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Short communication

The use of legume and grass cover crops induced changes in ion accumulation, growth and physiological performance of young olive trees irrigated with high-salinity water

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ABSTRACT

The impact of intercropping with two different forage crops (legume and grass) on the shoot growth, ion accumulation and some physiological traits of 3-old year olive trees irrigated by high-salinity water (120 mM NaCl) was assessed under greenhouse conditions. Olive plants were subjected to one of the following treatments: TC (control); TG – olive plants intercropped with grass (Oats: *Avena sativa* L) and TL – olive trees intercropped with legume (Berseem Clover: *Trifolium alexandrinum* L). Results showed that sodium root:leaf ratio and nitrogen concentration in olive leaves were significantly increased in TL treatment. Olive shoot length and maximum quantum yield of photosystem II (Fv/Fm) were significantly improved by using Berseem Clover as a cover crop. Negative correlation was observed between the total chlorophyll content and the leaf Na^+ concentration. On the other hand, polyphenol content was significantly increased in olive leaves when oats were used as cover crops, in relation to the high N-deficiency observed in olive leaves of TG treatment.

1. Introduction

Salt affects one third of the worlds irrigated surface, especially in arid, semiarid and coastal regions (Munns, 1993; Tabatabaei, 2006). Saline areas occupy 1 billion ha and are increasing worldwide (Munns, 1993). Salinity problems in crop production will become worse in areas with rapidly growing human population and limited water resources, which force growers to use poor quality water for irrigation. Soil salinity is a serious threat limiting crop production since it adversely reduces the overall productivity of the ecosystem (Munns, 2002).

In Tunisia, nearly 100 000 ha of irrigated lands were deeply affected by salinization and 75% of the soils were in the range of medium to highly sensitive to salinity. Irrigated lands by saline water were mostly occupied by forage crops resistant to salinity. Forages produced by irrigation with saline water provide additional income sources for farmers in marginal lands (Stenhouse and Kijne, 2006). In Tunisia in areas irrigated with saline water (4–7 g/l) the most commonly used forage species were Berseem and Oats. Agarwal et al. (2010) reported that most salt-tolerant genotypes of Berseem (*Trifolium alexandrinum L.)* were sometimes used in salinized areas where they showed significant reductions in biomass productions only at salinity levels of 70 and 140 mM NaCl. According to Murty et al. (1984), Oats (*Avena Sativa L.*) are considered to be a moderately salt-tolerant species compared with other cereal or forage crops. More recently NSW Department of Primary Industries (2017) found that oats show significant reduction in biomass when salinity exceeds 40 mM.

In a large Tunisian agricultural area, olive is the most cultivated fruit crop, with approximately 70 million trees covering 1600 thousand hectares of land. Traditionally, olive tree has been grown under rainfed conditions, since it is a crop well adapted to the semiarid and arid Mediterranean climates, and able to overcome periods of intense drought, while still producing a reasonable yield. However, the surface of irrigated olive orchards has increased considerably during the last years, motivated by the improvement in the yield when irrigated, and also by the spectacular increase of olive oil prices. But, increasing irrigated areas is very difficult for the olive industry, due to water scarcity and the increased competition with non agricultural uses (Fereres et al., 2003). Olive trees (Olea europaea L.) that are broadly cultivated in the Mediterranean region have an intermediate tolerance to salinity stress. In fact, the exposure to high sodium for a prolonged period of

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time severely reduces the yield (Gucci and Tattini, 2010; Fernández, 2014). Tolerance to NaCl in olive trees is mostly related to the salt exclusion mechanism at the root level, which prevents Na^+ accumulation in leaf tissues, as well as to the ability of the olive tree to maintain an adequate K^+/Na^+ ratio (Chartzoulakis, 2005; Kchaou et al., 2010). When olive is irrigated with water containing NaCl, growth reduction and onset of damage are more correlated to Na^+ than Cl^- accumulation in leaves (Kchaou et al., 2010; Bader et al., 2015). As a result, the intercropping of olive trees with salt-tolerant forages may be a promising solution to increase olive productivity when irrigated with saline water.

The aim of this work was to investigate the effect of intercropping olive trees with two forage crops (grass: Oats, and legume: Berseem Clover) on sodium and nitrogen accumulation in olive roots and leaves of Chemlali olive cultivar, irrigated with high salinity water that is frequently used in the arid region of Tunisia. Furthermore, we evaluated changes in some plant physiological (Fv/Fm, chlorophyll content, total phenols) and growth (shoot length) traits induced by the cover crops. To our knowledge, this is the first report that addressed the above issues, testing the possibility to manage ecologically salinity and increasing saline water productivity.

2. Material and methods

2.1. Plant material and culture conditions

Trials were conducted in the specialized station of the Olive Tree Institute of Sousse, Tunisia (35°49′34″N; 10°38′24″E). Uniform 3 year-old self-rooted olive trees (*Olea europaea* L. cv Chemlali) of about 1.5 m height were transplanted into 30-l pots filled with a mixture of soil, sand and manure (1:1:1, v/v/v).

Plants were grown for three months of adaptation inside a plastic green house (5 m × 3 m) that was opened in its extremes during the whole experiments, trying to alter as less as possible sunlight irradiance, air temperature and relative humidity but safe from undesired rain. Average temperature and humidity during the experiments inside a plastic green house were 23 °C and 68% respectively.

Forage seeds (4 g) were cultivated in November 2015, and all olive plants were irrigated with tap water until Jaunary 2016. Afterward, to avoid salinity shock, all olive plants were progressively irrigated with saline water for 15 days to reach the final salinity concentration of 120 mM NaCl. The exposure period starts at 120 mM NaCl.

The experimental design was a completely randomized one with three treatments and six replicates per treatment:

- Control plants (TC): olive trees grown without any associated cover crop.
- TG: olive trees intercropped with grass (Oats: *Avena sativa* L)
- TL: olive trees intercropped with a legume (Berseem Clover: *Trifolium alexandrinum* L).

2.2. Growth measurements

Before salinity exposure, the trees were marked just below the shoot tip in order to distinguish new growth from old growth. Three shoots per tree (6 trees per treatment) were selected, the length of the main-shoot was measured (M1, in cm). At the end of the exposure period (90 days after salinity application: DASA), the length of the main-shoot (in cm) was also measured (M2) and the final shoot growth was calculated as M2-M1 (in cm).

2.3. Mineral analyses

Mineral analyses were carried out at the end of the experimentation at 90 DASA on leaf and root dry materials which were obtained at 400 °C after drying at 65 °C until constant weight. The mineralization of the samples was obtained after a digestion process in 1 N nitric acid solution (HNO_3^-). The sodium (Na^+) content was measured using the flame emission photometry (Jenway PFP7, Bibby Scientific limited, Staffordshire, UK). Leaf N content was determined using the Kjeldahl method. All the values reported represent the means of at least 6 replications.

2.4. Chlorophyll a fluorescence measurement

Chlorophyll a fluorescence parameters were measured using a fluoremeter FIM 1500, ADC (Fluorescence Induction Monitor 1500, Anatyticol Developement Limited), according to the method described by Angelopoulos et al. (1996). Briefly, measurements were performed on 12 fully expanded mature leaves without visible injury symptoms. Leaves were darkened for 30 min prior to measurement. After 30 min of dark adaptation, the maximal quantum efficiency of photosystem II (Fv/Fm; Fv = Fm-F0) was obtained. F0 and Fm were respectively the minimum and maximum fluorescence yields in dark adapted samples, with all *Psi*I reaction centers fully open or closed (Faraloni et al., 2011).

2.5. Total chlorophyll content

Chlorophylls were extracted in 80% acetone from a fresh leaf sample (0.5 g) and absorbance was determined at 663 and 647 nm (Tekaya et al., 2016). Data were expressed as mg/g fresh weight. All reported values represent the means of at least 6 replications.

2.6. Total phenol content

10 ml of methanol was used for the extraction of phenolic compounds from fresh leaves (0.5 g). Tubes were placed on a shaker at 200 rpm for 24 h. After centrifugation (5000*g* for 10 min), leaf extracts were stored at −20 °C. The extracts were filtered thought a 0.45 μm syringe filter prior to analysis (Taamalli et al., 2012). Total phenols were quantified using the method of Montedoro et al. (1992), the Folin–Ciocalteau reagent (Merck Schuchardt OHG, Hohenbrunn, Germany) was added to the methanolic extracts. The absorbance was then read at 765 nm. All reported values represent the means of at least 6 replications.

2.7. Statistical analyses

All statistical analyses were performed using the SPSS 16.0 for Windows statistical package (SPSS, Chicago, IL, USA). The data were subjected to one-way analysis of variance (ANOVA). In order to determine significant differences ($P \leq 0.05$) between treatments, comparison between means was performed using the Tukey-HSD tests.

3. Results

3.1. Effect of using cover crops on olive plant growth

Measured at the end of the experiment (after 90 DASA), the lowest values of shoot length were reported in TC (1.5 cm) (Fig. 1). Shoot length increased significantly to reach 2.2 cm and 6.4 cm in TG and TL treatments, respectively.

3.2. Changes in sodium and nitrogen contents

Sodium content in TC olive roots was about 0.26%, it increased significantly using the forage cover crops (Fig. 2A). Compared to TC, the sodium content in olive roots increased significantly to 0.4 and 0.59% in TG and TL treatments respectively. In contrast, the intercropping with grass and legume decreased significantly the olive leaf Na^+ by about 8.7% and 31.7% in TG and TL treatments respectively (Fig. 2A). Sodium root: leaf ratio was 45.75 in TC, it increased