Effect of plant growth regulators and sodium chloride on indirect organogenesis of three cultivars of potato plant (*Solanum tuberosum* L.) via in vitro culture

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Abstract. The results indicate the significant superiority of Lizita on the Arnova and Safari cultivars in their response to callus induction and shoot formation after four weeks from culture, reached 31.00% and 30.19%, respectively. But the Safari cultivar gave less response to callus induction and shoot formation (23.33% and 16.76%, respectively). The results also showed the significant superiority of the combination of 3.0 mg.L^{-1} NAA + 1.0 mg.L^{-1} BA on the other combinations in the percentage of callus initiation, reached 71.00%, while the two combinations of 3.0 mg.L^{-1} 2,4-D + 1.0 mg.L^{-1} Kinetin and 3.0 mg.L^{-1} NAA + 1.0 mg.L^{-1} Kinetin gave the least response to callus induction (5.89% and 6.77%, respectively). The combination of 0.2 mg.L^{-1} NAA+1.5 mg.L^{-1} BA gave the highest percentage of shoots and a significant difference from other combinations. But the combination of 0.2 mg.L^{-1} 2,4-D+1.0 mg.L^{-1} BA gave the lowest percentage of shoots, reaching 18.80%. The results showed the significant superiority of interaction between the combination of 0.2 mg.L^{-1} NAA + 1.5 mg.L^{-1} BA and Lizita cultivar in the percentage of indirect shoots, which reached 41.67%. The interaction between with the combination of 0.2 mg.L^{-1} NAA + 0.5 mg.L^{-1} BA and Safari cultivar gave the lowest of percentage of shoots, reached 14.72%. The interaction between 0.0 mmoL.L^{-1} NaCl and Lizita cultivar gave the highest percentage of shoot formation, reaching 42.11%. While the interaction between 80 mmoL.L^{-1} NaCl and Safari gave the lowest percentage of shoot formation, which reached 11.78%. Lizita cultivar was significantly superior to the Arnova cultivar in the percentage of shoot formation when each was cultured on MS medium supplemented with NaCl at 100 mmoL.L^{-1}, reached 16.54% and 13.39%, respectively.
Key Words: benzyl adenine, callus, Kinetin, organogenesis, shoot proliferation.

Abbreviations used in the paper: BAP: 6-benzyl amino purine; NAA: α – naphthalene acetic acid; 2,4-D: 2,4-Dichlorophenoxyacetic acid; NaCl: Sodium chloride.

Introduction. The potato plant (*Solanum tuberosum*) belongs to the Solanaceae family. *S. tuberosum* is a vegetable crop, which ranks fourth in the world in terms of economic importance (production and consumption) after the wheat *Triticum aestivum* L., corn *Zea mays* L. and rice *Oryza sativa* L. (Chen et al 2007). Many people in the world depend on potato as a staple food as a good source of energy. The potato crop is rich in carbohydrates, starches, vitamins and minerals (Zamotaeva 1997). The auxins and cytokinins are the most used in the plant tissue culture technique for the important role in stimulating the growth of callus and the initiation of shoots and roots (George et al 2008). Auxin is responsible for cell division and elongation, and root formation (Devlin & Witham 1998). Cytokinins are an organic compounds produced by the plant that encourages cell division, tissue differentiation, breaking apical dominance and shoot formation of callus tissue (Hopkins 1999). Wadi (2007), explained that the addition of 0.5 mg.L^{-1} BAP and 2.0 mg.L^{-1} NAA to MS medium led to the induction of potato callus cv. Desiree. Khalafalla et al (2010), found that MS medium supplemented with 5.0 mg.L^{-1} TDZ (Thidiazuron) gave high number and longest of shoot of potato cv. Almera. Highest number of shoot/callus of potato plant cv. Alpha (5±1.6), were observed on MS medium supplemented with 5.0 mg.L^{-1} BAP + 1.0 mg.L^{-1} IBA (Abdelaleem 2015). Iraqi soil in the areas of the Mediterranean and the South suffer from the problem of salinization by 75%

(Al-Zubaidi 1989). Potato is a medium-sized crop sensitive to salinity. The critical limit tolerant of potato plant for the salinity ranges from 1.6 to 2.5 $dS.m^{-1}$ (Mass & Hoffman 1977). Potato micro propagation is a method used in many countries, including France, the Netherlands, India and South Korea, to acquire tolerant plants for salinity (Najjar 1993). As these studies were interested in finding a mechanical way to tolerate plants for salinity at the cellular level. The cells of the salinity are selected for the purpose of multiplication and the production of plants that can tolerate high salinity levels (Shah et al 2003). Zhang et al (2001) noticed a decrease in callus growth rate when different concentrations of salinity were added to MS medium. Al-Hagdow et al (1999) studied the effect of different concentrations of sodium chloride (0, 40, 80 mmoL) in propagating two varieties of potato plant (Srerra and Russet Burbank) via in vitro culture. They found that the Srerra was more tolerant than the Russet Burbank cultivar.

The aim of the present study is to produce potato plants tolerated for salinity by indirect organogenesis technique.

Material and Method. The study was carried out in the laboratory of Plant Tissue Culture at the Faculty of Agriculture, University of Basra.

The source of explants. Tubers of three certified Dutch cultivars of potato plant (Lizeta, Arnova and Safari) brought from the Horticulture Station - potato seed production project in Hindia district. The tubers of three cultivars were washed with running water to remove the dust and then left to dry. It then, tubers was incubated at temperature of 20-27°C in the dark for two weeks to break the rest phase and initiation and vegetative growth of buds. The sprouts grew to a length of 2-3 cm.

Surface sterilization of explants. The sprouts were excised from the tubers of three cultivars and placed in sterilization solution (Raravan) at 10% for 10 minutes. Then these sprouts were kept in antibiotic solution containing 100 $mg.L^{-1}$ Tetracycline and Rifampicin for 10 minutes. These explants were rinsed with sterile distilled water for 3 times. The sprouts were sterilized with 20% commercial chlorax solution containing 1.05 % sodium hypochlorite, and a drop of Polysorbate 20 for 15 minutes. Explants were rinsed in sterile distilled water for 3 times.

The medium preparation for callus induction. We used full strength MS (Murashige & Skoog 1962) basal medium supplied with sucrose 30,000 $mg.L^{-1}$, Thiamine-HCl 0.4 $mg.L^{-1}$, Adenine sulphates 40 $mg.L^{-1}$, Nicotinic acid, Biotin and Pyridoxine-HCl 0.5 $mg.L^{-1}$. Various combinations of growth regulators (2,4-D+BAP or Kinetin, and NAA+BAP or Kinetin 3.0+1.0 $mg.L^{-1}$ for each of them, respectively), have been added to prepared medium. The pH of the media was adjusted to 5.7 with 0.1 N NaOH or HCl after adding 6% agar, and before autoclaving at 1.04 $Kg.cm^{-2}$ for 15 minutes. All media were dispensed in culture tubes containing 15 mL medium cultures. Buds after reaching a length of 5-7 cm were cut into several nodal segments. Those nodal segments were cultured this medium mentioned above. The cultures incubated at a temperature of 27±1°C and darkness. The percentage of response to callus induction was recorded after four weeks from culture.

Shoot proliferation. In the first experiment, different combinations of growth regulators were added 0.2+0.5 $mg.L^{-1}$ and 0.2+1.5 $mg.L^{-1}$ NAA and BAP respectively, and 0.2+1.0 $mg.L^{-1}$ 2,4-D and BAP respectively to this medium components mentioned above. In the second experiment, sodium chloride with different concentrations (0, 80, 100, 120, 140 and 160 $mmoL.L^{-1}$), NAA at 0.2 $mg.L^{-1}$ and BAP at $mg.L^{-1}$, were added to the same of medium components. 100 mg of calli were cultured on those media mentioned above, and incubated at a temperature of 27±1°C and light intensity under 1000 Lux light intensity provided by white fluorescent lamps for 16 hrs. The percentage of shoot proliferation was recorded after four weeks from culture.

Statistical design and analysis. Completely randomized design was used with ten replicates. The data were subjected to the analysis of variance and mean values were compared using revised LSD at 5% (Snedecor & Cochran 1986).

Results and Discussion. The results in Table 1 indicate the significant superiority of Lizita on the Arnova and Safari cultivars in their response to callus induction after four weeks from culture reached 31.00% (Figure 1). But the Safari cultivar gave less response to callus induction (23.33%).

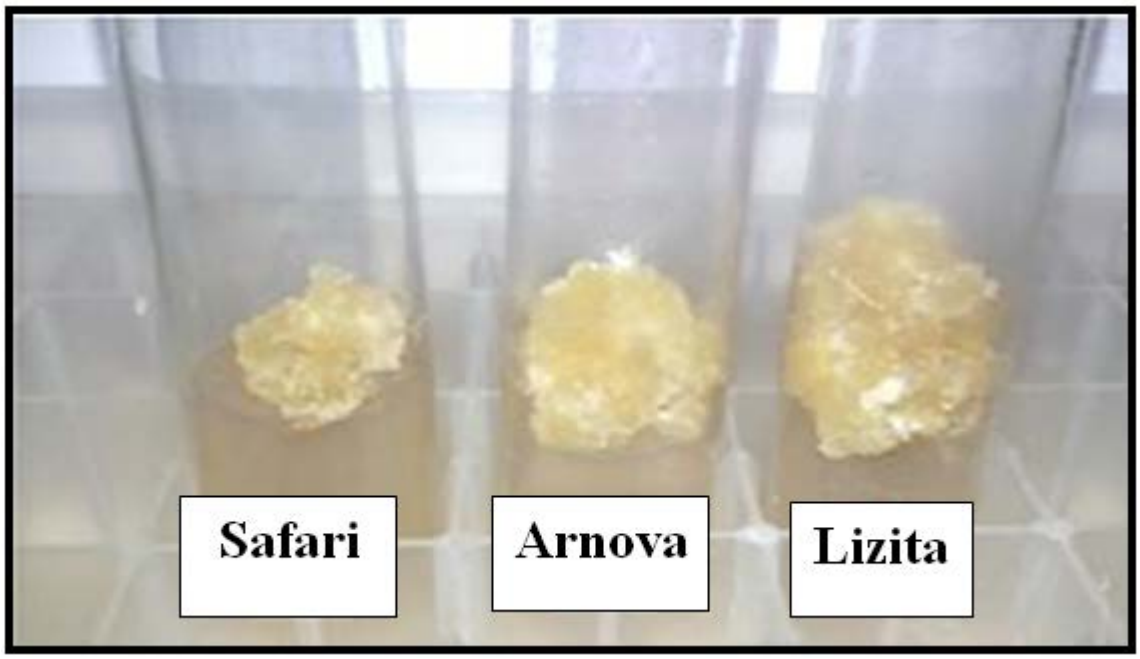

Figure 1. Callus induction of three cultivars of potato plant cultured on MS medium supplemented with 3.0 mg.L^{-1} NAA and 1.0 mg. L^{-1} BAP after four weeks of culturing.

The reason for the difference between the three cultivars is due to their differences in genetic characteristics. The results from the same table also showed the significant superiority of the combination of 3.0 mg.L^{-1} NAA + 1.0 mg.L^{-1} BAP on the other combinations in the percentage of callus initiation reached 71.00%. While the two combinations of 3.0 mg.L^{-1} 2,4-D + 1.0 mg.L^{-1} Kinetin and 3.0 mg.L^{-1} NAA + 1.0 mg.L^{-1} Kinetin gave the least response to callus induction (5.89% and 6.77%, respectively). The interaction treatment between the combination of 3.0 mg.L^{-1} NAA + 1.0 mg.L^{-1} BAP and Lizita gave the highest percentage to response of callus formation reached 76.67% (Table 1). While the interaction treatment between the combinations of 3.0 mg.L^{-1} 2,4-D + 1.0 mg.L^{-1} Kinetin and Safari gave the Lowest percentage to response of callus formation (4.00%).

Similar results to the present study were obtained by Khalafalla et al (2010). The increased response of nodal segment to callus formation is due to the effectiveness and role of auxin (NAA) in increasing cell and enlargement division. NAA at 3.0 mg.L^{-1} concentration is the optimum for callus induction (Rao & Singh 1991). Cytokinin (BAP) is effective in containing three double bonds compared with other types, as well as benzyl ring, which makes it more effective. It also has a physiological effect on cell division (Sudarmonowati et al 2009).

Table 1

Effect of cultivar, combinations of plant growth regulators and interaction between them on the percentage of the explant response to callus induction of potato plant after four weeks of culturing

Cultivar	*Concentrations of plant growth regulators (mg.L^{-1})*				*Mean of cultivar*
	3.0 2,4-D + 1.0 BA	*3.0 2,4-D + 1.0 Kinetin*	*3.0 NAA + 1.0 BAP*	*3.0 NAA + 1.0 Kinetin*	
Lizita	29.33	8.67	76.67	9.33	31.00
Arnova	22.33	5.00	69.00	6.33	25.66
Safari	17.33	4.00	67.33	4.67	23.33
Mean of plant growth combination	22.99	5.89	71.00	6.77	-
R-L.S.D P≥0.05	Cultivar	Combination	Interaction (cultiv. + comb.)	-	-
	1.35	1.56	2.70	-	

Effect of plan growth regulators on shoot proliferation. The Table 2, showed the significant superiority of Lizita on Arnova and safari cultivars in their response to indirect shoot proliferation after four weeks from culture (30.19%). While the Safari cultivar gave the lowest percentage of indirect shoot reached 16.76%. The difference in the percentage of shoots between cultivars is due to the genetic variation between them. Also from the same table it is noted that the combination of 0.2 mg.L^{-1} NAA+1.5 mg.L^{-1} BAP gave the highest percentage of shoots and a significant difference from other combinations. But the combination of 0.2 mg.L^{-1} 2,4-D+1.0 mg.L^{-1} BAP gave the lowest percentage of shoots reached 18.80%. The results showed the significant superiority of interaction between the combination of 0.2 mg.L^{-1} NAA + 1.5 mg.L^{-1} BAP and Lizita cultivar in the percentage of indirect shoots reached 41.67%. The interaction between with the combination of 0.2 mg.L^{-1} NAA + 0.5 mg.L^{-1} BAP and Safari cultivar gave the lowest of percentage of shoots reached 14.72% (Table 2). Similar results were obtained by Abdelaleem (2015). The cause of shoot formation is the balance between auxins and cytokinins through their effect on cellular differentiation and organ formation (Ramakrishnan et al 2005). The results of this study are similar to those of Davies (2004), who referred to the role of plant growth regulators in the proliferation of shoots in plant tissues. The level of plant hormones in the explants and concentrations of growth regulators added to medium have a major role in organogenesis (Davies 2004).

Table 2

Effect of cultivar, combinations of plant growth regulators and interaction between them on the percentage of the explant response to shoot proliferation of potato plant after four weeks of culturing

Cultivar	*Concentrations of plant growth regulators (mg.L^{-1})*			*Mean of cultivar*
	0.2 NAA + 0.5 BAP	*0.2 NAA + 1.5 BAP*	*0.2 2,4-D + 1.0 BAP*	
Lizita	27.22	41.67	21.67	30.19
Arnova	23.89	35.00	19.72	26.20
Safari	14.72	20.56	15.00	16.76
Mean of plant growth combination	21.94	32.41	18.80	-
R-L.S.D P≥0.05	Cultivar	Combination	Interaction (cultiv. + comb.)	-
	2.10	2.10	5.07	-

Effect of concentrations of sodium chloride on shoot proliferation. The callus tissue did not grow in the high levels of sodium chloride 120, 140 and 160 mmoL.L^{-1} (Figure 2, B).

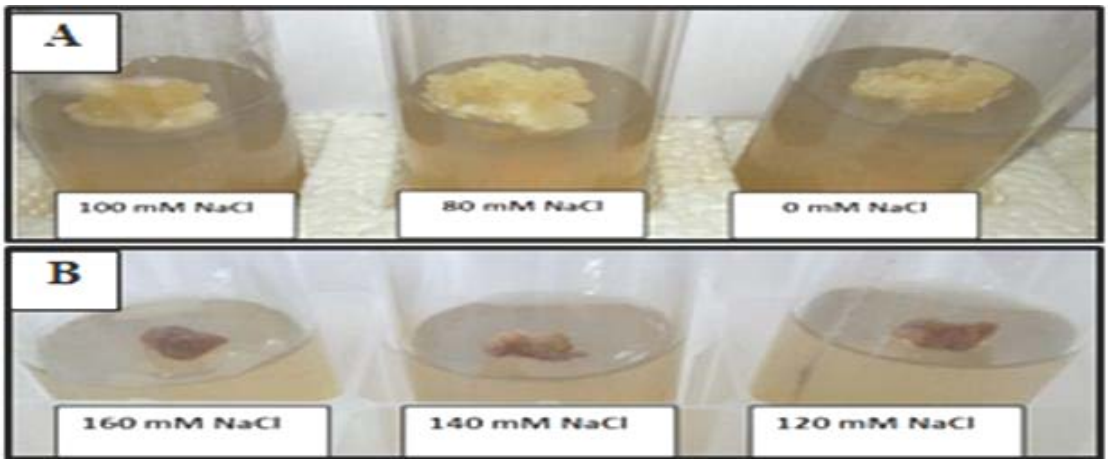

Figure 2. A - Growth and multiplication of callus of potato plant cv. Lizita cultured on MS medium supplemented with NaCl at 0, 80 and 100 mmoL.L^{-1}. B - Growth failed and browning of callus of potato plant cv. Lizita cultured on MS medium supplemented with NaCl at 120, 140 and 160 mmoL.L^{-1}.

The Table 3 showed the significant superiority of control treatment (0.0 mmoL.L^{-1} NaCl) on NaCl at 80 mmoL.L^{-1} treatment in the percentage of shoot formation reached 37.04% (Figure 3). But the interaction between 0.0 mmoL.L^{-1} NaCl and Lizita cultivar gave the highest percentage of shoot formation reached 42.11%. While the interaction between 80 mmoL.L^{-1} NaCl and Safari gave the lowest percentage of shoot formation reached 11.78% (Table 3).

Table 3

Effect of cultivar, concentration of sodium chloride and interaction between them on the percentage of the explant response to shoot proliferation of potato plant after four weeks of culturing

Cultivar	*Concentrations of sodium chloride (mmoL.L^{-1})*		
	0	*80*	*Mean of cultivar*
Lizita	42.11	23.30	32.70
Arnova	37.77	16.42	27.10
Safari	31.23	11.78	21.50
Mean of NaCl concentration	37.04	17.17	-
R-L.S.D P≥0.05	Cultivar	Concentration of NaCl	Interaction (cultivar + NaCl)
	1.227	1.002	1.735

The Figure 4 shows that Lizita cultivar was significantly superior to the Arnova cultivar in the percentage of shoot formation when each was cultured on MS medium supplemented with NaCl at 100 mmoL.L^{-1} reached 16.54% and 13.39%, respectively.

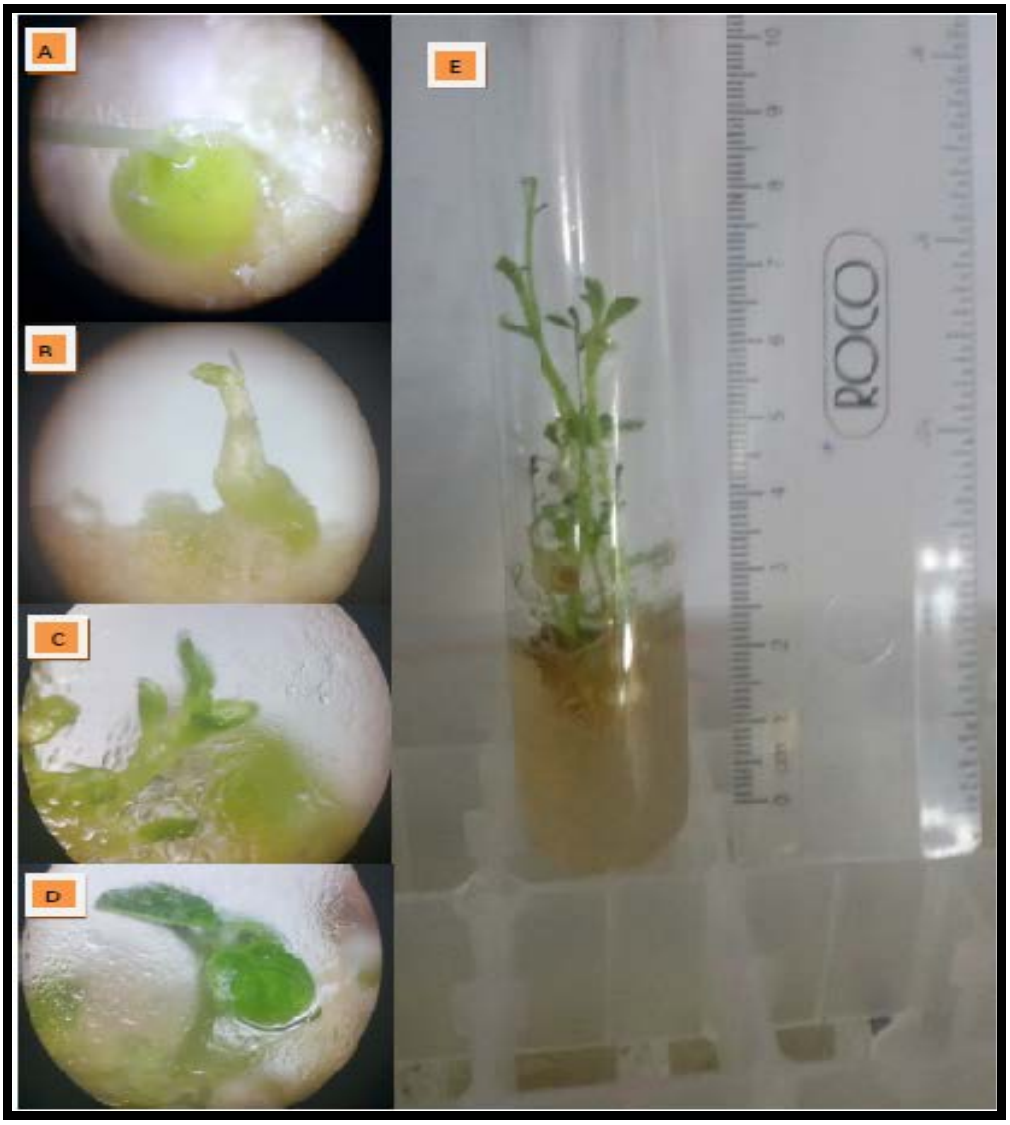

Figure 3. A, B, C, D and E - Indirect shoot proliferation from potato callus cv. Lizita cultured on MS medium supplemented with NaCl at 80 mmoL.L^{-1} concentration.

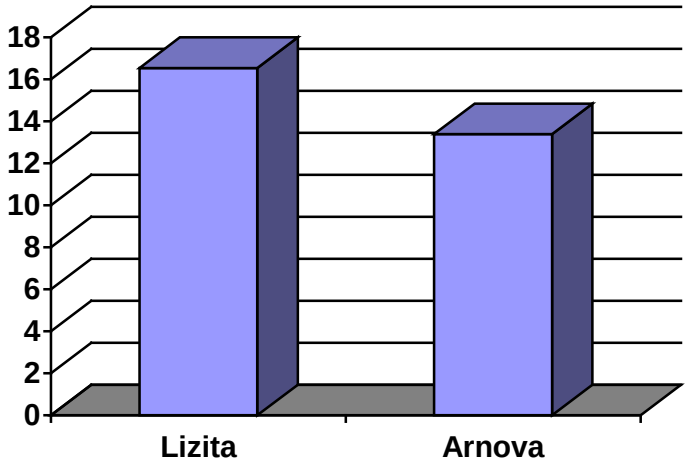

Figure 4. Effect of NaCl at 100 mmoL.L^{-1} concentration on the percentage of the explant response to shoot proliferation of potato plant after four weeks of culturing.

The decrease in percentage of shoot proliferation with increased sodium chloride concentration is due to the negative effects of salinity in cell division and differentiation, growth and physiological processes as well as the low efficacy of certain enzymes especially the peroxidase enzyme (Pahlich et al 1978).

Conclusions. Combination of 3.0 $mg.L^{-1}$ NAA + 1.0 $mg.L^{-1}$ BAP gave the highest response to the callus formation of three potato cultivars. NAA at 0.2 $mg.L^{-1}$ and BAP at 1.5 $mg.L^{-1}$ concentrations gave the highest percentage to shoot formation. The indirect shoot formation decreased with increased concentration of sodium chloride which added to the MS medium.

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