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Screening three cultivars of mutant Potato with sodium azide for salt tolerance

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Abstract

In vitro non- mutated and mutated plants with sodium azide at a concentration of 1 m mol for 30 minutes of three cultivars of Potato (Arnova, Provento and Emma) were screened to salt tolerate. Plants cultured in MS medium supplimented with different levels of sodium chloride (8, 10, 12 and 14 ds m^{-1}) with comparison treatment (electrical conductivity 6 ds m^{-1}). The cultures were incubated at a temperature of 25 ± 2 and 16 hours day $^{-1}$. The data were taken on the morphological characteristics of the vegetative growth (plant height, number of shoots and nodes) and the fresh weight of the vegetative group and its content of Na+, K+, Ca++ and amino acid proline. The results showed that the cultivars differed significantly in the morphological characteristics (plant height, number of nodes and the fresh weight of the vegetative growth, Arnova cultivar superior with rates 5.55 cm, 5.72 plant node $plant^{-1}$ and 236 mg, respectively. Salinity also caused a decrease in the rates of these traits by increasing the salinity levels in addition to its effect in increasing the accumulation of Na+ and decreasing K+, while it had no significant effect on the accumulation of Ca++ and the amino acid proline. Mutation had no significant effect on the accumulation of Ca++ and proline, while an increase K^+ in Provento cultivar at all salinity levels compared to 6 ds m^{-1} and a decrease in the Na^+ content at 6, 8 and 14 ds. m^{-1}.

Key words: Potato, Screening, morphological characteristic , salinity. proline.

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I. INTRODUCTION

Potato (*Solanum tuberosum L.*) belongs to the Solanaceae family, which is one of the most important vegetable crops in the world in terms of economic importance and is considered a main food for many peoples of the world as it is a good source of energy, rich in carbohydrates and starch, in addition to containing many minerals, vitamins and amino acids (Matlob et al., 1989). Potato crop is one of the important crops in Iraq, with its consumption increasing, focusing on its quality and increasing its productivity.. However, this productivity is not commensurate with the market need, which necessitated resorting to imports from Arab and international markets to fill the deficit (Report production of Cotton, Maize and Potato, 2013). In addition to the many attempts to improve the productivity of the potato crop in the cultivated areas in Iraq, but they did not meet the required degree of success because the Iraqi soil suffers from the problem of salinity, especially the central and southern, as 75% is affected by salt, especially in areas where potato cultivation (Al-Zubaidi 1989).

Salinity is considered one of the stress that leads to a disruption in the physiological processes of the plant by reducing the availability of soil water and causing an imbalance in the water balance inside the plant. Because salinity occupies large areas of Iraq and the need for genotypes that can withstand various stresses, it was necessary to employ modern breeding methods such as plant tissue culture technique and expanding the base of genetic variations using the mutagenesis technique (Ahmed et al., 2010: Gomez et al., 2019; Al Husseini, 2016). Both types of mutagens were widely used to stimulate the desired changes and thus obtain salinity-tolerant cell lines in a short period and in specific areas without being restricted to time and place.

Several studies indicated the role and effectiveness of Sodium azide as a chemical mutagenic and its effect in improving the ability of crops to resist them against biotic and a biotic stresses and developing resistance in sensitive crops to improve yield and specific characteristics against pathogens (Olawuyi and Okoli, 2017), through a role in causing a point mutation in the plant genome produces a protein that has a different function compared to the non-mutant plant, where the resulting mutant plant can survive under unfavorable conditions (Khan et al., 2009).

Zaman et al. (2015) evaluated the salt tolerance of eight potato cultivars (Desire, Cordinal, Kroda, Asterix, Challenger, Sh-5, Sant and Hermis) by culturing the axillary bud in MS medium supplemented with different concentrations of sodium chloride salt (0, 10, 20, 40, 60, 80 and 100) m mo lL $^{-1}$ found that Kroda cultivar was the most tolerant to salinity compared to the rest of the cultivars, Desiree, Cordinal and Sh-5 cultivars were moderately tolerant to salinity. While Asterix was the most sensitive to salinity.

Sudhersan et al. (2012) exposed twenty-five cultivars of potato which *in vitro* propagated to different levels of NaCl 0, 13, 17, 34, 51 and 68 mg L^{-1}.. The study showed that there are seven sensitive cultivars to salinity, 12 cultivars are highly sensitive and 6 cultivars are moderately sensitive, depending on phenotypic markers. In a study conducted by Khairallah and Jawad (2017) to evaluate the response of eight potato cultivars Almondo, Arizona, Buren, Everest, Riviera, Rudolph, Sever and Sylvana grown *in vitro* using a single node cuttings in MS medium supplemented with

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different concentrations of NaCl (0, 50 , 100 , 150 , 200) mmol The results showed that Riviera cultivar is resistant and tolerant to salinity, followed by Arizon and Buren cultivars. Due to the importance of the potato as a strategic crop, many laboratories are conducting research to improve its production under a biotic stresses by using chemical and physical mutagens (Rahman and Kaul, 1989; Al-Qurainy and Kan, 2009) and it was concluded that Sodium azide was very effective in causing genetic mutation for a wide range of crops, therefore, the aim of the research to screen and evaluate three cultivars of potato treated with Sodium azide under the influence of different levels of sodium chloride salt and comparing them with non- mutated plant and study morphological markers of the vegetative system and its content from ions and the amino acid proline which associated with salt tolerance .

II. MATERIALS AND METHODS

The experiment was conducted in the laboratories of the Genetic Engineering Department of the Center for Biotechnology and Food Technology / Agricultural Research Department / Ministry of Science and Technology 2021/2022.

In vitro propagated plants were mutated with sodium azide at a concentration of 1 m mol for 30 minutes of three cultivars of potato, namely Arnova, Provento and Emma. Mutated and non-mutated plants were cut into stem cuttings (1-2 cm long, containing 1-2 nodes) They were grown in vassal tubes containing 15 ml of MS medium (Murashige and Skooge, 1962) supplemented with indole acetic acid (IAA), sucrose and agar (for solidification of the medium) in the amount of 1, 30000 and 7000 mg L^{-1}, respectively, which were supplied with different levels of sodium chloride at electrical conductivity 8, 10, 12 and 14 ds. m^{-1} with the control treatment with electrical conductivity was 6 $ds.m^{-1}$ .The experiment was conducted with five replicates (an average stem cutting. per replicate) for each treatment. The cultures were incubated in the growth chamber at a temperature of 25±2°C and illumination for 16 hours day^{-1}. Data were taken on the morphological markers of the vegetative growth (plant height, number of shoot, number of nodes, fresh and dry weight of the vegetative growth) after 30 days.

Determination of ions content: 150 mg dry weight vegetative growth 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) .

Determination of proline content: 30 mg dry weight of vegetative growth was taken and mixed with 800 micro liter sulpha salicylic acid (3%) then digested and centrifuged at 2000 rpm min-1 for 10 minute. 0.5 ml from acetic acid and 0.5 ml ninhydrin solution adding to mixture solution (0.5 ml) then incubated in path water for 30 min. Red layer isolated by adding 2 ml toluene then colored intensity was determined by measured optical density using microplate reader spectrophotometer at wavelength 520 nanometer. According to proline using a standard curve of 1-100 microgram). Concentrations of proline was measured as follow as:

Micromole proline gm^{-1} vegetative dry weight = [(microgram proline / ml × ml toluene)/115.5/ micro mol)] / (dilution factor).

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statistical analysis

Factorial experiments were conducted using a completely randomized design (C.R.D) with five replications for *in vitro* culture experiments and three replicates to estimate ions and the amino acid proline in the vegetative growth .The data were statistically analyzed using the statistical program Genstat, the means were compared using the Least Significant Difference (LSD) test at a probability level of 5%.

III. RESULTS AND DISCUSSION

Effect of salt levels and mutation on morphological characteristics

plant height (cm)

The results in table 1 showed that a significant effect on plant height trait .The cultivar Emma in non- mutant treatment gave the highest average at level 6 ds m^{-1} reached 11.60 cm, followed by the cultivar Arnova with a rate of 11.20 cm, which differed significantly from the rest of the interactions, while plants of the same cultivar in the same treatment gave the lowest rate of plant height at 14 ds. m^{-1}, which is 1.10 cm. The decrease in plant height by increasing the salinity levels in the growth medium may be explained into a decrease in water stress, which negatively affects the efficiency of plants by absorbing water and the necessary nutrients in the process of division and elongation of stem tissues, which leads to a decline in plant height (Azeve do, 2009).

Shoot numbers (shoot. plant $^{-1}$)

It was observed in table 1 that in Non- mutant treatment , the cultivar Arnova at the salt level of 8 ds.m^{-1} gave an average of 1.20 shoot.$plant^{-1}$.Whereas, the cultivar Emma failed to give shoots when cultured at 6 ds. m^{-1} (comparison treatment) and 14 ds.m^{-1} , As for the mutant treatment, the two cultivars Arnova and Emma in 6 ds. $m^{-1}$and Arnova cultivars at 8 ds.m^{-1} gave the highest rate of 1.2 shoot.$plant^{-1}$, respectively.

Node number (Node .plant $^{-1}$)

As for the number of nodes a significant decrease in the number of nods was observed with an increase in the level of salinity in MS medium (table 1) and it showed the significantly excelled of the comparison treatment (6 dsm^{-1}) by giving high rates in both of mutated (7.20, 7.90 and 6.90 node..$plant^{-1}$) and non-mutated (8.00, 7.60 and 7.00 node $plants^{-1}$) for all cultivar Arnova, Provento and Emma respectively. It was followed by the salt level of 8 ds.m^{-1} in the comparison treatment at non- mutated at an average of 7.20 for Arnova and 7.20, 5.80 and 5.00 nods for Arnova, Emma and Provento cultivars, respectively, while the lowest average number of nods was 0.80 nods.$plant^{-1}$ for Emma at level 14 ds.m^{-1} in a non- mutated treatment.

Fresh weight of vegetative growth (mg)

Table 1 was showed that the significant decrease in the fresh weight of the vegetative growth with an increase in the salt levels. In the treatment non- mutated, the interactions did not differ significantly between them, except for the treatment of Arnova cultivar at 6 ds.m^{-1} (control treatment), which gave the highest average fresh weight of 238.00 mg, which differed from the cultivar Emma grown at the salt level of 14 ds. m^{-1} at a rate of 46.00 mg fresh weight, As

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for the mutated treatment, the Arnova cultivar appeared to be excelled at 6 and 8 ds.m^{-1} with rates of 434.00 and 410.00 mg fresh weight, respectively.

Table (1) Effect of cultivars and mutation with Sodium azide interaction with salt levels on the morphological characteristics of vegetative growth after 30 days..

Mutation	Cultivars	Salt levels dS m $^{-1}$				
		6	8	10	12	14
Plant height (cm)						
Non- mutation	**Arnova**	11.20	6.52	5.40	3.40	2.64
	Riviera	10.55	3.30	3.06	2.70	2.20
	Provento	11.60	3.70	3.20	1.54	1.10
Mutation	**Arnova**	10.10	5.80	4.40	3.96	2.10
	Riviera	6.72	5.16	3.24	2.74	2.36
	Provento	6.20	4.10	3.90	2.64	1.66
LSD 0.05	**2.12**					
NO. Shoot plant^{-1}						
Non- mutation	**Arnova**	0.00	1.20	0.60	0.60	0.60
	Riviera	0.30	0.80	0.60	0.60	0.60
	Provento	0.20	1.00	1.00	0.60	0.00
Mutation	**Arnova**	1.20	0.40	0.40	0.40	0.40
	Riviera	0.80	0.80	0.80	0.80	0.20
	Provento	1.20	1.20	1.00	0.80	1.00
LSD 0.05	**0.77**					
NO. nodes plant^{-1}						
Non- mutation	**Arnova**	7.20	7.20	6.80	5.20	5.20
	Riviera	7.90	4.60	3.40	3.40	3.00
	Provento	6.90	4.20	4.00	2.00	0.80
Mutation	**Arnova**	8.00	7.20	5.40	2.60	2.40
	Riviera	7.60	5.00	4.60	3.60	3.00
	Provento	7.00	5.80	4.20	3.00	3.00
LSD 0.05	**2.19**					
Fresh weight (mg)						
Non- mutation	**Arnova**	216.00	204.00	200.00	198.00	194.00
	Riviera	186.00	172.00	152.00	144.00	134.00
	Provento	238.00	168.00	136.00	86.00	46.00
Mutation	**Arnova**	434.00	410.00	176.00	168.00	160.00
	Riviera	226.00	226.00	170.00	142.00	140.00
	Provento	182.00	172.00	162.00	116.00	90.00b
LSD 0.05	**145.16**					

It appears from the results that salinity caused a decrease in the rate of plant height, number of shoot, number of nods, and the fresh weight of the vegetative growth by increasing their levels. the decrease in plant growth may be associated with a decrease in its water content due to the importance of water in cell division and elongation, causing most of the cell's vital processes to stop as a result of lack of water and thus its negative impact on its growth (Ahmed, 1984). on

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the other hand, the positive effect of mutated with sodium azide appears at levels 6 and 8 ds.m^{-1} in improving the plant's tolerance to stress when compared to non- mutated treatment (Figure 1), this may be due to the fact that the mutagen (sodium azide) may have caused a change at the genetic level, and thus was reflected in the studied traits, as the mechanism by which sodium azide works is the production of an azide compound, which interferes with the DNA of the cell nucleus, causing a point mutation, suggesting that SA works Substitution of GC with AT subsequently causes some changes in the production of amino acids (AL-Qurainy and Khan, 2009). Also, some studies (Salim et al., 2009, Dubey et al., 2017) indicated that the mechanism of action of Sodium azide is based on the production of an organic metabolite (β-azidoalanine [N3–CH2–CH(NH2)–COOH]) leading to chromosomal aberrations at lower rates compared to other substances.

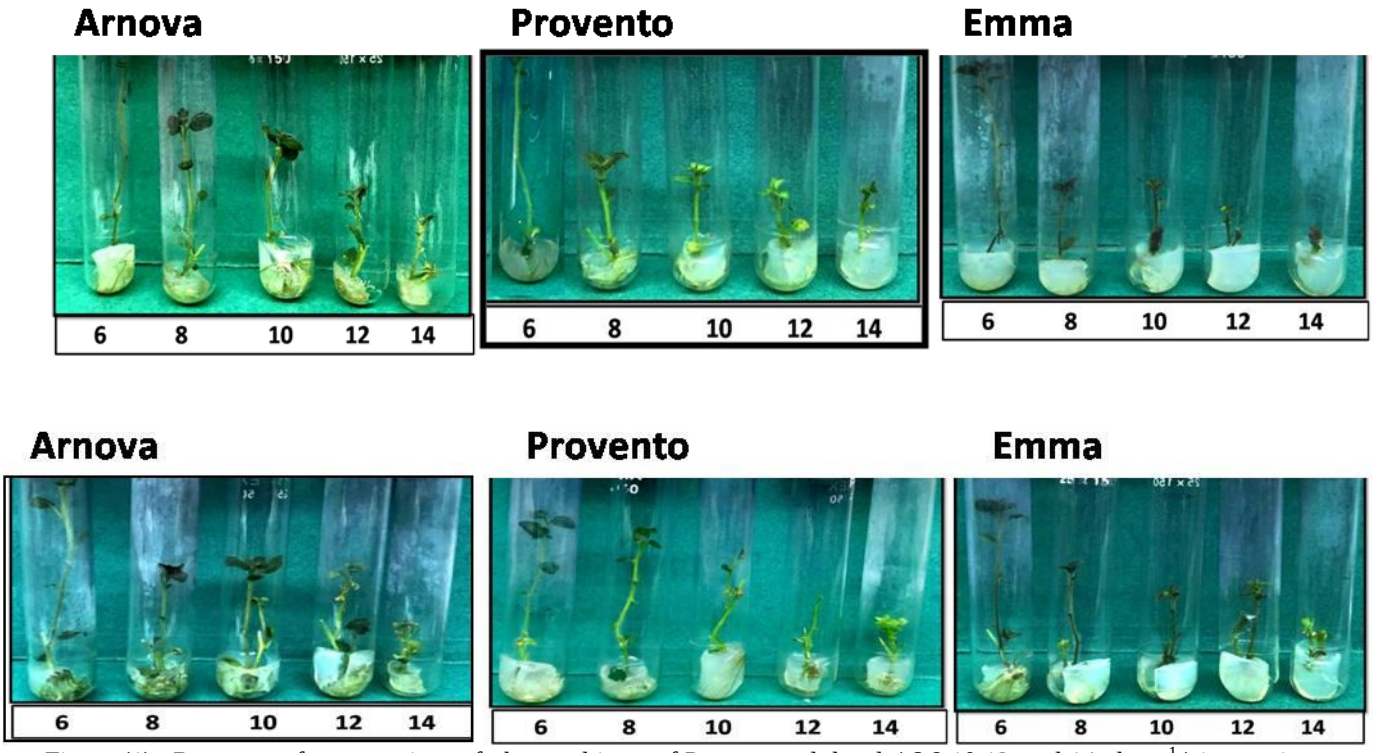

Figure (1). Response of stem cuttings of three cultivars of Potato to salt levels(6,8,10,12, and 14 d sm^{-1}) interaction with mutation (1 m mol NaN_3 for 30 minute) up : Non- mutated treatment down : Mutated treatment.

Effect of salinity levels and mutagen on ions and amino acid proline

Sodium Ions (Na+) (mg g^{-1} vegetative dry weight)

It was noted in the table 2 that in the Arnova cultivar, the concentration of **Na+** decreased in the salt levels 6, 8 and 14 in the mutated treatment, while it increased in the two salt levels 10 and 12 ds.m^{-1}. As for the Provento cultivar, the **Na+** concentration increased at all salinity levels compared with the control treatment (6 ds.m^{-1}) in which the **Na+** concentration decreased in the mutated treatment. the interaction also shows a decrease in the content of Na+ of the vegetative growth at all salt levels except for the level of 14 ds.m^{-1} in the mutated treatment compared to the non-mutated treatment.

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Potassium ions (K+) (mg g^{-1}veggetative dry weight)

In table 2, it was noted that the mutation led to an increase in the K+ content of the Provento cultivar at all salinity levels in compared with the 6 $ds.m^{-1}$, while the potassium accumulation was significantly decreased in the vegetative growth for all cultivars and for all salinity levels except at 14 ds. m^{-1} .

Calcium Ions (Ca++) (mg g-1 vegetative dry weight)

The table 2 showed that no significant effect appeared between the cultivars and mutation at different levels of salt on the content and accumulation of **Ca++** ions in the vegetative growth.

The results show that the cultivars differed significantly in their content of Na+, k+ and **Ca++** ions, and this difference may be due to the nature of the studied cultivars, which is reflected in their ability to take and transfer ions within tissues. Salinity had a significant effect on the concentration of Na+ and k+, while it had no effect on the concentration of Ca++, in which it was observed that Na+ concentrations increased with an increase the levels of salt, which was associated with the variation in K+ concentrations between the increase and decrease in the tissues of the vegetative system of the plants. At levels 8, 10, and 12 ds. m^{-1}, an increase in its concentration was followed by an increase in its concentration at 14 $ds.m^{-1}$. This may explain that the cells in the tissue of the vegetative system are working to allow these ions to enter it to increase its osmotic potential, causing the accumulation of these ions in Cytoplasm and its cell vacuoles as a result of salt stress and since Na+ ions are dominant in the medium, they have negatively affected the process of absorption and entry of K+ ions into cells as a result of competition between these two ions (Greenway and Munns, 1980). These results were in agreement with the results of many researchers (Munns, 1993; El-Dehemawy, 2009; Queiros et al., 2011; Nikam et al., 2015) who indicated an increase in the content of Na+ ions and the inhibitory effect of Na+ on the K+uptake system or K+ leakage from the cell, (Munns, 1999). Munns , (2002) indicated that halophytes and some non-halal plants work to regulate osmosis by concentrating salt in their tissues. However, the accumulation of these quantities becomes more toxic to sensitive plants than non-saline plants, and thus they are unable to modify their internal osmotic. As for the mutagenesis effect, it appears from the results that it had a significant effect in increasing the concentrations of Na+ ions and decreasing the concentrations of K+ ions, with the exception of the salt levels 8 and 14 $ds.m^{-1}$. This may be attributed to the fact that mutagenesis of the mutagen, whether physical or chemical, may affect the mechanism of ion absorption through the damage it causes to cellular membranes, thus affecting membrane permeability, or through the mutagenic effect on the ion transport system. The mutation did not affect the calcium ion accumulation in the cells. Mutation did not affect the Ca++ accumulation in the cells.

Table (2) Effect of mutation and salt levels on sodium (Na +) , potassium (K+) , calcium (Ca ++) ions (gm gm-1 vegetative growth dry weight) and proline (gm gm-1 c vegetative growth dry weight)for three cultivars (Arnova , Riviera and Provento) after 30 days..

Mutation	Cultivars	Salt levels dS m^{-1}				
		6	8	10	12	14
Na+ (gm gm-1 vegetative growth dry weight						
Non- mutation	Arnova	3.48	14.45	25.20	28.81	52.86

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	Riviera	2.47	16.21	19.14	32.15	54.38
	Provento	3.34	16.21	27.77	42.96	36.84
Mutation	Arnova	1.94	10.54	25.53	36.33	39.61
	Riviera	2.23	18.86	23.42	42.32	53.17
	Provento	3.30	13.13	21.16	30.89	42.29
LSD 0.05	31.01					
K+ (gm gm-1 vegetative growth dry weight)						
Non- mutation	Arnova	17.07	8.11	7.16	6.36	11.60
	Riviera	12.71	8.59	8.54	9.50	12.02
	Provento	17.73	9.85	13.85	16.13	12.66
Mutation	Arnova	7.71	7.88	6.84	7.39	6.55
	Riviera	14.70	23.49	15.33	16.71	17.14
	Provento	13.36	7.98	7.53	7.97	17.01
LSD 0.05	**9.41**					
Ca++ (gm gm-1 vegetative growth dry weight)						
Non- mutation	Arnova	3.22	2.37	3.12	3.15	3.44
	Riviera	2.66	2.90	2.55	3.61	3.94
	Provento	2.12	2.94	3.03	3.41	2.52
Mutation	Arnova	2.58	2.56	3.07	3.08	2.58
	Riviera	2.36	2.75	2.77	2.78	2.34
	Provento	2.31	2.50	2.58	2.49	3.10
LSD 0.05	**N.S**					
Proline micromole proline (gm-1 vegetative growth dry weight)						
Non- mutation	Arnova	43.10	50.90	17.80	48.90	44.70
	Riviera	57.50	48.10	48.80	53.60	57.10
	Provento	55.80	54.00	54.00	59.00	42.90
Mutation	Arnova	55.00	45.30	50.70	54.00	59.10
	Riviera	59.60	47.30	48.10	59.00	39.90
	Provento	54.70	48.60	57.40	50.90	54.90
LSD 0.05	**N.S**					

Plant content of the amino acid proline

The levels of salt and mutation no significant effect on the content and accumulation of the amino acid proline in the vegetative growth of the plants for all cultivars.

There are many roles that the amino acid proline plays in the salinity tolerance of plants under., as it has a role as a protective factor for enzymes (Solomon et al., 1994) and organelles in the cytoplasm (Van Rensburg et al., 1993), as well as being a carbon and nitrogen storage compound and a free radical scavenger. on membrane and protein stability (Chinnusamy et al., 2005). Although there are no significant differences between the cultivars treated with different levels of sodium chloride salt, when looking at the results it appears that the rates of proline accumulation in the tissues of vegetative growth at salt levels (8, 10, 12 and 14 ds .m^{-1} have been compared to some extent to their levels at 6 ds.m^{-1} (control treatment), where the accumulation of proline in cells is a common response in plants under salt stress (Szabados and Savouré, 2010) due to the high rates of its synthesis and low oxidation (Kumar et al., 2003). Hence its contribution to creating an equilibrium between the vacuole and the cytoplasm under stress conditions (Delauney and

Verma, 1993). It also appears that the rates of proline in the mutated treatment converged with its rates in the non-mutated treatment, in spite of there were no significant differences.

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