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Original article

The study of salt tolerance in regenerated plants from the roots of tobacco (*Nicotiana rustica* L.)

F. Sadat Fatehi, A.A. Ehsanpour*

Department of Plant and Animal Biology, Faculty of Biological Sciences and Technologies, University of Isfahan, Isfahan, Iran

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Extended abstract

Introduction

Soil salinity as a limiting factor for plant growth and development and one of the environmental stresses that has attracted the attention of scientists. NaCl Reduces seed germination percentage, root length, fresh weight and dry weight of seedlings and fresh weight of hypocotyl. Salinity stress inhibits plant growth and development and reduces photosynthesis, respiration and protein synthesis in susceptible species. When plants are exposed to environmental stresses such as salinity and drought, the balance between the production of reactive oxygen species (ROS) and the activity of interfering systems in the clearance of these radicals by antioxidants is disturbed and ultimately oxidative damage. the reactive oxygen species (ROS) accumulates in the leaves and lead to the oxidation of important cellular constituents such as protein, chlorophyll, lipid and nucleic acids. Salt tolerance increases if free radicals are produced through the intensification of the antioxidant system. There is a relationship between oxidative depletion and increased tolerance to salt and other environmental stresses and the efficiency of the antioxidant system. Plants use complex antioxidant systems that reduce the oxidative damage caused by ROS to cellular parts. This system controls the amount of ROS under both natural and environmental stressful conditions, without which plants can not convert solar energy in to chemical energy All plant body cells have the ability to regenerate, that is, to proliferate or create a new plant. In fact, regeneration is the basis of plant tissue culture, which means creating a complete plant with roots and stems from undifferentiated plant cells. Regeneration of plants by culturing undifferentiated cells in this in vitro is a clear reason for the flexibility of plant cells that occurs in response to specific environmental signals.

Materials and methods

In this study, root regenerated plants as well as unregenerate plants from tobacco roots were cultured in MS medium containing concentration of zero, 100, 200 Mm Nacl were grown 4 a weeks and then growth indicators of including fresh and Dry weight, photosynthetic pigments content including total chlorophyll and carotenoids, concentration sodium and potassium, total phenol content, proline content, total antioxidant level, total ROS, Lipid peroxidation, auxin content as well as RAPID-PCR analysis Got it.

* Corresponding author: Ali Akbar Ehsanpour; E-Mail: ehsanpou@sci.ui.ac.ir

Results and discussion

It was observed that fresh and dry weight of regenerated plants increased significantly over non-regenerated plants showed. It also increased the amount of photosynthetic pigments and reduced sodium and increased potassium, decreased total ROS and MDA and increased total antioxidant and auxin also relative to non-regenerated plants were observed in salt conditions, results obtained from RAPID-PCR analysis it showed somatic variation in regenerated plants comared to non-regenerated plants. Present research suggests that regenerated plants can enhances improved salinity resistance growth indices in saline conditions. In general, the results show that regenerated plants in both non-stress and salinity stress improved growth, physical and biochemical indices compared to non-regenerative plants. It was also found that regenerated plants R2 had better performance under salinity stress than plants regenerated plants R1 and non-regenerated plants. The occurrence of somaclonal variations between regenerated and non-regenerated plants has also been identified.

Conclusion

I n the present study, from regenerated and non regenerated tobacco roots were cultured in MS medium containing concentration of zero, 100, 200 Mm Nacl were grown 4 a weeks**.** The photosynthetic pigments were incresed while, sodium content reduced but potassium was increased, Total ROS decreased and MDA increased, total antioxidant and auxin also relative to non-regenerated plants were observed in salinity conditions. Results obtained from RAPID-PCR analysis showed somaclonal variation in regenerated plants comared to non-regenerated plants. Present data suggested that regenerated plants from root improved salinity tolerance and growth parameters in saline conditions.

Keywords**:** Regeneration, Resistance to NaCl salt, Salinity stress, Somatic variation, Tabacco plant

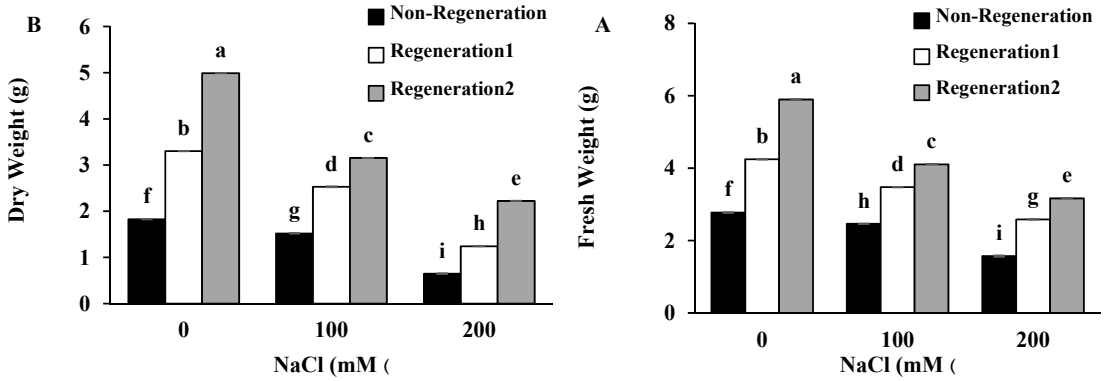

Fig.1. Effect of salinity on wet (A) and dry weight (B) The data are based on the average of three. Similar letters indicate not significant based on Duncan's test (P≤0.05).

Table 1. Comparison of the average fresh weight of regenerated and non-regenerated plants under salinity stress.

	Salinity (mM)		
	0	100	200
	g		
Non Regeneration	2.771^{f}	2.462^{g}	1.591^{i}
Regeneration1	4.242^{b}	3.475^{d}	2.186^{h}
Regeneration2	5.894^{a}	4.103^{c}	3.166^{e}

Means with dissimilar letters or letters show significance based on Duncan's test.

Table 2. Comparison of the average Dry weight of regenerated and non-regenerated plants under salinity stress.

	Salinity (mM)		
	0	100	200
	g		
Non Regeneration	1.829^{f}	1.52^{g}	0.649^{i}
Regeneration1	3.3^{b}	2.53^{d}	1.243^{h}
Regeneration2	4.988^{a}	3.154^{c}	2.224^{e}

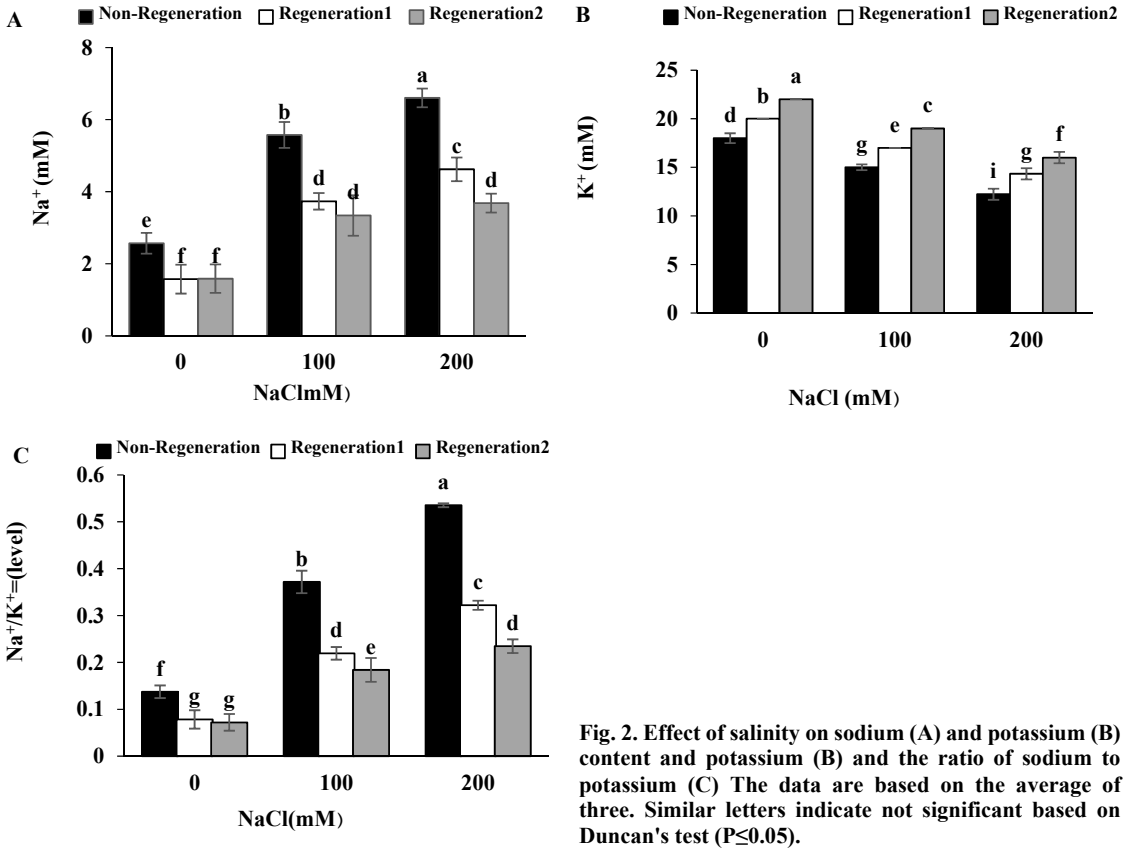

Fig. 2. Effect of salinity on sodium (A) and potassium (B) content and potassium (B) and the ratio of sodium to potassium (C) The data are based on the average of three. Similar letters indicate not significant based on Duncan's test (P≤0.05).

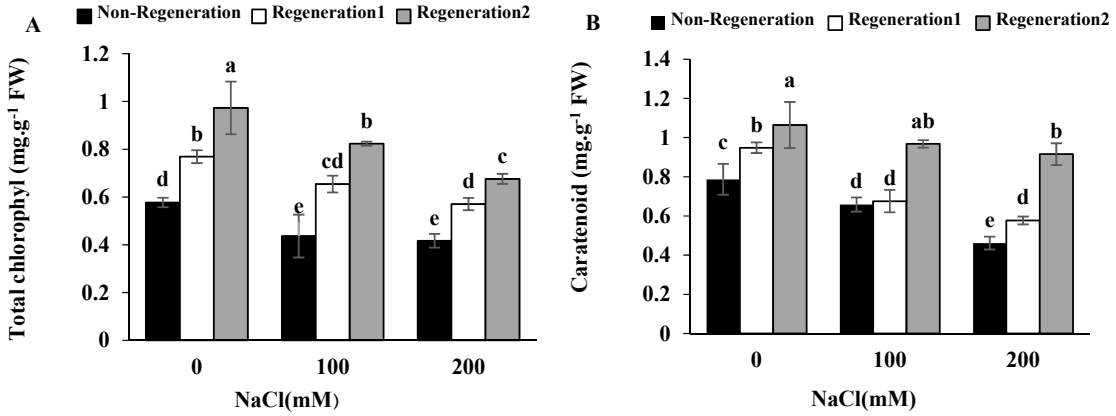

Fig. 3. Effect of salinity on chlorophyll content(A) and carotenoid (B) The data are based on the average of three. Similar letters indicate not significant based on Duncan's test (P≤0.05).

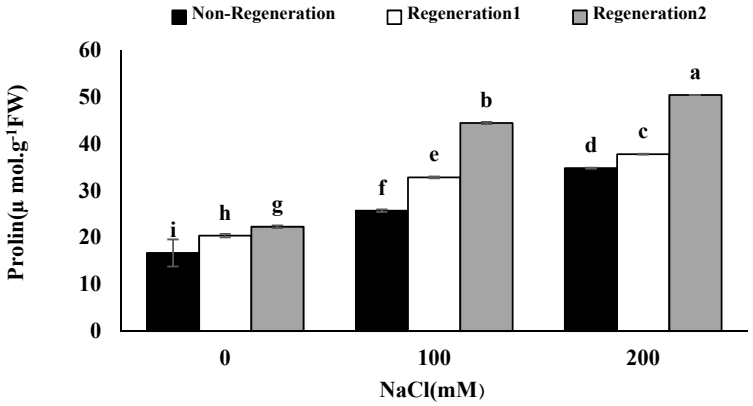

Fig. 4. Effect of salinity on proline content. The data are based on the average of three. Similar letters indicate not significant based on Duncan's test (P≤0.05).

Table 3. Comparison of the average Prolin of regenerated and non-regenerated plants under salinity stress

	Salinity (mM)		
	0	100	200
	µmol g^{-1} fresh tissue		
Non Regeneration	16.661^{i}	25.674^{f}	34.748^{d}
Regeneration1	20.358^{h}	32.780^{e}	37.739^{c}
Regeneration2	22.24^{g}	44.404^{b}	250.355^{a}

Means with dissimilar letters or letters show significance based on Duncan's test.

Table 4. Comparison of the average Total antioxidants of regenerated and non-regenerated plants under salinity stress.

	Salinity (mM)		
	0	100	200
	Relative percentage of total antioxidants		
Non Regeneration	0.244^{d}	0.555^{cd}	0.927^{bc}
Regeneration1	0.776^{cd}	1.388^{b}	2.478^{a}
Regeneration2	0.308^{d}	1.354^{b}	1.395^{b}

Means with dissimilar letters or letters show significance based on Duncan's test.

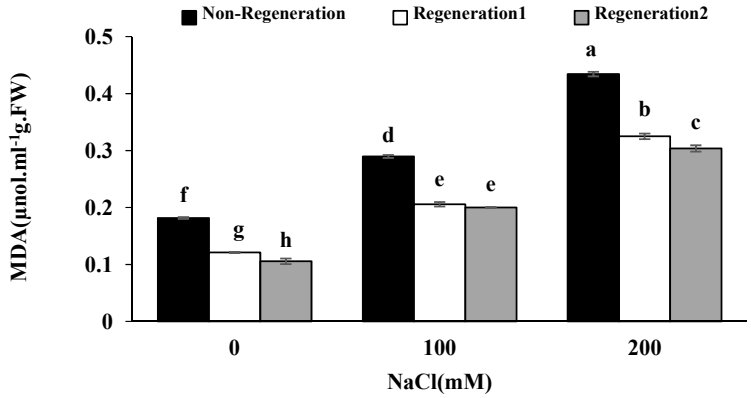

Fig. 5. Effect of salinity on lipid peroxidation. The data are based on the average of three. Similar letters indicate not significant based on Duncan's test (P≤0.05).

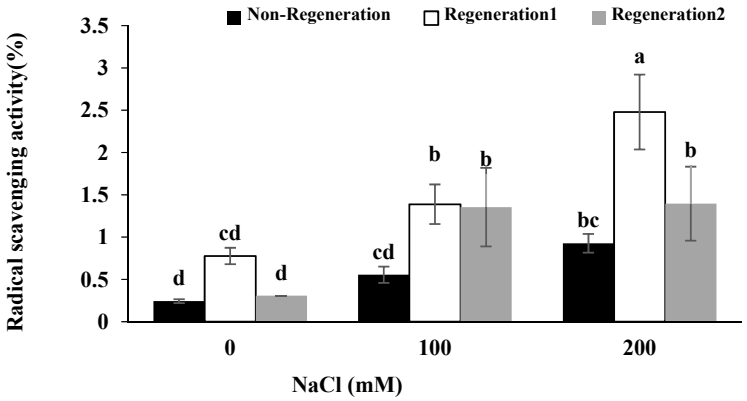

Fig.6. Effect of salinity on total antioxidants. The data are based on the average of three. Similar letters indicate not significant based on Duncan's test (P≤0.05).

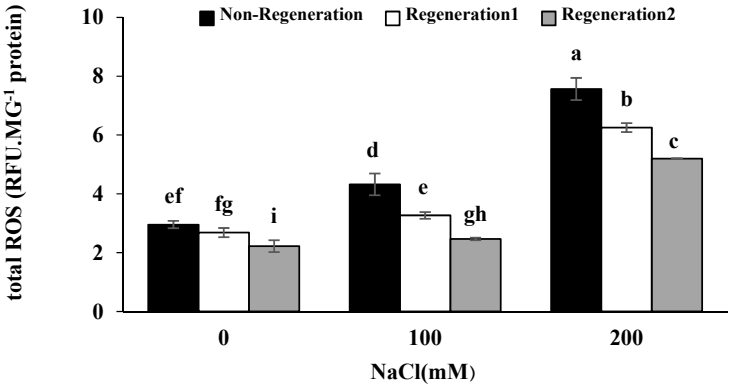

Fig. 7. Effect of salinity on total ROS The data are based on the average of three. Similar letters indicate not significant based on Duncan's test (P≤0.05)

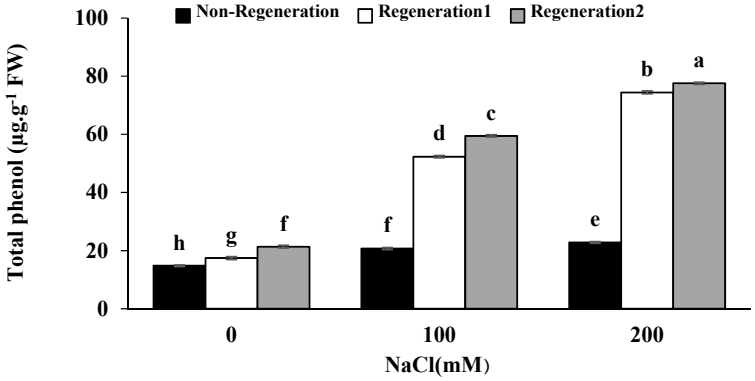

Fig. 8. Effect of salinity on total phenol content The data are based on the average of three. Similar letters indicate not significant based on Duncan's test (P≤0.05)

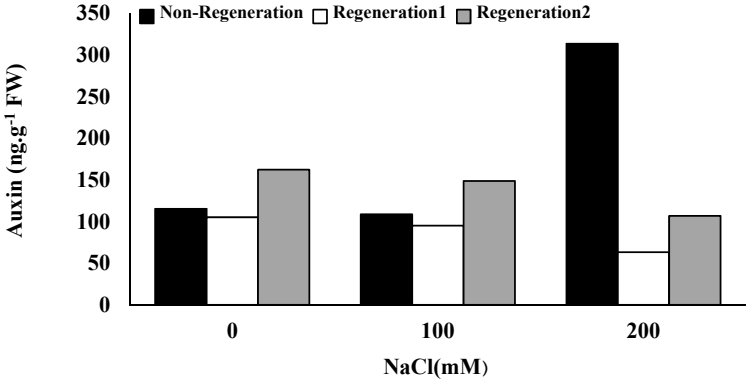

Fig. 9. Effect of salinity on leaf auxin content of regenerated and non-regenerating plants

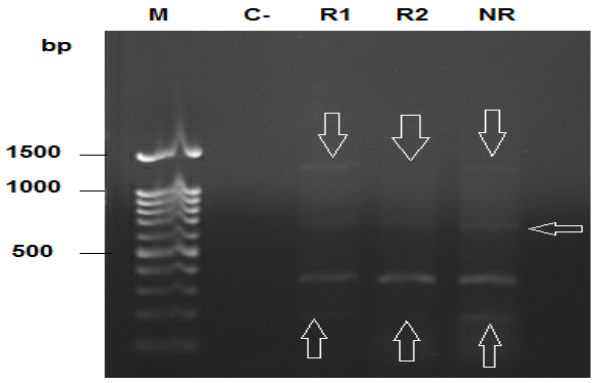

Fig. 10. Results of RAPID-PCR analysis (OPAA-20 primer) in regenerated and non-regenerated plants: Marker, C: Negative Control, R1: Regeneration Plant Line 1 (Regeneration1), R2: Regeneration plant 2, NR: Non-regeneration plant. The arrow indicates modified bands