I.J.A.B.R, VOL. 5(2) 2015: 181-186 ISSN 2250 – 3579

INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

SCREENING FOUR POTATO CULTIVARS FOR SALT TOLERANCE

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ABSTRACT
Experiments were conducted to study the effect of salt stress (6, 8, 10 and 12 dS/m) on membrane stability of leaves (% injury), callus growth parameters and ions accumulation in four potato varieties (Arnova, Provento, Burren and Riviera) under in vitro condition. Results indicated that significant differences was found among cultivars in % injury with range 8.23 to 50.59 %, Riviera had the lowest % injury with an average of 8.23, 10.67, 11.97 and 21.37 % at 6, 8, 10 and 12 dS/m respectively and considered as a salt tolerant genotype, while Arnova had the highest % injury with an average of 40.55, 50.59 and 50.58 at 8, 10 and 12 dS/m respectively and considered as a salt sensitive genotype. Potato varieties showed differential response to different EC levels, Riviera and Provento had the highest fresh weight and Relative Growth Rate at all salt levels. Salt levels decreased callus fresh weight, Relative Growth Rate with increasing EC levels. The results based on ions accumulation in callus tissue revealed that the genotypes were significantly different from each other in their accumulation of K^+, Na^+ and Ca^{++} under salt stress.

KEYWORDS: Membrane Stability, Potato, Salt Stress, Tissue Culture.

INTRODUCTION
Millions hectares of dry land agriculture are considered as salt affected area. The most common salts responsible for toxicity and associated with saline soils are NaCl. Salinity is a serious problem, affect plant growth and productivity in many crop. Salinity causes many adverse effects on plant growth by altering metabolic processing resulting in decreased stomata conductance and respiration, decreased water potential, ion imbalances and toxicity of specific ions (Yousif, 2002; Yousif and Al Kaaby, 2006; Wahid *et al*., 2007). Many parameters can be used to determine tolerance to salinity stress such as cell membrane injury, ion accumulation, fresh and dry weight. Cell membrane injury caused by stress based on electrolyte leakage from the cells, Arvin and Donnelly (2008) wrote that electrolyte leakage test has been widely used to assess the level of plant tolerance to various stresses. Sodium and chloride are the major ions accumulated in plant tissue that subjected to salt stress and caused many physiological disorders like plasmolysis and limit plant growth (Balal *et al*., 2011; Gao *et al*., 2015). According to Maas and Hoffman (1977) Potato has been classified as a moderately salt sensitive with thresholds ranging from 1.5 to 3.0 dS/m. Cell and tissue culture techniques have been used as approach to evaluate potato plants for salt tolerance (Rahman *et al*., 2008; Benavides *et al*., 200 ; Queiros *et al*., 2007; Aghaei *et al*., 2008; Sajid and Aftab, 2014) by using different material resources such as node cuttings, root tip segment and callus growth culture (Zhang and Donnelly, 1997; Morpurgo, 1991; Naik and Widholm, 1993). No information available for salt tolerance of 4 widely Iraqi potato cultivars. To understand salt tolerance mechanism of these cultivars, we tested their level of tolerance based on electrolyte leakage. Another objective of the present study was to assess *in vitro* screening for salt tolerance using physiological parameters.

MATERIALS & METHODS
The experiments were conducted at Genetic Engineering Department in Agricultural Research Directorate in Ministry of Science and Technology/Iraq. Potato varieties (Arnova, Provento, Burren and Riviera) were micro-propagated on MS (Murashige and Skoog, 1962) nutrient medium; pH was adjusted to 5.7 prior to autoclaving at 121ºC for 20 minutes.

Membrane stability
Artificial saline water with NaCl was prepared and electrical conductivity *(*EC*)* levels (6, 8, 10 and 12 dS/m) was adjusted with electrical conductivity meter (EC-meter). Salt tolerance was evaluated by the method described by Arvin and Donnelly (2008) based on electrolyte leakage from leaves. 15 leaves (approximately 150 mg) from *in vitro* plantlets (4 weeks old plantlets) rinsed many times with distilled/deionized water (DD water) to remove electrolytes. Leaves were placed in 15 ml of different levels of osmotic stress (6, 8, 10 and 12 dS/m NaCl) in test tubes (2.5 × 15 cm) and the control samples were submerged in DD water. Samples were incubated at 10ºC for 24 hours in the dark, and then washed five times with DD water. Tubes were warmed to 25ºC, shaken well by hand and EC level was read using an EC-meter. Following the initial reading, leaves samples were killed by autoclaving for 15 minutes, then cooled to 25 ºC and final EC values were measured. Electrolyte leakage or cell membrane stability was calculated as the %injury as follows:

$$\% \text{ Injury} = 1- [1- (T1/T2)/ 1- (C1/C2)] \times 100$$

Where T and C refer to the EC values of salt stress treated and control treatments respectively; 1and 2 refer to the initial and final EC respectively.

***In vitro* screening for salt tolerance**

For callus induction, internodal segments (1-1.5 cm) were excised from four week old *in vitro* plantlets. 10 explants were cultured in Petri dishes containing MS basal medium with 3% sucrose, 8% agar and 0.1, 100, 0.5, 0.5, 2, 2 mg/l of Thiamine –HCL, Inositol, Glycin, Nicotinic Acid, BA and 2,4-D respectively. After 4-6 weeks callus were fragmented into small pieces (150mg) and planted in the previous medium supplemented with different levels of NaCl to generate EC at 8, 10, 12 dS/m, the EC of the control treatment (MS basal medium, without adding NaCl) was 6 dS/m. Calli were selected and again subcultured with freshly prepared medium every 21 days.

All cultures were incubated in a growth room chamber at 25 ± 2°C under photoperiod 16 h light and 8 h dark.

After 60 days several characteristics were recorded .These include

1-Callus color

2-Callus fresh weight (mg): callus was divided into pieces of 150 mg (initial fresh weight), 5 pieces were placed in Petri dishes containing MS medium supplemented with different levels of EC (6, 8, 10 and 12 dS/m) after one month final callus fresh weight were recorded.

3-Relative Growth Rate (RGR) was calculated following the formulae of Lutts *et al.* (1998):

RGR (mg x 10^{-2}/gm callus fresh weight/day) = $\ln W_2 - \ln W_1 / \Delta t$

Where

W_1 refers to initial fresh weight.

W_2 refers to final fresh weight.

Δt refers to the time for culturing (30 day).

4-Water content estimated according to Forooghian and Esfarayeni (2013)

Relative Water Content of callus = [(Wet weight of callus – Dry weight of callus) /Wet weight of callus] × 100

Callus dry weight was determined after 2 days oven dried at 60°C.

5-Determination of ions content

150 mg dry weight callus was placed in beaker containing 9 ml digesting mixture (10 Nitric acid: 4 Perchloric acids: 1 sulfuric acid). The beakers were heated up to 60 ºC until the solution became colorless then the digestion diluted with distilled water. Concentrations of Ca^{++}, Na^{+} and K^{+} were measured using Atomic Absorption Spectrophotometer (Shimadzo AA-670) according to the manufacturer's recommendation.

Three replication were made in all determination except % injury (6 replication were used in each treatment). Results were statistically analyzed using GenStat program and means were separated using Duncan's test at a probability level of 5%.

RESULTS & DISCUSSION

Assessment % injury

In this study we tried using leaves in the middle of 4 weeks old plantlets with excluding upper and lower leaves to avoid the error caused by the sampling position of the leaf. Cell membranes control the rate of ions movement in and out of cells, under stresses plant membranes structure is seriously impair and this cause increasing in permeability and losing of integrity (Blokhina *et al*., 2003). Therefore, the ability of plants to maintain membrane integrity (less 50% injury) under salt stress determines as salt tolerant.

Results in table 1 revealed that in all cultivars, %injury significantly increased with increasing salt levels. Differences in %injury among studied cultivars under salt stress were observed, Riviera cultivar showed lower injury value and considered as salt tolerant genotype, Burren and Provento showed less than 50% injury in all salt levels, while Arnova variety showed greater than 50% injury at 10 and 12 dS/m and considered as a salt sensitive genotype.

Similar results were reported by Arven and Donelly (2008) this may be due the capacity of these varieties to accumulate sugars during stress, sucrose interact with cellular membranes to increase the stability of the lipid layers (Bajji *et al*., 2002).

TABLE 1. Effect of salt levels and potato varieties on %Injury of leaves.

Salt levels dS/m	Varieties			
	Arnova	Burren	Provento	Riviera
6	24.31de	20.98bcd	12.71abc	8.23a
8	40.55g	29.66def	20.46bcd	10.67a
10	50.59h	32.13efg	25.15def	11.97ab
12	50.58h	34.11fg	28.09def	21.37cd

Means followed by the same letters are not significantly different (P<0.05) according to Duncan's test.

***In vitro* selection of salt- tolerant callus**

Genetic variation generated by somaclonal variation can be exploit it by subjecting tissue to salt stress and selection cell cultures that resistance to salt. The results in Table 2 showed that callus growth different among genotypes in the control treatment (salt-free media), the highest callus fresh weight was recorded in Riviera (2098.6 mg) followed by Provento (1530.7mg), Arnova (531.5 mg) and Burren (506.3) which means that genotypes variance for their response to callus induction. This result is in line with previous findings for potato varieties (Forooghian and Esfarayeni, 2013; Sajid and Aftab, 2014). Callus fresh weight significantly decreased with increasing NaCl levels in all tested varieties. Callus color in the control treatment ranged from green to greenish – white, while the color at 12 dS/m ranged from yellowish –brown to browning for all cultivars except Riviera which had green calluses with little brown area (table 2). Brownish color indicating cell necrosis and usually use as an indicator of tissue culture intolerance to osmotic stress (Bouiamrine and Diouri, 2012). Decreased in *callus* proliferation and the change in callus color with increasing of NaCl concentration are in agreement with the many studies (Rahnama and Ebrahimzadeh, 2004; Sajid and Aftab, 2012; Sajid and Aftab, 2014) and this may be due that cell division would decrease and less starch is produced (Forooghian and Esfarayeni, 2013).

TABLE 2. Effect of salt levels and potato varieties on callus fresh weight and callus color

Salt levels dS/m	Fresh weight of callus (mg)				Callus color			
	Arnova	Burren	Provento	Riviera	Arnova	Burren	Provento	Riviera
6	531.5 ab	506.3 a	1530.7 e	2098.6 f	Greenish–yellow	Greenish	Greenish-white	Green
8	446.2 a	475.1 a	1192.9 de	1542.6 d	Browning	Yellow	Little green with brown	Green
10	304.9 a	442.1 a	963.0 bcd	1348.1 de	Yellowish – brown	Yellow with Browning	Browning	Green with little brown area
12	292.6 a	266.3 a	737.3 bc	1028.3 cd	Yellowish – brown	Browning	Browning	Green with little brown area

Means followed by the same letters are not significantly different (P<0.05) according to Duncan's test.

Increasing salt levels decreased Relative Growth Rate (RGW) in all cultivars (Fig.1). Comparing the cultivars effect on this parameter revealed that the highest value was related to Provento cultivar at all salt levels and the lowest values of this parameter were recorded in Arnova cultivar with an average of 3.218, 2.173, 1.615 and 1.681 mg x 10^{-2}/gm callus fresh weight/day at 6, 8, 10 and 12 dS/m respectively. The reduction in RGW is well documented in literature (Lutts et *al.*, 1998; Queiros *et al*., 2007; Yousif, 2002).

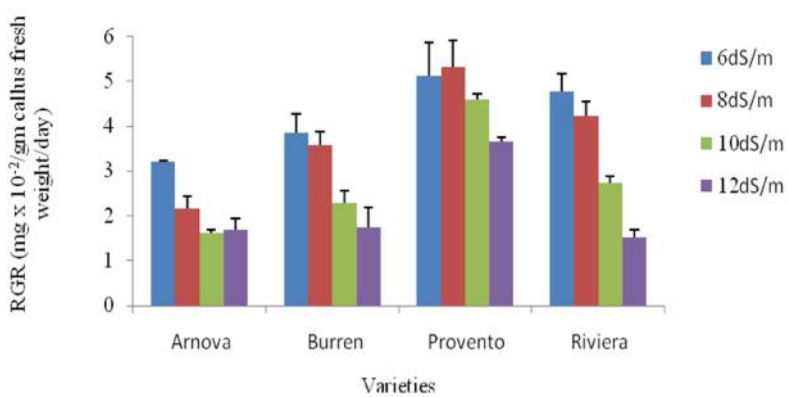

FIGURE 1. Effect of salt levels and potato varieties on callus Relative Growth Rate.

The data for Relative Water Content of callus is given in Figure 2. The results showed that salt levels did not affect on Relative Water Content in Burren tissue, while there were increased in water content at 8 dS/m for Arnova and Riviera. Adverse effect of salt was found in Relative Water Content of callus at 12 dS/m in all varieties except Burren. Queiros *et al*. (2007) observed that the water content was decreased in salt-tolerant calli growing on medium supplemented with NaCl and they explained the reduction in the water content may be due to the high osmotic pressure of the culture medium with high salt concentration. The result presented in Figure 3 shows that although the overall trend is a reduction in K^+ with increasing salt stress, but K^+ concentrations at 8 dS/m are higher than in callus grown without salt in all varieties except Burren. The effect of salt on K^+ accumulation varied by varieties and the highest K^+ concentration related with Provento and Riviera varieties compared with others in all salt levels. Variations in callus Ca^{++} concentrations according to varieties and salt (Fig. 4) were observed. It is worth noticing that the pattern of Ca^{++} accumulation in response to salt levels interfered with K^+ accumulations. lowest sodium accumulations were observed at 6 dS/m (without NaCl salt), that means Na^+ content of all varieties increased as medium salinity increased but the pattern of increase Na varies at different salinity levels (Fig 5).

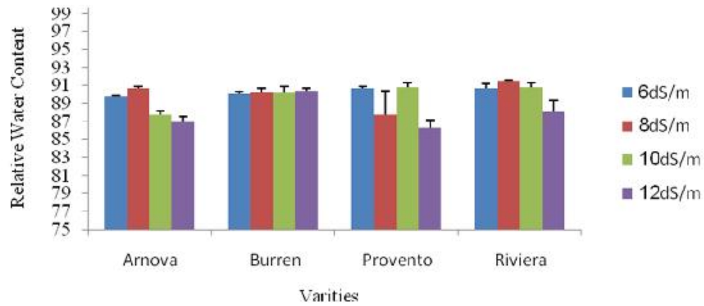

FIGURE 2. Effect of salt levels and potato varieties on Relative Water Content of callus

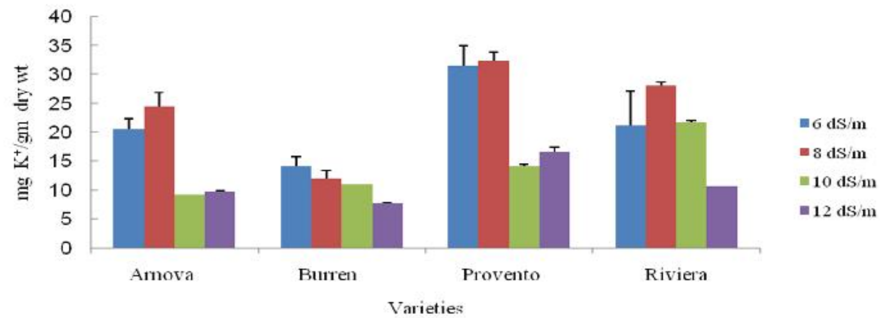

FIGURE 3. Effect of salt levels on K^+ concentration in callus dry weight of potato varieties

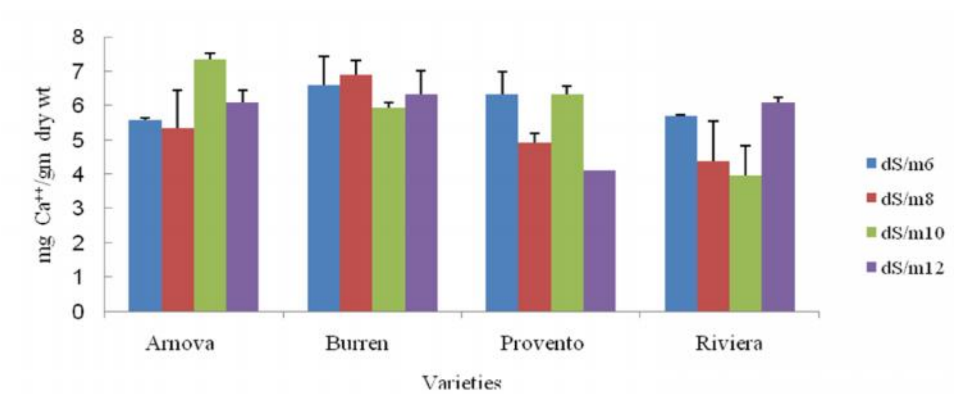

FIGURE 4. Effect of salt levels on Ca^{++} concentration in callus dry weight of potato varieties

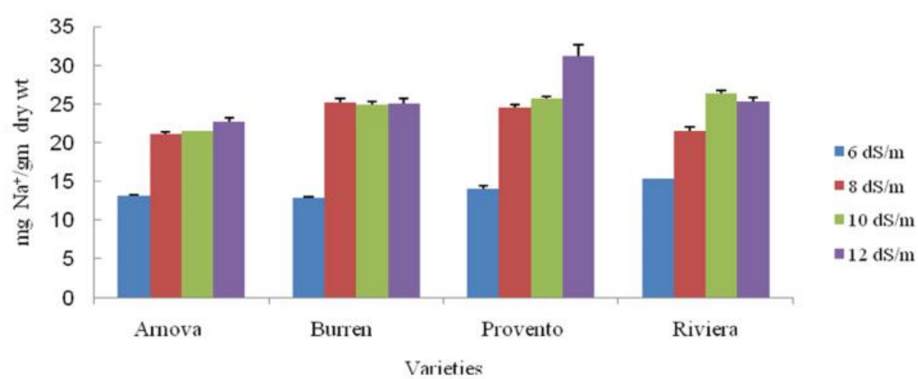

FIGURE 5. Effect of salt levels on Na+ concentration in callus dry weight of potato varieties

Statistical analysis revealed that all the genotypes were significantly different from each other in their accumulation of K^+, Na^+ and Ca^{++} under control treatment, that means ions accumulation is genetically controlled. Many studies have been documented that the response of different potato cultivars to salt stress is genotype dependent (Daneshmand *et al*., 2010; Jaarsma *et al*., 2013; Shah *et al*., 2011). It is clear from the results that saline conditions (salt levels) appears to involve an alteration in the ionic composition and all varieties were not behaved in a similar manner, this results are also consistent with many studies (Daneshmand *et al*., 2010; Javed, 2002; Nagi and Hafith, 2009; Yousif, 2002; Yousif and Al Kaaby, 2006) this might be related to competence of cell membranes which affected in ions exchange within cultivated tissues. From other hand there was a direct link between NaCl concentrations in the medium and Na content of the calli, the high concentration of this toxic ion may caused unstable the cellular membranes by displacing the K^+ and Ca^{2+} and caused unstable the cellular membranes and increase in membranes permeability and loss of their integrity (Blokhina *et al*., 2003; Hasegawa *et al*., 2000).

CONCLUSION

According to the results, the evaluation of salt tolerance could be based on membrane stability, callus growth parameters and ion concentrations. Depends on these parameters, Riviera ranked as the most tolerant to salinity followed by Provento, Burren and Arnova.

ACKNOWLEDGMENT

The author is highly acknowledged Soil and Water Resources Center/ Agricultural Research Directorate/ Ministry of Science & Technology for help in analyzing ions.

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