Effect of salt stress on some chemicals characteristics of callus for three cultivars of potato plant (*Solanum tuberosum* L.)

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Abstract. The callus of Lizita cultivar gave the highest significant value in its content of proteins, proline and malondialdehyde (MDA) compared to the Arnova and Safari cultivars as it reached 16.23 mg g^{-1} dw, 3.05 µg g^{-1} dw and 2.04 nmol g^{-1} fw, respectively. Also the callus gave the highest significant value in its content of ascorbic acid, total dissolved carbohydrates, proline and MDA when cultured on MS medium supplemented with 80 mmol L^{-1} of NaCl as it reached 12.91 mg $100g^{-1}$ fw, 32.45 mg g^{-1} dw, 3.41 µg g^{-1} dw and 2.46 nmol g^{-1} fw, respectively. But it gave the lowest significant value in its content of total proteins when cultured on MS medium supplemented with 80 mmol L^{-1} of NaCl, reached 12.57 mg g^{-1} dw. The interaction between Lizita cultivar and 80 mmol L^{-1} gave the highest significant value in total dissolved carbohydrates; proline and MDA accumulation reached 33.18 mg g^{-1} dw, 4.15 µg g^{-1} dw and 3.17 nmol g^{-1} fw, respectively. While the Lizita cultivar and 0.0 mmol L^{-1} NaCl treatment gave the highest significant value in total proteins content reached 18.21 mg g^{-1} dw. The callus of Lizita cultivar gave the highest significant value in its content of proteins, proline and MDA compared to the Arnova cultivar when cultured on MS medium supplemented with 100 mmol L^{-1} of NaCl, as it reached 12.6 mg g^{-1} dw, 5.04 µg g^{-1} dw and 4.61 nmol g^{-1} fw, respectively.

Key Words: ascorbic acid, benzyl adenine, in vitro, lipid peroxidation, MS salts.

Introduction. Salinity is one of the environmental and abiotic stresses that reduce the availability of water in soil and change the ionic balance in plants (Rhoades et al 1992). The agricultural land in Iraq, where potato crop is grown is affected by the problems of salinity by 75% (Al-Zubaidi 1989). Some studies related to tissue culture have focused on reducing the salinity problems affecting plant growth and improving their salinity tolerance. This is done by selecting salinity-tolerant cells for use in producing tolerant plants for salinity (Shah et al 2003). Forooghian & Esfarayeni (2013) found that the callus of Agria cultivar was more tolerant to salinity compared to the other two cultivars (Sante and Savalan) when they were cultured in MS medium supplemented with NaCl at 0, 50, 75 and 100 mmol. Also, the fresh and dry weight of callus decreased with increased sodium chloride concentration. Salinity has an important role in stimulating antioxidant enzymes that reduce damage caused by oxidation. Ascorbic acid is one of the most important non-enzymatic antioxidants (Parida & Das 2005). Some studies noted the increased concentration of ascorbic acid in callus tissue of potato with increased sodium chloride when added in MS medium. They also explained that ascorbic acid works to remove free radical damage in cells treated with high concentrations of sodium chloride (Queiros et al 2007; Mohamed et al 2010). Carbohydrates play an important role in adapting plant tissues to environmental stress. The starch turns into simple dissolved sugars that regulate the osmotic cells and protect the cell membranes from damage (Levitt 1980; Bray 1993). One of the studies was to multiplication of potato callus that found increased total and non-reducing sugars with increasing the concentration of sodium chloride to 50 mmol, while reducing sugars increased by NaCl concentration at 75 mmol (Silva et al 2001). The increased sodium chloride salt in the growth medium leads to reduced protein content in plant tissues and increased proline formation (Helal & Mengel 1979). Proline (amino acid) is the most organic compounds accumulation when the plant is exposed to salt stress (Cherry 1994). This amino acid increases the acidity of

the cells and removes free radicals, as well as the balance of the osmotic pressure of cells (Hossain et al 2014). The fat oxidation of cell membranes is used as a test to estimate the sensitivity of plant tissues to oxidative stress (Wang & Han 2009). Lipid peroxidation is the oxidation of fat to the compound of malondialdehyde (MDA) when exposed to salt stress (Gawel et al 2004). The aim of the study is to know the effect of salt stress on some chemical characteristics of callus of three potato cultivar by in vitro culture technique.

Material and Method. The study was carried out in the laboratory of Plant Tissue Culture at the Faculty of Agriculture, University of Basra. Tubers of three certified Dutch cultivars of potato plant (Lizita, Arnova and Safari) brought from the Horticulture Station for Potato Seed Production Project. The tubers of three cultivars were washed with running water to remove the dust and then left to dry. Then the tubers was incubated at a 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. These sprouts were excised from the tubers of the three cultivars and were 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. These sprouts was then sterilized with 20% commercial chlorax solution containing 1.05% sodium hypochlorite, and a drop of Polysorbate 20 (Tween 20) for 15 minutes. These explants were rinsed in sterile distilled water 3 times. Full strength MS (Murashige & Skoog 1962) basal medium supplied with substances referred in Table 1 were used. 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 in the medium mentioned above. The cultures were incubated at a temperature of 27±1°C and darkness conditions. The callus was formed after four weeks from culture. 100 mg of callus was cultured on the same MS medium components supplemented with sodium chloride with different concentrations (0, 80, 100, 120, 140 and 160 mmol L^{-1}). The cultures were incubated at a temperature of 27±1°C in darkness. Callus grew in treatments at 0, 80 and 100 mmol L^{-1} after 4 weeks from culture. But the callus did not grow in the treatments at 120, 140 and 160 mmol L^{-1} so it was excluded from the experiment.

Table 1

Organic compounds added to MS medium

Substance	*Quantity (mg L^{-1})*
Sucrose	30000
Thiamine-HCl	0.4
Adenine sulphates	40
Nicotinic acid	0.5
Biotin	0.5
Pyridoxine-HCl	0.5
NAA	3.0
BA	1.0

The studied chemical characteristics:

1. Ascorbic acid (mg 100 g^{-1} fresh weight): 1.0 gram of fresh weight of potato callus for the purpose of estimating ascorbic acid according to the method described in A.O.A.C (1975).
2. Total dissolved carbohydrates (mg g^{-1} dry weight): Carbohydrates are estimated in Modification of Phenol Sulphuric Acid Colorimetric Method described by Dobois et al (1956).

3. Total proteins (mg g^{-1} dry weight): The percentage of nitrogen was estimated in the Microkieldhal method according to the method described by Page et al (1982). The percentage of protein was estimated according to the following equation:
   Total proteins (%) = %Nitrogen X 6.25
4. Proline content (µg g^{-1} dry weight): Proline content is estimated according to the method described by Troll & Lindsley (1955).
5. Lipid peroxidation (nmol MDA g^{-1} fresh weight): MDA is estimated according to the method described by Hodges et al (1999).

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).

Abbreviations. BA: 6-benzyl adenine.
NAA: ∝-naphthalene acetic acid.
MDA: Malondialdehyde.

Results and Discussion

Ascorbic acid (Vitamin C). The results shown in Table 2 indicate significant differences between the treatments in ascorbic acid content of callus of three potato cultivars. The Arnova cultivar was significantly superior on Lizita and Safari cultivars in the ascorbic acid content of callus, reached (10.72, 9.79 and 9.12 mg 100 g^{-1} fw, respectively). The reason for the difference between the potato cultivars in the ascorbic acid content in the callus is due to the genetic variation between them. The same table shows that the concentration of sodium chloride at 80 mmol L^{-1} was significantly different from the control treatment in ascorbic acid content of callus (12.91 and 6.84 mg 100 g^{-1} fw, respectively). The reason for the increase in the ascorbic acid content in callus is due to the salinity which has led to the induction of antioxidants synthesis that have increased its content (Sairam & Tyagi 2004). Or the high content of ascorbic acid is due to its role as an antioxidant in reducing damages of free radicals as well as its contribution to the bio-activities of cells (Shigeoka et al 2002). The results of the study were agreed with the results of other studies conducted on potato callus (Queiros et al 2007; Mohamed et al 2010). The interaction treatment between Arnova cultivar and 80 mmol L^{-1} was significantly different on the other treatments in ascorbic acid content reached 14.00 mg 100 g^{-1} fw. While the interaction treatment between Lizita and 0 mmol NaCl gave the lowest content of ascorbic acid in the callus, which was 6.08 mg 100 g^{-1} fw (Table 2). Figure 1 indicates no significant differences between the callus of Lizita and Arnova in ascorbic acid content when cultured on MS medium supplied with 100 mmol L^{-1} NaCl, reached 16.61 and 15.80 mg 100 g^{-1} fw, respectively.

Table 2

Effect of cultivar, concentration of sodium chloride and interaction between them on the ascorbic acid (mg 100g^{-1} fw) of potato plant callus after four weeks of culture

Cultivar	*Concentrations of sodium chloride (mmol L^{-1})*		
	0	*80*	*Mean of cultivar*
Lizita	6.08	13.50	9.79
Arnova	7.45	14.00	10.72
Safari	7.00	11.24	9.12
Mean of NaCl concentration	6.84	12.91	-
R-L.S.D P≥0.05	Cultivar	Concentration of NaCl	Interaction (Cultivar + NaCl)
	0.92	0.89	1.68

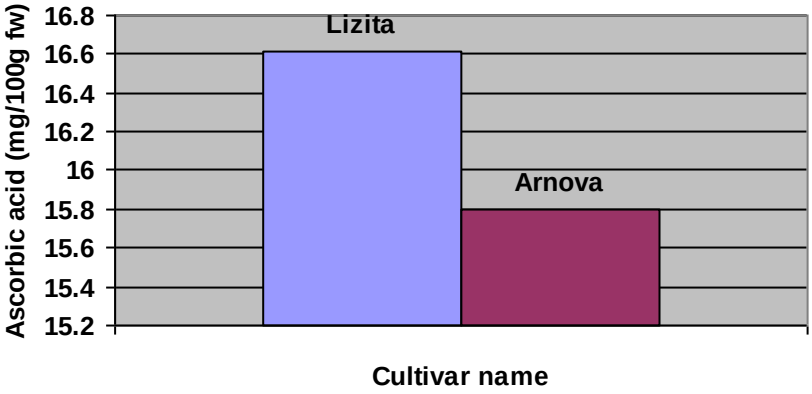

Figure 1. Effect of 100 mmol L^{-1} NaCl on ascorbic acid content in callus of Lizita and Arnova cultivars after four weeks from culture (RLSD 0.05 = Non-significant).

Total dissolved carbohydrates. The Table 3 shows no significant differences between the three potato cultivars in total dissolved carbohydrates content of callus. But 80 mmol L^{-1} NaCl was significantly different on control treatment in total dissolved carbohydrates of callus reached 32.45 and 24.35 mg g^{-1} dw, respectively. The high concentration of total dissolved carbohydrates is due to its degradation into reducing and non-reducing sugars that work with proline to regulate the osmotic potential in the cells while exposed to salt stress (George et al 2008). The results of the study were agreed with the results of other studies conducted on callus (Al-Kaby et al 2010). The interaction treatments between 80 mmol L^{-1} NaCl and the three potato cultivars were significantly different on 0.0 mmol L^{-1} NaCl and three cultivars in total dissolved carbohydrates of callus. The interaction between Lizita cultivar and 80 mmol L^{-1} gave the highest value in total dissolved carbohydrates content of callus reached 33.18 mg g^{-1} dw. While the interaction between 0.0 mmol L^{-1} and Safari cultivar gave the lowest value reached 23.57 mg g^{-1} dw (Table 3).

Table 3

Effect of cultivar, concentration of sodium chloride and interaction between them on the total dissolved carbohydrates (mg g^{-1} dw) of potato plant callus after four weeks of culture

Cultivar	*Concentrations of sodium chloride (mmol L^{-1})*		
	0	*80*	*Mean of cultivar*
Lizita	24.99	33.18	29.09
Arnova	24.50	32.18	28.34
Safari	23.57	31.98	27.78
Mean of NaCl concentration	24.35	32.45	-
R-L.S.D P≥0.05	Cultivar	Concentration of NaCl	Interaction (Cultivar + NaCl)
	N.S.*	1.62	2.85

N. S.* - Non-significant.

The results showed no significant differences between callus of Lizita and Arnova cultivars in total dissolved carbohydrates when cultured on MS medium supplemented with 100 mmol L^{-1} NaCl (Figure 2).

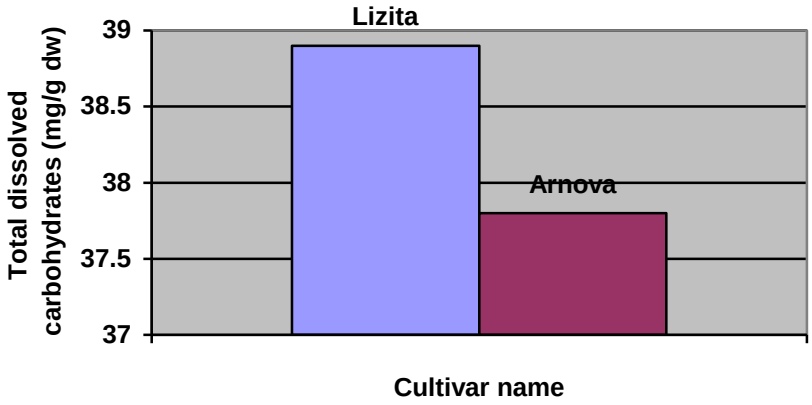

Figure 2. Effect of 100 mmol L^{-1} NaCl on total dissolved carbohydrates content in callus of Lizita and Arnova cultivars after four weeks from culture (RLSD 0.05 = Non-significant).

Protein content. Callus of Lizita cultivar was significantly superior on Arnova and Safari cultivars in proteins content reached 16.23 mg g^{-1} dw (Table 4). But the callus of Safari cultivar gave the lowest significant value in protein content (12.28 mg g^{-1} dw). The significant differences between potato cultivars are due to genetic variation among them. The 0.0 mmol L^{-1} NaCl treatment gave the highest significant value in proteins content reached 16.40 mg g^{-1} dw. While the 80 mmol L^{-1} NaCl treatment gave the lowest significant value in proteins content reached 12.57 mg g^{-1} dw (Table 4). The reasons for the low protein content in the callus exposed to salt stress were due to the lack of effective conversion of amino acids to proteins and inhibition of the activity of certain enzymes in callus tissue (Bowler et al 1992; Soloman et al 1994; Mobaraky 2001). The low protein content is also due to high levels of salinity leading to an increase in Reactive Oxygen Species (ROS) production, which negatively affects protein synthesis (Makela et al 2003). The interaction between Lizita cultivar and 0.0 mmol L^{-1} NaCl gave the highest significant value in protein content of callus reached 18.21 mg g^{-1} dw. While the interaction between Safari cultivar and 80 mmol L^{-1} of NaCl gave the lowest significant value in protein content reached 10.31 mg g^{-1} dw (Table 4).

Table 4

Effect of cultivar, concentration of sodium chloride and interaction between them on the total protein content (mg g^{-1} dw) of potato plant callus after four weeks of culture

Cultivar	*Concentrations of sodium chloride (mmol L^{-1})*		
	0	*80*	*Mean of cultivar*
Lizita	18.21	14.25	16.23
Arnova	16.74	13.17	14.95
Safari	14.25	10.31	12.28
Mean of NaCl concentration	16.40	12.57	-
R-L.S.D P≥0.05	Cultivar 1.07	Concentration of NaCl 1.03	Interaction (Cultivar + NaCl) 1.83

Figure 3 indicates that the callus of Lizita cultivar is significantly superior on the callus of Arnova cultivar in the total protein content when cultured on MS medium supplemented with 100 mmol L^{-1} of NaCl (12.60 and 8.80 mg g^{-1} dw, respectively). The reason for this may be due to the fact that Lizita cultivar is more tolerant to salt stress when compared to the Arnova cultivar.

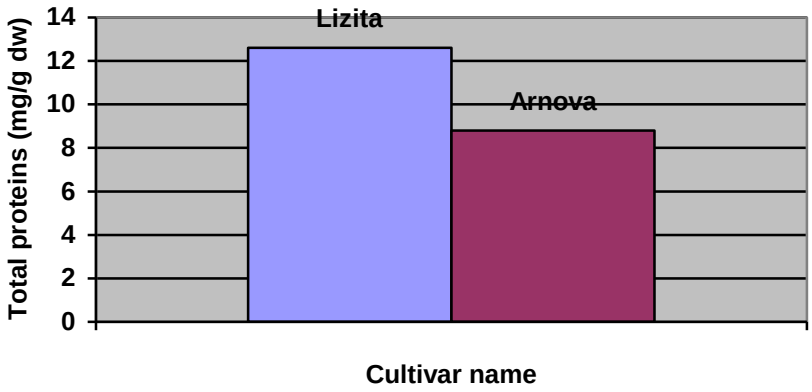

Figure 3. Effect of 100 mmol L^{-1} NaCl on total proteins content in callus of Lizita and Arnova cultivars after four weeks from culture (RLSD 0.05≥1.73).

Proline content. Callus of Lizita cultivar was significantly different on the Arnova and Safari cultivars in proline content, recording 3.05 µg g^{-1} dw (Table 5). While the Safari cultivar gave the lowest value in proline content (2.19 µg g^{-1} dw). The genetic variation between cultivars led to significant differences between them. The 80 mmol L^{-1} NaCl treatment gave the highest significant value in proline content of callus reaching 3.41 µg g^{-1} dw. But the control treatment gave the lowest value in proline content which reached 1.61 µg g^{-1} dw (Table 5). Increased proline accumulation with increased sodium chloride concentrations is due to the deficiency of the activity of proline oxidase (Girija et al 2002). The increased accumulation of proline in the callus is for the purpose of increasing tolerance to salt stress (Ahmed et al 2009). The results of the study are consistent with many other researchers' findings (Amini & Ehsanpour 2005; Cardenas et al 2006). The callus of Lizita cultivar cultured on MS medium supplemented with 80 mmol L^{-1} NaCl, gave the highest significant value in proline content (4.15 µg g^{-1} dw). But the callus of Safari cultivar cultured on MS medium supplemented with 0.0 mmol L^{-1} of NaCl, gave the lowest significant value in proline content reaching 1.38 µg g^{-1} dw (Table 5).

Table 5

Effect of cultivar, concentration of sodium chloride and interaction between them on the total proline content (µg g^{-1} dw) of potato plant callus after four weeks of culture

Cultivar	*Concentrations of sodium chloride (mmol L^{-1})*		
	0	*80*	*Mean of cultivar*
Lizita	1.95	4.15	3.05
Arnova	1.51	3.09	2.31
Safari	1.38	3.01	2.19
Mean of NaCl concentration	1.61	3.41	-
R-L.S.D P≥0.05	Cultivar	Concentration of NaCl	Interaction (Cultivar + NaCl)
	0.08	0.08	0.14

The callus of Lizita cultivar was significantly different in proline accumulation compared to Arnova cultivar, when cultured on MS medium supplemented with 100 mmol L^{-1} of NaCl which recorded 5.04 and 4.19 µg g^{-1} dw, respectively (Figure 4). The reason for the increased accumulation of proline in the Lizita cultivar is due to nitrogen compounds involved in the synthesis of proline instead of proteins (Makela et al 2003).

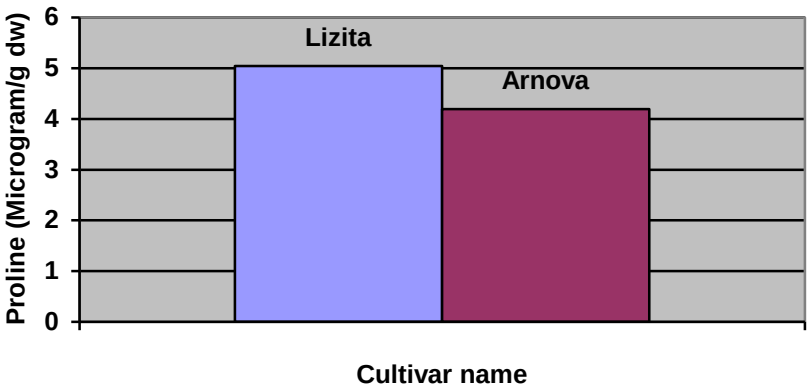

Figure 4. Effect of 100 mmol L^{-1} NaCl on proline content in callus of Lizita and Arnova cultivars after four weeks from culture (RLSD 0.05≥0.52).

Lipid peroxidation. Table 6 shows that callus of Lizita cultivar gave highest significant value in MDA accumulation in comparison with Arnova and Safari cultivars, which recorded 2.04, 1.99 and 0.85 nmol MDA g^{-1} fw, respectively. While the main effect of 80 mmol L^{-1} of NaCl gave the highest significant value in MDA accumulation reaching 2.46 nmol MDA g^{-1} fw. But the control treatment (0.0 mmol L^{-1} of NaCl) gave the lowest value in MDA accumulation which reached 0.79 nmol MDA g^{-1} fw (Table 6).

Table 6

Effect of cultivar, concentration of sodium chloride and interaction between them on the lipid peroxidation (nmol (MDA) g^{-1} fw) of potato plant callus after four weeks of culture

Cultivar	*Concentrations of sodium chloride (mmol L^{-1})*		
	0	*80*	*Mean of cultivar*
Lizita	0.90	3.17	2.04
Arnova	0.87	3.11	1.99
Safari	0.60	1.09	0.85
Mean of NaCl concentration	0.79	2.46	-
R-L.S.D P≥0.05	Cultivar	Concentration of NaCl	Interaction (Cultivar + NaCl)
	0.03	0.03	0.05

The reason for increased MDA accumulation with increased sodium chloride concentrations was due to lower antioxidant synthesis and increased free radical concentration which led to lipid peroxidation in cellular membranes (Esfandiari et al 2007). The results of the present study are agreed with the results of other studies conducted on callus (Yasar 2003; Queiros et al 2007; Kusvuran et al 2013). The callus of Lizita cultivar gave the highest significant value in MDA accumulation with 3.17 nmol MDA g^{-1} fw, when cultured on MS medium supplemented with 80 mmol L^{-1} of NaCl. While the callus of Safari cultivar cultured on MS medium supplemented with 0.0 mmol L^{-1} of NaCl, gave the lowest value of MDA accumulation (0.60 nmol MDA g^{-1} fw). Figure 5 shows that callus of Lizita cultivar gave the highest significant value of MDA accumulation when cultured on MS medium supplemented with 100 mmol L^{-1} of NaCl, which reached 4.61 nmol MDA g^{-1} fw. While the callus of Arnova cultivar gave the lowest value of MDA accumulation reaching 4.05 nmol MDA g^{-1} fw (Figure 5).

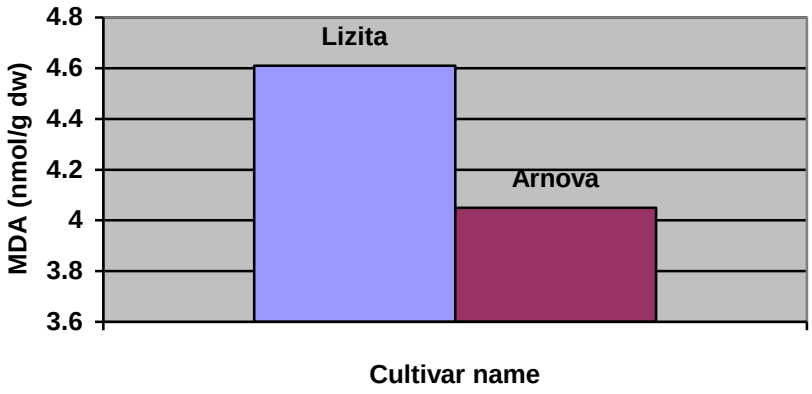

Figure 5. Effect of 100 mmol L^{-1} NaCl on malondialdehyde (MDA) accumulation in callus of Lizita and Arnova cultivars after four weeks from culture (RLSD 0.05≥0.42).

Conclusions. Potato cultivars differed significantly in their tolerance to salt stress. Lizita was more tolerant to salt stress than Arnova and Safari cultivars. Ascorbic acid and total soluble carbohydrates, proline and MDA increased their content in the callus when was exposed to 80 mmol L^{-1} of sodium chloride. While the content of proteins decreased when sodium chloride concentration increased to 80 mmol L^{-1} of NaCl. Lizita cultivar is more tolerant to salt stress than Arnova cultivar when cultured on MS medium supplemented with 100 mmol L^{-1} of sodium chloride.

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