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Salt Stress Sensitivity of Chokeberry (*Aronia melanocarpa* L.) *in vitro* and *in vivo* Conditions

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HIGHLIGHTS

- Results show that the chokeberry plant is sensitive to salty environments.
- Plant fresh and dry weights decreased with increasing salt concentration.
- Increasing salt concentration decreased chlorophyll content.
- It may be recommended not to use saline soil or irrigation waters in chokeberry cultivation.

Abstract

In this study, the reactions of the chokeberry plant, which has attracted attention with its high antioxidant content and importance in human nutrition in recent years, to salt stress *in vitro* and *in vivo* conditions have been investigated. In this context, morphological, physiological, and biochemical reactions of plants at different salt levels *in vitro* and *in vivo* conditions have been studied. Salt concentrations *in vitro* were 1/3 dilute MS, 7/10 dilute MS, MS (control), MS + 1 gL^{-1} NaCl, MS + 3 gL^{-1} NaCl, MS + 6 gL^{-1} NaCl, MS + 8 gL^{-1} NaCl, MS + 9 gL^{-1} NaCl; *in vivo*, it was applied in the form of 25 mm NaCl per week with irrigation water to chokeberry lings planted in 2 liter pots containing peat: pearlite mixture in a ratio of 2:1 *in vivo* condition. The experiment was terminated by determining the salt levels in the soil from the moment the damage to the leaves due to salt stress began. According to the research results, *in vitro* conditions in 1/3 dilute MS, 7/10 dilute MS, MS (control) medium, no damage to explants occurred, MS +1 gL^{-1} NaCl and MS + 3gL^{-1} NaCl doses of shoot tips and leaves browning, MS + 6 gL^{-1} NaCl, MS + 8 gL^{-1} NaCl, MS + 9 gL^{-1} NaCl caused death in the medium. Browning of the shoot tip and leaves has occurred in plants during salt application under *in vivo* conditions. As a result of salt applications, plant height, plant dry weight, root length, chlorophyll content, protein content decreased in parallel with the increase in dose, and there was no change in leaf relative water content and proline content.

Keywords: Chokeberry, Salt stress, *In vitro, In vivo*

1. Introduction

Soil salinity is the most important abiotic stress factor after drought in world agriculture and prevents plant growth, especially in arid and semi-arid regions. Today, approximately 45% of the world's agricultural areas

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are exposed to drought stress, and with this drought, salinity occurs in 6% of agricultural areas. Salt stress, which negatively affects the fertility of soils and causes large areas to be left out of agriculture, has become an important problem that limits agricultural production areas today. This stress factor, which constitutes important agricultural constraints, affects plant morphology and anatomy, while also limiting growth and development (D'anna et al. 2003; Keutgen and Keutgen 2003). Salinity affects different physiological and metabolic processes in plants. Influence of these processes causes various symptoms in plants such as decrease in leaf area, increase in leaf thickness and wilting, absorption of leaves, root and shoot necrosis and decrease in stem length. Especially in salt stress, plant nutrient uptake decreases, and ionic imbalances occur in the cells. In addition, the resulting oxidative stress can affect the metabolic activities of the cell and activate antioxidant enzyme activities (Greenway and Munns 1980).

Under abiotic stress conditions, the rate of photosynthesis in plants decreases as a result of the decrease in leaf relative moisture content and leaf water potential (Lawlor 2002). It is known that the main reason for the decrease in photosynthesis rate in saline conditions is due to stomatal limitation (Cornic and Massacci 1996). Under salt stress, the decrease in photosynthetic rate and internal CO_2 concentration due to the closure of stomata prevents photosynthetic metabolism. The closure of stomata during salt stress also negatively affects the nutrition of plants (Oren et al. 1999).

The use of tissue culture methods is increasing day by day in physiological studies on plant stress, as they provide fast and effective results. Tissue culture technique is used advantageously and effectively in the evaluation of selected genotypes and in determining the resistance of fast-growing and developing varieties to some stress conditions such as cold, drought and salinity (Kaya 1988). Nowadays, it is very popular to use abiotic stress resistant plants using tissue culture techniques. Because *in vitro* conditions are more controllable than *in vivo* conditions and intensive work can be done in limited areas (Shiyab et al. 2003).

In a study, "Camarosa" and "Chandler" strawberry cultivars decreased leaf and root fresh weight, leaf area and leaf number, while decreasing stomatal conductance and transpiration rate under saline conditions (Turhan and Eriş 2007). In another study, the responses of four different strawberry genotypes to different NaCl concentrations (0, 25, 50, 75 and 100 mM) were examined under *in vitro* conditions, and it was determined that high salt concentration reduced the multiplication coefficient and affected calcium and sodium concentrations as well as dry weight (Torun et al. 2007). In another study, it was determined that 500, 1000 and 1500mgL^{-1} salt applications inhibited vegetative growth in "Kabarla" and "Gloria" strawberry cultivars plants irrigated with Hoagland nutrient solution, and although dry leaf weight decreased in high dose applications, dry leaf weight increased in low dose applications (Yilmaz and Kina 2008).

In the *in vitro* study using "Sweet Charlie" and "Benihoppe" strawberry varieties, the salt and alkali tolerance indices of the varieties were determined, and it was determined that the rooting rate, average number of roots and root length decreased by limiting the *in vitro* rooting ability in both stress factors (Zhao et al. 2017).

The effects of different NaCl levels (0, 50, 100, 150mM) on "Viking" aronia variety plants in the *in vitro* culture medium were investigated. The ½MS medium compared to the full MS medium performed better at almost all growth parameters at every NaCl concentration. The average highest plant height was recorded in the control treatment (4.77cm) of the ½MS medium; the lowest plant height was recorded in 150mM NaCl treatment (1.67cm) of the MS medium. The lowest leaf area was determined at 150mM NaCl concentration, and it reduced 68.8% in the MS medium and 73% in the ½MS medium. Rooting was highest in the control groups (100%) in both media; a significant gradual decrease occurred in 50 and 100mM NaCl concentration, and rooting did not occur in 150mM NaCl treatment (Nas et al. 2023).

Among the rest of small fruits, chokeberry is one of the most important small fruits in terms of containing the highest percentage of antioxidants. Chokeberry classified as ornamental's shrub as well as decorative autumn coloration that making her very popular (Hirvi and Honkanen 1985). Chokeberry classified between most fruit in terms of antioxidants, where the antioxidants percentage in chokeberry is higher than that is in apple, banana, elderberries and others. The high levels of flavonoids and anthocyanin in aronia is higher than those found in cranberries and five times more. In addition to their containment on an antioxidants and

polyphenols and they also contain on mineral and vitamins. As well as the chemicals content in chokeberry it has been alleged that it reduces some disease such as the potential cancer and heart disease. To supply a natural red color in products with poor color stability, chokeberry juice has been increasingly used in the food industry. Commercially, chokeberry is fundamentally used for juice either alone or blended with other fruit juices such as apple or grape. Mainly aronia juice is commercially, used either alone or blended with other juices such as apple and grape. Food coloring, tea, syrup and fruit spread coloring all these uses includes other uses for chokeberry. The uses of aronia juice have different from zone in Europe to other. In Russia apple and chokeberry juices are combined and fermented to producing or giving red wine. Either in Europe the juice often blended with apple juice to give juice a blush in Lithuania have been using chokeberry juice alone or blended with other fruits so as to produce dessert wines. Reports from the Ukraine describe chokeberry as improving the color, tannin level and sugar of grape wines (Smith and Ringenberg 2003). For chokeberry benefits prevent urinary tract infection and weight control, there are others benefits relate in chokeberry fruit such as treat inflammations, hypertension as well as can be very beneficial in cases of arthritis, cardiovascular conditions and other diseases. Chokeberry also contributes to strengthening immunity, blood vessels, lower blood pressure levels, and chokeberry also delays the natural aging process. Therapeutically they show positive effect in the anti-inflammatory and anti-oxidative activity and, also in the treatment several of neoplasms (Kowalczyk et al. 2003).

As a result of the literature review, studies on salinity stress of chokeberry plants under *in vitro* and *in vivo* conditions are quite limited and insufficient. For this reason, the aim of the study was to add new information to the literature and to determine the cultivation limits by measuring the responses of the aronia plant to different levels of salty growing conditions.

In this study, the responses of chokeberry (*Aronia melanocarpa*) plants grown under *in vitro* and *in vivo* conditions to different salt levels were examined in terms of morphological, physiological and biochemical aspects.

2. Materials and Methods

The study was conducted in 2022 in the Plant Biotechnology Laboratory and greenhouses of the Department of Horticulture. In the study, "Viking" chokeberry saplings were used as plant material.

In the experiment, the morphological and biochemical responses of chokeberry saplings propagated *in vitro* to different NaCl doses both *in vitro* and *in vivo* were examined.

2.1. Growing plants under in vitro conditions

For the surface sterilization of cuttings taken from one-year-old shoots, it first started by removing the leaves and leaflets on the shoots. The shoots were divided into 3-4 cm long single-node pieces and left under tap water for 30 minutes. The cuttings taken into the sterile cabinet were first kept in ethyl alcohol (70%) for 1-2 minutes and then rinsed with sterile pure water. It was sterilized for 10-20 minutes in 10% sodium hypochlorite solution containing a few drops of Tween-20. Then, after rinsing with sterile pure water, the sterilized cuttings were transferred to the shoot development medium (Mendi et al. 2003). The prepared cuttings were planted in culture tubes with 10 mL volume media solidified with 8 gL^{-1} agar containing 1.0 mgL^{-1} GA_3, 3% sucrose, MS essential minerals and vitamins. The cultured cuttings were left to develop under 1500-3000 lux white fluorescent light in a growth room at 25±1°C. After 21 days, new shoots were transferred to the medium containing 1.0 mgL^{-1} BA, and when the required number of plants was reached, the plants were transferred to the medium containing IBA (1.0 mgL^{-1}) during the rooting stage.

Shoots developed from cuttings were cultured in MS medium containing 2 mgL^{-1} BAP to ensure reproduction (Ružić et al. 2000). The plants, which were kept in the propagation medium for four weeks, were removed from the culture medium at the end of the 4th week and transferred to the new propagation medium. After the sufficient number of plants was reached, the obtained plants were placed in the rooting medium (Figure 1).

Figure 1. "Viking" chokeberry variety shoot propagation.

2.2. Application of salinity treatments

After reaching a sufficient number of plants, the obtained plants were cultured in MS medium containing 2 mgL^{-1} IBA. Rooted plants after 4 weeks were used for salt applications. For the proliferation treatments, salinity stress was achieved bud shoot tips, from the proliferation medium, which were placed in the standard medium with eight different concentrations of NaCl (1/3 dilute MS, 7/10 dilute MS, MS (control), MS + 1 gL^{-1} NaCl, MS + 3 gL^{-1} NaCl, MS + 6 gL^{-1} NaCl, MS + 8 gL^{-1} NaCl, MS + 9 gL^{-1} NaCl). ECs of these medium were measured and the results are given in Table 1.

Table 1. EC values of *in vitro* environments

Media	EC (mmhos/cm)
1/3 dilute MS	400
7/10 dilute MS	2500
MS (control)	4800
MS+1 gL^{-1} NaCl	6250
MS+3 gL^{-1} NaCl	9000
MS+6 gL^{-1} NaCl	13000
MS+8 gL^{-1} NaCl	15000
MS+9 gL^{-1} NaCl	16500

2.3. Growing plants under in vivo conditions

In the *in vivo* conditions, 25 mM NaCl was applied weekly along with irrigation water to chokeberry saplings planted in 2 liter pots containing a 2:1 peat:perlite mixture. The experiment was concluded by determining the salt levels in the soil from the moment the damage to the leaves due to salt stress began.

In the study, shoot tips of the "Viking" chokeberry variety were used as explants (Figure 3.4). Under *in vitro* culture conditions, aronia plants were treated with 8 different salt concentrations (1/3 dilute MS, 7/10 dilute MS, MS (control), MS + 1 gL^{-1} NaCl, MS + 3 gL^{-1} NaCl, MS + 6 gL^{-1} NaCl, MS + 8 gL^{-1} NaCl, MS + 9gL^{-1} NaCl) were applied.

2.4. Observations and measurements made in the experiment

In determining the salinity index, scores from 1 to 4 are given according to the damages listed below (Sivritepe et al. 2008).

1: No damage to plants,
2: Browning on the shoot tip and leaves,
3: Browning on the entire leaf and stem,
4: Death in plants,

In leaf samples taken from plants in September, photosynthesis efficiency (Arıkan 2017), membrane permeability in leaf discs (Lutts et al. 1996) and leaf relative water content (LRWC) (Kaya and Higgs 2003) were determined in mature leaves using the Li-Cor 600 device, stomatal conductance (Arıkan 2017), chlorophyll-a, chlorophyll-b and total chlorophyll contents in the leaf were determined (Arnon 1949). Protein determination in plants under salt stress was determined according to the "Bradford" (Bradford 1976) method, and proline determination was made spectrophotometrically by the acid-ninhydrin method (Bates et al. 1973).

The fresh weight of roots and plants was determined with the help of precision scales. Dry weights were determined with a precision balance after drying the same samples in an oven at 72ºC for 48 hours (Sanchez et al. 2004).

Plant height was determined by measuring the section from the root collar to the extreme growth point of the plant with the help of a ruler.

At the end of the applications, the areas of the leaf samples taken from the plants were determined using the Adobe Photoshop program (Ipek et al. 2019).

Photosynthetic efficiency (μmol CO_2 $m^{-2}s^{-1}$) was determined during the growing period with the Li-Cor 600 device on a total of 10 leaves of 5 randomly selected plants from each repetition of the applications in the last week of June, July, August and September.

2.5. Experimental design and statistical analysis

The study was carried out under *in vitro* conditions with a total of 240 plants, with 3 replicates in each application and 10 plants in each repetition. In *in vivo* conditions, a total of 60 plants were used in 3 replicates in each application and 10 plants in each repetition. The data obtained from the study were subjected to ANOVA at 5% significance level in the SPSS program, T test and Duncan multiple comparison test was applied to evaluate the differences between the applications.

3. Results

3.1. Effects of salt applications on chokeberry plants in vitro

Explants taken from chokeberry plants were damaged to varying extents by salt applications during their stay in the *in vitro* condition. No damage occurred in explants in 1/3 dilute MS, 7/10 dilute MS and MS (control) medium, browning of shoot tips and leaves at MS + 1 gL^{-1} NaCl and MS + 3 gL^{-1} NaCl doses, MS + 6 gL^{-1} NaCl, deaths occurred in, MS + 8 gL^{-1} NaCl, MS + 9 gL^{-1} NaCl medium (Figure 2).

Figure 2. NaCl application to chokeberry *in vitro* conditions, from left to right 1/3 MS, 7/10 MS, MS, 1 gL^{-1} NaCl, 3 gL^{-1} NaCl, 6 gL^{-1} NaCl, 8 gL^{-1} NaCl, 9 gL^{-1} NaCl

The effects of salt applications on plant height, plant fresh and dry weights were found to be statistically significant. Applications with the longest plant height; It occurred in 7/10 dilute MS medium (4.40 cm), followed by 1/3 dilute MS (3.76 cm) and control applications (3.68 cm). After this application, plant heights decreased with the increase in salt concentration (Table 2).

Table 2. Effects of salt applications on plant height, plant fresh and dry weight, root length, leaf number and leaf area.

Treatments	Plant Height (cm)	Plant fresh weight (g)	Plant dry weight (g)	Root length (mm)	Leaf number	Leaf area (cm^2)
1/3 dilute MS	3.76 b*	0.286 a	0.040 a	15.21 a	11.3 a	2.96 c
7/10 dilute MS	4.40 a	0.282 a	0.044 a	13.72 a	12.5 a	4.42 b
MS (control)	3.68 b	0.242 a	0.039 a	11.92 a	13.0 a	4.97 b
MS+1 gL^{-1} NaCl	3.09 c	0.229 a	0.036 a	14.88 a	11.4 a	5.57 a
MS+3 gL^{-1} NaCl	1.80 d	0.081 b	0.015 b	1.85 b	7.3 b	2.65 c

*Means separation within the column by Duncan's multiple range test and means marked with different letters (a, b, c...) indicate a significant differences $P < 0.05$

There was no difference in terms of plant fresh and dry weight between 1/3 dilute MS, 7/10 dilute MS, MS and MS + 1 gL^{-1} NaCl medium, but there was a significant decrease in the MS + 3 gL^{-1} NaCl dose. The effects of the treatments on root length, number and area of leaves were also found to be statistically significant. There was no difference in root length and number of leaves between 1/3 dilute MS, 7/10 dilute MS, MS and MS + 1 gL^{-1} NaCl medium, and a significant decrease occurred at the MS + 3 gL^{-1} NaCl dose. The maximum leaf area was determined in MS + 1 gL^{-1} NaCl medium, followed by MS and 7/10 MS medium, and the least occurred in 1/3 dilute MS and MS + 3 gL^{-1} NaCl dose (Table 2).

Significant effects of the applications on chlorophyll contents have also been determined. While total chlorophyll contents were close to each other in 1/3 MS, 7/10 MS and MS medium, chlorophyll contents decreased significantly in parallel with the increase in salt dose. There was no difference in chlorophyll-a contents in 1/3 MS, 7/10 MS, MS and MS + 1 gL^{-1} NaCl medium, and an increase occurred at the MS + 3 gL^{-1} NaCl dose. On the other hand, no difference was found in chlorophyll-b content between 1/3 MS, 7/10 MS, MS and MS + 1 gL^{-1} NaCl medium, and a significant decrease was determined at the MS + 3 gL^{-1} NaCl dose (Table 3).

Table 3. Effects of salt applications on chlorophyll, protein and proline contents

Treatments	Total chlorophyll (gL^{-1})	Chlorophyll-a (gL^{-1})	Chlorophyll-b (gL^{-1})	Protein* (μg protein g^{-1} FW)	Prolin (μg prolin g^{-1} FW)
1/3 dilute MS	117.46 a*	39.69 b	62.02 a	91.55 b	20.94
7/10 dilute MS	117.49 a	39.70 b	62.03 a	111.31 a	19.36
MS (control)	117.60 a	39.75 b	62.11 a	113.66 a	23.82
MS+1 gL^{-1} NaCl	82.26 b	39.73 b	62.09 a	106.17 ab	23.32
MS+3 gL^{-1} NaCl	71.85 c	43.87 a	29.48 b	14.93 c	20.42
					N.S.**

*Means separation within the column by Duncan's multiple range test and means marked with different letters (a, b, c...) indicate a significant differences $P < 0.05$.
**N.S.: Non-significant

The effects of salt applications on protein content were found to be statistically significant, while their effects on proline content were found to be insignificant. While protein contents were close to each other in 1/3 MS, 7/10 MS, MS and MS + 1 gL^{-1} NaCl medium, a significant decrease occurred at MS + 3 gL^{-1} NaCl dose (Table 3).

3.2. Effects of salt applications on aronia plants in vivo

As a result of salt application *in vivo*, browning of shoot tips and leaves occurred in plants (Figure 3). The effect of salt applications on plant height *in vivo* was found to be statistically insignificant. With salt application, stem diameter, root length, leaf area, plant fresh and dry weight, and root fresh and dry weight decreased significantly compared to the control (Table 4).

Figure 3. Effects of salt applications on chokeberry plants *in vivo*

Table 4. Effects of salt applications on some morphological features *in vivo.*

Treatments	Plant height (cm)	Stem diameter (mm)	Root length (mm)	Leaf area (cm^2)	Plant fresh weight (g)	Plant dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Control	52.62	7.13 a*	41.95 a	4.63 a	33.76 a	17.92 a	33.00 a	19.14 a
Salt	51.25	6.11 b	26.55 b	3.61b	16.23 b	8.95 b	15.44 b	6.90 b
	N.S**							

*Means separation within the column by t test and means marked with different letters (a, b, c...) indicate a significant differences $P < 0.05$.
**N.S.: Non-significant

Table 5. Effects of salt applications on some physiological characteristics *in vivo*

Treatments	Membrane permeability	LRWC (%)	Protein (µg g^{-1} FW)	Prolin (µg g^{-1} FW)	Total chlorophyll (gL^{-1})	Chlorophyll-a (gl^{-1})	Chlorophyll-b (gl^{-1})
Control	155.4 b*	61.70 a	18.37 a	9.21 b	88.23 a	29.49	39.03 a
Salt	226.2 a	67.37 a	12.61 b	13.06 a	80.64 b	30.13	29.49 b
		N.S.**				N.S.**	

	Photosynthesis efficiency (µmol CO_2 $m^{-2}s^{-1}$)	Stoma conductance (mmol $m^{-2}s^{-1}$)
Control	0.379 a	0.077 b
Salt	0.238 b	0.111a

*Means separation within the column by t test and means marked with different letters (a, b, c...) indicate a significant differences $P < 0.05$.
**N.S.: Non-significant

The effects of the treatments on leaf relative water content and chlorophyll a content were found to be statistically insignificant, but their effects on membrane permeability, protein, proline, total chlorophyll and chlorophyll-b, photosynthesis efficiency and stomatal conductance were found to be significant (Table 5). Salt application increased membrane permeability and stomatal conductance compared to the control, and

decreased protein, proline, total chlorophyll and chlorophyll-b content and photosynthesis efficiency compared to the control.

4. Discussion

Salt applications have harmful effects on plants both *in vitro* and *in vivo*. This shows that the chokeberry plant is sensitive to salty environments. There is no information in the literature about the sensitivity of the chokeberry plant to salt. In this respect, the results obtained are valuable as they are the first data on the subject.

Salt applications negatively affected the underground and above-ground development of plants. According to Özelçi (2020), salinity shrinks the main stems, reduces the formation of side branches and causes the death of newly formed juicy branches, and also tries to inhibit cambium activity, which increases its concentration in the middle part. In addition, the applications also reduced total chlorophyll, photosynthetic activity and protein contents. Similar effects of salt on cultivated plants have been determined in many studies. In a study conducted under *in vitro* conditions on "Myrobolan 29C" rootstock, it was determined that rooting speed, number of roots, root length, plant height, fresh plant weight, dry plant weight and chlorophyll content decreased significantly with the increase in salt concentration (Ipek et al. 2019). These results show that environmental salinity reduces water potential and the ability of plants to take up water. This rapidly reduces the rate of cell expansion in growing tissues (Munns 2011). In another study, it was determined that salt applications prevented vegetative growth in "Kabarla"and "Gloria" strawberry varieties irrigated with Hoagland nutrient solution, and dry leaf weight decreased in high dose applications (Yilmaz and Kina 2008). In another study, in an *in vitro* study using "Sweet Charlie" and "Benihoppe" strawberry varieties, the salt and alkali tolerance indices of the varieties were determined and it was determined that the rooting rate, average number of roots and root length decreased by limiting the *in vitro* rooting ability in both stress factors (Zhao et al. 2017). Similar effects of salt applications have been detected on lemon (Sharma et al. 2013), *Aloe vera* (Moghbeli et al. 2012) and citrus rootstocks (Bahmani et al. 2012).

The fact that salt reduces the development of above- and below-ground organs may be due to the inhibitory effects of salt on metabolic activities related to cell division, differentiation and elongation. Additionally, salinity's reduction of endogenous auxin levels (Khan et al. 1976) may be another effective factor.

Plant fresh and dry weights decreased with increasing salt concentration. Similar results were obtained in studies conducted on the subject in different plant species (İpek et al. 2019; Moghbeli et al. 2012; Sharma et al. 2013; Ghaleb et al. 2010). This result is explained by the fact that high NaCl levels inhibit leaf expansion, largely due to inhibition of cell division rather than cell expansion (Chartzoulakis and Klapaki 2000).

Increasing salt concentration decreased chlorophyll content. Chartzoulakis and Klapaki (2000) on quince, (Sivritepe et al. 2008) on grapes, Harb et al. (2002) on banana; similar results were obtained by İpek et al. (2019) on "Myrobolan 29C" rootstock. These results can be explained as chlorophyll biosynthesis is inhibited by salt application. The depressive effect of stress conditions on the absorption of some ions involved in chlorophyll formation, such as Mg and Fe, causes chlorophyll suppression in leaves and/or an increase in some growth inhibitors. Ethylene or abscisic acid production (Hanafy Ahmed et al. 2002), which increases aging that may occur in case of salt stress, is also effective here.

The negative effects of salt applications on chlorophyll content also negatively affect all processes related to photosynthesis. In this case, the negative impact of characteristics such as number of leaves, leaf area, underground and above-ground growth and protein content can be attributed to this. Salinity affects different physiological and metabolic processes in plants. Affecting these processes causes various symptoms in plants such as decrease in leaf area, increase in leaf thickness and wilting, absorption of leaves, root and shoot necrosis and decrease in stem length (Parida and Das 2005).

The effects of salt applications in *in vitro* and *in vivo* conditions are generally parallel. Salt applications significantly reduced plant fresh weight, plant dry weight, leaf area, total chlorophyll, chlorophyll-b and protein contents in both environments. Salt applications significantly reduced plant height *in vitro*, while a

decrease occurred *in vivo*, but no statistical difference was detected. The highest salt concentration increased chlorophyll-a content *in vitro* compared to the control, while it increased *in vivo*, but no statistical difference was detected. While the applications did not have significant effects on proline content *in vitro*, they significantly increased *in vivo*.

5. Conclusions

As a result of the data obtained, varying degrees of damage occurred to chokeberry plants from salt applications *in vitro*. While there was no damage to the explants in 1/3 dilute MS, 7/10 dilute MS, MS (control) medium, browning of the shoot tips and leaves was observed at the doses of MS + 1 gL^{-1} NaCl and MS + 3 gL^{-1} NaCl, and MS + 6 gL^{-1} NaCl, deaths occurred in plants in, MS + 8 gL^{-1} NaCl, MS + 9 gL^{-1} NaCl medium.

Salt applications caused harmful effects on chokeberry both *in vitro* and *in vivo*. From this perspective, it can be said that the aronia plant is sensitive to salty growing environments. In this respect, it may be recommended not to use saline soil or irrigation waters with high salt content in chokeberry cultivation, or to cultivate it by reclaiming the soil and irrigation water of the land where it will be grown.

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