Environmental and Experimental Botany 71 (2011) 184–191

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Effects of agronomic application of olive mill wastewater in a field of olive trees on carbohydrate profiles, chlorophyll a fluorescence and mineral nutrient content

Beligh Mechri[a,b,*], Hechmi Cheheb[b], Olfa Boussadia[b], Faouzi Attia[b], Fethi Ben Mariem[b], Mohamed Braham[b], Mohamed Hammami[a]

[a] Laboratoire de Biochimie, USCR Spectrométrie de Masse, UR Nutrition et Désordres Métaboliques, Faculté de Médecine, Monastir, Tunisia
[b] Laboratoire d'Ecophysiologie, Institut de L'Olivier de Sousse, Tunisia

ARTICLE INFO

Article history:
Received 23 October 2010
Received in revised form
28 November 2010
Accepted 2 December 2010

Keywords:
Olive mill wastewaters
Arbuscular mycorrhizal fungi
PSII efficiency
Soluble carbohydrate

ABSTRACT

Olive mill wastewater (OMW) management is a serious environmental issue for the Mediterranean area where there is the most production of olive oil. OMW contains a high organic load, substantial amounts of plant nutrients but also several compounds with recognized toxicity towards living organisms. Moreover, OMW may represent a low cost source of water. We studied the influence of irrigation with OMW (amounts applied: 30, 60, 100 and 150 $m^3\ h^{-1}$) in a field of olive trees on root colonization, photosynthesis, chlorophyll fluorescence, leaf nutrient concentration and soluble carbohydrate. The soil fatty acid methyl ester (FAME) 16:1ω5 was used to quantify biomass of arbuscular mycorrhizal (AM) fungi and the root FAME 16:1ω5 analysis was used as index for the development of colonization in the roots. Agronomic application of OMW decreased significantly the abundance of the soil FAME 16:1ω5 and the root FAME 16:1ω5 in the soil amended with 60, 100 and 150 $m^3\ ha^{-1}$ OMW. Decreased root FAME 16:1ω5 due to OMW amendment was associated with a significant reduction of tissue nutrient concentrations in the olive trees. The highest application of OMW to the soil reduced significantly the olive trees uptake of N, P, K, Ca, Mg, Fe, Cu, Mn and Zn. Land spreading of OMW increased concentration of soluble carbohydrate in the olive leaves, mostly due to decreased sink demand for carbon by the root. In the olive trees amended with 150 $m^3\ ha^{-1}$ OMW, net CO_2 uptake rate (A), quantum yield of photosystem II electron transport (Φ_{PSII}), maximal photochemical efficiency of photosystem II (Fv/Fm), photochemical quenching (q_p) and the electron transport rate (ETR) were significantly depressed, whereas non-photochemical quenching (NPQ) was found to increase. Taken with data from experiments in field conditions, our results suggest that agronomic application of OMW alters the functioning of arbuscular mycorrhizas and can even disrupt the relationship between AM fungi and olive trees.

1. Introduction

The industrial olive oil sector generates large quantities of solid and liquid wastes and by-products in these countries during a short period of time (November–February). Around 30 million m^3 of olive mill wastewater (OMW) are produced annually in the Mediterranean area, causing environmental concern (Casa et al., 2003). Some characteristics of OMW are favourable for agriculture, since this effluent is rich in organic matter, nitrogen, phosphorous, potassium and magnesium (Paredes et al., 1999; Rinaldi et al., 2003). The organic fraction of the OMW contains a complex consortium of phenolic substances, some nitrogenous compounds (especially amino acids), organic acids, sugars, tannins, pectins, carotenoids, polyphenols and almost all of the water soluble constituents of the olives (Mulinacci et al., 2001; Lesage-Meessen et al., 2001). The inorganic fraction contains chloride, sulfate, and phosphoric salts of potassium as well as calcium, iron, magnesium, sodium, copper, and other trace elements in various chemical forms. The inorganic constituents at the concentration levels found in OMW are not toxic; quite the reverse, they may potentially serve as good sources of plant nutrients and thereby rendering this effluent potentially suitable for recycling as a soil amendment (Rinaldi et al., 2003). Additionally, in organic and sustainable farming, the nutritional value of OMW as well as its potential herbicidal activity (Ghosheh et al., 1999), and ability to induce suppression against soil-borne plant pathogens are of extra value (Kotsou et al., 2004).

Abbreviations: OMW, olive mill wastewater; FAME, fatty acid methyl ester; AM fungi, arbuscular mycorrhizal fungi; A, net carbon dioxide uptake rate; PSII, photosystem II; Fv/Fm, maximal photochemical efficiency of photosystem II; Φ_{PSII}, quantum yield of photosystem II electron transport; NPQ, non-photochemical quenching; q_p, photochemical quenching; ETR, electron transport rate; QA, primary electron acceptor of PSII.

* Corresponding author at: Laboratoire de Biochimie, USCR Spectrométrie de Masse, UR Nutrition et Désordres Métaboliques, Faculté de Médecine, Monastir, Tunisia. Tel.: +216 73 462 200; fax: +216 73 460 737.
E-mail address: beligh.mechri77@yahoo.fr (B. Mechri).

0098-8472/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.envexpbot.2010.12.004

The role of AM fungi in the stimulation of growth and nutrient uptake of many host plants is well documented (Smith and Read, 1997; Jeffries et al., 2003). Olive plants are known to host AM fungi and can be colonized by different species, including *Glomus mosseae*, *Glomus clarum*, *Glomus caledonium*, *Glomus monosporum*, *Glomus intraradices and Glomus viscosum* (Jamil Mohammad et al., 2003; Calvente et al., 2004; Porras-Soriano et al., 2009). Mycorrhiza can enhance the uptake of several nutrients by plants, including P (Smith and Read, 1997), N (Hawkins et al., 2000; Xiao et al., 2010), K (Porras-Soriano et al., 2009), Ca (Rhodes and Gerdemann, 1978), Zn (Subramanian and Charest, 1997; Subramanian et al., 2009), Cu, Mn, and Fe (Miransari et al., 2009). In exchange of translocating mineral nutrients from the soil to the host plant, AM fungi receive carbon from their host plant (Smith and Read, 1997; Nakano-Hylander and Olsson, 2007). AM fungi, in addition to being functionally related as a group, share certain common biochemical characteristic distinguishing them from most other fungi (Morton and Benny, 1990). One of these is the abundance of the fatty acid 16:1ω5 in both the phospholipids of the membrane and in the neutral lipids (Johansen et al., 1996). This fatty acid has been demonstrated to be of use in the detection and biomass quantification of AM mycelium growth in soil (Olsson et al., 1995). This fatty acid accumulates in roots during AM fungus colonization (Graham et al., 1996; Olsson et al., 1995), and the amount accumulated is correlated to microscopically estimated measures of total root colonization (Olsson et al., 1997). We used this approach to understand the abundance of AM fungi in soil and also the olive trees root colonization following land spreading of OMW.

Phenolics compounds are able to influence plant growth and root colonization (Siqueira et al., 1991). However, the role of phenolics in the AM symbiosis is not well established. Some exogenously applied phenolics may stimulate indigenous populations of AM fungi in the field resulting in increased plant growth and yields (Siqueira et al., 1991). However, phenolic compounds may also inhibit the establishment of AM symbioses and decrease the host growth (Leadir et al., 1997). Thus, addition of OMW to soil might result in increased colonization of root by AM fungi. However, these residues contain several to 10 g of total polyphenols per liter (Fountoulakis et al., 2002; Sabbah et al., 2004), which may suppress plant growth directly and/or through the decrease of AM symbiosis.

All plants absorb light with their pigments and a small part of the absorbed light can be re-emitted as fluorescence that originates mainly from chlorophyll a molecule of photosystem II (PSII). The maximum fluorescence emission amounts to approximately 3%, the minimum to approximately 0.6% of the absorbed light (Krause and Weis, 1991). Studies of the photosynthetic activity in different organisms are often of particular interest, and by using chlorophyll fluorescence measurements, photosynthetic activity can easily be detected. Factors causing damage to the photosynthetic system will therefore be recognized through changes in the different fluorescence parameters (Van Kooten and Snel, 1990). Analysis of chlorophyll a fluorescence transients has provided evidence for abiotic stresses, like light intensity (Haldimann et al., 1996), drought (Correia et al., 2006), temperature (Fracheboud et al., 1999), herbicide (Bigot et al., 2007) nutrient deficiency (Morales et al., 2000) and heavy metals (Mallicka and Mohnb, 2003)

PSII is essential to the regulation of photosynthesis because it catalyses the oxidation of water into oxygen and supports electron transport (Geiken et al., 1998). Koves-Pechy et al. (1998) and Shrestha et al. (1995) have reported on a beneficial role of mycorrhizal symbiosis on PS II activity and net gas exchange, respectively.

In view of the above background, the following questions were addressed: (i) Is agronomic application of OMW in a field of olive trees beneficial or detrimental to the growth of AM fungi? (ii) If land spreading of OMW has an effect (positive effect or negative effect) on the abundance of AM fungi, does A, Φ_{PSII}, leaf nutrient concentration, and the pool of soluble carbohydrate in the olive trees change?

2. Materials and methods

2.1. Characteristics of the OMW used for irrigation

The original OMW used in the present study was obtained from an olive oil production plant located in the city of Ouled Jaballah, Tunisia, which uses discontinuous process for extraction of olive oil. The main characteristics of the OMW were: pH: 5.1; electrical conductivity (EC): 9.1 dS m^{-1}; salinity: 6.37 g l^{-1}; chemical oxygen demand (COD): 93 g l^{-1}; N: 1340 mg l^{-1}; P: 720 mg l^{-1}; K: 6200 mg l^{-1}: phenols: 8400 mg l^{-1}.

2.2. Experimental design

Trees located at Ouled Jaballah, Tunisia, North latitude 35°12′, East longitude, 10°59′, spaced 12 m × 12 m apart were selected in 2004 for the experiments. The climate of this region is typical Mediterranean, semiarid to arid, with an average rainfall of 200 mm $year^{-1}$ and an average annual temperature of 18–20 °C. Physico-chemical characteristics of the olive trees soil used in this study were as follows: pH (H_2O) 8.53; EC: 0.44 dS m^{-1}: sand: 78.1%; clay: 12.85%; silt: 5.1%; organic C: 0.37%; N: 0.042%; Olsen P: 20.86 mg kg^{-1}; exchangeable K: 0.43 (meq 100 g^{-1}). The experiment included four levels of OMW application (TC: 0 m^3 ha^{-1}, T1: 30 m^3 ha^{-1}, T2: 60 m^3 ha^{-1}, T3: 100 m^3 ha^{-1} and T4: 150 m^3 ha^{-1}). Threes plots (576 m^2 each: 24 m × 24 m; 4 olive trees per plot) were designed for each treatment (12 olive trees per treatment). The application of OMW as soil amendment was realized on December 2004 in one application.

2.3. Roots, leaves and soil sampling

Roots and leaves samples were collected at after approximately one year of agronomic application of OMW (February 2006). A composite root sample was collected from each plot at 10 to 20 cm depth. The roots were divided into two subsamples, one of them stored at −20 °C for later fatty acid measurements (root colonization) and the other used for soluble carbohydrate measurements. From each plot, approximately 50 mature leaves were collected in paper bags and stored in a portable ice chest. Once in the laboratory, leaves were washed with 0.03% Triton X-100 and rinsed in deionized water. Dry mass of leaves from each plot was recorded after drying in a hot air oven at 70 °C to a constant weight and grinding to a fine powder. Soil sample was collected at this time. All sampling events included the collection of soil samples from each plot at 0 to 20 cm deep. In the laboratory, soil subsamples were sieved (<2 mm) and used for fatty acids measurements (abundance of AM fungi).

2.4. Soil and root fatty acid methyl ester (FAME) analysis

Lipids were extracted from the soil samples (3 g) as well as the root samples (30 mg) using the EL-FAME method (Schutter and Dick, 2000). Briefly, a mild alkaline hydrolysis (0.2 M KOH in methanol) was used to extract whole cell fatty acids (FA). The FAME extraction residue was dissolved in hexane. The hexane layer was transferred to a clean tube, and the hexane was evaporated off, after which FAMEs were resuspended in 0.5 ml of hexane–methyl tert-butyl ether (1:1) for analysis. Individual FAMEs were analyzed on a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector and a HP-5MS column (95% dimethyl–5% diphenyl polysiloxane, length 30 m × 0.25 mm). The temperature was programmed to increase from 170 to 270 °C at a rate of