.

Micronutrients

For the preparation of this stock solution 100 folds (100X) of the particular salt needed per liter of the medium was weighed accurately and dissolved in 750 ml of distilled water. The stock solution was made up to the mark (1000 ml) by further addition of distilled water.

Iron sources

It was made 100 times the final strength of the solution in 1 liter of distilled water. Two chemicals, FeSO_4 and Na_2EDTA , were dissolved in 750 ml of distilled water in a conical flask by heating in a water bath and the final volume was made up to 1 liter by further mix of distilled water.

Vitamins/ organics

The following vitamins and amino acids were kept in the present research for the preparation of MS medium: Pyridoxine HCl (Vitamin B6), Thiamine HCl (Vitamin B1), Myoinositol (Inositol), Glycine and Nicotinic acid (Vitamin B3). Each of the above vitamins/organics except myoinositol was prepared as stock solution separately in 100 times the BM by dissolving in distilled water.

Preparation of culture media from stock solutions

To prepare 1 liter of MS (Murashige and Skoog, 1962) medium the following steps were followed

1. About 400 ml of distilled water was taken in a flask.
2. One hundred ml of macronutrients, 10 ml of micronutrients, 100 ml of irons and 10 ml of vitamins were taken from each of these stock solutions into a 2-litre beaker on a heater cum magnetic stirrer.
3. One hundred ml of myoinositol was added frequently to the solution and dissolved.
4. 30 ml of sucrose was added to this media and gently mix to dissolve completely.
5. The required volume of hormone solutions was directly added to the solutions in the beakers.
6. The solution was poured into a 1000 ml measuring cylinder and the volume was made up to 1000 ml with the addition of distilled water.
7. pH of the die medium was adjusted to 5.8 with a digital pH meter with the help of adding 0.1 N NaOH or 0.1 N HCl as necessary.

Sterilization

To ensure safety condition under *in vitro*, all instruments, glass wares and culture media were purified by autoclaving.

Sterilization of culture medium

The glass of ampules containing the medium was autoclaved under 15 psi of pressure at 121°C for 20 minutes. After autoclaving the culture media transferred to cool under normal temperature.

Culture methods

Explants selection

Fresh and disease-free seed micro tuber of potato varieties (Diamant, Cardinal and Granula) was selected and healthy sprouted shoots were separated.

Explants treatment

The separated explants were taken in a small beaker. The explants were sterilized as follows:

- a. Distilled water was poured up to 1/3 of the beaker and two drops added and shaken for 10 minutes. Then the water was wasted.
- b. Then the explants were washed with 70% ethanol for 30 seconds.
- c. After discarding the ethanol, the explants were washed with 0.05%
- d. Mercuric Chloride (HgCl_2) for 20 minutes and then cleanse in distilled water 4 times to wash HgCl_2 .

Then the explants were air-dried in the Laminar Air Flow Chamber for ten minutes and were transferred on MS media. This process was made for each variety.

Inoculation

The culture was injected with the treated explants in the dark at $25 \pm 2^\circ\text{C}$ for shoots induction. After 3-6 weeks of inoculation, sprouted shoots of the responsive cultivars started to produce potato plant. *In vitro* potato plants were treated as an explants culture medium.

Explants transfer (Incubation)

The size of Explant at least one node were transferred to a culture solution (MS basal solid medium), and were incubated in a temperature-controlled room at $25 \pm 2^\circ\text{C}$ under a 16 hours light photoperiod with a lighting power of about 2000-3000 lux for plant developed. Day-to-day observations were- carried out to note the response.

Subculture

Successful shoot formation became evident when small green leaves began to emerge; it was the first sign of regeneration. These tiny leaves when improved in their actual shape were transferred into fresh media containing the salt level combination or the best one among them for further proliferation and development. Subcultures were carried out regularly at an interval of 15 days.

Treatment details

The present research consisted of two factors:-

Factor A: 3 varieties of potato; denoted as:

V_1 = Diamant, V_2 = Cardinal and V_3 = Granula

Factor B: 6 levels of salt (NaCl) concentration; denoted as:

T_1 = MS medium without NaCl (control) T_4 = MS medium with 2% NaCl

T_2 = MS medium with 1% NaCl T_5 = MS medium with 2.5% NaCl

T_3 = MS medium with 1.5% NaCl T_6 = MS medium with 3% NaCl

So, there were a total of 18 (6x3) treatment combinations.

a) Shoot length (cm)

The length of the shoot proliferated after 28 days was recorded from the base to the tip. The mean values of the data provided were the shoot length.

b) Number of leaves plantlet⁻¹

At the time of emerging, the amount of leaves was recorded. The amount of leaves of sapling was counted by subtracting the sum of leaves at setting time from the total number of observed leaves and it was recorded after 28 days of culture and the mean was calculated.

c) Number of nodes/shoot

The numbers of nodes per shoot were counted after 28 days of subculture.

d) Days required for root initiation

The records for the days to root start were recorded by regular visual observation. The mean values of the data provided the days required for root initiation.

e) Number of roots plant let

The quantity of roots per plant let generated over days was counted and the mean values of the data provided were the amount of roots/plant let.

f) Root length (cm)

The length of the roots (cm) proliferated over days (28) was recorded from the base of the plant let to the angle of the root using a 15 centimeter scale. The mean values of the data counted were the root size.

Statistical analyses

Data collected on different measurement under test were statistically analyzed to check the impact of the experimental results. The analysis of variance was represented and means were compared by Duncan’s Multiple Range Test (DMRT) for assimilation of results. The significance of the difference between the pair of means was evaluated at 1% level of significance using MSTAT-C computer set packages.

RESULT AND DISCUSSION

The research was conducted to assess the performance of dissimilar potato varieties under the different salt stresses for *in vitro* plant let initiation. The results of the test have been existed and discussed distinctly under the following conditions.

Days required for shoot initiation

Effect of variety

The types of varieties shown important changes in the days required for shoot initiation. Cardinal took the highest number of days (19.17) and was statistically similar to Diamant (18.72). In contrast, Granula took least number of days (17.32) (Fig. 1). The results agree with Zaman *et al.*, (2014); Khenifiet *et al.*, (2011) and observed *In vitro* screening of types potato varieties.

Effect of different levels of NaCl concentration

Different levels of NaCl concentration significantly influenced the days needed for shoot initiation. Without NaCl concentration took the shortest days (16.76). The other treatments required intermediate days for shoot initiation between the highest and the lowest extremes (Fig.1). The treatment MS medium with 3% NaCl concentration took the meaningfully the highest days (20.52) followed by (19.81) days at MS medium with 2.5% NaCl concentration that were statistically alike with each other while MS medium.

Interaction effect of variety and different levels of NaCl concentration

The relations effects between cultivars and different levels of NaCl concentrations showed significant differences on days essential for shoot beginning. The determined total of days (20.86) required was in Cardinal with MS medium with 3% NaCl concentration followed by (20.81) days in Diamant with the same concentration that is statistically same with each other and so on (fig.1). In contrast, the minimum days (15.96) required was saw in Granula with x MS medium without NaCl concentration followed by (16.86) days Diamant cultivar with concentration MS medium without NaCl.

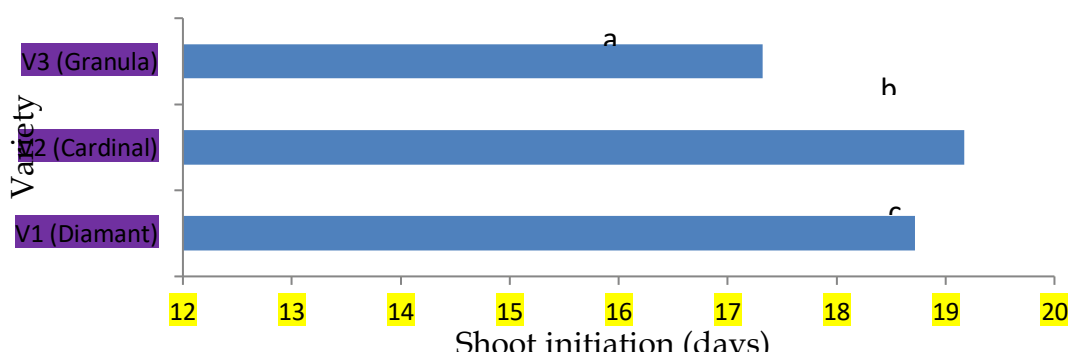


Fig. 1: Effect of variety on days required for shoot initiation.

Values shown different letter(s) type meaningfully at 1% level of probability evaluated by DMRT.

Percentage of shoot initiation Effect of variety

Significant variations were found among the different varieties in respect to ratio of shoot start. Granula varieties produced significantly the highest level of shoot (50.04%) starting while Cardinal produced the lowest amount of shoots (40.89%) (Fig.2) .

Effect of different levels of NaCl concentration

The percentage of shoot beginning shown significant differentiates due to the impact of type of concentration levels of NaCl. The treatment MS medium without NaCl concentration produced the highest percentage of the shoot (61.18%) followed by (49.71%) of the shoot with MS medium with_1% NaCl statistically significant with each other and so on (Fig.2). In contrast, MS medium with3% NaCl concentration produced the lowest percentage of shoot (37.14%). The results agree with Elwan (2007); Rahnama and Ebrahim zadeh (2005) and they observed when NaCl level increase then percent shoot start decrease.

Interaction effect of variety and different levels of NaCl concentration

The relation effects between different cultivars and concentrations of different levels of NaCl showed significant effects on the percentage of shoot growing. The highest percentage of shoot formation (69.27%) was observed in Granula cultivars with MS medium without NaCl treatment concentration followed by (64.11%) with MS medium without NaCl concentration in cultivars. Diamant, with the same concentrations in Cardinal percentage of the shoot (50.14%) and so on. In difference, the lowest (35.32%) was found in a cardinal cultivar with MS medium with 3% NaCl concentrations.

Effect of variety of Shoot length (cm)

Different varieties showed significant level in shoot size. Granula produced the tallest shoot (3.91 cm) followed by Diamant (3.82 cm) both were ranked same while Cardinal produced the shortest shoot length (3.63 cm). The different length of the shoot may be influenced due to the different cultivars.

Effect of different levels of NaCl concentration

Different concentrations levels of NaCl showed significant differences in shoot length. The treatment MS medium without NaCl concentration produced significantly the longest shoot (4.90 cm) in followed by MS medium with1% NaCl level concentration produced shoot (4.27 cm) and so on.

(Fig.2). In difference, the shortest shoot (2.93 cm) was with MS media with 3% NaCl level concentration.

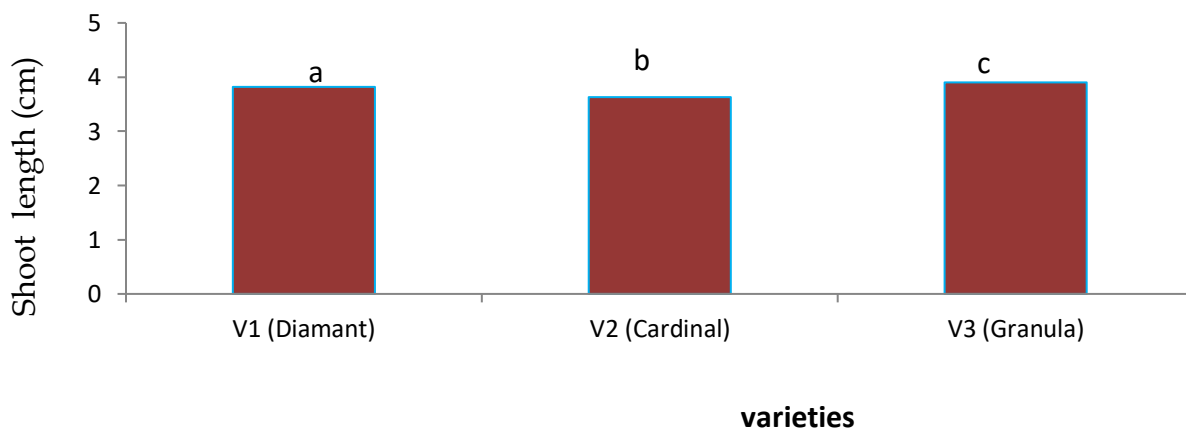


Fig.2: Effect of variety on shoot length.

Results shown different letter(s) type considerably at 1% level of probability analyzed by DMRT.

Interaction effect of variety and different levels of NaCl concentration

The related effects between varieties and the concentration of different levels of NaCl on shoot size also showed significant differences. The extended shoot (5.47 cm) was produced by the management combination of MS medium without NaCl concentration with Granula in followed by (4.76 cm) was formed by the same management combination with Diamant, (4.48 cm) was produced by the treatment combination of MS medium without NaCl with Cardinal and so on (Fig.3). But the shortest one (2.80 cm) was in the treatment arrangement of MS medium with 3% NaCl with Granula and (2.82 cm) was in same management combination with Cardinal varieties of field result with control and low level of salt stress is good.

Table 1.Effects of types of absorptions of NaCl on days necessary for shoot beginning, percentage of shoot start, shoot size (cm).

Varieties	Treatments	Days necessary for shoot start	Percentage of shoot start	Shoot length (cm)
V ₁ (Diamant)	T ₁ (MS medium without NaCl)	16.86 efg	64.11 b	4.76 b
	T ₂ (MS medium with1% NaCl)	17.90 cde	49.15 cde	4.44 b
	T ₃ (MS medium with1.5% NaCl)	18.43 bcd	44.46 efg	3.72 cd
	T ₄ (MS medium with2% NaCl)	18.95 bc	39.31 ghi	3.65 cde
	T ₅ (MS medium with2.5% NaCl)	19.39 abc	38.49 hi	3.17 fg
	T ₆ (MS medium with3% NaCl)	20.81 a	38.77 hi	3.17 fg
V ₂ (Cardinal)	T ₁ (MS medium without NaCl)	17.46 def	50.14 cd	4.48 b
	T ₂ (MS medium with1% NaCl)	17.18 defg	46.10 def	3.97 c
	T ₃ (MS medium with1.5% NaCl)	18.95 bc	39.36 ghi	3.46 def
	T ₄ (MS medium with2% NaCl)	19.89 ab	36.21 i	3.59 cdef
	T ₅ (MS medium with2.5% NaCl)	20.68 a	38.21 hi	3.47 def
	T ₆ (MS medium with3% NaCl)	20.86 a	35.32 i	2.82 g
V ₃ (Granula)	T ₁ (MS medium without NaCl)	15.96 g	69.27 a	5.47 a
	T ₂ (MS medium with 1% NaCl)	16.02 fg	53.88 c	4.38 b
	T ₃ (MS medium with 1.5% NaCl)	15.92 g	47.68 def	3.74 cd
	T ₄ (MS medium with 2% NaCl)	16.76 efg	48.68 cdef	3.79 cd
	T ₅ (MS medium with 2.5% NaCl)	19.36 abc	43.40 fgh	3.26 ef
	T ₆ (MS medium with 3% NaCl)	19.88 ab	37.33 i	2.80 g
LSD _{0.01} value	1.34	4.99	0.40	
CV (%)	3.30	4.93	4.80	
Level of significance	**	**	**	

In a column values having different letter (s) differ significantly at 1% level of probability analyzed by DMRT. ** denotes significant at 1% level of probability

Number of leaves plantlet⁻¹ effect of variety

Different cultivars showed significant variability in amount of leaves plant let. Granula produced the highest amount of leaves (3.34) followed by diamant produced leaves (3.16) with significant difference, while Cardinal produced the lowest number (2.92) of leaves plant let (Fig. 4). The finding of the present study is similar those Khenifi *et al.*, (2011); Rahman *et al.*, (2008) quantity of leaves varies in different varieties.

Effect of different levels of NaCl concentration

Different applications of NaCl showed significant differences in amount of leaves plant let. The maximum amount of leaves (4.12) was produced by MS medium without NaCl treatment combination followed by (3.62) in MS medium with 1% NaCl treatment combination and so on (Table 3). In compare, the minimum amount (2.29) of leaves was produced with MS medium with 3% NaCl treatment combination.

Interaction effect of variety and different levels of NaCl concentration

The related effects between varieties and different concentration levels of NaCl also showed important differences in a amount of leaves plant let. Diamant x MS medium without NaCl had the highest number (4.42) of leaves followed by (4.26) leaves with Granula at the same treatment of MS medium with no NaCl concentration that ranked equal, (3.69) leaves with Cardinal at the treatment MS medium without NaCl concentration. In contrast, the lowest number of leaves (2.05) in Cardinal with MS medium with 3% NaCl followed by (2.26) leaves with Diamantat MS medium with 3% NaCl treatment combinations. The results observed the maximum number of leaves in lower salt levels with different cultivar of potato.

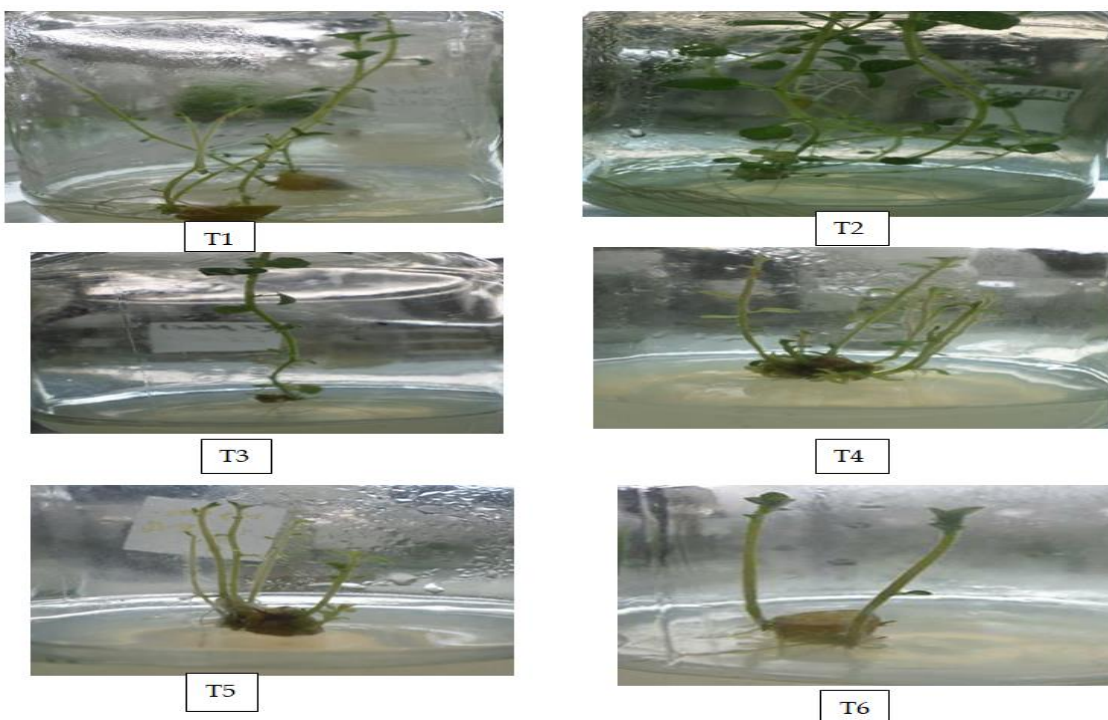


Plate 2. Effect of different levels of NaCl concentration on shoot regeneration of “Cardinal”.

- | | |
|---|---|
| T ₁ = MS medium without NaCl | T ₄ = MS medium with 2% NaCl |
| T ₂ = MS medium with 1% NaCl | T ₅ = MS medium with 2.5% NaCl |
| T ₃ = MS medium with 1.5% NaCl | T ₆ = MS medium with 3% NaCl |

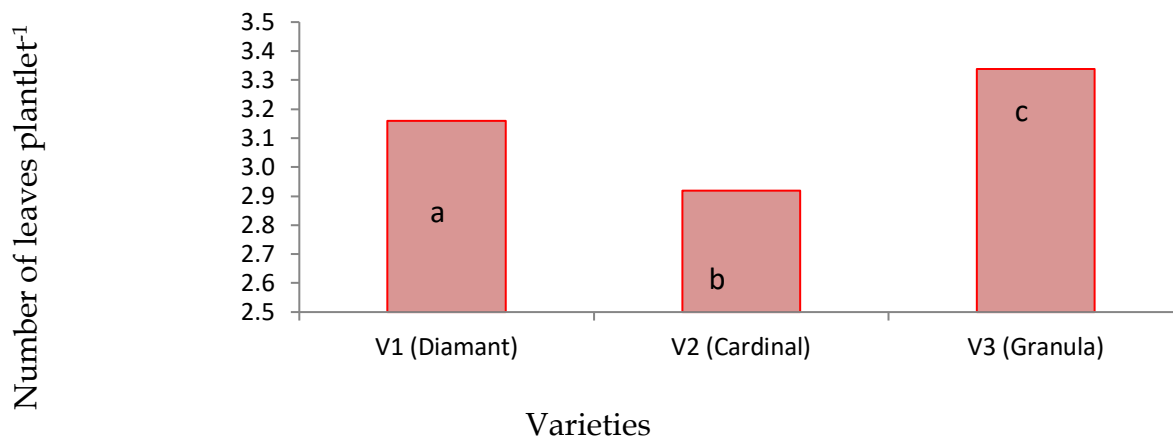


Fig.3.Effect of variety on number of leaves plantlet⁻¹.

Values shown different letter(s) types of significantly at 1% level of probability analyzed by DMRT

Number of nodes per shoot Effect of variety

Different cultivars showed significant variability in amount of nodes per shoot. Granula produced the maximum amount of nodes (3.26) followed by Diamant (3.06) significantly difference, while Cardinal produced the lowest number (2.91) of nodes per shoot (Fig. 5). Zaman *et al.*, (2014) observed in experiment eight cultivars significant in number of nodes similar with each other.

Effect of different levels of NaCl concentration

Different concentrations of the levels of NaCl showed substantial differences in amount of nodes per shoot. The maximum amount of nodes (3.83) was produced by MS medium without NaCl concentration treatment followed by (3.71) nodes at MS medium with 1% NaCl treatment combination that ranked equal. In contrast, the minimum number (2.19) of nodes was produced with MS medium with 3% NaCl treatment combination followed by (2.54) nodes at MS medium with 2.5% NaCl treatment combination (Table 3). The results agree with Zaman *et al.*, (2014) observed nodes number varies in different salt stress.

Interaction effect of variety and different levels of NaCl concentration

The related effects between varieties and different levels of NaCl concentrations also showed important differences on amount of nodes per shoot. Granula x MS medium without NaCl had the maximum number (4.35) of nodes followed by (3.73) nodes with same cultivar at the treatment of MS medium with 1% NaCl concentrations and so on. In contrast, the lowest number of nodes (2.00) produced in Cardinal with MS medium with 3% NaCl concentration followed by (2.28) nodes with Diamant at same MS medium with 3% NaCl treatment combination.

Effect of variety

The effect of types of varieties on plant initial weight varies significantly. Granula produced the highest fresh weight (0.74 g). The lowest plant initial weight was observed in Cardinal (0.66 g) followed by Diamant (0.68 g) that statistically similar to each other.

Effect of different levels of NaCl concentration

The effect of different concentrations levels of NaCl on plant initial weight showed significant differences. The different levels of NaCl in respect to MS medium without NaCl supplied the maximum initial weight of plant (0.91 g), followed by MS medium with 1.5% NaCl (0.80 g) significantly differences (Table 3). The lowest weight of plant (0.48 g) was observed with MS medium with 2.5% NaCl followed by MS medium with 3% NaCl concentration.

Interaction effect of variety and different levels of NaCl concentration

The related effects between varieties and concentrations of various levels of NaCl showed major differences on the plant initial weight. The highest in the plant initial weight (0.95 g) was observed in Granula at MS medium without NaCl followed by (0.94 g) in Cardinal with MS medium with 1% NaCl concentration, (0.93 g) in Diamant with MS medium without NaCl concentration but statistically equal ranked. In compare, the lowest plant fresh weight (0.46 g) was found at MS medium with 2.5% NaCl with Cardinal followed by (0.48 g) fresh weight in Granula cultivar on MS medium with 3% NaCl level.

Effect of variety on Days required for root initiation

The time obligation for root start was significantly influenced by the different varieties. Diamant required the maximum number of days for root beginning (16.03) while the lowest number of days (14.66) was needed for Cardinal followed by (15.12) days in Granula cultivar.

Effect of different levels of NaCl concentration

Different concentrations of levels of NaCl on root initiation showed significant differences. The highest days (17.65) required for root in MS medium with 3% NaCl followed by (17.17) days the MS medium with 2.5% NaCl level concentration. In contrast, the lowest amount of days (13.24) was required at the MS medium with 1% NaCl treatment combinations followed by MS medium with 1% NaCl (14.37) days but significantly differences.

Number of roots plantlet (effect of variety)

Substantial differences were observed in different cultivars for the amount of roots per plant let. Granula produced the maximum number of roots (7.34) followed by (6.51) roots in Diamant and those significantly difference while the lowest amount (5.62) roots was found from Cardinal. The *in vitro* redevelopment and reproduction potentiality were maximum in the cultivar Granula followed by Diamant and Cardinal.

Effect of different levels of NaCl concentration

The effects of different concentrations of levels of NaCl on the number of roots showed significant differences. The maximum number of roots (8.94) appeared with MS medium with 1% NaCl concentrations followed by (7.05) at MS medium without NaCl concentrations and the lowest number of roots (4.66) was observed in MS medium with 3% NaCl concentration (Table 4).

The observation was also similar to those of Khrais *et al.*, (1998); Etehadnia (2009) and observed that the salt level increase then the number root decrease.

Interaction effect of variety and different levels of NaCl concentration

Distinct variations were found in respects to roots per plant let due to the interaction effect of cultivars and different concentrations of level of NaCl. The highest number of roots (10.18) was observed with Granula at MS medium with 1% NaCl followed by (8.98) roots on the cultivar Diamant at MS medium with 1% NaCl concentration. However, the lowest amount of roots (4.27) was observed with Cardinal × MS medium with 3% NaCl followed by (4.69) roots on the same cultivar at MS medium with 2.5% NaCl concentration. The observation was also similar to those Khrais *et al.*, (1998); Etehadnia (2009) and observed the effect of cultivar and different levels of NaCl concentration.

Effect of variety of Root length (cm)

Significant differences were observed in the root length of different cultivars. The maximum size (5.11 cm) of root after 28 days was detected in Granula. The smallest size (3.69 cm) of root was found in Cardinal followed by (4.00) cm root with Diamant those are significantly difference. The results agree with Zaman *et al.*, (2014) and observed root length higher at specific cultivars.

Effect of different levels of NaCl concentration

Different concentrations levels of NaCl on the root size after 28 days also showed significant difference. The elongated root length (5.67 cm) appeared in MS medium with 1% NaCl concentrations followed by (5.35) cm in MS medium without NaCl concentrations when the smallest root size (3.63 cm) was found in MS medium with 3% NaCl concentration followed by (3.64 cm) in MS medium with 2.5% NaCl concentration that ranked equal. The other managements produced intermediate root size. The present study has similar results to those of Zaman *et al.*, (2014); Khenifiet *al.*, (2011) and observed that the highest regenerated plant root length and was at the lowest level of NaCl and control.

Interaction effect of variety and different levels of NaCl concentration

There were significant difference between the collaboration effects of cultivars and different levels of NaCl concentration. The tallest root (7.79 cm) was observed after 28 days in Granula x MS medium with 1% NaCl concentration followed by (6.37 cm) in the same cultivar at MS medium without NaCl concentration and so on. In contrast, the smallest root (2.73 cm) was found in Cardinal with MS medium with 3% NaCl followed by (2.96 cm) in Diamant in MS medium with 3% NaCl concentration.

Table-2: The related effects between varieties and different concentrations of NaCl on days required for root initiation, number of roots plantlet⁻¹, root length (cm)

Varieties	Treatments	Days necessary for root initiation	Number of roots plantlet ⁻¹	Root length (cm)
	T ₁ (MS medium without NaCl)	14.74 d	7.51 d	5.16 c
	T ₂ (MS medium with 1% NaCl)	13.68 e	8.98 b	4.89 cd
V ₁ (Diamant)	T ₃ (MS medium with 1.5% NaCl)	14.52 d	6.72 f	3.78 g
	T ₄ (MS medium with 2% NaCl)	15.91 c	5.76 g	3.86 g
	T ₅ (MS medium with 2.5% NaCl)	18.58 a	5.25 h	3.35 hi
	T ₆ (MS medium with 3% NaCl)	18.66 a	4.76 i	2.96 jk
	T ₁ (MS medium without NaCl)	13.71 e	6.49 f	4.53 ef
	T ₂ (MS medium with 1% NaCl)	12.55 f	7.66 d	4.33 f
V ₂ (Cardinal)	T ₃ (MS medium with 1.5% NaCl)	13.52 e	5.71 g	3.76 g
	T ₄ (MS medium with 2% NaCl)	14.52 d	4.83 i	3.56 gh
	T ₅ (MS medium with 2.5% NaCl)	16.57 bc	4.69 i	3.23 ij
	T ₆ (MS medium with 3% NaCl)	17.14 b	4.27 j	2.73 k
	T ₁ (MS medium without NaCl)	12.77 f	7.17 e	6.37 b
	T ₂ (MS medium with 1% NaCl)	13.51 e	10.18 a	7.79 a
	T ₃ (MS medium with 1.5% NaCl)	15.02 d	8.25 c	4.68 de
V ₃ (Granula)	T ₄ (MS medium with 2% NaCl)	15.97 c	7.64 d	4.25 f

	T ₅ (MS medium with 2.5% NaCl)	16.36 c	5.86 g	4.34 f
	T ₆ (MS medium with 3% NaCl)	17.16 b	4.95 hi	3.25 hij
LSD value		0.72	0.29	0.31
CV (%)		2.39	2.45	3.22
Level of significance		**	**	*

In a column values given different letter (s) differ significantly at 1% level of probability analyzed by DMRT

T₁= MS medium without NaCl T₄= MS medium with 2% NaCl

T₂= MS medium with 1% NaCl T₅= MS medium with 2.5% NaCl

T₃= MS medium with 1.5% NaCl T₆= MS medium with 3% NaCl

CONCLUSION

The research was conducted to assess the effects of different concentrations and combinations of levels of NaCl on the *in vitro* condition of potato cultivars. Diamant, Cardinal and Granula. The experiment consisted of two different sub-experiments (shoot regeneration and root regeneration) with sprouted potato micro tuber as explants and six different levels of NaCl concentration. Among the cultivars, Granula performed better than others in respects to the least amount of days (17.32) essential for shoot initiation, the maximum percentage shoot (50.04%) initiation, the tallest shoot size (3.91 cm), the highest number of leaves (3.34) per plantlet, the maximum amount of nodes (3.26) per shoot, maximum fresh weight of plant (0.74 g), the maximum amount of roots (7.34) per plant let and the maximum size of root (5.11 cm) while days necessary for root initiation Cardinal took the minimum amount of days (14.66). In the most of parameters “Cardinal” showed the lowest result and “Diamant” showed intermediate result. Among them the different managements, the result revealed that different level of NaCl concentration had significant influence on all the parameters. Among the concentrations six different levels of NaCl, the treatment MS medium without NaCl concentration showed best performance in respects to shortest days (16.76) required for shoot initiation, the maximum percentage shoot (61.18%) initiation, the largest shoot length (4.90 cm), the maximum amount of leaves (4.12) per plantlet, the maximum amount of nodes (3.83) per shoot and maximum fresh weight of plant (0.91 g). In contrast the treatment MS medium with 1% NaCl in respects to the shortest days (13.24) required for root initiation, the highest number of roots (8.94) per plantlet and the highest length of root (5.67 cm). Among the treatment MS medium with 3% NaCl concentration showed the lowest performance in respects to the highest days (20.52) required for shoot initiation, the lowest percentage shoot (37.14%) initiation, the shortest (2.93 cm) shoot length, the lowest number of leaves (2.29) per plantlet, the lowest number of nodes (2.19) per shoot, the highest days (17.65) required for root, the lowest number of roots (4.661) and the shortest root length (3.63 cm). In contrast, Cardinal with MS medium with 3% NaCl showed lowest performance over others in respects to lowest percentage shoot (35.32%) initiation, the lowest number of leaves (2.05) per plantlet, the lowest number of nodes (2.00) per shoot, the lowest number of roots (4.27) and the shortest root length (2.73 cm) while at same cultivar at MS medium with 2.5% NaCl concentration the lowest weight (0.46 g) of plant. From the study, it may be concluded that, Granula cultivar performed the best among the three potato cultivars against the *in vitro* salt stress. The cultivars performed best under non-saline to 1% saline condition.

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