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Polyploidy increases tolerance to salt stress in Anise hyssop (*Agastache foeniculum* [Pursh.] Kuntze)

SEYYEDEH FARAHNAZ TALEBI¹, MOHAMMAD JAMAL SAHARKHIZ², MARYAM JAFARKHANI KERMANI³, YAVAR SHARAFI^{4,*}

¹ Department of Horticultural Sciences, Faculty of Agriculture, Shiraz University, Shiraz, Iran

² Medicinal Plants Processing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

³ Department of Tissue and Cell Culture, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran, P.O. Box: 31535-1897

⁴ Department of Horticultural Sciences, Shahed University, Tehran, Iran

*Corresponding author. E-mail, y.sharafi@shahed.ac.ir

Abstract. Salinization is one of the most serious environmental problems in agriculture. Polyploid induction could increase abiotic stress tolerance in plants. In this study, the effect of different NaCl concentrations (0, 50, 100 and 150 mM) was studied on diploid (2x) and tetraploid (4x) plants of anise hyssop (*Agastache foeniculum*) *in vitro*. The results indicated that salt stress reduced survival percentage, stem length, and leaf and shoot number in both tetraploid and diploid plants. However, tetraploid plants had better survival and growth rates compared with diploids. The highest antioxidant enzyme activity was observed in the plants treated with 100 mM NaCl, while increasing the salinity to 150 mM NaCl lowered the activity of antioxidant enzymes significantly. Essential oil content in diploid and tetraploid plants decreased as the concentration of NaCl was elevated. Also, salinity stress affected the chemical composition of essential oil in both diploid and tetraploid plants. In conclusion, the results indicated that tetraploids showed greater tolerance to salt stress compared with diploids, and polyploidy might be a useful breeding method in anise hyssop to amplify its tolerance to salt stress under soil salinity.

Keywords: anise hyssop, essential oil, polyploidy, salt tolerance.

Abbreviations: EO - essential oil; ROS - reactive oxygen species; O₂⁻ - superoxide radicals; H₂O₂ - hydrogen peroxide; OH[•] - hydroxyl radicals; SOD - superoxide dismutases; CAT - catalases; APX - ascorbate peroxidases; GST - glutathione S-transferases; GPX - glutathione peroxidases.

INTRODUCTION

Anise hyssop (*Agastache foeniculum*) from the family Lamiaceae is an important medicinal plant. The essential oil (EO) of anise hyssop is mainly

biosynthesized in its leaves and flowers which contain significant amounts of methyl chavicol. In medicinal plants, secondary metabolites are fundamentally produced by genetic pathways, although environmental factors also strongly influence their biosynthesis (Zhang, 2015). Biotic and abiotic environmental factors, specifically salinity and drought conditions, affect growth parameters, medicinal plants' survival, and their essential oil yield (Heidari et al. 2008, Heydari et al. 2020, sharafi et al. 2017). Podda et al. (2013) stated that salinity is one of the most important abiotic stresses in agriculture affecting the plant growth and agricultural productivity. High levels of soil salinity have toxic effects on the absorption of nutrients from the root system in the plant through osmotic processes which, in turn, reduces essential oil production and modifies their composition in medicinal and aromatic species (Sarmoum et al. 2019). It is essential to determine the environmental factors under which medicinal and aromatic plants offer higher yields and improve quality. High salinity can disturb essential physiological processes due to factors such as water deficits, nutritional imbalance, hyper-osmotic stress, ion imbalance, metabolic disorders, and appearance or disappearance of some proteins which may eventually lead to death (Meng et al. 2016). These culminate in reduction of growth, yield, and quality of plants. Therefore, the over expression of genes encoding the biosynthetic enzymes may increase proline concentration in plant cells (Apse and Blumwald, 2002; Rabiei et al. 2011). On the other hand, oxidation reactions from choline to glycine betaine enhance plant resistance to salinity (Apse and Blumwald, 2002). Saline stress increases production of reactive oxygen species (ROS) including superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^*) which cause oxidative damage to different cellular components including membrane lipids, proteins, and nucleic acids (Hasanuzzaman et al., 2020). Plants use low molecular mass antioxidants such as ascorbic acid, superoxide dismutase (SOD), catalases (CAT), ascorbate peroxidases (APX), glutathione S-transferases (GST) and glutathione peroxidases (GPX) to scavenge ROS (Apse and Blumwald, 2002). Several mechanisms have been developed in plants under salt stress, one of which is the control of ion movement across tonoplasts to maintain a low Na^+ concentration in the cytoplasm (Brini and Masmoudi, 2012). Apse and Blumwald (2002) showed that plants could use several strategies to keep a high K^+/Na^+ ratio in the cytosol to control the entry of Na^+ ions into and out of cells.

Polyploidy has been used in horticulture as a breeding tool to improve morphological, physiological, and physio-biochemical characteristics (Kermani et al. 2003,

Talebi et al. 2017). Some polyploids are tolerant to environmental stresses such as drought (Li et al. 2009), heat (Zhang et al. 2010), nutrient-poor soils (Kolar et al. 2014), and salinity (Mouhaya et al. 2010, Podda et al. 2013). This increased tolerance may be related to duplicate gene expression or simply associated with evolutionary processes. Meanwhile, few studies have specifically reported the relationship between ploidy level and abiotic tolerance in plants (Podda et al. 2013). Polyploidy plants had enabled better adaptation to some detrimental environmental conditions (Parisod et al., 2010) and enhanced tolerance to a range of abiotic stresses and biotic, such as soil salinity (Chao et al., 2013). Polyploidy improved resistance to salt stress in rice (Tu et al., 2014), and citrus tetraploid genotypes (Mouhaya et al., 2010). Salt resistance in polyploidy plants was related to reduced sensitivity of plasma membrane K^+ -permeable channels in the meristem root zone and increased sensitivity of Ca^{2+} -permeable channels in the elongation and mature root zones to H_2O_2 (Liu et al., 2019).

Omami et al. (2006) reported that CAT is one of the major antioxidant enzymes which breaks down H_2O_2 to oxygen and water. Chao et al. (2013) reported that autopolyploidy induces resistance to salinity and may represent an adaptive outcome of the enhanced K^+ accumulation of plants with higher ploidy. Bagheri and Mansouri (2014) found that polyploidy raised protein and sugar content under saline conditions. In another study, Munns (2002) suggested that the soil salt reduced water absorption and growth rate which could be due to loss of cellular turgor pressure and hormonal signals produced by the roots. When the amounts of salt rise to toxic levels in the plant cell, it is transported to leaves, which results in reduction of the photosynthetic leaf area and premature leaf senescence (Munns, 2002). In salt-tolerant plants, there is a low rate of Na^+ and Cl^- transport to leaves where these ions are sorted in vacuoles in a way to prevent their build-up in cytoplasm, cell walls, and avoid salt toxicity (Greenway and Munns, 1980).

Aromatic plants that are salt stress tolerant should also maintain their growth and secondary metabolite production (Aziz et al. 2008; Ahmadi et al. 2013). Tabatabaie et al. (2007) showed that abiotic stress changed the quantity and quality of essential oil and thus reduced the market value of the *Mentha piperita* plants. Aziz et al. (2008) reported that essential oil yields of Peppermint (*Mentha piperita* L.), Pennyroyal (*Mentha pulegium* L.), and Apple mint (*Mentha suaveolens* Ehrh.) diminished under salt stress, compared with controls.

Currently, there is no information available regarding the effects of salt stress on induced polyploid anise hyssop plants compared with diploid parents. Accord-

ingly, the purpose of this study was to compare the effect of salt stress on tetraploid and diploid plants by measuring growth rate, antioxidant enzyme activity, and essential oil content of this plant.

MATERIALS AND METHODS

The tetraploid ($2n=4x=36$) and diploid ($2n=2x=18$) explants of anise hyssop (*Agastache foeniculum* [Pursh.] Kuntze) that were used in this study were obtained from our previous study (Talebi *et al.* 2017). These plants were grown under greenhouse conditions (16/8 h light/dark cycle, 21°C and 15°C day/night temperature and 60 % humidity). The tetraploid and diploid explants were cultured on an

Murashige and Skoog medium medium containing 0.6 mg/l 6-benzylaminopurine (BAP) and 0.2 mg/l 1-naphthaleneacetic acid (NAA) and sub-cultured every four weeks (Fig. 1). The cultures were incubated under controlled conditions of temperature ($25\pm 2^\circ\text{C}$), light (2000- 2500 lux for 16 h/d provided by fluorescent tubes), and 60-70% humidity.

Adaptation of micropropagated plantlets was carried out in pots filled with sand and vermiculite (1:1, v:v) in a greenhouse. Initially, all plants were irrigated with a nutrient solution with half strength Hoagland's for 4 weeks and then irrigated every 3 days with full-strength Hoagland's solution containing salt (NaCl) at 0, 50, 100, and 150 mM (Hoagland and Arnon 1950). The cultures were then incubated under a photoperiod of 16 hr light and 8 hr dark, light intensity of 2000- 2500 lux, and at a temperature of 21°C day and 15°C night and 60%

humidity. Morphological traits such as survival percentage and plant growth (leaf and shoot number, stem length) were measured.

Essential oil content was measured after three months. This content was determined using hydro-distillation by placing the aerial parts of dried plants (10 g) in a modified Clevenger apparatus for 3 hours (Ozturk *et al.* 2004) whereafter the essential oil content (w/w %) was calculated. The composition of essential oil was analyzed by GC-MS (Agilent Technologies 5977A GC/MSD System, USA) analysis, using a fused silica capillary HP-5 column (30 m \times 0.32 mm i.d.; film thickness 0.25 μm with an Agilent gas chromatograph series 7890A equipped with a flame ionization detector (FID). The injector and detector temperatures were kept at 250°C and 280°C, respectively. Nitrogen was used as carrier gas at a flow rate of 1 ml/min; oven temperature program was 60210°C at the rate of 4°C.min, which was then programmed to 240°C at the rate of 20°C.min, and finally, held isothermally for 8.5 min. The split ratio was 1:50 and the GC-MS analysis was carried out by Agilent gas chromatograph equipped with fused silica capillary HP-5MS column (30 m \times 0.25 mm i.d.; film thickness 0.25 μm) coupled with 5975C mass spectrometer. Helium was used as carrier gas with an ionization voltage of 70 eV. Ion source and interface temperatures were 230°C and 280°C, respectively. Finally, the mass ranged from 45 to 550 amu (atomic mass unit). The activity of antioxidant enzymes such as CAT and POD was measured according to the method of Chance and Maehly (1955). Experiments were analyzed in a factorial design based on a completely randomized design. Analysis of variance was performed and comparisons of means were conducted using Duncan's multiple range test (DMRT) at the 0.01 or 0.05 levels of probability. All analyses were performed using SAS and MSTATC software.



Figure 1. Micropropagation of tetraploid and diploid plants of anise hyssop.

RESULTS

It was observed that the survival percentage of diploid and tetraploid plants decreased with elevation of NaCl concentrations. The diploids survived at 100mM NaCl, while tetraploids were able to survive at a higher salt concentration of 150 mM (Fig. 2, 3). Diploid plants did not tolerate 150 mM NaCl and died under these conditions, while 21% tetraploid plants survived at 150 mM NaCl.

The results revealed that stem length, leaf and shoot number significantly declined in tetraploid and diploid plantlets of anise hyssop under salt stress. In diploids and tetraploids, the highest stem length and number

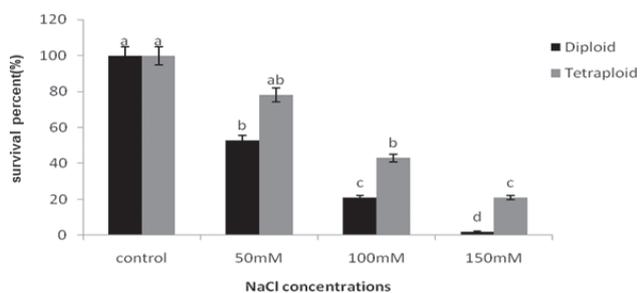


Figure 2. Effect of salt stress on survival percent in diploid and tetraploid plants.



Figure 3. Effect of salt stress on survival of diploid and tetraploid plants.

of leaves and shoots was observed in the control, while the lowest stem length and leaf and shoot number was detected at 150 mM NaCl (Figs. 4, 5, 6).

The results indicated that CAT activity was enhanced at 50 and 100 mM NaCl treatments in diploids and tetraploids of Anise hyssop. Although the CAT activity decreased at 150 mM NaCl in both diploids and tetraploids, it remained higher in 150 mM NaCl treatment compared with the control (Table 1). Fig 8? illustrates that the plants treated with 100 mM NaCl had the highest POD activity. However, the activity of antioxidant enzymes was higher in the tetraploid plants (Table 1).

The essential oil content extracted from the diploid and tetraploid plants is displayed in Fig. 7. The results indicated that salinity reduced the essential oil content in diploid and tetraploid plants as compared with essential oil produced in control plants. The maximum essential oil percentages in diploid (1.37%) and tetraploid (2.82%) plants were obtained from control plants. The minimum essential oil content was observed in 150 mM

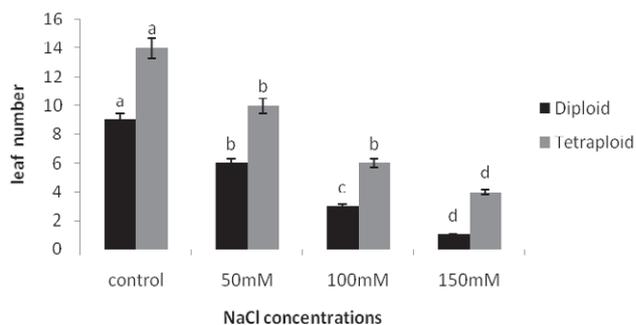


Figure 4. Effect of salt stress on leaf number in diploid and tetraploid plants.

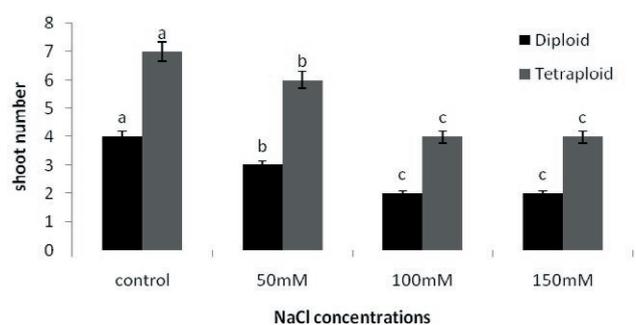


Figure 5. Effect of salt stress on shoot number in diploid and tetraploid plants.

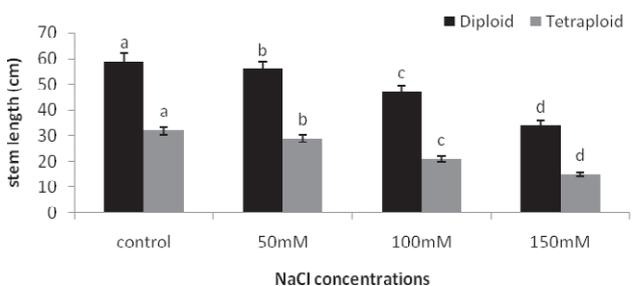


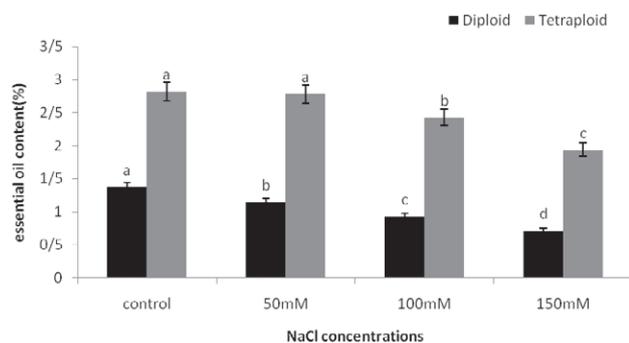
Figure 6. Effect of salt stress on stem length in diploid and tetraploid plants.

NaCl in diploid (0.71%) and tetraploid (1.97%) plants. The reductions in essential oil content were greater in diploids than in tetraploids under salt conditions.

The results of components identified through gas chromatography (GC/MS) in diploid and tetraploid plants are reported in Table 2. In tetraploid plants, with an increase in salt stress, the percentage of methyl chavicol, anisaldehyde, and β -caryophyllene rose, while the percentage of α -Thujene, Terpinene, and Germacrene D did not change. However, several other constituents decreased at the maximum salt concentration tested.

Table 1. Influence of different concentrations of NaCl on selected antioxidant enzyme activity. Means with the same letters in each column are not significantly different at $p < 0.01\%$.

POD activity ($\mu\text{mol min}^{-1} \text{mg}^{-1} \text{protein}$)		CAT activity ($\mu\text{mol min}^{-1} \text{mg}^{-1} \text{protein}$)		Treatment
Tetraploid (%)	Diploid (%)	Tetraploid (%)	Diploid (%)	
1.17 d	0.67d	2.31 d	1.07 d	Control
1.33 b	0.84 b	2.43 b	1.24 b	50mM
1.62 a	1.16 a	2.50 a	1.34 a	100mM
1.21 c	0.71 c	2.37 c	1.14 c	150mM

**Figure 7.** Effect of salt stress on essential oil content in diploid and tetraploid plants.

Data revealed that the percentage of all chemical constituents of essential oil in the diploid plants decreased with elevated NaCl concentration. In contrast, the changes in the essential oil constituent levels in the tetraploid plants were relatively lower than in diploid plants under the salt stress conditions.

DISCUSSION

According to the results of this study, salt stress reduced survival percentage and plant growth in tetraploid and diploid plants. The main reason for this reduction may be attributed to suppression of growth due to changes in developmental pathways under saline conditions. Salt stress reduced leaf growth and leaves exhibited wilting and chlorosis in diploid plants (Meng et al. 2011, Wang et al. 2013). Studies of Munns (2002) showed that plants treated under saline conditions had decreased water availability as well as sodium chloride toxicity. Munns (2002) reported that salt-induced drought stress decreased the ability of the plant to absorb water and nutrients from the soil. The ability of

plant cells to prevent Na^+ transport into the growing tissues is critically important for maintaining metabolic processes during cell growth against the toxic effects of Na^+ (Khorasaninejad et al. 2010). Khorasaninejad et al. (2010) reported that reduction in dry weight under salinity stress may be related to inhibition of hydrolysis of reserved foods and their translocation to the growing shoots. Similar decreases in growth parameters under salt stress were found in *Salvia officinalis* (Ben Taarit et al., 2009), thyme (Ezz El-Din et al. 2009), and basil (Said-Al Ahl and Mahmoud, 2010).

In this study, the highest activity of antioxidant enzymes was observed in the plants treated with 100 mM NaCl. Increasing salinity beyond 100 mM NaCl significantly decreased the activity of antioxidant enzymes. Under salt stress conditions, reactive oxygen species (ROS) increase in chloroplasts (Meng et al. 2016). Generally, salt stress results in an increased accumulation of ROS, such as H_2O_2 , which may act as a signal molecule during stress conditions, which in turn induces gene expression encoding antioxidant enzymes (Breusegem et al. 2001). Tseng (2007) showed that salt stress tolerance in cabbage was enhanced with the production of cuprozinc-superoxide dismutase (Cu/Zn SOD) and catalase (CAT) in chloroplasts. The levels of plant hormones such as abscisic acid (ABA) increase with high salt concentrations. ABA plays an important role in the mechanism of salt tolerance (Omami et al. 2006). Chao et al. (2013) found that autopolyploid plants have greater tolerance to salinity compared with diploids, which could be related to the enhanced K^+ in the tetraploid plants. Meng et al. (2016) reported that salt stress facilitated increased H_2O_2 production, antioxidative enzymes, non-enzymatic antioxidants, and protein activity in tetraploid plants compared with diploid plants. On the other hand, gene expression and synthesis of plant hormones such as ABA grow under salt conditions (Riddle et al. 2010). Tu et al. (2014) found that tetraploid rice showed less root growth inhibition, accumulated a higher proline content and lower malondialdehyde (MDA) content, and exhibited a higher frequency of normal epidermal cells than diploid rice did under salt conditions. The response of salt-tolerant organisms to salinity stress involves synthesis and accumulation of osmo-protective compounds, which are small, non-toxic compounds and can stabilize proteins, cellular structures and increase the osmotic pressure of the cell (Yancey et al. 1982). The high levels of proline and glycine betaine were correlated with improved tolerance to salinity (Apse and Blumwald, 2002). Similar results were observed in *Melissa officinalis* (Ozturk et al. 2004), *Majorana hortensis* (Shalan et al. 2006), *Thymus vulgaris* (Najafian et al. 2009), and *Mentha pulegium* (Queslati et al. 2010).

Table 2. The effect of salt stress on essential oil composition in diploid and tetraploid plants. ** Means followed by the same letter in each row are not significantly different by LSD test ($P < 0.05$).

Compounds	Diploid NaCl (mM)				Tetraploid NaCl (mM)			
	control	50mM	100mM	150mM	control	50mM	100mM	150mM
α -Thujene	0.52 ^a	0.51 ^b	0.46 ^c	0.44 ^d	0.64 ^a	0.61 ^c	0.63 ^b	0.64 ^a
α -Pinene	0.61 ^a	0.59 ^b	0.54 ^c	0.52 ^d	0.42 ^a	0.41 ^b	0.34 ^c	0.27 ^d
Camphene	0.52 ^a	0.50 ^b	0.42 ^c	0.39 ^d	0.41 ^a	0.41 ^a	0.32 ^b	0.22 ^c
1 -Octen-3-ol	0.28 ^a	0.17 ^b	0.11 ^c	0.09 ^d	0.35 ^a	0.31 ^b	0.23 ^c	0.22 ^d
3-Octanone	0.37 ^a	0.31 ^b	0.22 ^c	0.16 ^d	0.14 ^a	0.09 ^b	0.00 ^c	0.00 ^c
Sabinene	0.18 ^a	0.14 ^b	0.7 ^c	0.00 ^d	0.30 ^a	0.23 ^b	0.11 ^c	0.02 ^d
β -Pinene	0.52 ^a	0.44 ^b	0.39 ^c	0.21 ^d	0.34 ^a	0.26 ^b	0.16 ^c	0.06 ^d
3-Octanol	0.04 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.10 ^a	0.05 ^b	0.00 ^c	0.00 ^c
myrcene	0.04 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.06 ^a	0.00 ^b	0.00 ^c	0.00 ^c
p-Cymene	0.63 ^a	0.54 ^b	0.49 ^c	0.36 ^d	0.84 ^a	0.84 ^a	0.75 ^b	0.73 ^c
1 ,8Cineole	3.24 ^a	3.23 ^b	3.18 ^c	3.13 ^d	3.05 ^a	2.98 ^b	2.87 ^c	2.86 ^d
Limonene	2.69 ^a	2.61 ^b	2.56 ^c	2.53 ^d	3.02 ^a	2.92 ^b	2.94 ^c	2.93 ^d
γ -Terpinene	0.37 ^a	0.29 ^b	0.15 ^c	0.07 ^d	0.32 ^a	0.30 ^b	0.29 ^c	0.32 ^d
Trans-sabinene hydrate	0.04 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.04 ^a	0.00 ^b	0.00 ^b	0.00 ^b
Cis-linalool oxide	0.08 ^a	0.03 ^b	0.00 ^c	0.00 ^c	0.05 ^a	0.00 ^b	0.00 ^b	0.00 ^b
Trans-linalool oxide	0.05 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.06 ^a	0.00 ^b	0.00 ^b	0.00 ^b
Linalool	0.55 ^a	0.46 ^b	0.43 ^c	0.39 ^d	0.61 ^a	0.53 ^b	0.47 ^c	0.42 ^d
1 -Octen-3-yl acetate	0.28 ^a	0.19 ^b	0.12 ^c	0.09 ^d	0.37 ^a	0.31 ^b	0.27 ^c	0.26 ^d
α -Campholenal	0.02 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.02 ^a	0.00 ^b	0.00 ^b	0.00 ^b
Camphor	0.04 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.07 ^a	0.03 ^b	0.00 ^c	0.00 ^c
Trans-pinocarveol	0.27 ^a	0.16 ^b	0.08 ^c	0.00 ^d	0.30 ^a	0.26 ^b	0.18 ^c	0.09 ^d
Trans-verbenol	0.04 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.02 ^a	0.00 ^b	0.00 ^b	0.00 ^b
Pinocawone	0.03 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.03 ^a	0.00 ^b	0.00 ^b	0.00 ^b
Borneol	0.52 ^a	0.47 ^b	0.42 ^c	0.39 ^d	0.28 ^a	0.22 ^b	0.19 ^c	0.11 ^d
Terpinen-4-ol	0.02 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.05 ^a	0.00 ^b	0.00 ^b	0.00 ^b
Methyl chavicol	78.77 ^a	78.73 ^b	78.68 ^c	78.61 ^d	81.11 ^d	81.13 ^a	81.13 ^b	81.15 ^a
Piperitone	0.35 ^a	0.26 ^b	0.18 ^c	0.03 ^d	0.20 ^a	0.14 ^b	0.09 ^c	0.00 ^d
Anisaldehyde	0.68 ^a	0.54 ^b	0.43 ^c	0.34 ^d	0.81 ^b	0.80 ^c	0.82 ^{ba}	0.82 ^a
Bornylacetate	0.54 ^a	0.47 ^b	0.31 ^c	0.28 ^d	0.42 ^a	0.37 ^b	0.33 ^c	0.26 ^d
β -Bourbonene	0.58 ^a	0.56 ^b	0.51 ^c	0.45 ^d	0.44 ^a	0.40 ^b	0.39 ^c	0.37 ^d
β -Caryophyllene	0.72 ^a	0.65 ^b	0.41 ^c	0.35 ^d	0.61 ^c	0.56 ^d	0.63 ^b	0.65 ^a
(E)- α -Bergamotene	0.05 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.03 ^a	0.00 ^b	0.00 ^b	0.00 ^b
α -Humulene	0.04 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.02 ^a	0.00 ^b	0.00 ^b	0.00 ^b
Germacrene D	0.24 ^a	0.17 ^b	0.00 ^c	0.00 ^c	0.30 ^a	0.30 ^a	0.29 ^b	0.30 ^a
β -Selinene	0.02 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.03 ^a	0.00 ^b	0.00 ^b	0.00 ^b
Valencene	0.02 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.03 ^a	0.00 ^b	0.00 ^b	0.00 ^b
Bicyclgermacrene	0.20 ^a	0.13 ^b	0.00 ^c	0.00 ^c	0.21 ^a	0.17 ^b	0.08 ^c	0.00 ^d
β -Bisabolene	0.02 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.01 ^a	0.00 ^b	0.00 ^b	0.00 ^b
γ -Cadinene	0.04 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.02 ^a	0.00 ^b	0.00 ^b	0.00 ^b
δ -Cadinene	0.04 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.06 ^a	0.00 ^b	0.00 ^b	0.00 ^b
Spathulenol	0.33 ^a	0.26 ^b	0.23 ^c	0.10 ^d	0.45 ^a	0.39 ^b	0.36 ^c	0.27 ^d
Caryophyllene oxide	0.30 ^a	0.33 ^b	0.25 ^c	0.07 ^d	0.48 ^a	0.42 ^b	0.31 ^c	0.27 ^d
Globulol	1.45 ^a	1.29 ^b	1.13 ^c	0.57 ^d	1.72 ^a	1.72 ^a	1.67 ^b	1.67 ^b

In our study, salinity reduced the essential oil content in diploid and tetraploid plants compared with control plants. Data showed that treatment of tetraploid plants with different concentrations of NaCl had a different response in terms of essential oil composition and production. In the diploid plants, the percentage of all chemical constituents of essential oil decreased with elevation of NaCl concentration. Aziz *et al.* (2008) found that essential oil synthesis in peppermint was very sensitive to stress. Further, Olfa *et al.* (2009) reported that essential oil content in marjoram (*Origanum majorana*) was reduced consistently with rising salt concentration. Salinity stress requires additional energy for plant cells; therefore, the amount of carbon for growth and flower initiation and essential oil synthesis is reduced during stress (Cheesman 1988). Reductions in essential oil content could be due to decreases and changes in photosynthesis systems, essential oil biosynthesis and metabolic pathways (Aziz *et al.* 2008). However, Belaqziz *et al.* (2009) reported that oil content of *Thymus maroccanus* did not change with elevation of salt concentration.

The results of the present investigation demonstrated that anise hyssop is sensitive to salt stress. However, tetraploid plants were more resistant to salt stress than diploids. This was most probably due to the bigger cell size and fewer cells in the unit area in tetraploids compared with diploids (Comai, 2005). Thus, the responses of polyploid plants may differ in terms of morphological, physiological, cellular and biochemical aspects (Shafeizargar *et al.* 2013). Riddle *et al.* (2010) reported that polyploidy induction increased chromosome number, DNA content, gene expression, and enzyme activity per cell. In addition, according to our previous study, the polyploid plants of anise hyssop had a larger stomata size and density, chloroplast number, morphological features (leaf length and width, distance between the nodes, leaf area, plant height, fresh and dry weight, and spikes length), and physio-biochemical characteristics (net photosynthesis, protein content, catalase and peroxidase activity) (Talebi *et al.* 2017). Thus, tetraploid plants could naturally tolerate salt stress better than diploid plants. According to Zhang *et al.* (2015), the response of the autotetraploid apple seedlings to salt stress was better than that of the diploid. Other reports have also suggested that polyploidy induction is an efficient way to increase abiotic stress tolerance in *Spathiphyllum wallisii* (Van Laere *et al.* 2010), *Dendranthema nankingense* (Liu *et al.* 2011), *Brassica rapa* L. (Meng *et al.* 2011), and *Nicotiana benthamiana* (Deng *et al.* 2012).

CONCLUSION

According to the results obtained in the present study, salt stress reduced survival percentage, stem length, leaf and shoot number in tetraploid and diploid plants. The minimum growth rates were detected at 150 mM NaCl in both diploids and tetraploids. However, since tetraploid plants had higher rates of growth compared with diploids, they showed a higher percentage survival and growth compared with diploids under salt stress conditions. The highest activity of antioxidant enzymes for the two ploidy levels was observed in the plants treated with 100 mM NaCl. Tetraploid plants were more resistant to salt stress than diploids. Increasing salt concentration caused a significant reduction in the essential oil content in both tetraploid and diploid plants. Nonetheless, tetraploid plants showed different responses under different salinity stress conditions when the percentage of essential oil composition was measured. In the diploid plants, the percentage of all chemical constituents of essential oil decreased with increasing NaCl concentration. The results of our work suggest that in Anise hyssop, tetraploid plants have a better protective mechanism than diploid plants against saline conditions.

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