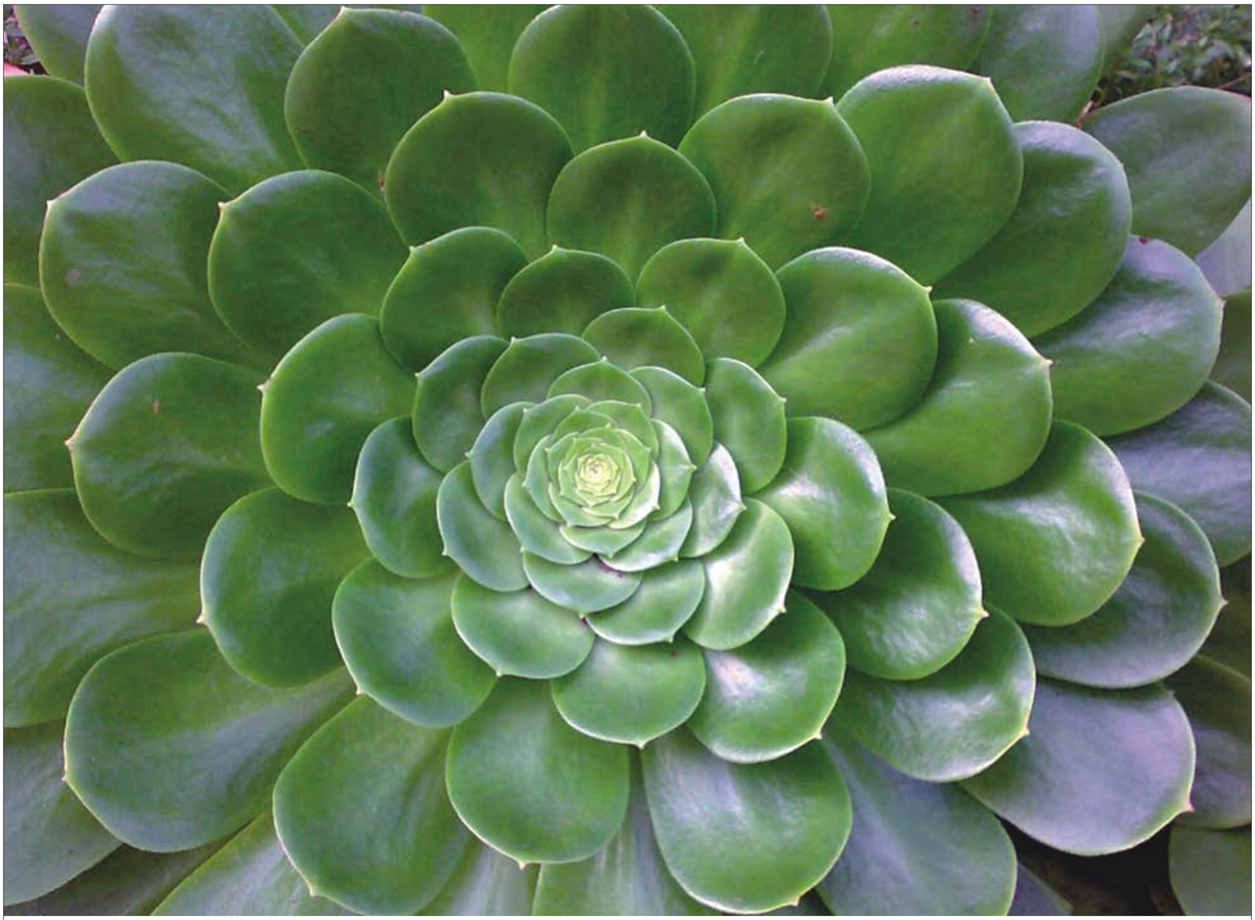

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Research Article
Morphological Characteristics of Different Mastic Tree (*Pistacia lentiscus* L.) Accessions in Response to Salt Stress under Nursery Conditions

[1]Giuseppe Cristiano, [1]Giuseppe De Mastro, [1]Mariano Fracchiolla, [1]Cesare Lasorella, [2]Vincenzo Tufarelli, [1]Barbara De Lucia and [1]Eugenio Cazzato

[1]Department of Agricultural and Environmental Sciences, University of Bari "Aldo Moro", 70126 Bari, Italy
[2]Section of Veterinary Science and Animal Production, Department of DETO, University of Study of Bari "Aldo Moro", 70010 Valenzano, Italy

Abstract
Background and Objective: *Pistacia lentiscus* L. (Anacardiaceae mastic tree or lentisk) is a bush playing an important role in the Mediterranean maquis ecosystem. For this purpose, a study on different accessions of mastic tree was carried out in Southern Italy to define the variability of characters related to morphology and survival under salt stress conditions. **Methodology:** This was study evaluated under nursery conditions the growing responses of 10 accessions of *P. lentiscus* to 6 different levels of salinization obtained by adding 0, 200, 400, 600, 800 and 1000 g of salts 100 L^{-1} of substrate, respectively. The following parameters were recorded; plant height, survival of plants and total root length, total biomass, number of leaves per plant and leaf area. **Results:** The results showed that *P. lentiscus* can well tolerate salt at high concentrations when grown under nursery conditions. Furthermore, among the investigated germplasm, a different plant accessions response to salt stress was observed. **Conclusion:** Thus, by identifying of mastic tree accessions more tolerant to salty soil and the revegetation of degraded agricultural lands can be attained.

Key words: Accessions, germplasm, morphology, *Pistacia lentiscus*, salinity

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Corresponding Author: Eugenio Cazzato, Department of Agricultural and Environmental Sciences, University of Bari "Aldo Moro", Bari, Italy

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pistacia lentiscus L. (Anacardiaceae, mastic tree or lentisk) is a bush playing an important role in the Mediterranean maquis ecosystem. Further, mastic tree is a rustic, drought resistant evergreen species having a high ability to resprout after cutting or fire and a horizontal growth assuring protection against the erosion of soil (Mulas *et al*., 1998, Tattini *et al*., 2006).

Spontaneous populations show to be characterised by a natural variability in ability of seed germination as a consequence of the high incidence of partenocarpy and ovary abortion. The availability of selected ecotypes having high seed germination for possible use in sustainable forestry or anti-desertification programs is therefore significant. Recently, afforestation and revegetation, programs for abandoned, degraded lands are encouraged by EU (Murillo *et al*., 2005) and frequently make a pressure on nurseries for a massive availability of young plants. Nursery production of native Mediterranean shrubs and trees in Southern Italy has shifted from bare root plant production to container growing systems in last two decades (Stellacci *et al*., 2013). To date, the choice of species in the Mediterranean urban green spaces is oriented towards the native plants such as mastic tree to encourage environmentally sound landscaping practices (De Lucia and Cristiano, 2015).

Salinity is one of the major abiotic stresses that affect plant production and growth in many arid and semi-arid areas (Gebauer *et al*., 2004, Barakat *et al*., 2013). Many semi-arid regions are characterized by too saline soil and water where, net evaporation is high and soil washing is negligible due to limited freshwater associated with scarce rainfall and poor drainage of soil (Barakat *et al*., 2014). Further, salinity may affect the growth of ornamental shrubs by reducing growth and leaf expansion resulting from osmotic effects or toxicity due to the high concentration of Na^+ and Cl^- typical of saline water (EPA, 1992). However, to the best of the knowledge, there are few studies investigating the response of *P. lentiscus* germplasm to saline stress in nursery production. It was recommended that ecotypic differentiation enabled *P. lentiscus* to grow in different habitats (Shaviv, 1978). The germplasm therefore, allows to evaluate the species-response to different stresses of abiotic origin. In mountains or in hilly lands of warm Mediterranean areas the cover by *Pistacia lentiscus* may be more or less total and uniform, principally in areas having desertification risk. Although, the protective role played by lentisk in the maquis is well known, little information is available about its potential capacity to colonize new soils since, the knowledge of spreading mechanisms of the species is scanty. Thus, in the present study, it was evaluated the growing responses of 10 different accessions of *P. lentiscus* to 6 levels of salinity under nursery conditions.

MATERIALS AND METHODS

The study was conducted at the greenhouses of the Campus of the Department of Agro-Environmental and Territorial Science, University of Bari "Aldo Moro", Italy. A total of 2 kg of fruits from each 10 mastic tree accessions were collected from individual tree in 10 different sites in Apulia and Basilicata regions (Southern Italy) from the end of November and the beginning of December, 2014.

Table 1 shows the main characteristics of the 10 sites and the different mastic tree accessions examined (n = 10). After harvesting, the fruits were stripped by manual squeezing using appropriate sieves and perforated with jets of water for the pulp removal.

The seeds were air-dried at ambient temperature, until the attainment of a moisture of 6-7% and subsequently stored at the temperature of 4°C. The seeding was made using alveolate containers in a greenhouse. The production phase of the mastic tree plants ended 2 months after seeding when, the transplantation was performed in a same substrate subjected to 6 different salinity levels. The substrate for the transplantation was constituted by a mixture (50:50 v/v) of a ground air-dried soil and a commercial substrate for potting. The soil was a sandy-clay having total N of 1.6‰ and a field

Table 1: Altitude, latitude, longitude, soil EC_e, pH and total N of the sites where the 10 accessions were collected

Accession	Site	Altitude (m a.s.l.)	Latitude	Longitude	Ece (dS m^{-1})	pH	N‰
A1	Cala dei Ginepri	20	40°46′ N	17°37′ E	0.45	7.99	2.86
A4	S. Maria d'Irsi 2	39	40°46′ N	16°21′ E	0.41	8.04	2.13
A5	Lido Marini (LE)	10	39°51′ N	19°11′ E	0.78	7.69	1.18
A6	Manfredonia (FG)	5	41°35′ N	15°49′ E	0.60	7.90	2.77
A8	Matera	32	40°38′ N	16°35′ E	0.72	8.07	1.42
A9	Noicattaro (BA)	10	41°20′ N	16°58′ E	0.56	8.07	3.39
A10	Scanzano 1 (MT)	0	40°14′ N	16°44′ E	2.6	8.13	1.51
A12	Specchia (LE)	23	39°55′ N	18°17′ E	0.27	8.01	1.82
A14	Tolve 2 (PZ)	40	40°44′ N	16°20′ E	0.88	7.92	0.43
A15	Torre Lapillo (LE)	3	40°17′ N	17°50′ E	0.99	7.98	1.23

Table 2: Chemical characteristics of the substrates used as treatments

Substrates/salinity levels	Salts content (g 100 L^{-1})	ECe (dS m^{-1})	pH
S0	0	1.7	7.95
S1	200	8.2	7.60
S2	400	12.5	7.35
S3	600	17.1	7.32
S4	800	21.5	7.27
S5	1000	25.1	7.22

capacity and wilting point of 24.9 and 15.5% of dry weight, respectively. The commercial substrate had the following characteristics (on dry matter): 45% of organic carbon, 0.5% of organic N, 60% of organic matter, pH of 6 and electrical conductivity EC_e<1.5 dS m^{-1}. The mixture obtained had total N of 2.2‰ and a field capacity and wilting point of 35.6 and 22.5% of the dry weight, respectively. This mixture was subjected to 6 different levels of salinization (S0, S1, S2, S3, S4 and S5) obtained by adding 0, 200, 400, 600, 800 and 1000 g of salts 100 L^{-1} of substrate, respectively (Table 2). The salts used were a mixture of NaCl and $CaCl_2$ in a weight ratio 1:1.

Plastic pots (40×60×6 cm) having a hole necessary for the harvesting of leaching fraction in a special bottle were filled with the 6 substrates; subsequently, 20 plants for each accession were transplanted. A split plot experimental design was used with four replicates, the 'salinity level' and 'accessions' were tested in the main plots and in the sub-plots, respectively.

Plants were growth under salinized substrates 87 days. Irrigation was performed with water of good quality EC_w = 0.5 dS m^{-1}. The irrigations were performed manually distributing water on the soil surface when the 35% of the available water lost by evapotranspiration and estimated by subsequent weightings. The volume of water for each irrigation was performed in order to limited percolation required to fully humidify the whole profile of substrate. The following parameters were recorded; plant height (measured every 20 days), survival of plants and total root length, total biomass (on dry weight), number of leaves per plant and leaf area, measured using a leaf area meter (LI COR 3100-USA) at the end of the trial.

Data obtained were subjected and after angular transformation of the percentage values to ANOVA according to a split-plot design. The differences between means were evaluated with Student-Newman-Keuls (SNK) test.

RESULTS

Plants survival: The survival percentage of plants recorded at the end of the trial showed differences between the 10 accessions (Table 3). A depressing effect of saline treatments on plant's survival was found and it was significant only at the higher doses of salinity (from S3 to S5 treatment, corresponding to an ECe value of 17.1, 21.5 and 25.1 dS m^{-1}, respectively). Furthermore, the accessions investigated showed a different plant survival in particular, the higher percentages were recorded by A12 and A10 accessions (96.9 and 90.6%, respectively) whereas, the lower value was found in A1 (75.0%). It is interesting to underline the higher salinity tolerance to the A12 and A10 accessions compared to the other accessions particularly, significant applying the higher salt doses (significance of interaction salinity×accessions) (Table 3).

Table 3: Mass and Hoffman model used for relative survival of 10 accessions used for experiment evaluated on Ec_{e50}

Accessions	Equation	EC_{e50} (dS m^{-1})
A1	y = 100-5.18 (x-11.8)	21.5
A4	y = 100-5.5 (x-13.78)	22.9
A5	y = 100-3.17 (x-12.39)	28.2
A6	y = 100-4.70 (x-12.38)	23.0
A8	y = 100-3.04 (x-9.26)	25.7
A9	y = 100-5.39 (x-16.59)	25.9
A10	y = 100-3.13 (x-15.24)	31.2
A12	y = 100-1.56 (x-17.23)	49.3
A14	y = 100-4.23 (x-11.47)	23.3
A15	y = 100-1.84 (x-3.89)	31.1

Table 4: Plant survival (%) in response to different salinity levels of the accessions of mastic tree

	Salinity level						
Accessions	S0	S1	S2	S3	S4	S5	Mean
A1	100.0	100.0	96.9	71.9	50.0	31.3	75.0
A4	100.0	100.0	100.0	93.8	56.3	34.4	80.7
A5	100.0	96.9	96.9	90.6	68.8	59.4	85.4
A6	100.0	100.0	93.8	87.5	56.3	37.5	79.2
A8	100.0	96.9	93.8	84.4	62.5	46.9	80.7
A9	100.0	100.0	100.0	93.8	81.3	50.0	87.5
A10	100.0	100.0	100.0	93.8	81.3	68.8	90.6
A12	100.0	100.0	100.0	100.0	93.8	87.5	96.9
A14	100.0	96.9	96.9	75.0	56.3	43.8	78.1
A15	100.0	87.5	90.6	90.6	59.4	56.3	80.7
Mean	100.0	97.8	96.9	88.1	66.6	51.6	83.5
LSD	0.05						
Salinity (S)	6.0						
Accessions (A)	7.7						
S×A	15.8						

To characterize the behaviour of the accessions in terms of plants survival with increasing salinity of the substrate, it was applied the equation of Maas and Hoffman (1977) to the values of relative survival to obtain the critical threshold, slope and $EC_{e50\%}$ (Table 4). Results showed that the four less resistant accessions to salinity in terms of survival were A14, A6, A4 and A1 with also a relatively low values of critical threshold (11.47-13.78 dS m^{-1}) and a relatively high slope values (4.23-5.50%). Among these accessions, the $EC_{e50\%}$

Table 5: Roots length (cm) in response to different salinity levels of the accessions of mastic tree

	Salinity level						
Accessions	S0	S1	S2	S3	S4	S5	Mean
A1	24.0	18.5	16.3	15.1	9.1	9.8	15.5
A4	27.5	20.0	19.6	16.5	13.5	11.4	18.1
A5	24.3	20.4	17.7	12.1	11.9	9.8	16.0
A6	22.2	18.9	15.9	12.8	11.1	10.2	15.2
A8	23.3	18.9	17.8	14.1	10.0	8.4	15.4
A9	28.0	22.0	17.3	16.0	12.9	11.3	17.9
A10	29.7	23.0	24.9	18.9	13.6	9.4	19.9
A12	24.0	23.6	17.8	15.0	12.7	11.0	17.3
A14	22.0	19.6	17.5	11.5	11.9	9.2	15.3
A15	24.8	17.2	18.3	17.7	12.2	8.2	16.4
Mean	24.9	20.2	18.3	14.9	11.9	9.9	16.7
LSD	0.05						
Salinity (S)	1.3						
Accessions (A)	1.6						
S×A	ns						

Table 6: Plant height (cm) in response to different salinity levels of the accessions of mastic tree

	Salinity level						
Accessions	S0	S1	S2	S3	S4	S5	Mean
A1	19.0	14.6	10.0	8.9	8.0	5.5	11.0
A4	19.9	15.2	13.8	9.7	7.9	5.3	12.0
A5	16.4	14.4	9.8	7.4	6.6	4.9	9.9
A6	13.5	12.5	9.7	7.0	5.3	4.7	8.8
A8	16.9	13.9	12.7	9.8	6.6	5.3	10.9
A9	15.6	15.0	11.3	9.8	5.9	5.7	10.5
A10	19.2	17.1	14.6	11.8	6.9	5.0	12.5
A12	16.7	16.5	10.7	9.1	8.0	5.7	11.1
A14	15.0	11.5	9.7	6.9	4.5	4.9	8.7
A15	19.5	15.0	11.3	9.5	5.9	4.6	11.0
Mean	17.2	14.6	11.4	9.0	6.6	5.2	10.6
LSD	0.05						
Salinity (S)	0.7						
Accessions (A)	0.8						
S×A	1.7						

values varied from 21.5 (A1) to 23.3 (A14) dS m^{-1}. Moreover, along with the more tolerant accessions, it was noted a different response in the accessions A15 and A10 due to a low slope value in A15 (1.84%) and to an high value of critical threshold in A10 (15.24 dS m^{-1}) however, the estimated $EC_{e50\%}$ values resulted similar for both accessions. The accession A12 (Specchia) showed the highest capacity of survival with increasing salinity having a slope of 1.56 and a critical threshold of 17.23 dS m^{-1} and an estimated value of $EC_{e50\%}$ equal to 49.3 dS m^{-1}.

Plant height and roots length: A decrease of plant height in all investigated accessions and for each level of salinity was recorded (Table 5). In particular, the lowest plant height was found in A14 and A6 accessions (8.8 cm) conversely, the highest values were recorded in A10 and A4 accessions (12.3 cm). Moreover, a significant interaction S×A was found. The plant height decreased linearly (0.53 cm and R^2 = 0.994) for each ECe unit. Regarding roots length (Table 6) it was observed for each accession a linear decrease (R^2 = 0.997) when the salinity level increased (from 24.9 cm for S0 to 9.9 cm for S5 treatment, respectively).

Table 7: Total biomass (milligram per plant) on dry weight) of the accessions of mastic tree in response to different salinity levels

	Salinity level						
Accessions	S0	S1	S2	S3	S4	S5	Mean
A1	1656	1004	646	443	258	209	702.7
A4	1948	1270	1049	578	533	228	934.5
A5	1483	1012	637	379	286	188	664.1
A6	1059	835	620	336	257	158	544.0
A8	1478	1002	767	513	400	146	717.4
A9	1342	1007	814	465	320	255	700.2
A10	1647	1046	1093	601	335	157	813.1
A12	1550	1203	756	544	527	282	810.3
A14	1176	727	612	358	185	161	536.6
A15	1635	926	797	604	287	180	738.2
Mean	1497	1003	779	482	339	196	716
LSD	0.05						
Salinity (S)	61.8						
Accessions (A)	79.8						
S×A	162.9						

Total dry biomass: The total dry biomass of mastic tree accessions is reported in Table 7. Increasing salinity the production of dry biomass decreased in all accessions. As mean of all accessions, it varied from 1635 mg $plant^{-1}$ (S0) to 180 mg $plant^{-1}$ (S5). Considering the single accessions, it was possible to classify the accessions in three groups based on their productive performance, 1st group comprised 3 accessions having the highest production (A4, A10 and A12), the 2nd group with the lower production (A6 and A14) and the 3rd group including the accessions with medium-productive performance. A significant interaction S×A was detected in particular among the accessions in S0 treatment, the A4 showed the best biomass production whereas, under S4-S5 treatments the A12 accession reported the best performance.

Leaves number and leaf area: The effect of salinity level on leaf area and number of leaves per plant are shown in Table 8 and 9. As mean value of all accessions, the leaves number decreased significantly from 24.5-10.3 moving from S0 to S5 treatment. Further, among the investigated accessions, the higher leaves number was found in A4, A10 and A15 accessions whereas, the lower was reported in A6 and A14 accessions. Accordingly, leaf area decreased when salinity level increased (from 2.6 in S0 to 0.7 cm^2 in S5, respectively).

Table 8: Leaves per plant (n) of the accessions of mastic tree in response to different salinity levels

	Salinity level						
Accessions	S0	S1	S2	S3	S4	S5	Mean
A1	27.9	20.5	17.5	14.9	12.0	10.8	17.3
A4	26.0	22.0	20.2	15.7	15.1	11.3	18.4
A5	26.7	21.9	20.2	15.3	11.8	10.8	17.8
A6	19.7	16.8	14.9	12.0	9.1	9.0	13.6
A8	21.8	19.9	16.3	14.7	12.1	8.9	15.6
A9	23.2	21.6	19.0	13.9	12.7	12.0	17.1
A10	24.8	22.3	22.0	17.0	13.0	9.9	18.2
A12	24.2	21.3	19.5	16.2	13.7	11.3	17.7
A14	21.2	17.6	15.6	14.7	10.9	8.7	14.8
A15	29.2	21.7	19.1	15.6	12.3	10.5	18.1
Mean	24.5	20.6	18.4	15.0	12.3	10.3	16.8
LSD	0.05						
Salinity (S)	1.0						
Accessions (A)	1.3						
S×A	ns						

Table 9: Leaf area (cm^2) of the accessions of mastic tree in response to different salinity levels

	Salinity level						
Accessions	S0	S1	S2	S3	S4	S5	Mean
A1	2.5	2.1	1.4	1.3	0.8	0.6	1.5
A4	3.2	2.4	2.0	1.4	1.2	0.7	1.8
A5	2.5	2.1	1.4	1.1	0.8	0.7	1.4
A6	2.4	2.0	1.6	1.0	0.7	0.7	1.4
A8	3.1	2.1	1.8	1.4	0.9	0.6	1.7
A9	2.1	2.0	1.4	1.5	0.8	0.8	1.4
A10	2.4	1.9	1.8	1.3	0.8	0.7	1.5
A12	2.9	2.7	1.7	1.4	1.3	0.7	1.8
A14	2.4	2.0	1.5	1.2	0.6	0.6	1.4
A15	2.3	1.9	1.7	1.5	0.8	0.6	1.4
Mean	2.6	2.1	1.6	1.3	0.9	0.7	1.5
LSD	0.05						
Salinity (S)	0.14						
Accessions (A)	0.17						
S×A	ns						

DISCUSSION

The level of salinity caused a depressing effects on the examined parameters such as the plant survival percentage, productive traits as well as on the morphological parameters. This study results confirmed that the salinity is one of the major environmental factors limiting the growth and productivity of plants as previously reported in the available previous study (Parida and Das, 2005; Tattini and Traversi, 2008; Barakat *et al*., 2013). Thus, this study findings provide helpful information about the different accessions of mastic tree adaptation ability, survival and growth in nursery conditions under salt stress.

Regarding the ability of the plant to survive in a saline environmental condition, the 10 accessions can be characterized according to the model proposed by Maas and Hoffman (1977), resulting in a high difference among the investigated accessions as confirmed by the critical threshold values, slope and $EC_{es50\%}$. Therefore, *P. lentiscus* can be classified as "tolerant" to salinity in terms of relative survival during the growing early stage as also reported by Tattini *et al*. (2006) and recently by Cristiano *et al*. (2016).

Increasing salinity the production of *P. lentiscus* total dry biomass and the plant height decreased in all the investigated accessions. However, among accessions it was noted a different response to the salt stress in particular, the accessions A10, A12 and A15 resulted in higher tolerance to salinity based on their high value of $EC_{e50\%}$. This finding have been also observed recently by Cristiano *et al*. (2016) in the same *P. lentiscus* accession A12.

The responses of the accessions to salt stress may be presumably due to a different defence mechanism to sodium toxicity through its compartmentalization in roots and the subsequent elimination through the radical turnover determining the replacement of older with younger roots. This behaviour was previously observed by Ramoliya and Pandey (2002) in *Salvadora oleoides* and more recently by Cristiano *et al*. (2016) in *P. lentiscus*. As expected also, the salinity level led a significant reduction of both leaf area and number of leaves per plant. The same trend was observed in Cristiano *et al*. (2016) who worked on two different accessions of *P. lentiscus*.

CONCLUSION

In conclusion based on this study findings, the overall results of this study suggested that *P. lentiscus* can well tolerate salt also at high concentrations when grown under nursery conditions. Furthermore, among the investigated germplasm and a different plant accessions response to salt stress was observed. Thus, by identifying of mastic tree accessions more tolerant to salty soil and the revegetation of degraded agricultural lands can be attained.

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