Influence of *in vitro* Salinity on Growth and some Element Contents of Three Grape *(Vitis vinifera* L.) Cultivars

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Abstract:

Influences of *in vitro* salinity in form of sodium chloride (NaCl) and calcium chloride ($CaCl_2$) on growth and element contents of three grape cultivars (Thompson Seedless, Red Roomy and Beauty Seedless) were investigated. Shoot tip explants were cultured on Murashige and Skoog (MS) medium containing 1.0 mg/l benzylaminopurine (BAP). The experiment was conducted with four levels of NaCl (0, 50, 85 and 120 mM) and four levels of $CaCl_2$ (0, 1, 5 and 10 mM) and mixture of both salts with three levels [0, 60 (50 NaCl+10 $CaCl_2$) and 90 (85 NaCl+5 $CaCl_2$) mM]. The results showed that the growth parameters (proliferation, plantlet height, number of leaves, number of nodes, internode length, fresh and dry weight) significantly decreased with increasing NaCl concentrations up to 120 mM compared to the control treatment. However, application of $CaCl_2$ treatments counteracted this inhibitory effect on the growth parameters at 5 mM. $CaCl_2$ supply increased shoot Ca^{2+} content and decreased shoot Na^+ content. Increasing NaCl and $CaCl_2$ concentrations in the culture medium increased the contents of Na^+, Ca^{2+}, whereas K^+ content decreased compared to the control.

Keywords: *Vitis vinifera*, NaCl, $CaCl_2$, *in vitro*

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Introduction:

Salinity is one of the most serious environmental stresses influencing crop growth and productivity (Koca *et al*., 2007). The complex effects of salinity cause a reduction in the growth of which is due to osmotic effect or reduction in water absorption and specific effect of ion such as sodium and chlorine that particularly have toxic effects on fruit trees (Shani and Ali, 2005 and Marandi *et al*., 2009). The osmotic effect on growth is proportional to decrease in the osmotic potential of the soil solution, operates from low values of soil salinity, and reduces leaf water potential, transpiration and photosynthesis (Jaleel *et al*., 2007 and Khan and Panda, 2008).

Calcium supply to the saline soil solution regulates Na^+ uptake by plants. It can prevent the accumulation of toxic levels of Na^+ (Maas, 1993). Calcium plays an essential role in processes that preserve the structural and functional integrity of plant membranes, stabilize cell wall structures, regulate ion transport and selectivity, and control ion-exchange behavior as well as cell wall enzyme activities (Marschner, 1995).

Plant tissue culture provides useful information to elucidate response of salt stress; because *in vitro* conditions are more controllable than *in vivo* conditions and the large number of genotype can be evaluated in a limited space (Shiyab *et al*., 2003; Shatnawi, 2006; Cavagnaro *et al.* 2006 and Molassiotis *et al.,* 2006).

Research results of grape cultivars showed that with increasing concentrations of NaCl in the culture medium caused a reduction of vegetative growth (El-Sabrout 2003; Charbaji and Ayyoubi 2004; Mohammadkhani *et al.*, 2012 and Bybordi, 2012). However, application of $CaCl_2$ treatments counteracted this inhibitory effect of growth parameters (Sotiropoulos, 2007 on M4 apple rootstock; Lolaei *et al*., 2012 on olive; Arshi *et al*., 2005 on senna (*Cassia angustifolia*) and Amuthavalli *et al.*, 2012 on cotton. In addition, increasing NaCl and $CaCl_2$ concentrations in the culture medium under *in vitro* conditions caused increased concentration of Na and Ca in plantlets, whereas K concentration decreased in comparison to the control (El-Sabrout, 2003; Alizadeh *et al*., 2010; Sivritepe *et al*., 2010; Bybordi, 2012; Karimi and Yusef-Zadeh, 2013).

The present study aimed to *in vitro* evaluation for salt tolerance of some grape cultivars *via* study the effects of salinity on the morphological and biochemical aspects in the tested cultivars.

Materials and Methods:

The present experiment started on 2010 year and ended on 2014, at fruit crops orchard and tissue culture laboratory, Fruit Department, Faculty of Agriculture, Assiut University, to study the tolerance of three grape cultivars (Red Roomy, Thomson Seedless, and Beauty Seedless) to salt stress against different types and levels of salts (NaCl, $CaCl_2$).

Shoot tip explants (0.5-1.0 cm long) of three grape cultivars were cultured on (MS) Murashige and Skoog (1962) medium supplemented with 0.1 mg/l benzylaminopurine (BAP), 0.01 mg/l 3-indol butyric acid (IBA), 1.5 mg/l gibberellic acid (GA_3), 30 g/l sucrose, 0.1 g/l myo-inositol and 2.5 g/l Gelrite for solidification as an establishment medium and proliferated in the same medium

containing 1.0 mg/l BAP. For *in vitro* salt tolerance, proliferated shoots were cultured in MS medium supplemented with NaCl and $CaCl_2$ solutions as follows: a) 0, 50, 85 and 120 mM of NaCl; b) 0, 1, 5 and 10 mM of $CaCl_2$ and c) 0, 60 (50 NaCl+10 $CaCl_2$) and 90 (85 NaCl+5 $CaCl_2$) mM. Also, pH of the prepared medium was then adjusted to 5.6 – 5.8 using 0.1 N NaOH and HCl solutions. Medium was poured in 200 ml/jar and 25 ml medium/jar then jars were autoclaved at 1.5 kg/cm^2 pressure and 120°C temperatures for 20 minutes. The jars containing medium were left to be air-cooled for solidification. Incubation of culture jars were made in culture room at temperature of 24±2°C under florescent light for 16 hours/day for 4-6 weeks. Each treatment consisted of 15 repetitions (15 jars). The repetitions was divided into 5 replicates, each one consisted of 3 jars (repetitions).

Measurements:

The following measurements were evaluated throughout the experimental seasons:

A- Vegetative growth measurements:

1. Number of proliferated shoots/ explant.
2. Length of proliferated shoot (cm).
3. Number of leaves/shoot.
4. Number of nodes/shoot.
5. Length of internode (cm).
6. Plantlet fresh and dry weight (g).

B- Mineral composition:

Plantlets were collected and oven-dried at 70°C for 48 hours. The dried plantlets were ground using Wiley mill, and acid digested using H_2O_2 and H_2SO_4 method (Parkinson and Allen, 1975). The following minerals were determined.

1. Na^+ and K^+ were determined by flame photometer method (Williams and Twine, 1960).
2. Ca^{++} was determined by Perkin Elmer Atomic Absorption Spectrophotometer (Murphy and Riley, 1962).

The data of all minerals were expressed as mg/g dry weight.

Statistical analysis:

The experiments were set as a factorial experiment (3x9) with five replicates. Means compared with the Least Significant Difference (L.S.D.) at 5% level of probability (Steel and Torrie 1980).

Results and Discussion:

1-Effect of salinity on vegetative characteristics:

Data presented in Table (1) and Fig. (2) revealed that, there were a decrease in all vegetative growth characteristics under the study accompanied with the increase in NaCl concentration from zero to 120 mM. On the other hand, $CaCl_2$ has positive effect on vegetative characteristics compared to NaCl treatments. The data indicated that there were significant increases in growth parameters resulted from 1 to 5 mM $CaCl_2$ concentrations compared to 10 mM $CaCl_2$ concentration. In addition, $CaCl_2$ had an additive effect when using with the NaCl.

The number of new proliferated shoots per explant was 3.9, 2.6, 2.1 and 1.5 when NaCl concentration was used at 0, 50, 85 and 120 mM, respectively. For $CaCl_2$, the proliferation rate was significantly increased from 1 to 5 mM (3.7 to 4.7, consecutively). The highest multiplication (4.8 shoot/explant) occurred when Red Roomy and Beauty Seedless cul-

tivars were grown on MS medium supplemented with 5mM $CaCl_2$. As for conclusion, $CaCl_2$ induced the highest proliferation rate compared to NaCl or the mixture of both salts. In addition, There were gradual decreases in shoot length in response to the increase of NaCl concentration being the maximum at 120 mM NaCl (64.7% reduction in plantlet height). The differences among NaCl treatments were, statistically, significant. On the other hand, data showed insignificant increases in plantlet height resulted from 1 to 5 mM $CaCl_2$ concentrations. In general, insignificant differences were found among Thompson Seedless and Beauty Seedless cultivars; meanwhile Red Roomy showed the highest shoot length at all salinity treatments.

The data, also, indicated that there was a significant reduction of leaves per shoot produced as a result of using NaCl. Control treatment produced 3.8 leaf/shoot while 120 mM NaCl produced 2.1 (44.7 % reduction) leaf/shoot. Concerning of $CaCl_2$, the leaves number per shoot was 3.2 at 1 mM $CaCl_2$ increased to 3.9 at 5 mM $CaCl_2$. The highest number of leaves per shoot (4.3) was found in the Beauty Seedless cultivar at 5 mM $CaCl_2$. The same trend was found for the number of node per shoot with the highest value of (4.2 node/shoot) for Thompson Seedless cultivar. In addition, overall NaCl treatments decreased internode length compared to the control; however, such decrements were not significant at 50, 85 and 120 mM NaCl treatments (0.8 *vs*. 0.7, 0.6 and 0.6 cm, consecutively). Using $CaCl_2$ mixed with NaCl had no effect on average internode length compared with other salinity treatments. The highest value was 1.7 cm for Thompson Seedless grown in MS medium supplemented with 5 mM $CaCl_2$.

The data revealed that NaCl treatment at 50 mM had insignificant effect on plantlet fresh weight (2.5 g) compared to the control (3.2 g). NaCl treatments at 85 and 120 mM significantly reduced plantlet fresh weight 2.0 and 1.5 g, respectively compared to untreated plants. On the other hand, data in the same Table revealed that $CaCl_2$ at 5 mM significantly increased plantlet fresh weight (5.4 g) compared to the control (3.2 g) and 1 mM $CaCl_2$ (3.0 g). Generally, $CaCl_2$ treatments induced the highest plantlet fresh weight compared to NaC treatmentsl. The highest value was 6.5 g for Red Roomy cultivar when the medium was supplemented with 5 mM $CaCl_2$. Additionally, data indicated that plantlets grown on MS medium + NaCl were, negatively, influenced by salinity treatments based on the plantlet dry weight. The plantlets dry weights were found to be affected as a result of the least NaCl treatment (50 mM); such reduction was 19.2 % increased to 34.6 and 38.5% for 85 and 120 mM, consecutively. On the opposite side, the average dry weight of the three grape cultivars insignificantly increased from 1 to 5 mM $CaCl_2$ compared to the control. The average plantlets dry weights were 0.26 g *vs*. 0.20 and 0.26 g, respectively. As for conclusion, $CaCl_2$ induced the highest plantlet dry weight comparing to NaCl treatment or the mixture of the two salts.

The changes in morphological traits with increasing the salt concentration were reported in grape cultivars with numerous researchers (El-Sabrout, 2003; Charbaji and Ayyoubi, 2004; Mohammadkhani *et al.,*

2012; Fozouni *et al.,* 2012; Bybordi, 2012 and Karimi and Yusef-Zadeh, 2013). In addition, finding of Shibli *et al*. (2003) on almond, Naeniei *et al.* (2006) and El-Agamy *et al*. (2010) on pomegranate, Al-Darweesh (2006) on olive, Shiyab *et al.* (2003) and Perez-Tornero *et al*. (2009) on citrus and Sotiropoulos (2007), and Bahmani *et al*. (2012) on apple support the present data.

2- Effect of salinity on nutritional status:

Figure (1) revealed that the sodium content showed a tendency for positive response to NaCl treatments. Sodium contents were 2.11, 5.00, 8.02 and 12.82 mg/g dry weight when NaCl concentrations were used at 0, 50, 85, and 120 mM, respectively. The differences among NaCl treatments were statistically significant. On the other hand, for $CaCl_2$ treatments, there was insignificant effect among all $CaCl_2$ treatments and the control, while using the mixture of the both salts showed a positive increase in sodium content compared to the control. Potassium content of shoots of the tested grape cultivars was greatly reduced with the increase of NaCl concentrations. For calcium content, the data indicated that the effect of NaCl treatments on calcium content showed a significant negative response. Concerning of $CaCl_2$ treatments, the calcium content showed a tendency for positive response to $CaCl_2$ treatments. Calcium contents were 4.61, 5.11, 6.00 and 7.46 mg/g dry weight when $CaCl_2$ concentrations were used at 0, 1, 5, 10 mM, consecutively. The differences among $CaCl_2$ treatments were statistically significant. In addition, NaCl mixed with $CaCl_2$ at 60 mM, regardless of the effect of the cultivars, recorded the highest value of calcium content (11.72 mg/g dry weight) compared to all tested salinity treatments.

The results indicated that plantlets Na^+ content in the studied grape cultivars showed a tendency for positive response to all salinity treatments. On the contrary, K^+ and Ca^{2+} contents of shoots of the tested grape cultivars were reduced with the increase of NaCl concentrations, while using $CaCl_2$ induced increased in K and Ca contents. These results were in agreement with those reported by several authors (Troncoso *et al*., 1999, El-Sabrout, 2003, Sivritepe *et al*., 2010, and Bybordi, 2012) on grape, Shibli *et al*. (2003) on almond; Naeniei *et al.*(2006) and El-Agamy *et al*. (2010) on pomegranate; Sotiropoulos (2007) on apple rootstocks M4 and Habibi and Amiri (2013) on citrus but in disagreement with Alizadeh *et al*. (2010) on grape and Ahmed *et al*. (2005) on citrus.

Table (1): Effect of salinity on vegetative growth characteristics of some grape cultivars.

Characteristic	Treatment (B) / Cultivar (A)	0.0 Control	mM NaCl			mM CaCl2			mM Mix		Cultivar mean	L.S.D. 5%
			50	85	120	1	5	10	60	90		
Shoot/explant	Thompson Seedless	4.2	2.6	2.2	1.4	3.8	4.6	2.8	2.8	1.4	2.9	A= N.S B= 0.4 AB= N.S T= 0.8
	Red Roomy	4.0	2.8	2.0	1.4	4.0	4.8	2.4	3.0	1.2	2.8	
	Beauty Seedless	3.4	2.4	2.2	1.6	3.4	4.8	2.6	2.6	1.2	2.7	
	Treatment mean	3.9	2.6	2.1	1.5	3.7	4.7	2.6	2.8	1.3		
Shoot length (cm)	Thompson Seedless	3.0	2.6	1.9	1.4	2.4	2.4	2.2	1.9	1.5	2.1	A= 0.1 B= 0.2 AB= 0.3 T= 0.3
	Red Roomy	3.4	2.8	2.6	1.5	2.4	2.5	2.3	2.0	1.7	2.3	
	Beauty Seedless	3.7	2.7	2.2	0.7	2.2	2.6	1.6	1.3	1.3	2.0	
	Treatment mean	3.4	2.7	2.2	1.2	2.4	2.5	2.0	1.7	1.5		
Leaf/explant	Thompson Seedless	3.7	3.3	2.5	2.0	3.5	3.6	2.5	2.4	1.8	2.8	A= 0.1 B= 0.3 AB= 0.4 T= 0.4
	Red Roomy	3.7	3.3	3.1	2.2	3.1	3.8	2.9	2.6	2.0	3.0	
	Beauty Seedless	4.1	3.3	3.0	2.2	3.1	4.3	3.4	2.6	1.8	3.1	
	Treatment mean	3.8	3.3	2.9	2.1	3.2	3.9	3.0	2.5	1.9		
Internode length	Thompson Seedless	0.8	0.6	0.6	0.8	0.7	1.7	0.7	0.7	0.7	0.8	A = 0.2 B= 0.4 AB= N.S T= 0.6
	Red Roomy	0.8	0.8	0.7	0.6	0.9	0.7	0.7	0.7	0.6	0.7	
	Beauty Seedless	0.9	0.6	0.6	0.4	0.8	0.6	0.5	0.4	0.3	0.6	
	Treatment mean	0.8	0.7	0.6	0.6	0.7	1.0	0.6	0.6	0.6		
Fresh weight (g)	Thompson Seedless	3.1	2.9	2.5	1.9	3.0	5.3	3.4	3.2	1.9	3.0	A= 0.4 B= 0.6 AB= 1.0 T= 1.0
	Red Roomy	3.2	2.5	1.7	1.5	2.8	6.5	4.0	3.5	2.1	3.1	
	Beauty Seedless	3.3	2.2	1.9	1.1	3.3	4.4	1.8	3.6	1.5	2.6	
	Treatment mean	3.2	2.5	2.0	1.5	3.0	5.4	3.1	3.4	1.8		
Dry weight (g)	Thompson Seedless	0.23	0.25	0.19	0.14	0.22	0.19	0.18	0.19	0.20	0.20	A= N.S B= 0.04 AB= 0.07 T= 0.07
	Red Roomy	0.28	0.18	0.13	0.17	0.14	0.27	0.21	0.25	0.15	0.20	
	Beauty Seedless	0.27	0.20	0.20	0.16	0.23	0.31	0.18	0.29	0.11	0.22	
	Treatment mean	0.26	0.21	0.17	0.16	0.20	0.26	0.19	0.24	0.15		

T dunnett= the differences between control and each treatment were tested by Dunnett test

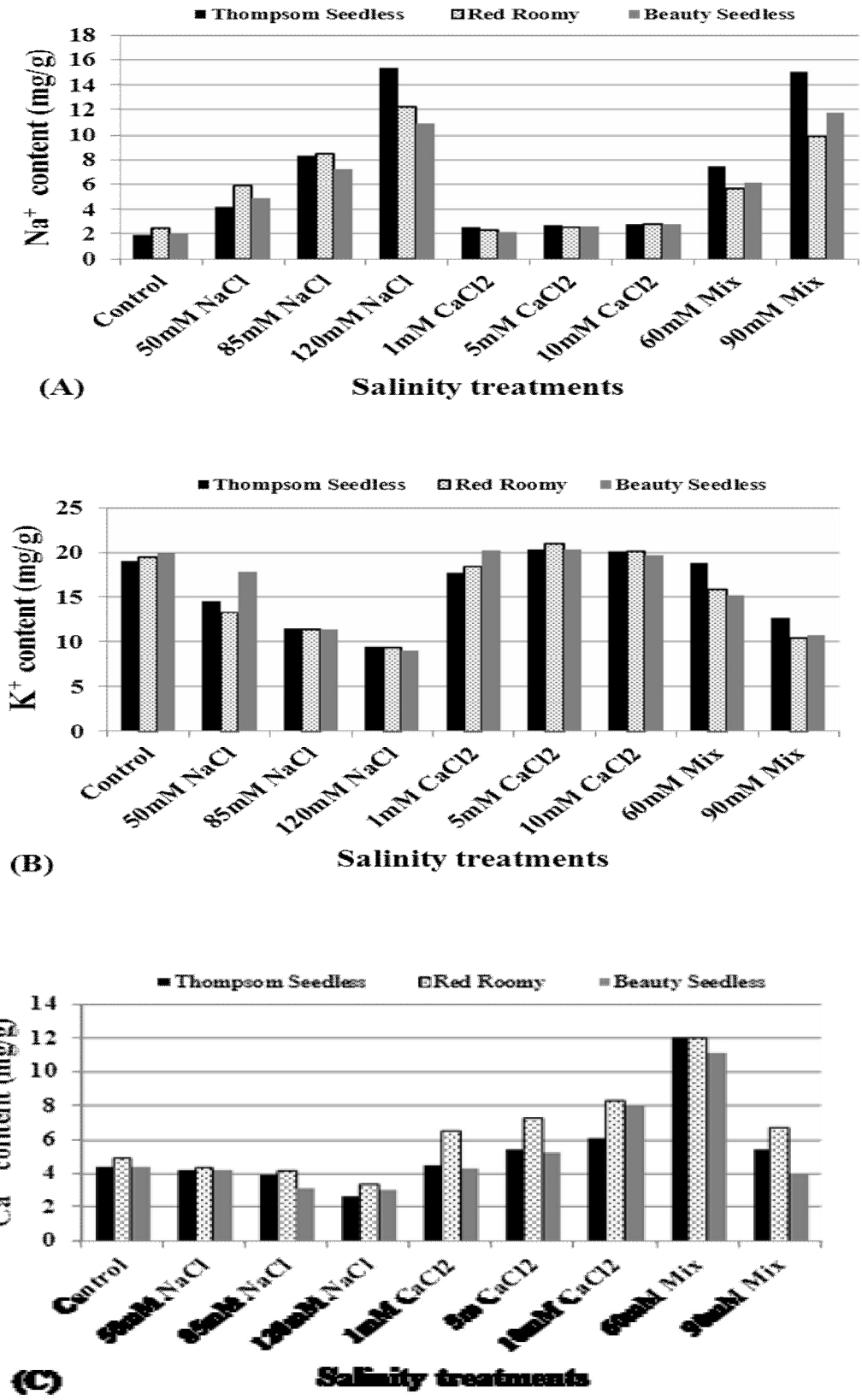

Fig. (1): Effect of salinity on nutritional status. A) Sodium content, B) Potassium content and C) Calcium content (mg/g dry weight).

Fig. (2): Growth comparison of three grape cultivars (A- Thompson Seedless, B- Red Roomy and C- Beauty Seedless) explants grown on MS medium supplemented with different levels of salinity**.**

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تأثير الملوحه معملياً على نمو ومحتوى بعض العناصر في ثلاثة أصناف من العنب

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الملخص:

تمت دراسة تأثير الملوحة معملياً في صورة كلوريد الصوديوم وكلوريد الكالسيوم على نمو والمحتوى من العناصر لثلاثة أصناف من العنب (البيوتي سيدلس والرومي الأحمر والبناتي الأبيض). تمت زراعة الأجزاء النباتية المنفصلة للقمم النامية لتلك الأصناف الثلاثة على بيئة موراشيجي وسكوج المحتوية على تركيز ١ملجم/لتر من البنزيل أمينوبيورين. تم تنفيذ التجربة بأربع مستويات من ملح كلوريد الصوديوم (صفر، ٥٠، ٨٥ وكذلك ١٢٠ ملليمولر)، أربعة مستويات من ملح كلوريد الكالسيوم (صفر، ١، ٥ وكذلك ١٠ ملليمولر) وكذلك مخاليط لكلا الملحين في ثلاثة مستويات (صفر، ٦٠، ٩٠ ملليمولر).

أوضحت النتائج أن صفات النمو (معدل التفريع، طول النبيتات، متوسط عدد الأوراق، عدد العقد، طول السلامية، الوزن الطازج والجاف) قد تناقصت بصورة معنوية مع زيادة تركيزات كلوريد الصوديوم حتى ١٢٠ ملليمولر مقارنة بالمعاملة القياسية. كما أوضحت النتائج، أن أضافة معاملات كلوريد الكالسيوم قاومت الفعل التثبيطي على صفات النمو عند تركيز ٥ ملليمولر. كما أن أضافة كلوريد الكالسيوم أدى إلى زيادة المحتوى من أيونات الكالسيوم وانقص محتوى أيونات الصوديوم بالمجاميع الخضرية. وبزيادة تركيزات كلوريد الصوديوم وكلوريد الكالسيوم في بيئة الزراعة المغذية، فإن المحتوى من كل من أيونات الصوديوم والكالسيوم قد أزدادت، بينما قل المحتوى من أيونات البوتاسيوم مقارنة بالمعاملة القياسية.