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Growth, nutrient acquisition, and physiological responses of three olive cultivars to induced salt stress

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ABSTRACT

Three olive (*Olea europaea* L.) cultivars Nabali Baladi (NB), Nabali Muhassan (NM), and Grossi Di'Espagna (GE) were evaluated under salt stress. Seedlings were treated with salinity induced by a 3:1 ratio of calcium chloride and sodium chloride to four concentration levels measured as electrical conductivity (EC) [1.2, 4.1, 7.0, and 14.0 dS/m] for 122 days. Olive seedlings varied in their response to salinity. In all treatments, NB had the highest root; stem and leaf dry weights had among the highest total plant dry weights, specific stem length (SSL) and relative water content (RWC). NB seedlings maintained the highest stomatal conductance at 7.0 dS/m and highest chlorophyll index at 14.0 dS/m. Olive seedlings that tolerated salt tolerance developed mechanisms of nutrient acquisition and distribution in the organs, by storing minimal amounts of sodium (Na^+) and chloride (Cl^-) in the stems and loading the most in the leaves and roots.

Introduction

The olive tree is an ancient hardwood recognized as a symbol of peace and prosperity in many cultures across the world. These archaic trees were cultivated by the residents of the Jordan Valley or Teleilat Ghassul as early as the Chalcolithic period (4500−3500 BC) (Bourke 2002).

Nabali Baladi (NB) is the most commonly grown olive cultivar in Jordan (Ateyyeh, Stosser, and Qrunfleh 2000). It is favored due to its table olive taste, and high oil yield of medium to light olive oil with a distinctive flavor (Zohary and Hof 1988). The tree is indigenous to Jordan and naturally adapted to arid conditions. Nabali Muhassan (NM), also known as Improved Nabali, is supposedly an improved cultivar of NB. The plant has rapid root propagation, faster growth, and larger fruits when compared to NB. However, the fruit has a lower oil content, poorer quality, poor pickling quality, and susceptibility to insects and diseases. Grossi Di'Espagna is an introduced cultivar in Jordan. It originated in Italy and is valued primarily for the size of its fruit. It is usually used for green olives in brine, but can also be used for black table olives.

The use of saline water for agricultural production has expanded significantly in the last decade, whereas it is generally accepted that around 10% of irrigated areas are affected by salinity (Dinar 2009). In order to use saline water efficiently for irrigation, there is a need to understand how salinity affects plants or tree populations and a need to monitor salinity levels to constantly ensure they remain within acceptable ranges (Szabolcs 1994). Salt tolerance is usually grouped into three categories: tolerant, moderately tolerant, and sensitive. Different cultivars fall into different salt tolerance levels (Bouaziz 1990; Al-Absi, Qrunfleh, and Abu-Sharar 2003). In that regard, salt tolerance of olive trees was reported to vary among cultivars (Gucci, Lombardini, and Tattini 1997).

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Plant growth reduction due to salinity is generally related to the osmotic potential of the soil solution at the root zone and to the accumulation of toxic ions that would be distributed throughout the tree. An imbalance of ions may disrupt photosynthesis, which is the main function of a plant (Parida and Das 2005). Due to competitive interactions and by affecting the selectivity of membranes to the ions, the uptake of many essential nutrients will be affected when sodium (Na^+) and chloride (Cl^-) ions increase in the soil solution. It is known that extracellular Na^+ reduces the intracellular potassium (K^+) influx, and as a result, will impair the acquisition of this essential nutrient by leaf, shoot, and root (Al-Absi, Qrunfleh, and Abu-Sharar 2003; Alleva et al. 2006).

Olive trees have developed a defense mechanism in dealing with toxic ions. Studies have shown that there is an increasing gradient of Na^+ and Cl concentration from the roots to the stems or apical organs of the tree by methods of exclusion and compartmentalizing (Chartzoulakis et al. 2005). This system prevents accumulation of toxic ions in the aerial parts of the plant, where photosynthesis occurs (Greenway and Munns 1980; Storey and Walker 1999). In order to assess the tolerance of plants to salinity stress, growth or survival of the plant is measured because it integrates the up- or downregulation of many physiological mechanisms within the plant (Niknam and Mccomb 2000).

In this study, our objective was to evaluate the salinity response of three olive cultivars from Jordan, namely Nabali Baladi (NB), Nabali Muhassan (NM), and Grossi Di'Espagna (GE). In particular, growth, nutrient acquisition, and physiological responses of these cultivars were characterized under salinity-induced levels, in order to identify the most successful candidate to be recommended for saline irrigation. We also examined the effect of salinity on the ability of the different cultivars to prevent toxic ion transport to the harmful portions of the plant. These criteria have been reported in multiple papers to have genotypic variability in response to salt stress (Tattini 1992; Chartzoulakis et al. 2002; Chartzoulakis 2005; Vigo, Therios, and Bosabalidis 2005) and could be used to assess the degree of salinity tolerance.

Materials and methods

This study was conducted at The Hashemite University of Jordan, Zarqa, located at 32°05′ N latitude and 36°06′ E longitude. One-year-old cuttings of the cultivars NB, NM, and GE were used for this experiment. All trees were acquired from the National Center for Agricultural Research and Extension (NCARE), Baqa', Jordan, on the same day. All young trees selected had an initial height between the ranges of 60 and 85 cm, and no more than three branches.

Before starting the experiment, olive seedlings were transferred to 5-L pots filled with soil. Plants were acclimatized to greenhouse environment for about two months and kept well watered with tap water. Greenhouse day temperatures were in the range of 20−30°C, and mean midday photosynthetic photon flux density was 365 μmol s^{-1} m^{-2} (measured by a quantum sensor (LI-250A; LICOR)). Uniform plants from each cultivar were assigned randomly to one of four irrigation treatments (1.2 (control), 4.1, 7.0, and 14.0 dS/m) using a completely randomized block design for 121 days. There were six experimental blocks, each containing a total of 12 plants (3 cultivars × 4 salinity levels).

Initial seedling traits

At the day when irrigation treatments were started and before applying treatments, two of the six randomized blocks were destructively harvested (three plants from each cultivar). Plants were removed from their soil and separated into root, stems, and leaves. Leaf area was determined. All plant parts were oven-dried at 68°C for three days, and dry weights were taken (data not shown).

Salinity treatments

A 3:1 ratio of calcium chloride and sodium chloride, respectively, were diluted in water to create the stock solution. Treatment solutions were created by adding stock solution to tap water until the desired salinity, measured by electrical conductivity (EC), was achieved. All readings were recorded using an

EC meter (Milwaukee SPEM500). At the same day when initial data were recorded, the remaining four blocks were subjected to salinity treatment (EC 4.1 dS/m) to prevent salt shock, except for the control that was watered with tap water (1.2 dS/m). The EC of the irrigated water continuously and gradually increased until each desired treatment was at the appropriate experimental level or desired EC reading (1.2 (control), 4.1, 7.0, and 14.0 dS/m). All treatments were applied manually every two days to the field capacity for the total duration of four months.

Growth parameters

Plant heights were measured at Day 0 and the final day i.e. Day 121. The accumulated plant height was measured by subtracting plant heights at Day 0 from plant heights at Day 121. Specific stem height was calculated as: stem height/ stem dry weight (cm/g), relative growth rate which was calculated using the equation of Gutschick and Kay (1995): $RGR = \ln W2 - \ln W1/(T2 - T1)$, where W2 was the final dry weight at Day 121 (T2), and W1 was the initial dry weight (DW) determined from initial data harvest on Day 1 (T1) began. Net assimilation rates (NAR) were calculated as: $NAR = M2 - M1/T2 - T1 \times \log L2 - \log L1/L2 - L1$, where M2 was the final dry weight at Day 121 (T2) and M1 was the initial DW determined from the initial DW recorded on Day 1 of the experiment (T1).

Relative water content (RWC) measurements were taken bi-weekly to measure water relations. Leaf discs from five of the youngest fully expanded mature leaves from the median portion of the stem of two randomly selected plants of each cultivar were used. RWC was calculated using the equation, fresh weight−dry weight/saturated weight−dry weight $(FW - DW/SW - DW)(100)$, where FW is the fresh weight, DW represents fresh weight sample oven-dried at 68°C, and SW represents the saturated weight of sample, which was immersed overnight in distilled water.

Chlorophyll content index was recorded using a compact Opticom CMM-200 Chlorophyll Content Meter every two weeks. In order to measure g_s a steady-state porometer (LI-1600; LI-COR, Lincoln, Nebraska) was used, every two weeks.

Final harvest analysis

At the end of the experiment on Day 121, all plants were destructively harvested into leaves, stems, and roots. Leaf area was recorded using the leaf area meter (LI-3050C; LI-COR, Lincoln, Nebr.). All plant parts were oven-dried at 68°C for three days, and DWs recorded after their weights were stable.

All dried plant samples (leaves, stems, and roots) were ground separately to a fine powder and sent to the NCARE, Baqa', Jordan, to be analyzed for their Na^+ and Cl^- contents.

Data analysis

Statistical analysis was performed using SAS 9.2 software for Windows (SAS Institute, Cary NC). Significant differences between values of all parameters were determined at $p \leq 0.05$ using Proc Mixed, PDIFF, analysis of variance, and Duncan's multiple range tests.

Results

Effect of salinity on plant growth characteristics

Regardless of the treatment, NB had accumulated the highest root (4.53 g), stem (8.60 g), leaf DWs (4.10 g) and the highest total plant DW (Table 1). NB also had the highest leaf area and specific stem length (SSL). When NB is compared to the other cultivars, DWs, leaf area, and SSL were significantly higher ($p \leq 0.0001$) at the end of the experiment.

Plant growth parameters show that there were always significant differences among treatments ($p \leq 0.0001$). Results indicate that as EC increases, DWs significantly decrease. This can be observed with roots, stems, and leaves. Higher values of root-to-shoot ratios indicate an increased root DW

Table 1. Growth traits of the three olive cultivars on Day 121 of the experiment.

Cultivar	Root DW (g)	Stem DW (g)	Leaf DW (g)	Shoot DW (g)	Total plant DW (g)	Roots/shoots	Specific stem length (cm/g)
Nabali Baladi	4.54 a[z]	8.60 a	4.10 a	12.54 a	17.11 a	0.39 b	85.19 a
Grossi Di'Espagna	3.72 b	3.09 c	2.07 c	5.24 c	8.52 c	0.46 a	82.97 b
Nabali Muhassan	3.20 c	6.89 b	3.69 b	10.37 b	15.73 b	0.61 b	79.19 c
SE	±0.50	±1.25	±1.05	±2.15	±1.70	±0.12	±1.20
Treatment							
Control	4.26 a	9.08 a	5.07 a	14.03 a	19.52 a	0.36 d	84 a
4.1 dS/m	3.99 b	7.65 b	3.40 b	11.23 b	16.17 b	0.42 c	86 a
7.0 dS/m	3.65 c	4.46 c	2.71 c	7.00 c	10.62 c	0.57 b	86 a
14.0 dS/m	3.39 d	3.58 d	1.96 d	5.26 d	8.84 d	0.69 a	72 b
SE	±0.13	±0.70	±0.89	±1.20	±1.12	±0.01	±4.0

[z]Means within the columns followed by the same letter are not significantly different.
Means ± SE were assisted at $p \leq 0.05$.
p-Value for cultivar, treatment, and their interaction was ≤ 0.0001 for all table parameters.

when compared to shoots (leaves and stems). Leaf area results indicate significant differences between different treatments of all cultivars. When irrigated with 14.0 dS/m solution, all cultivars experienced a severe DW loss (Table 1).

Total plant dry weight

NB plants were recorded to have among the highest total plant DWs under all treatments. NM has the highest total plant DW when watered with the control treatment; however, its total plant DW decreases by approximately 50% when watered with 4.1 dS/m (Table 2). This was not the case for NB where it did not suffer any significant losses when watered with 4.1 dS/m as seen with NM. In addition, NB had a significantly higher total plant DW (12.24 g) when compared to NM (7.64 g) and GE (6.12 g) under severe salt stress or 14.0 dS/m.

Specific stem length

NB plants had the greatest SSL under all treatments (Table 2). Only when NB plants were irrigated with 14.0 dS/m, they suffered a significant loss ($p \leq 0.0001$) but still had values twofold higher than those of NM ($p \leq 0.0001$), and were comparable to GE plants watered with tap water.

Table 2. Total plant dry weight, specific stem length, accumulated plant height, relative growth rate, relative water content, chlorophyll index, net assimilation rates, and stomatal conductance (g_s) in controls and salt-treated plants of three cultivars.

Treatment	Control 1.2 dS/m			4.1 dS/m			7.0 dS/m			14.0 dS/m		
	NB	GD	NM	NB	GD	NM	NB	GD	NM	NB	GD	NM
TDW (g)	24.0 b[z]	15.0 c	37.4 a	23.0 b	10.0 d	14.0 c	14.5 c	11.0 d	9.0 d	11.4 d	7.3 e	8.0 e
SSL (cm/g)	89.5 a	83.0 c	70.0 b	89.0 a	73.5 b	75.0 b	84.5 a	75.0 b	62.0 c	70.5 b	60.0 c	40.1 d
APH (cm)	14.0 c	15.5 h	20.5 a	12.8 e	13.5 d	15.5 b	11.0 f	10.0 g	6.9 h	6.2 i	5.1 j	4.5 k
RGR (mg · g^{-1}· d^{-1})	2.40 b	2.15 c	2.80 a	2.40 b	2.07 c	2.35 b	2.30 b	2.03 c	2.13 c	2.00 c	1.84 d	1.90 d
RWC (%)	89.5 a	88.0 ab	90.4 a	85.0 bc	85.3 bc	80.3 c	79.0 c	79.8 c	73.5 d	74.3 d	77.0 cd	69.1 e
CHI	92.0 a	93.5 a	90.0 a	84.5 c	83.5 c	72.5 e	75.5 d	70.8 e	60.5 f	70.3 e	95.5 f	51.0 g
NAR (mg · cm^{-2}· d^{-1})	0.315 b	0.310 b	0.330 b	0.355 a	0.310 b	0.325 b	0.339 a	0.29 b	0.293 b	0.210 c	0.200 c	0.198 c
gs (mmol ·m^{-2} ·s^{-1})	1260 a	1060 bc	1070 c	1430 a	1100 bc	1000 c	940 b	910 c	780 cd	740 d	710 d	510 ed

[z]Data are means ± SE of 4 replicates.
Rows marked with the same letter were not significantly at the $p \leq 0.05$.

Accumulated plant height

When irrigated with the control (14 cm) and 4.1 dS/m treatments, NB plants had among the lowest accumulated plant height (Table 2), but when irrigated with both 7.0 and 14.0 dS/m irrigation treatments, NB had significantly higher accumulated plant height than NM and GE. Even though NM plants accumulated the greatest plant height when irrigated with tap water, their height accumulation significantly decreased ($p = 0.002$) when irrigated with 4.1 dS/m. Besides, the accumulated plant height of NM plants was almost threefold shorter when watered with 14.0 dS/m.

Relative growth rate

Relative growth rate (RGR) of NB plants was among the highest with tap water irrigation (Table 2). There were no significant differences between NB plants watered with control, 4.1, and 7.0 dS/m treatments. When plants irrigated with 14.0 dS/m, NB had significantly higher RGRs than both GE and NM. In addition, NB plants irrigated with 14.0 dS/m also had similar RGR as GE plants irrigated with the control, or NM plants irrigated with 7.0 dS/m.

Relative water content

RWC of all plants decreased with the increase of salinity treatments. When compared to other cultivars under all irrigation treatments, NB plants had among the highest RWC (Table 2). At 7.0 and 14.0 dS/m, NM plants had among the lowest RWC (73 and 70%, respectively).

On Day 1 of the experiment, there were no significant differences of RWC ($p = 0.055$) between all plants of NB, GE, and NM (Figure 1). On Day 32 which is designated "month 2," RWC of NB plants showed no significant differences between plants watered with the control, 4.1, or 7.0 dS/m. At the end of the experiment (Day 121), NB maintained among the highest RWC rates for most irrigation treatments.

Chlorophyll index

NB cultivars maintained among the highest values on the chlorophyll index scale (Table 2) for all irrigation treatments. NB plants irrigated with 14.0 dS/m were about twofold higher than GE plants that irrigated with 14.0 dS/m ($p = 0.0001$) and close to threefold higher than NM plants that irrigated with 14.0 dS/m ($p = 0.004$).

On Day 1 of the experiment, there were no significant differences of the chlorophyll index ($p = 0.055$) between all plants of NB, GE, and NM (Figure 2A–C). At the end of the experiment, NB maintained among the highest chlorophyll index for most irrigation treatments, while NM plants had the lowest, especially at 14.0 dS/m (60) (Figure 2A–C).

Net assimilation rate and stomatal conductance (g_s)

Regardless of the treatment, NB plants maintained the highest NAR (Table 2). No significant differences in NAR ($p = 0.3345$) were recorded for all plants at 14.0 dS/m.

The highest rates of g_s were maintained by NB cultivars (Table 2) when irrigated with saline solutions: control, 4.1, and 7.0 dS/m. When plants were irrigated with 7.0 dS/m, NB maintained the highest rates at 920 mmol m^{-2} s^{-1} ($p = 0.004$) and had among the highest rates of g_s at 14.0 dS/m.

Ion content

Sodium and chloride content of NB plants was highest within the leaves. NB plants also had among the lowest ion content within stems (Table 3). All cultivars had same chloride content in their roots ($p = 0.1337$), while NM had the highest amount of sodium and chloride content in the stems. As
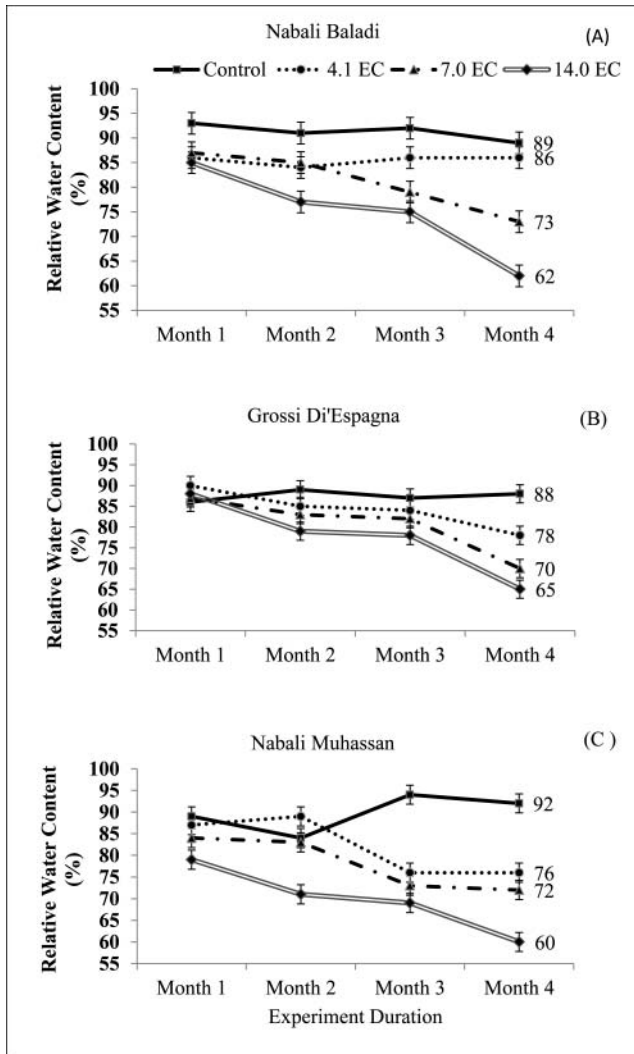
Figure 1. (A–C) Relative water content (%) in controls and salt-treated plants of three cultivars over entire length of experiment. Data are means ± SE of 4 replicates.

irrigation treatments increased in their salinity levels, the ion content in the leaves, stems, and roots increased significantly. Cultivars irrigated with 14.0 dS/m had the greatest amount of chloride and sodium in all plant organs ($p \leq 0.0001$) (Table 3). Under control treatments, NB plants expressed no significant differences in the roots stems and leaves ($p = 0.0934$) (Figure 3A). Comparing to the other cultivars, NB plants accumulated more sodium in the roots and leaves than in the stems. However, GE and NM plants exhibit more sodium in their stems (Figure 3A–C).

The amount of chloride in the roots increased almost fivefold when irrigated with 4.1 dS/m, (from 0.10 to 0.50%) and continued to increase to 0.60% when irrigated with 7.0 dS/m and to 0.75% when irrigated with 14.0 dS/m; the same pattern can be observed in the leaves and stems of NB (Figure 4A).

Chloride content of the stems of NM plants was almost twofold higher than that of GE and NB plants. The amount of chloride in the NM leaves was the same at 4.1, 7.0, or 14.0 dS/m treatments ($p = 0.2337$) but less than the control ($p = 0.005$) (Figure 4C). Under control treatment, NB and NM plants had the lowest amount of chloride in the roots, while GE plants had the largest ($p = 0.0003$) (Figure 4A–C). Chloride in the stems of NB and GE plants also maintained the lowest. However, NM had more chloride in their stems (0.45%) ($p = 0.005$).
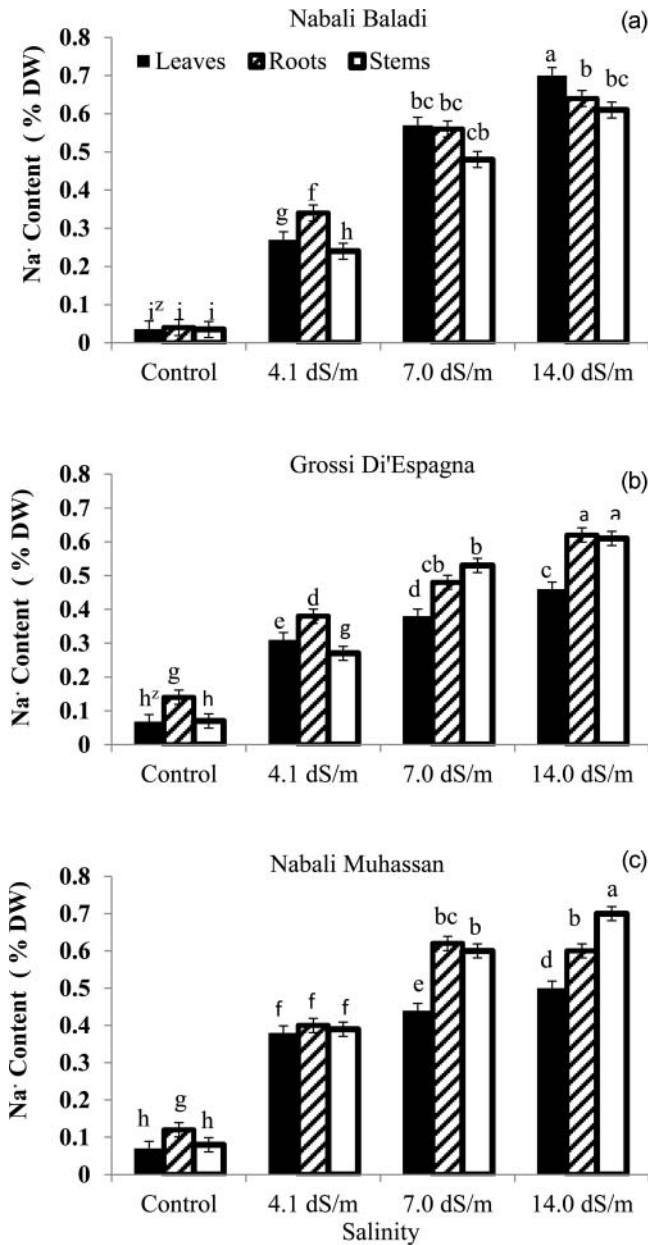
Figure 2. (A–C) Chlorophyll index of controls and salt-treated plants of three cultivars over entire length of experiment. Data are means ± SE of 4 replicates.

NB plants contained threefold less chloride in the stems than NM plants and less than GE plants ($p = .0067$). NB plants contained the largest amount of chloride ion in the leaves which was more than that in the leaves of NM plants, and also significantly ($p = 0.0040$) greater than that of GE plants.

Discussion

Plant growth assessment

Salinity stress has been previously shown to cause deleterious effects on many growth parameters in olive trees (Therios and Misopolinos 1988; Chartzoulakis et al. 2002; Chartzoulakis 2005; Vigo,

Table 3. Ion content of plants harvested on Day 121 of the experiment.

Cultivar	Leaves		Stems		Roots	
	Na$^+$	Cl$^-$	Na$^+$	Cl$^-$	Na$^+$	Cl$^-$
Nabali Baladi	0.39 a[z]	0.24 a	0.34 b	0.16 b	0.39 a	0.48 a
Grossi Di'Espagna	0.30 c	0.19 b	0.37 a	0.12 b	0.41 a	0.53 a
Nabali Muhassan	0.35 b	0.12 c	0.44 a	0.46 a	0.43 a	0.47 a
SE	±0.02	±0.01	±0.03	±0.12	±0.05	±0.07
Treatment						
1.2 dS/m	0.06 c	0.15 c	0.06 d	0.11 d	0.10 d	0.19 c
4.1 dS/m	0.31 b	0.19 a	0.30 c	0.23 c	0.37 c	0.45 b
7.0 dS/m	0.47 a	0.18 a	0.54 b	0.29 b	0.55 b	0.67 a
14.0 dS/m	0.55 a	0.20 a	0.64 a	0.35 a	0.62 a	0.66 a
SE	±0.08	±0.01	±0.12	±0.10	±0.15	±0.17

[z]Means within the columns followed by the same letter are not significantly different.
Means ± SE were assisted at $p \leq 0.05$.
p-Value for cultivar, treatment, and their interaction was ≤ 0.0001 for all parameters.

Therios, and Bosabalidis 2005). The effect of salinity on plants not fitted for survival in saline conditions is a 200 reduction in growth and yield (Mass and Hoffmann 1997).

Cultivars such as NB that maintained the highest DW under saline irrigation might be indicated as one of the most salt-tolerant olive cultivars. Similar reports were found by Therios and Misopolinos (1988) for cultivars Megaritiki and Chalkidikis, and by Tattini et al. (1995) for the cultivar Frantoio which were concluded to be the most salt-tolerant cultivars.

DWs of roots, stems, and leaves varied in their response to salinity. Roots under severe stress (14.0 dS/m) decreased by approximately 10%, while stem DWs decreased almost threefold and leaves had the largest decrease, dropping almost threefold less than leaves of plant irrigated with control treatments. These results indicate that the high salinity levels may actually alter the pattern of dry matter distribution favoring the roots. This phenomenon was also observed in kiwifruit (Chartzoulakis et al. 1995) and beans (Seeman and Critchley 1985).

SSL is a more accurate measurement with regard to plant assessment than height accumulation alone because it indicates a more stable plant (Chartzoulakis et al. 1995). Though NM plants are known to elongate faster as can be seen in Table 2, NB plants had the greatest SSL under all treatments (Table 2). This indicates that throughout all treatments, NB plants maintained the most desirable stronger and thicker stems and a wider and better water passage for the above plant parts.

The decline in leaf growth is the earliest response to glycophytes exposed to salt stress (Munns and Termaat 1986). This is caused in part to the ion accumulation in the leaves as an initial response to salt stress (Greenway and Munns 1980). However, NB's had no significant differences between irrigation treatments in leaf DW, which indicate a faster adaptation response to salt stress than the other 220 cultivars.

With regard to plant height accumulation, NB plants had slow but steady rates of growth. When compared to the other cultivars at high salt levels, NB plants had significantly higher elongation rates. NB plants had lower growth rates at 14.0 dS/m, which indicated their ability to resolve salinity irrigation up to 7.0 dS/m, as seen in Table 2. NB had the least affected rates of the growth parameters. It can be determined that the NB plants expressed genotypic variation that lessen the salinity effect, leading to the conclusion that when using growth parameters as selection criteria, NB plants are the most salt-tolerant.

Relative water content

Therios and Misopolios (1988) reported that olive trees treated with saline water decrease their water uptake. This is caused mainly by the decreased osmotic potential in the solutions containing the sodium chloride (NaCl) (Gucci, Lombardini, and Tattini 1997). Based on the results of our study, all
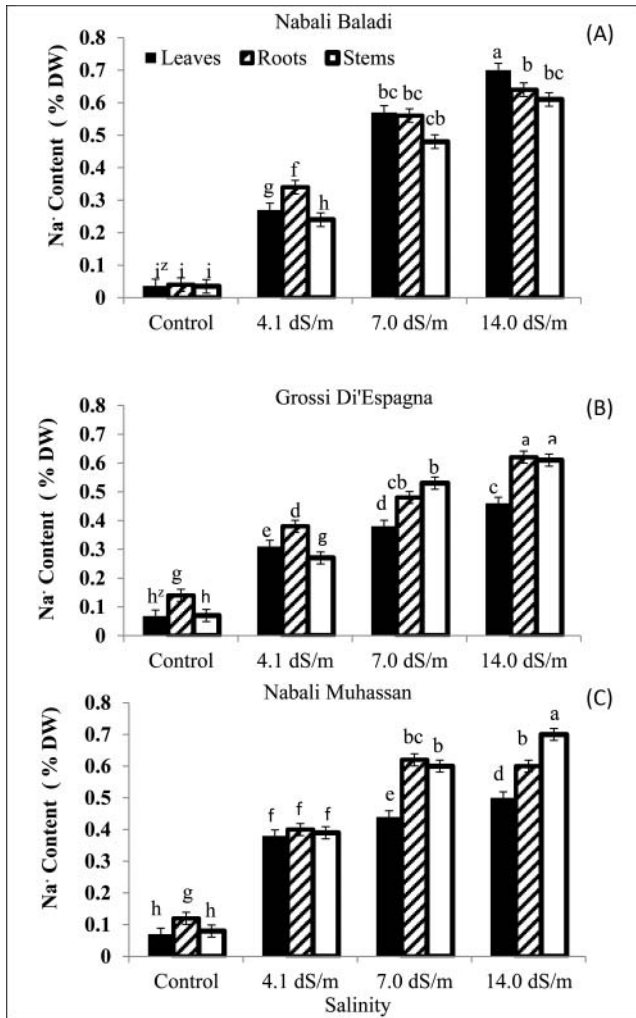
Figure 3. (A–C) Sodium content of controls and salt-treated plants of three cultivars. Data are means ± SE of 4 replicates. ^{z}Columns marked with the same letter were not significantly different at the $p \leq 0.05$ probability level.

cultivars experienced a significant decrease in their RWC (Table 2). NB plants had among the highest RWC when compared to other cultivars under all irrigation treatments.

The early response of olive trees to salinity is the reduction in the leaf water potential and RWC, which is the case in most woody crops. Olive trees are known to be salt-tolerant, and this is proven when studies show that RWC changes occur at higher-level salinities when compared to changes in other fruit trees irrigated with saline water (Banus and Primo-Millo 1992). The decrease in the RWC is a result of high salt concentration of the external solution which caused osmotic stress and dehydration at the cellular level. RWC is a representation of the ability of olive trees to exclude these "osmotic stress ions" in order to maintain their proper water balance (White and Broadley 2001). Therefore, by observing cultivars with the greatest ability to maintain a stable RWC, we not only gain insight into the grade of their defense, but can also screen for genotypic variations in their tolerance levels.

Results show that with regard to time, GE did not start to show a significant decline until the third month (around Day 95) of the treatment (Figure 1B), while NB started to decline within the first month. This does not necessarily mean that GE is more salt-tolerant. In fact, it could mean quite the opposite. The first adaptive mechanism of olive trees with regard to salt stress as mentioned earlier is to drop its RWC (Ben-Ahmed et al. 2006). This means that NB (Figure 1A) had a faster response time
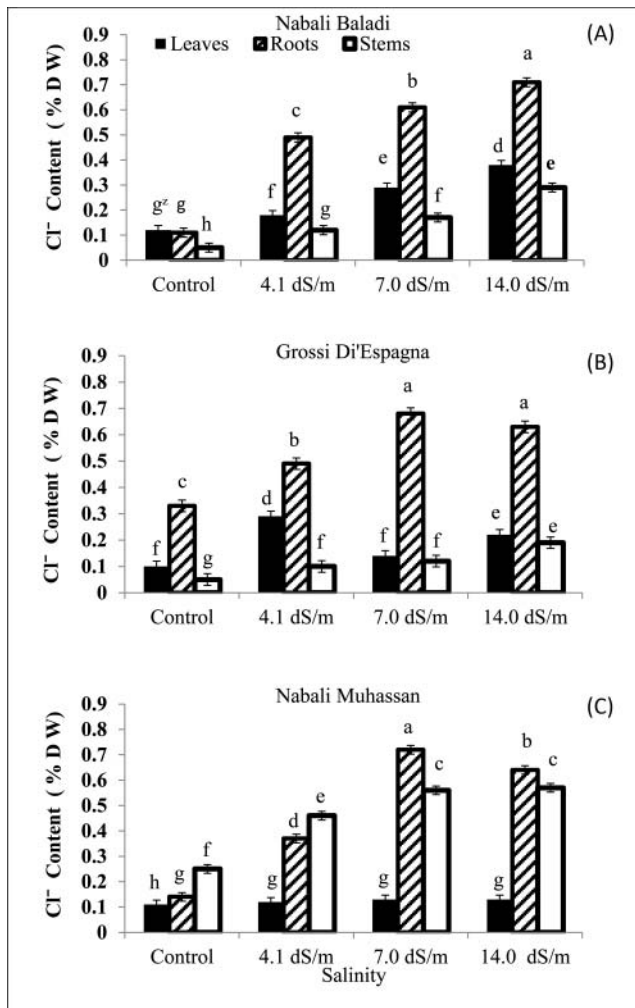
Figure 4. (A–C) Chloride content of controls and salt-treated plants of three cultivars. Data are means $\pm$ SE of 4 replicates. ^{z}Columns marked with the same letter were not significantly different at the $p \leq 0.05$ probability level.

to the salt stress than that of GE. NM (Figure 1C) also had a fast response time, but was not able to maintain its RWC above an acceptable level.

Based on their RWC, both GE and NB proved to be more salt-tolerant than NM, in co-ordinance with their ability to maintain an ideal or acceptable RWC at levels of severe salt stress. However, NB maintained the most stable and highest rates at a range of 70–75% which is deemed acceptable when irrigated with 7.0 dS/m.

Stomatal conductance

It has been concluded that beyond a certain salinity threshold solution concentrations can alter plant morphology and cause stomata to become less responsive to environmental changes (Loreto and Bongi 1987). Plants that are able to maintain increased levels of g_s are more adapted to survival and can tolerate higher levels of salt stress. NB and GE maintained a relatively high conductance rate through all treatments, with the exception of the severe salt stress of 14.0 dS/m (Table 2). Loreto and Bongi (2003) stated that perhaps the main limiting factor of photosynthesis is the inhibition of the g_s, due to the low chloroplast carbon dioxide levels that would cause inhibited stomata. One possible reason for the

closure of stomata may be the osmotic effect of salinity-induced abscisic acid or ABA accumulation, which can cause a reduction of g_s, intercellular carbon dioxide, chlorophyll content, and rubisco activity (Giorio, Sorrentino, and D'Andria 1999).

These results indicate that at severe salt stress levels, all plants would not be able to survive for very long. However, at a salinity concentration of 7.0 dS/M, NB and GE are comparatively functional, and are able to maintain an acceptable rate of g_s, while NM cannot. In addition, NB cultivars maintained the highest rates of g_s when irrigated with tap water, 4.1 and 7.0 dS/m. NB plants also maintained among the highest rates of g_s when irrigated with 14.0 dS/m. That would lead to the conclusion that NB in co-ordnance with g_s would be considered the most salt-tolerant cultivar, especially at levels of 7.0 dS/m or less.

Chlorophyll index

A decrease in chlorophyll content and photosynthesis seems to be a direct result of the g_s rates. Salinity is known to reduce net g_s and thus photosynthetic rate (Tezara et al. 2002; Burman, Garg, and Kathju 2003). The results of the chlorophyll content (Table 2) indicate that NM begins to lose its chlorophyll when treated with a salinity that is generally accepted as an appropriate irrigation level (7.0 dS/m). By observing the chlorophyll content of plants one can gain greater insight into the salt tolerance mechanisms of a plant. At high levels of salinity, chlorophyll is degraded (Malibari et al. 1993; Salama et al. 1994). NM has the lowest chlorophyll index rate under all treatments. Their values were especially low at irrigation treatments with 14.0 dS/m (60). Excess salt in photosynthetic material also causes shrinkage of thykaloids and stacking of adjacent membranes of grana. Ionic imbalances can also cause the reduction of chloroplasts (Blumwald 2000).

Ion content

Osmotic adjustment or accumulation of solutes by cells is a process by which water potential or a cell can be decreased without accompanying decrease in cell turgor (Taiz and Zeiger 2002). Osmotic adjustment in plants subjected to salt stress can occur by the accumulation of organic or inorganic solutes. In some cases, accumulation of solutes is so high that it goes beyond the limits of regulation of cytoplasm content with associated impairments of growth (Pitman 1984), which may be expected in severe salt stress such as that of 14.0 dS/m.

Plants deal with ions in two ways. These ways are termed ion exclusion or inclusion. In plants that exclude ions, a major mechanism of salt tolerance is to keep the salt ions away from the meristems, particularly in the aerial parts including the actively expanding leaves that are photosynthesizing (Bartolini, Mazuelos, and Troncoso 1991). In these plants, either accumulation of organic solutes and a variety of inorganic ions must be increased to achieve osmotic balance. In contrast, plants that undergo inclusion of ions under salt stress, the principal mechanism is to use Na^+ and Cl^- ions with osmotic adjustment at the organ, tissue, or cellular level (Greenway and Munns 1980; Wyn Jones 1981).

Salt tolerance in glycophytes is associated with the ability to limit the uptake and/or transport of ions mainly Na^+ and Cl^- from the root zone to aerial parts (Greenway and Munns 1980). This would keep harmful ions away from the photosynthesizing leaves. Thus, indicating that olive trees should exercise methods of inclusion. Our data clearly indicate that although the olive tree cultivars should be excluding ions, there is still an increase in ion content of all plant organs of all cultivars (Table 3). Both Na^+ and Cl^- ions increased within plant tissue. While this seems counterintuitive to the notion of "exclusion," it should be mentioned that excluding means limiting. At high concentrations of salt stress, the plant is not able to properly exclude the ions (Seemann and Critchley 1985). This can be seen in Table 3, with plants watered at EC levels of both 7.0 and 14.0 dS/m. Cultivars treated with moderate salinity stress indicate significantly less ion accumulation in the leaves than in the roots for all cultivars (Table 3). However, at higher levels of salinity, ions begin to accumulate in the leaves.

To elaborate further, all cultivars exhibited increased sodium and chloride ions at the root level (Table 3), in all treated plants. This would explain the ion exclusion at the roots, and that the plants

attempt to maintain the ions away from the actively photosynthesizing leaves, or aerial parts. As the salinity levels become more severe, there is a dramatic increase in the amount of ion in the roots, indicating more active exclusion which as seen with NB plants shows a fivefold increase between plants irrigated with control and 14.0 dS/m (Figures 3 and 4).

When trying to determine salt tolerance success, it is imperative to not assume that plants with the least amount of accumulated ion are most successful. It is more important to observe where the ions are distributed (Marin, Benlloch, and Fernandez-Escobar 1995). Na$^+$ ions of NB were found mostly in the roots throughout most treatments (Figure 3A). In severely stressed plants (14.0 dS/m), however, NB experienced more sodium in the leaves than in the roots, which coincides with the trends mentioned earlier and that was not the case for GE and NM (Figure 3B and C).

In NB plants, Cl$^-$ (Figure 4A) were significantly higher in the roots of all treated plants. Chloride ions were also significantly higher in the leaves when compared to the stems, which again fits the known and accepted trends of salt ion exclusion. This same pattern was observed for GE olive plants for all treatments. Notably, NB exclusion of chloride to the leaves (Figure 4A) is not as prevalent as that of sodium ions (Figure 3A), which may suggest a more efficient exclusion method for chloride than for sodium (Tester and Davenport 2003). GE (Figure 3B) also contains a significantly larger amount of sodium in the roots than in stems or leaves, which again suggests a more sufficient exclusion method for chloride ions. NM is drastically different than the other two cultivars. Surprisingly there was a very large amount of chloride ions found in the stems (Figure 4C). This is negative due to the fact that stems are the least favorable place for the ions to exist. Ion exclusion and compartmentalization at the root level regulates ion concentration in the xylem sap preventing accumulation of potentially harmful toxic ions in the aerial parts (Flowers and Yeo 1989; Drew, Hole, and Picchioni 1990). If they are in the roots they are kept at a far distance from the photosynthesizing material, if they are in the leaves they display toxic effects and may soon be dropped, but the stems are active transport sites and exhibit no physical mechanism of exclusion (Heimler et al. 1995). The lack of chloride ions in the leaves and the increased amount in the stem may predict some type of failure in the plant to export its toxic ions successfully to the leaves of the plant.

The noticed differences among cultivars at high salinity fits with the general findings that differences in the capacity for sodium retention in the roots reflects differences in salt tolerance (Prat and Fathi-Ettai 1990; Reiman 1992). When using ion content exclusion as a method of salt tolerance screening, NB is the most salt-tolerant, while NM is the least.

Conclusions

Increased salinity leads to reduction of all plant growth parameters (DWs of the roots, stems, and leaves). Results indicate that the high salinity levels may actually alter the pattern of dry matter distribution favoring the roots. Under salt stress, wider and better water passage for the above plant parts could be achieved by plants by maintaining stronger and thicker stems. With regards to plant height accumulation, the slow but steady rates of growth could be an adaptation in salt-tolerant plants. Reducing leaf RWC is an early response in salt stress olives. Maintaining sufficient stomatal conductance under moderate salt stress varies among olive cultivars.

The results of the stomatal conductance coincide with the results of chlorophyll content, which prove the strong relationship between them in the salt-stressed olive plants.

Salt-tolerant olives have more efficient exclusion method for chloride to the leaves than for sodium. Salt-tolerant olive plants exhibited more sodium and chloride ions in the root than in stems or leaves. This would explain the ion exclusion at the roots, and the plants attempt to maintain the ions away from the actively photosynthesizing leaves, or aerial parts.

NB was able to maintain the best rates for most of the parameters. In addition, with regard to ion content, NB was the most efficient, and followed the pattern of successful ion exclusion methods usually affiliated with salt tolerant plants.

Perhaps NB's success can be attributed to its long-standing residency in the country of Jordan as the most popular and widely grown tree. It is perfectly adapted to living and surviving in such semiarid environment, which may allow for less energy consumption than the non-native GE, or the cultivar NM. More studies would be needed to discern the effect of salinity on crop yield of olives which would only be possible with older trees in the field. Other studies that would also be useful would include prolonged irrigation of saline solutions to observe and analyze long-term effects.

Acknowledgments

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