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Rootstock influences the response of pistachio (*Pistacia vera* L. cv. Mateur) under saline stress condition

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Abstract-Effects of saline irrigation water were studied on the behaviour of 5-year-old female trees of 'Mateur' variety grafted on *Pistacia vera* L. and *Pistacia atlantica* Desf. rootstocks for three successive years. Different irrigation water qualities were used: (i) fresh water (ECw: 1.95 dS/m); (ii) moderately saline water (ECw: 5 dS/m); and (iii) saline water (ECw: 12 dS/m). The following parameters were assessed: Individual trunk cross-sectional area, scion shoot growth, stomatal density, leaf area, chlorophylls and water contents. Results showed that scion axillary shoot lengths, internode lengths and bud number decreased significantly at the highest level of saline water treatment mainly in the thrird year of study. Trees grafted on *P. atlantica* rootstock showed a slight early growth advantage compared to those having *P.vera* as rootstock. Stomatal density was affected by both salinity and rootstocks. The highest stomata density was obtained with severe saline treatment (ECw: 12 dS/m). During the three years of study, the highest and the lowest stomata density on the abaxial leaf surface were recorded on *P. atlantica* and *P. vera* rootstocks, respectively.Leaf chlorophyll content, relative water content and leaf area decreased by increasing salinity. The most important reductions in chlorophyll content, leaf area and relative water content were observed on *P.vera* rootstock. *P. atlantica* rootstock induced the lowest decline in chlorophyll content suggesting higher salt tolerance due to the maintenance of higher cell turgor. Agricultural practice based on its use as rootstock may lead to better rusticity of *P. vera* varieties.

Keywords:pistachio, salinity, rootstocks, growth parameters, chlorophyll content, RWC, leaf area.

1. Introduction

Salt stress imposes a major environmental threat to agriculture.Its adverse impacts are getting more serious problem in regions where saline water is used for irrigation. In order to reduce salinity impairement, the technique of tree grafting is used in recent years. This traditional environmentally friendly approach allows adaptation to different environmental conditions, and provides improved scion growth and fruit yield (Storey and Walker, 1999). Grafting salt-sensitive plants onto salt-tolerant rootstocks allows to overcome salinity hazards as has been shown in apple (Yin *et al*., 2010), grape (Jogaiah *et al*., 2014), mango (Dayall*et al*., 2014) and pistachio (Tavallali *et al*., 2008).Most pistachio plantations all over the world are grown on saline soils (EC>6 dS/m) and irrigated with low quality and saline water. In Tunisia, pistachio has becoming one of the most important commercial nuttree (Ghorbel et al., 1998) due to its tolerance to salts. The use of salt tolerant rootstock was demonstrated to be a valid strategy in increasing the salt tolerance of pistachio (Ferguson et al., 2002). In order to overcome the serious facts of salinity, special attention should be given to understandthe mechanisms adopted by particular rootstocksto avoid toxic salt effects (Colla et al. 2010).It has been stated that *P.vera*rootstocksis sensitive to moderately tolerant to salinity compared to *P.atlantica* and *P.intergerrima*(Picchioni et al., 1990; FAO, 2002).Besides its use assand stabilizer,*P. atlantica* can grow under severe conditions of arid and semi-arid areas (Benhassaini et al., 2007).The effect of salt on pistachio growth has been studied ondifferent rootstocks (Tavalli, 2008; Karimi, 2012). Previous study showed that vegetative growth of pistachio was adversely affected by salinitythrough a

reduction of apical and axillaryshoot lengths, vegetative bud number and internodes lengthat 12 dSm^{-1}(Mehdi et al., 2010). The reduction of vegetative growthby salt may be a result of a combination of osmotic and specific Na and Cl ion effects (Correia et *al.*, 2010).Under salt conditions, the plant water uptake decreases due to the reduction of water potential and the toxic effect of specific ions at the cellular level (Ebert, 2000). Salinity tolerance mechanisms in plants act to either withstand cellular dehydration or minimize water loss to maintain a suitable water status for development.In the same way, salinity of irrigation water induces the partial or total closure of stomata in order to preserve the plant water status. This phenomenon is always accompanied by a decrease in stomata density and guard cell length to limitates leaf gas exchanges.It causes a reduction in stomatal conductance, photosynthesis and transpiration (Lefi and Ben Hamid, 2014). Ferguson et al. (2002) studied the response of *P.atlantica*,*P. integerrima* and UCB-1 rootstocks to salinity stress.They reported that increasing salinity provoked a reduction in leaf area that was more pronounced on*P. integerrima* rootstock.This paper describes the effects of saline irrigation water and rootstock(*P. vera* and *P. atlantica*) on leaf growth parameters of Mateur pistachio variety.For this purpose, leaf area, stomatal density, leaf water and chlorophyll contents were followed during three consecutive years under different quality of irrigation water.

2. Materials and methods

2.1. Experimental site and plant material

This study was conducted in the "Taous" experimental orchard of the Olive Tree Institute situated at 26 km in the north of Sfax. Five-year-old cv.'Mateur' female pistachio trees were grafted on *Pistacia vera* L. and *Pistacia atlantica* Desf. rootstocks. Tree spacing distance was 6x6 m and plants were irrigated three times a monthduring three consecutive years.The amount of water supplied to trees was estimated according toPenman-Monteith-FAO equation (Doorenbos and Pruitt, 1977).The following irrigation treatments were applied: fresh water, ECw = 1.95 dS m^{-1} (control C); moderately saline water, ECw = $5dSm^{-1}$ (moderately saline water T_1), and highly saline water, ECw = 12 dS m^{-1} (high saline water T_2).The fresh water used was that supplied by the Tunisian National Water Carrier (C).The moderately saline water (T_1) was provided from the local reservoir situated in the area of the experimental orchard. The higher salinity treatment (T_2) was appliedby addingsodium chloride (NaCl) to the irrigation well water (T_1) up to 12dS m^{-1} according toSparks (2002) formula: Salt (mg L^{-1}) = 640 x EC (dS m^{-1}). Thephysicochemical poperties of the different irrigation waters used were described in the table 1. The soil quality of the experimental orchard is a shallow sandy-clay (table 2).

Table 1. Physiochemical properties of irrigation waters used

Properties	Fresh water	Moderately saline water	High saline water
pH	7.4	7.6	7.6
Sodium (mg L^{-1})	154.1	600	1200
Potasium (mg L^{-1})	290.3	500	620
Calcium (mg L^{-1})	90.2	273	326
Chloride (mg L^{-1})	326.5	1138	1689

Table 2. Soil characteristics of the experimental orchard

Characteristics	Values
Sand (%)	44
Clay (%)	47
Silt (%)	9
Electical conductance (dS m^{-1})	3.25
pH of saturated soil solution	8.2
Organic matter (%)	1.5
Total Nitrogen (%)	0.41
Potassium (%)	147
Phophorus (%)	0.22
Sodium (ppm)	620
Chloride (ppm)	570

2.2. Growth measurements

Tree growth was followed by the measurements of scion axillary shoot lengths, internode lengths and bud number as well as rootstock trunk cross-sectional area (TCSA) at the end of each year of experimentation.

2.3. Stomatal density and leaf area

Samples of five leaflets from the mid-section of current year shoots were collected from five trees per rootstock every two months. Leaf area was measured with a WinDIAS Colour Image Analysis System (Delta-T Devices Ltd, Cambridge, UK). Stomatal density was assessed using an optical Leitz microscope (Leitz DIA LUX 22EB) equipped with a digital camera (Hitachi KP-D 40 Color Digital). Stomata were counted with the analysis software program for image analysis (Delta-T Devices Ltd., Cambridge, UK). Leaf imprints were taken from abaxial leaf surfaces, using nail polish, on 3 leaves per treatment (3 separate areasper leaf). These imprints were later examined under a high magnifications (250×) microscope, and stomatal density (number of cells per surface area) was determined.

2.4. Relative water content

The relative water content (RWC) in the leaves was measured as follows: leaf sample was weighed to determine its fresh weight (FW) and then placed in distilled water for 24 h until full turgor.After this period, the leaf was removed from water, wiped with the filter paper and weighed (TW) before being placed in an oven at 80°C during 48 h.Samples were then weighed again to determine the dry weight (DW).RWC was determined according to Clarke and McCaig (1982) equation:

$$RWC = (FW - DW) / (TW- DW) \times 100$$

were FW: Fresh Weight
DW: Dry Weight
TW: Turgor Weight

2.5. Chlorophyll contents

The chlorophyll content in the leaves was estimated spectro-photometrically in a known aliquot 80 percent acetone extract. The absorbance was measured at 645 and 663 nm for the estimation of chlorophyll a, chlorophyll b and total chlorophyll. The following formulaes suggested by Mackinney (1941) were used for the estimation of thedifferent fractions of chlorophyll:

$$\text{Chlorophyll a}= 12.7\ (\text{Abs. at } 663 \text{ nm})-2.69\ (\text{Abs. at } 645 \text{ nm})\times V/1000 \times W$$

$$\text{Chlorophyll b}= 22.9\ (\text{Abs. at } 645 \text{ nm})-4.68\ (\text{Abs. at } 663 \text{ nm})\times V/1000 \times W$$

$$\text{Total chlorophyll}= 20.2\ (\text{Abs. at } 645 \text{ nm})+8.02\ (\text{Abs. at } 663 \text{ nm})\times V/1000\times W$$

Where, Abs: Absorbance; V: Final volume of chlorophyll extract (mg), W: Fresh weight of the leaf extract (g).

2.6. Statistics

All data were subjected to one way ANOVA analyses using SPSS software of Windows. Mean differences were determined by Duncan's multiple range tests at $p \leq 0.05$. The comparisons in terms of the growth and chlorophyll content data were performed between all treatments and rootstocks.

3. Results and discussion

3.1. Growth parameters and stomata density

Table 3 represents values of all growth parameters measured during the whole period of study.

3.1.1. Shoot length

During the first year, the apical and axillary shoot lengths of trees grafted on *P. atlantica* were either not affected by moderately saline water (table 3). Trees budded on *P.vera* showed a significant decrease in apical shoot length since EC=5dSm^{-1} of salinity whereas axillary shoot length decreased only at EC_w=12dSm^{-1}. The apical shoot length was affected by high salinity treatment (12 dSm^{-1}) for trees grafted on *P.atlantica* rootstock while axillary shoot length did not exhibit significant change on *P. vera* rootstock. During the second year of salt water irrigation, apical and axillary shoot lengths were not affected by salinity on *P. atlantica* rootstocks while a significant reduction in apical shoot

length was recorded at both salt treatments on *P.vera* rootstock. In the last year of study, apical and axillary shoot lengths were declined significantly at both salt treatments on *P. v*era rootstock but remained unchanged on *P.atlantica* rootstock with an exception of a significant decrease in length of axillary shoots at EC_w=12dSm^{-1}.
Growth reduction is a mechanism necessary for the survival of plants exposed to abiotic stress (Zhu et al., 2008). In this study, shoot growth of trees grafted on *P. atlantica* appeared less affected by water salinity than those growing on *P. vera* rootstock. These findings confirm the best performance of *P. atlantica* rootstock under saline conditions (Ferguson et al., 2002).

3.1.2. Abaxial stomata density and leaf area

Stomata and leaf area are characters which influence transpiration rate, stomatal conductance and photosynthesis to a great extent and play an important role on growth and development of trees.
Salt-treated trees grafted on *P.atlantica* showed no significative variation in leaf abaxial stomata density and leaf area during the first year of study. Similar results were recorded on *P.vera* except of a significant decrease in leaf abaxial stomata density under 12dSm^{-1}salinity treatment.
After two and three years of irrigation with saline water, leaf area decreased significantly for all pistachio trees; Similarly, water salinity treatments induced significant reductions in leaf stomata density on both rootstocks after two years of experimentation. At the end of trial period, this parameter varied differently on each rootstock. Indeed, trees grafted on *P.vera* showed a significant decrease of their leaf abaxial stomata density (27.3 % lower than control) at EC=5 dSm^{-1} and a slight but not significant increase at EC=12dSm^{-1} (37.4% higher than control). On *P.atlantica* rootstock, leaf stomata density did not show significant change at EC=5 dSm^{-1} but exhibited a significant reduction at EC=12 dSm^{-1} (41.5 % lower than control).
It was demonstrate that low stomatal density may be beneficial for tolerant pistachio rootstock prescribed to be used in marginal environments in terms of salinity (Ferguson et al., 2002).Many studies have shown that decreasing stomatal density and lowering stomata movement were controlled by an hormonal message from the roots, the abscisic acid (Zorb et al., 2013). This stomata density reduction may induce a decrease in leaf transpiration and consequently inhibiting photosynthesis (Wilkinson and Davies, 2002) and leaf expansion (Munns et al., 2006). In this study, trees grafted on *P. atlantica* exhibited greater stomatal density reductions than ones budded on *P.vera*, reflecting its higher eco-physiological adaptation to salinity. That joined the literature to explain the agricultural practice based on the use of *P. atlantica* as a rootstock of *P. vera* for a better rusticity to the abiotic constraints as salinity. Ben Hamed and Lefi (2015) allotted the good performance of pistachio trees grafted on *P. atlantica* rootstock to the adaptive ecophysiological characteristics related particularly to root growth.

3.1.3. The trunk cross-sectional areas (TCSA)

The TCSA of stocks was significantly higher in trees having *P.atlantica* as rootstock during the tree years of study. Both saline water treatments induced no significant variation in this parameter for *P.atlantica* rootstock during the two first years of experimentation. A significant reduction was recorded, however, in the last year. The TCSA of *P. vera* stocks decreased significantly at all salinity treatments during the second and third year of study.

3.2. Water status: Relative water content (RWC)

Pistachio trees treated with fresh water (C) maintained high RWC on both *P. vera* and *P. atlantica* rootstocks. Under salt stress (T_1 and T_2), the RWC was significantly reduced, especially for trees budded on *P. vera* at moderately and saline water treatment all over the experimental period. In fact, RWC has been as lower as the salt stress became more severe (Fig. 1). RWC reduction is due to an increase in osmolarity in the cytoplasm causing osmotic stress and cellular dehydration.
During the two first years of experiments, trees grafted on *P.atlantica* showed high RWC values for all salinity treatments (Fig. 1). Neverthless, these values decreased only by salinity up to 12 dSm^{-1} in the last trial year. It can be related to the wild nature of *P.atlantica* rootstock. In term of cell turgor, Lefi and Ben hamed (2014) reported that under severe salinity (EC=12dSm^{-1}), the water status of studied pistachio trees was widely affected in *P. vera*, whereas, *P. atlantica* maintained high leaf turgor compared to control. A more favorable hydration status in *P.atlantica* revealed a limiting transpiration

mechanism (Porcel*et al*., 2012) due to osmotic adjustment, which maintains the osmotic balance between the cytoplasm and vacuole preventing the efflux of water from the cytoplasm (Ben Ahmed *et al*., 2008).

Table 3: Sodium chloride effect on Apical shoot length (Ap SL), Axillaries shoot length (Ax SL), trunk cross-sectional area (TCSA), Leaf area and abaxial stomata density (Ab SD) of *P.vera* and *P.atlantica* rootstocks during three years of study.

Experimental period	Rootstock	Salt treatments	AP SL (cm)	Ax SL (cm)	TCSA (cm^2)	Leaf area (cm^2)	Ab SD (N/mm^2)
First year	*P.vera*	C	41.6^{bc}±20	10.5^{bc}±4.6	142.2^{h}±13.0	37.0^{d-f}±9.4	361.2^{bc}±38.2
		T1	34.9^{d}±26.3	12.4^{ab}±6.3	165.5^{gh}±13.6	32.4^{e-i}±4.1	325.0^{cd}±3.2
		T2	27.3^{d-f}±19.34	8.7^{c-e}±3.2	150.6^{gh}±12.7	29.6^{d-f}±19.4	298.9^{d}±22.6
	P.atlantica	C	40.4^{bc}±21.1	12.7^{ab}±5.8	210.4^{fg}±13.0	41.0^{cd}±3.4	394.3^{ab}±59.1
		T1	54.5^{a}±13.6	15.0^{a}±5.1	262.9^{ef}±13.5	44.2^{bc}±2.0	425.0^{a}±4.2
		T2	49.0^{ab}±18.1	9.6^{cd}±1.9	226.3^{f}±14.0	$38.\ 4^{c-e}$±2.8	384.5^{ab}±72.5
Second year	*P.vera*	C	12.7^{h} ±3.2	5.6^{f}±1.7	302.8^{c-e}±14.0	49.6^{b} ±3.2	208.3^{ef}±27.2
		T1	6.4^{ij} ±1.4	4.9^{f}±2.0	256.0^{f}±13.0	$29.\ 4^{gh}$ ± 1.4	144.9^{gh}±13.0
		T2	4.6^{j}±4.3	5.2^{f}±1.8	270.1^{f}±12.4	26.0^{i}±4.3	115.4^{ij}±27.8
	P.atlantica	C	16.0^{f-h}±6.1	6.1^{f}±1.9	366.6^{b-e}±14.7	60.6^{a}±4.4	228.4^{e}±23.4
		T1	16.5^{f-h}±8.1	5.8^{f}±1.9	366.9^{b-e}±13.7	44.6^{bc}±3.3	168.3^{f-h}±13.7
		T2	19.6^{ef}±8.3	5.0^{f}±1.1	365.9^{b-e}±15.0	33.9^{d-h}±8.3	113.7^{ij}±16.6
Third year	*P.vera*	C	19.0^{e-g}±2.2	7.5^{de}±3.2	410.2^{bc}±15	50.5^{b}±4.72	187.4^{e-g}±14.2
		T1	14.4^{h-j}±3.5	5.8^{f}±2.6	290.3^{d-f}±15	34.0^{d-h}±3.5	136.2^{h-j}±15
		T2	11.6^{h-j}±5.5	5.7^{f}±2.8	257.5^{ef}±15.3	26.6^{i}±5.2	257.5^{ef}±15.3
	P.atlantica	C	29.8^{c-e}±2.1	8.7^{c-e}±4.4	777.9^{a}±16.3	$61.\ 5^{a}$±2.08	190.0^{e-g}±15.9
		T1	26.9^{d-f}±8.2	6.7^{de}±3.8	422.1^{b}±16.8	34.6^{d-g}±8.15	150.1^{g-i}±21.6
		T2	27.8^{d-f}±1.5	4.7^{f}±2.9	390.02^{b-d}±15.9	29.0^{gi}±1.5	111.1^{i}±17.2

All values represent average per plant±standard deviation (n≥22). Values on the same column having a same letter are not significantly different at p≤0.05.

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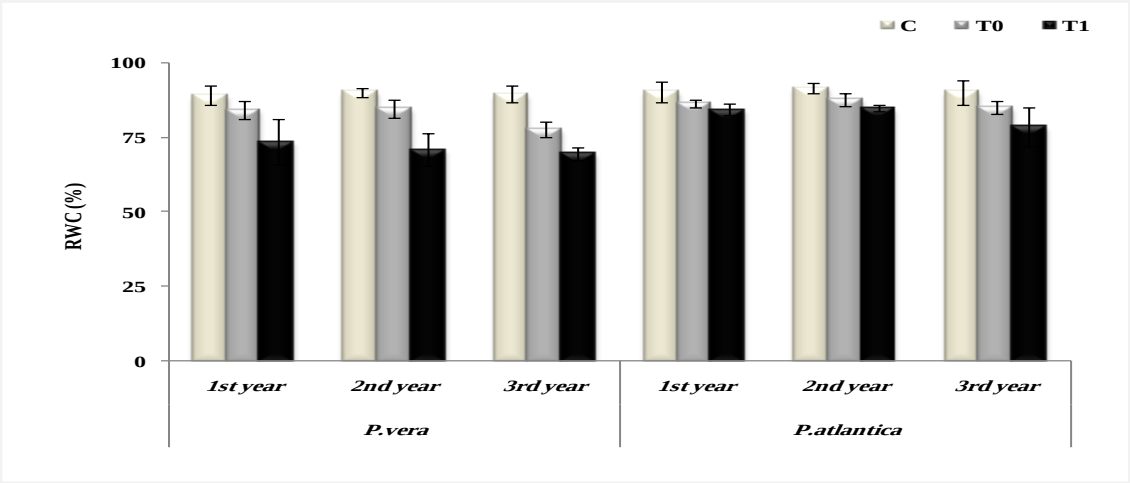

Figure 1. Sodium chloride effect on leaf relative water content of Mateur pistachio variety grafted on *P. vera* and *P. atlantica* rootstocks. Data are the mean of three replicates.

3.3. Chlorophyll content

By increasing salinity levels from 1.95 to 12 ds/m, chlorophyll a, b and total chlorophyll were reduced all over the period of study (Fig.2 A, B, C). Maximum reduction was observed when trees were exposed to high salinity level (12 dS/m). Decrease in chlorophyll contents induced by salinity in different pistacia species have been reported earlier (Behboudian et al. 1986; Ranjbar et al. 2002). Reduction in chlorophyll content under salt stress may be due to chlorophyll degradation and/or reduced rate of synthesis, together with a decrease in thylakoid membrane stability (Parida and Das,

2005). Mumtaz khan et al. (2014) suggested that decrease in chlorophyll concentration of salt treated trees could be attributed to the increase activity of chlorophyll degrading enzyme, chlorophyllase.
Mean values of data showed that trees grafted on *P.vera* had the maximum reduction of these three photosynthesis pigments under salinity stress comparing to onces budded on *P.atlantica* (Fig. 2). Our results were in agreement with Ferguson et al. (2002) results who reported that chlorophyll contents decreased with increasing salinity in 'Kerman' cultivar grafted on *P.atlantica*, *P.integerrima* and *UCB1* rootstocks and these rootstocks can be tolerant to salinity by saving chlorophyll contents and photosynthetic efficiency of leaf at higher levels of salinity.

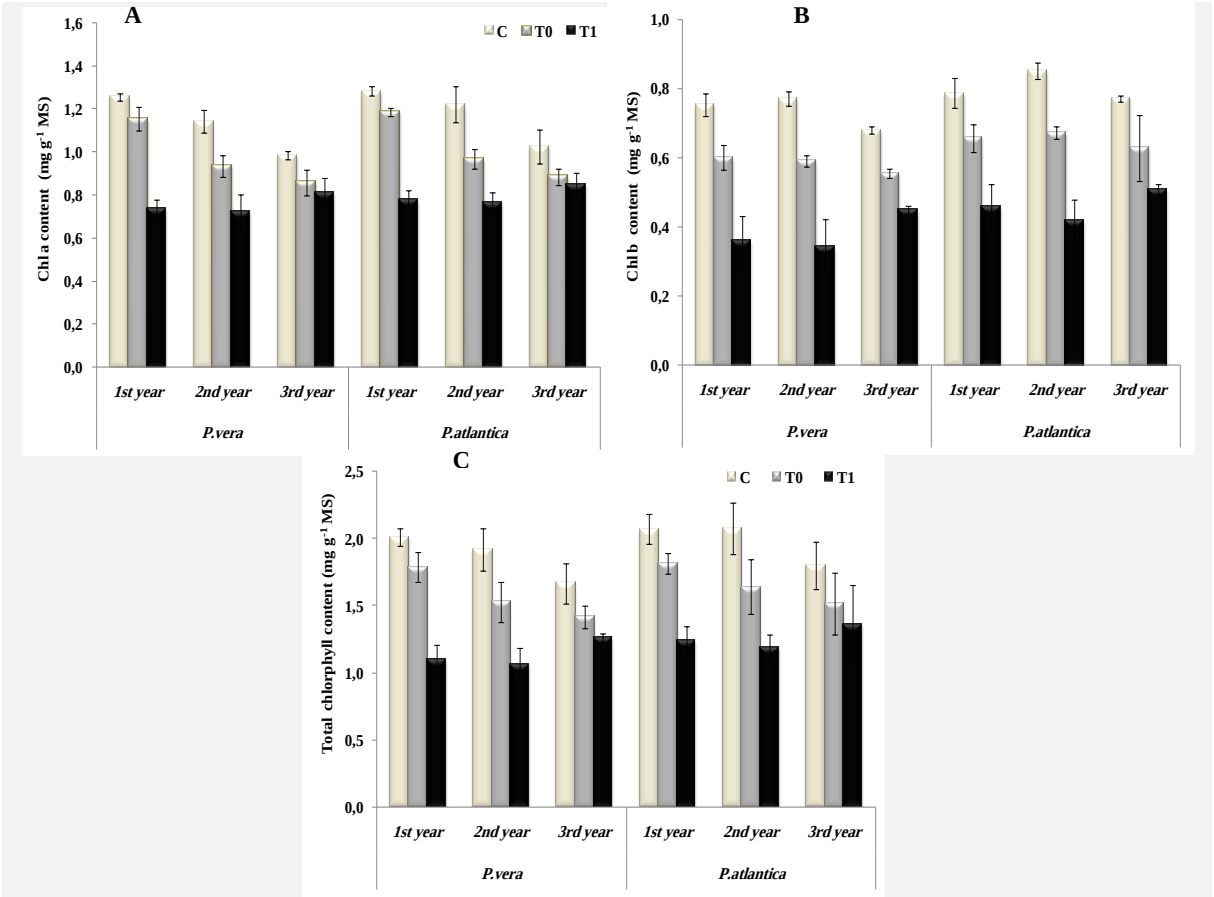

Figure 2. Sodium chloride effect on chlorophyll a (A), chlorophyll b (B) and total chlorophyll contents (C) of Mateur pistachio variety grafted on *P.vera* and *P.atlantica* rootstocks. Data are the mean of three replicates.

4. Conclusion

This study has highlighted the relatively salt tolerance of pistachio trees grafted on two local rootstocks *P.vera* and *P.atlantica*. Based on the criteria used for judging salt tolerance inthe present study, *P.atlantica* rootstocks could be considered as the most salt tolerant rootstockand would be well adapted to arid regions of Tunisia wherethe water may be moderately saline. The higher salt tolerance of this rootstock could clearly be due to higher ability tolimit transpiration by reduction of leaf area and stomata density, greater potential to maintain high cell turgor and finally less reduction in shoot length and trunk diameter.

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