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In vitro evaluation of salinity tolerance of two selected dwarf Mahaleb (*Prunus mahaleb* L.) genotypes

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Abstract

Salinity is one of important restrictive factors in plants growth and crop production at many world places that looked it long time ago. Sweet and sour cherry are sensitive plants to salinity tolerance in fruit trees. This study was conducted to determin tolerance rate to salinity stress of two dwarf selected mahaleb genotypes during 2013-2014. This experiment was laid out in a factorial experiment in a completely randomized design with three replications. A first factor, genotype in two level (DM-171 and DM-249) and second factor NaCl in 4 levels (0, 50, 100 and 150 mM). Results showed that salinity had significant effect on shoot length and diameter, chlorophyll fluorescence content, proline content and soluble sugar. With increasing salinity level, shoot length and diameter and chlorophyll fluorescence content reduced and proline content and soluble sugar increased. Our results revealed that there wasn't significant differences between two mahaleb genotype (except chlorophyll). Genotype DM-249 had more chlorophyll fluorescence than DM-171, but there was significant difference between NaCl concentrations in both genotypes. The maximum proline content (9.7 mM FW) obtained in the highest salinity level (150 mM NaCl) and the minimum proline content (0.6 mM FW) obtained in the control. The highest content of soluble sugars (0.24 mg/g FW) was found in leaves under highest NaCl salinity while there were no significant change among DM-249 and DM-171 ($P < 0.05$).

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Introduction

Salinity is an important environmental constraint to crop productivity in arid and semi-arid regions of the world (Foolad, 1996). As about 30 percent of world lands affected by salinity (Noitsakis *et al*, 1997). A high salinity concentration at root zone, may often occur in Mediterranean areas during the long summer season, as a result of high temperatures and both reduced water availability and quality of irrigation water (Massai *et al*, 2004). This is while most of temperate zone fruit trees are salt sensitive and salinity significantly reduce their yield (Boland *et al*, 1993).

Salinity tolerance for Prunus species have been expressed 1.5-1.7 ds/m, and higher than associated with leaves sunburn, yield reduction, and senescence before maturity (Ottman and Byrne, 1998). One of proper ways for reduce destruction effects of soil and water salinity is using of cultivars that can grow in salt conditions and have sufficient yield (Foolad, 1996). There is wide variety in cultivars tolerance and woody plants genotypes to salinity (Kozlowski, 1997). Research on sweet cherry showed that induced salinity reduced growth and chlorophyll content in shoots and induced oxidative stress (Erturk *et al*, 2007). Cell lines affected by salinity and manitol had smaller cells compared with other cell lines (Ochatt and Power, 1989). Ca^{2+} acts as a cell membrane protector against the adverse effects of Na^{1+} (Lucchesini and Vitagliano, 2011). Studies on Prunus cultivars showed that both growth and starch content of in vitro root cultures were affected by salt concentration also, a significant inverse correlation was found between salt tolerance and starch accumulation in the maturation zone of root tips (Andreu *et al*, 2011).

In vitro response of two Prunus cultivars including Nemared and GF677 showed that by increasing KCl concentrations, the number of shoots per explant was not significantly affected for both rootstocks (Sotiropoulos *et al*, 2006). NaCl effect on bitter almond showed that elevated salinity resulted in reduction in shoot growth and rooting (shibli *et al*, 2003). Study on berry seed germination showed that seeds from different genotypes showed wide variability to the salinity (vijayan *et al*, 2002). Study of salinity effects on MM106 rootstocks showed that explant growth affected seriously by salinity treatments (Bahmani *et al*, 2012). Determination of proline concentration in fruit trees showed that the proline concentration in root tissues and root exudates from all rootstocks increased as salt concentration in the medium increased, following a trend similar to that of whole plant tissues (Marin *et al*, 2009). The aim of the present study was evaluation of salinity tolerance of two dwarf selected Mahaleb genotypes by in vitro culture.

Materials and methods

Current season shoots of two dwarf Mahaleb genotypes (DM-249 and DM-171) were excised from trees of the Mahaleb from Collection Khorasan Razavi Agriculture and Natural Resources Research Center.

Sterilization of explants

For sterilization, shoots were placed under running tap water for two hours and submerged in 0.1% mercury chloride solution for two minute. Shoots were rinsed three times in sterile distilled water and then explants with 5 cm length (at least two bud) were prepared.

Experiment mothod

Explants were individually transferred to culture tube containing 15ml of the sterile distilled water. Cultures were maintained at 24±3°C and 16:8 h photoperiod of cool-white light at 2000-2500 lux. After 14 days, uniform developed explants were excised and transferred to the jars containing 15 ml of the Murashige and Skoog (MS) basal medium. The medium were supplemented with 30 gL^{-} Sucrose, 2mg L^{-} Benzyl Adenine (BA) and 6g L^{-} Agar. The pH of the media was adjusted to 5.7 ±0.05 with HCl 0.1N or NaOH 0.1N prior to sterilization by autoclaving at 121°C for 15min. The explants were maintained at the same conditions described above for 30 days.

Induction of salinity

Uniform developed explants were selected and transferred to the MS media containing different concentrations of NaCl namely 0, 50, 100 and 150mM. No plant growth regulator was added to these media. The incubation conditions were the same as described above. After 20 days, at the end of experimental period, shoot length and diameter, Chlorophyll florescence, proline and soluble sugars content were recorded.

Shoots length and diameter

Measured using Callipers (±1 mm)

Chlorophyll fluorescence

After a 30-min dark period in ambient conditions in the laboratory, chlorophyll fluorescence was measured using a pulse-amplitude modulated Fluorometer. Measurements of minimal (F0) and maximal (Fm) fluorescence yields allowed determination of the optimal quantum yield (Fv/Fm), the ratio (Fm – F0)/Fm being used to calculate the maximal potential efficiency of PS II of dark adapted leaves (Maxwell and Johnson, 2000).

Determination of proline content

0.5 g of fresh leaves was homogenized with 10 ml of 95% ethanol and 5 ml of 75% ethanol. The mixture was centrifuged at 1500 RPM for 15 min. above phase separated and 10 ml distilled water, 5ml ninhydrin (1.25gr ninhydrin + 30 ml acetic acid + 20ml of 6M phosphoric acid) and 5 ml acetic acid glacial added and placed in boiling water bath for 45 min at 100°C. After samples to be cooled, 10mg toluene added to it and shaked intensely. The light absorption of above phase was estimated at 520 nm using spectrophotometer. The proline concentration was determined using a standard curve. Free proline content was expressed as mM FW of leaves (Bates *et al* 1973).

Determination of soluble sugars content

0.5 g of fresh leaves homogenized with 5 ml of 95% ethanol. One-tenth ml of preserved alcoholic extract in refrigerator mixed with 3 ml anthrone (150 mg anthrone, 100 ml of 72% sulphuric acid, W/W). The samples placed in boiling water bath for 10 minutes. After samples to be cooled, the light absorption of samples was estimated at 625 nm using spectrophotometer. Contents of soluble sugar were determined by using glucose standard and expressed as mg/g FW of leaves (Irigoyen *et al*, 1992).

Statistical analysis

The experiment was carried out as a factorial experiment based on a completely randomized design (CRD) with two factors and 3 replications per treatment and 5 jars per replication. The first factor was the different dwarf mahaleb genotypes, and the second was the different concentrations of NaCl (0, 50, 100 and 150 mM). Statistical analysis of the data was carried out by MSTATC. The results subjected to an analysis of variance (ANOVA) and difference among treatments means were compared by using Duncan's multiple range test at P≤ 0.05.

Results

Shoot length

The mean values of shoot length of the mahaleb genotypes at different levels of salinity are presented in Table 1. Shoot length of mahaleb showed a decrease by increasing salinity but no significant change was found in both genotypes (P < 0.05). Shoot length was longest in control (3.2cm).

Table 1. Effect of salinity on shoot length of selected dwarf Mahaleb genotypes.

Genotype	Salinity (mM)				Mean
	0	50	100	150	
DM-249	3.1^{a}	2.8^{ab}	2.3^{bc}	1.7^{d}	2.5^{A}
DM-171	3.2^{a}	3.1^{a}	2.5^{b}	1.9^{cd}	2.7^{A}
Mean	3.2^{A}	2.9^{A}	2.4^{B}	1.8^{C}	

Means followed by the same letter are not significantly different at 5% level according to Duncan test.

Table 2. Effect of salinity on shoot diameter of selected dwarf Mahaleb genotypes.

Genotype	Salinity (mM)				Mean
	0	50	100	150	
DM-249	4.9^{a}	4.5^{a}	2.6^{bc}	1.8^{cd}	3.4^{A}
DM-171	5.1^{a}	5.0^{a}	3.3^{b}	1.5^{d}	3.7^{A}
Mean	5.0^{A}	4.7^{A}	3.0^{B}	1.7^{C}	

Means followed by the same letter are not significantly different at 5% level according to Duncan test.

By contrast, shoot length of concentration 150mM, were shorter, measuring 1.8 cm. Among the different NaCl concentrations applied, 50 mM NaCl did not significantly reduce the shoot length of the explants. Our results confirm by Bahmani *et al* (2012) who reported in apple rootstock (MM106), Elevated salinity from 20 (control) to 40, 80, 100 and 120 mM NaCl resulted in reduction in shoot growth (shoot number, length and fresh weight) and rooting (rooting percentage, root number and length). And sayed & gabr, (2013) who reported shootlet length (cm) and the number of leaves per shootlet in *Solidago altissima* Gray were depressed upon increasing of salts mixture in medium.

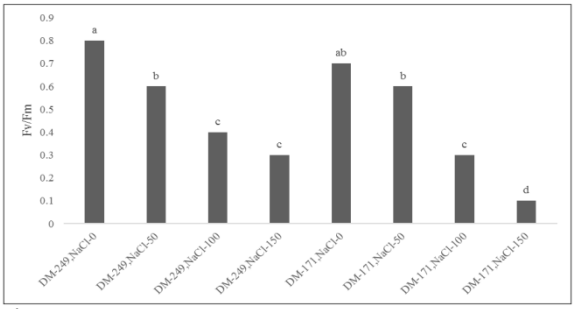

Fig. 1. Effect of selected dwarf Mahaleb genotypes and different concentrations of NaCl on chlorophyll fluorescence content.

Shoot diameter

After 3 weeks in the proliferation culture media, the shoot diameter were significantly affected by the NaCl treatments. As shown in Table 2, the shoot diameter decreased with increasing of NaCl concentrations from 50 to 150 mM. Among the different NaCl concentrations applied, 50 mM NaCl did not significantly reduce the shoot diameter of the explants. At 150 mM NaCl the shoot diameter were adversely effected. Results showed that there were no significant difference between DM-249 and DM-171. Shoot diameter was longest in control (5mm). But, shoot diameter of concentration 150mM, were shorter, measuring 1.7 mm ($P < 0.05$).

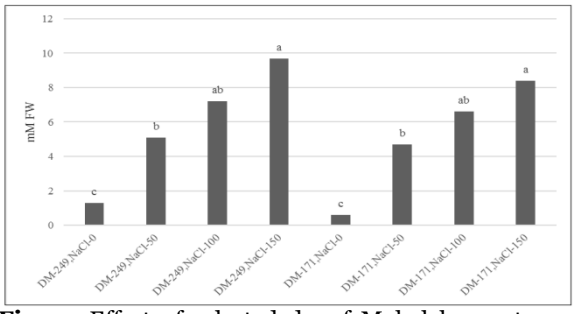

Fig. 2. Effect of selected dwarf Mahaleb genotypes and different concentrations of NaCl on proline content.

Chlorophyll fluorescence

Chlorophyll fluorescence Maximal quantum yield of PS II (Fv/Fm) showed significant difference for both genotypes (DM-249 was better than DM-171) and different concentration of NaCl ($P < 0.05$). Results showed that with increasing salt concentration, the amount of chlorophyll fluorescence has decreased. This ratio decreased when plant was in the presence of 150Mm NaCl (0.1) as compared to control (0.8) (Figure1). These results confirm by Puteh and *et al* (2013) who reported the minimum fluorescence (Fo) of the studied genotypes significantly increased under water stress, whereas the maximum quantum yield (Fv/Fm) and maximum primary yield (Fv/Fo) of PSII were significantly declined.

Proline content

Free proline content in leaves of mahaleb showed an increase by increasing salinity but no significant change was found in both genotypes ($P < 0.05$). Salt stress caused a manifold increase in free proline content of this genotypes in DM-249 and DM-171. The maximum proline content (9.7 mM FW) obtained in the highest salinity level (150 mM NaCl) and the

minimum proline content (0.6 mM FW) obtained in the control (Figure2). Above results confIrm by Sayed and Gabr (2013) and Marin *et al* (2009) who reported the proline content of the exudates increased with the NaCl concentration of the culture medium.

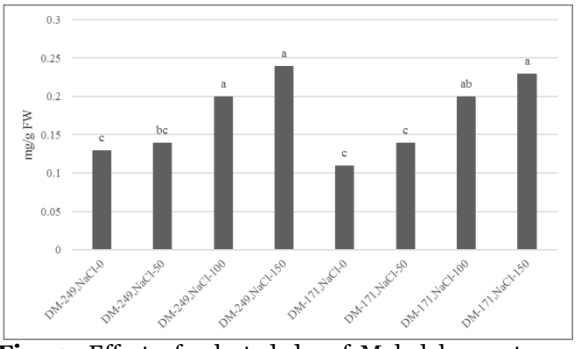

Fig. 3. Effect of selected dwarf Mahaleb genotypes and different concentrations of NaCl on solution sugar content.

Soluble sugars

Soluble sugars content in leaves significantly affected by salinity. With increasing salt concentration, the amount of soluble sugars increased. Results showed that the highest content of soluble sugars (0.24 mg/g FW) was found in leaves under highest NaCl salinity ($P < 0.05$) while there were no significant change among DM-249 and DM-171 (Figure3). Our results confirm by Pattanagul and Thitisaksakul (2008) on effect of salinity stress on growth and carbohydrate metabolism in three rice (Oriza sativa L.) cultivars differing in salinity tolerance.

References

Andreu P, Arbeloa A, Lorente P, Marin JA. 2011. Early Detection of salt stress tolerance of Prunus rootstocks by excised root culture. American Society for Horticultural Science **46(1),** 80-85.

Bahmani R, Gholami M, Mozafari A, Alivaisi R. 2012. Effects of salinity on in vitro shoot proliferation and rooting of Apple rootstock MM.106. World Applied Sciences Journal **17(3),** 292-295.

Bates IS, Waldern RP, Tear ID. 1973. Rapid determination of free praline for water stress studies. International Journal of Plant and Soil Science **39,** 205-207.

Boland AM, Mitchell PD, Jerie PH. 1993. Effect of saline water combined with restricted irrigation on peach tree growth and water use. Australian Journal of Agriculture Research **44,** 799-816.
http://dx.doi.org/10.1071/AR9930799

Erturk U, Sivritepe N, Yerlikaya C, Bor M, Ozdemir F, Turkan I. 2007. Responses of the Cherry rootstock to salinity in vitro. International Journal for Experimental Botany. Biologia Plantarum **51(3),** 597-600.

Foolad MR. 1996. Response to selection for salt tolerance during germination in tomato seed derived from PI 174263. Journal of American Society for Horticultural Science **121(6),** 1006-1011.

Irigoyen JJ, Emerich DW, Sanchez-Diaz M. 1992. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (Medicago sativa L.). Journal of Plant Physiology **84,** 55-60.
http://dx.doi.org/10.1111/j.13993054.1992.tb08764.x

Kozlowski TT. 1997. Response of woody plants to flooding and salinity. Journal of Tree Physiology **1,** 1-29.

Lucchesini M, Vitagliano C. 2010. Effects of NaCl and CaCl2 in Prunus cerasifera tissue culture. International Society for Horticultural Science 336: II International Symposium on In Vitro Culture and Horticultural Breeding.

Marin JA, Andreu P, Carrasco A, Arbeloa A. 2009. Determination of proline concentration, an abiotic stress marker, in root exudates of excised root cultures of fruit tree rootstocks under salt stress. Scientific Information Database. Revue des Regions Arides, 722-727.

Massai R, Remmorini D, Tattini M. 2004. Gas exchange, water relations and osmotic adjustment in two scion/rootstock combination of Prunus under

various salinity concentration. International Journal of Plant and Soil Science **259,** 153-162.

Maxwell K, Johnson GN. 2000. Chlorophyll fluorescence a practical guide. Oxford Journals. Science & Mathematics. Journal of Experimental Botany **51(345),** 659-668.

Noitsakis B, Dimassi K, Therios I. 1997. Effect of NaCl induced salinity on growth, chemical composition and water relation of two almond (Prunus amygdalus L) cultivars and the hybrid GF677 (Prunus amygdalus- Prunus persica). International Society for Horticultural Science **449,** 641-648.

Ochatt SJ, Power JB. 1989. Cell wall synthesis and (saline) sensitivity in cultured Colt Cherry (Prunus avium × Pseudocerasus) Protoplasts. Journal of Plant Cell Reports **8,** 365-367.

Ottman Y, Byrne DH. 1988. Screening rootstock of Prunus for relative salt tolerance. International Society for Horticultural Science **23,** 375-378.

Pattanagul W, Thitisaksakul M. 2008. Effect of salinity stress on growth and carbohydrate metabolism in three rice (Oriza sativa L.) cultivars differing in salinity tolerance. Indian Journal of Experimental Biology **46,** 736-742.

Puteh AB, Saragih AA, Ismail MR, Mondal MA. 2013. Chlorophyll fluorescence parameters of cultivated (Oryza sativa L. ssp. indica) and weedy rice (Oryza sativa L. var. nivara) genotypes under water stress. Australian Journal of Crop Science **7(9),** 1277-1283.

Sayed S, Gabr A. 2013. Responses of solidago altissima Gray to different salinity levels during in vitro propagation. Middle-East Journal of Scientific Research **14(12),** 1676-1684. http://dx.doi.org/10.5829/idosi.mejsr.2013.14.12.11021

Shibli RA, Shatnawi MA, Swaidat IQ. 2003. Growth, osmotic adjustment, and nutrient acquisition of bitter almond under induced sodium chloride salinity in vitro. Communications in soil science and plant analysis **34(13-14),** 1969-1979. http://dx.doi.org/10.1081/CSS-120023231

Sotiro poulos TE, Dimassi KN, Tsirakoglou V, Therios IN. 2006. Responses of two Prunus rootstocks to KCL induced salinity in vitro. International Journal for Experimental Botany. Biologia Plantarum. **50(3),** 477-480.

Vijayan K, Chakraborti SP, Ghosh PD. 2002. Screening of Mulberry (Morus spp.) for salinity tolerance through in vitro seed germination. Indian Journal of Biotechnology **3,** 47-51.