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Monitoring of mineral and polyphenol content in olive leaves under drought conditions: Application chemometric techniques

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ABSTRACT

Flavonoids and phenolic acids are of the most important bioactive compounds in plants owing to the physiological and ecological significance but the contents are dependent on a number of different factors, such as plant species, harvesting times, environmental conditions, soil and plant mineral content, extraction techniques, etc. Nutritional status of plants is of great importance for sustainable crop productivity and desired traits of plants. In this context, the present study was designed to investigate and correlate the plant nutritional status with total phenolic (TPC) and flavonoid contents (TFC). Results showed that the responses of both cultivars were different with respect to the element accumulation concentration and accumulation times. cv. Gemlik was not affected by irrigation periods whereas cv. Kilis Yaglik was influenced but after irrigation periods the samples were allowed to be in the same group and did not differ for cv. Kilis Yaglik.

1. Introduction

Among the abiotic stresses, water shortage is considered to be one of the major limitations for sustainable agriculture worldwide (Din et al., 2011; Anjum et al., 2012; Lefèvre et al., 2012). On a global basis, drought assumed to be the consequences of soil and/or atmospheric water deficits has substantial eco-physiological constraints to plant survival and to crop productivity and quality (Boyer, 1982). Herein, water deficits lead to the induced nutritional disorders which may be attributed as consequences of mineral availability, competition, uptake and translocation of minerals within the plant or specifically in different parts of plant or cells (Grattan and Grieve, 1999; Bartels and Sunkar, 2005; Munns and Tester, 2008; Yousif et al., 2010). Any disruption with respect to the ionic or cationic compositions within the plant may influence the normal or complete life cycle since the elements are specific and cannot be replaced by another one. Consequently, functional metabolisms are directly affected by the disruption deviating from optimal nutrition (Sharma, 2006). For example, increase in P uptake provides advantages of symbiotic relationship between the roots and mycorrhiza and subsequently enhances both the growth and tolerance of plants against drought and also uptake of P, Zn, Cu, Mn and Fe (Bagayoko et al., 2000). Therefore, the nutrient availability, uptake, transport, accumulation and especially interaction between nutrient supply are of the important factors regarding tolerance or growth and it is worthy to note that water deficiency does not affect plants in a way hence its impacts are extremely complex (Henckel, 1964). These effects are dependent on different plant species and even the chemo-types or varieties of the same species. Therefore, universal and uniform mechanism of acclimatization with respect to irrigation systems cannot be generalized and depending on this fact, research in plants responses to water deficit has been important interest for scientists for many years in order to develop, improve or explore drought-tolerant plant species or cultivars, genotypes within the same species for a sustainable agricultural production, especially in agrarian cultures worldwide (Hamrouni et al., 2001; Turk et al., 2004; Al-Barrak, 2006; Bettaieb et al., 2009; Bybordi, 2010).

Mineral deficiencies resulting from inadequate supply of elements disrupt plant metabolism and function and deficiencies or excessive amounts of one element may induce deficiencies or excessive accumulations of another (Taiz and Zeiger, 2008).

Olive trees (*Olea europaea*) are native to the eastern Mediterranean basin that spread westwards beyond Turkey into Europe. Olive is the most important crop for Kilis province (Turkey) and its surroundings, which are located in the south eastern region

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of Turkey. In the region, Kilis Yaglik and Gemlik cultivars are of the most common cultivated olives. Kilis Yaglik is native to the region but Gemlik was introduced to the region and cultivated in the recent years. Those cultivars are well-known as the table and oil olive cultivars and have significant economic value. Ripe fruits and edible oil extracted from the fruits of olive trees are the main parts used in Mediterranean cousins. Besides these edible uses, olive oil and leaves have been used as traditional medicine in this region. Clinical evidence on curing effects of olive leaf for some ailments such as lowering blood pressure, enhancing immune system, antibacterial, antifungal and anti-inflammatory have been proven. Because of its active polyphenols effects, olive has an important place in the Mediterranean diet (Ryan et al., 2001; Ferreira et al., 2007). Besides this it is obvious that the leaves are rich in polyphenols, especially in oleuropein, rutin, verbacoside, apigenin-7-glucoside and luteolin-7-glucoside, olive leaves have also been used by native people in folk medicine in these areas. (Benavente-Garcıa et al., 2000; Savournin et al., 2001). The quality, origin and variety of the plant material effects concentration of polyphenolic compounds in olive leaves (Campeol et al., 2003). The leaves of the olive varieties contain high amounts of polyphenols and flavonoids. Of those polyphenols, oleuropeins are of the most common components in the composition. The high content of antioxidative compound, oleuropein, is promising for the pharmaceutical industry in the future (Salah et al., 2012).

In addition to the secondary metabolite content and composition in commonly consumed plants, their mineral composition also has a vital importance for proper human health through modulation of enzymes and thus in an essential way affecting the biochemical and physiological processes in cellular metabolism (Łozak et al., 2002). From the view of recent scientific reports, desired quantity of mineral composition and contents in food and medicinal plants are advised for healthy life. However it is furthermore emphasized that excessive doses and accumulation of these elements, especially heavy metals, could cause serious health problems. To determine the mineral compositions of some food and medicinal crops, scientific studies have recently been enhanced. Most of the Turkish medicinal plants and products have also been studied for their mineral compositions, as well. In the recent years, we have focused on the determination of mineral contents of edible parts of the plants (Sekeroglu et al., 2012, 2011, 2008; Koca et al., 2009; Ozkutlu et al., 2007).

Application of chemo-metric approach in characterization of experimental samples has been extensively applied to quantitative evaluation of discrimination of variable results. In the present study, olive cultivars, experimental conditions and harvesting times in elemental concentrations and polyphenol contents were tested by analysis of variance (ANOVA) followed by the multiple comparison test of Duncan using SPSS. Due to the existence of different experimental factors, chemo-metric techniques including Principal Component Analysis (PCA) were applied for: (i) analytical evaluation of water scarcity between cultivars, (ii) identification of monthly elemental changes.

2. Material and methods

2.1. Experimental material and sampling locations

Two olive cultivars namely (Kilis Yaglik and Gemlik) were about 8 years old grown in the orchard of the Kilis province (Turkey). Kilis Yaglik is native to the region but Gemlik was introduced to the region and cultivated in the recent years. For irrigation system, 12-month field manipulation irrigation experiment in two olive cultivars. The cultivars were exposed to irrigation and non-irrigation (April–September) and natural climate conditions of the region (October–March). For the irrigated groups, the trees were irrigated twice each week applying drip irrigation and the second group was not irrigated.

2.2. Geology of the sampling areas

Kilis province are characterized with Mediterranean red soil, limeless brown forest soil, basaltic and colluvial soils. Mediterranean red forest soils are poor in organic matter and silica but rich in ferric oxide, calcium oxide and aluminum oxide. Basaltic soil and limeless forest soils have relatively high silica and ferric oxide contents but low aluminum oxide and organic matter. Colluvial soils include high content of calcium carbonate and calcium oxide and low content of silica, aluminum oxide and organic matter are lower (Kesici, 1994). Long-term average (1954–2013) of total precipitation were 496 for Kilis. Long term average temperature were 17 °C for Kilis.

2.3. Preparation of plant samples for mineral content

First of all the plant samples were cleaned and washed by deionized water, later air dried. Pre-dried samples were de-moisturized at 70 °C for 48 h in an oven and ground for chemical analysis. 0.2 g of ground samples was placed into burning cup, 5 ml HNO3 65% (Merck, Darmstadt, Germany) and 2 ml H_2O_2 30% (Merck, Darmstadt, Germany) were added immediately. After incinerating in a HP-500CEM MARS 5 microwave (crop. Mathews NC, USA) at 200 °C, the solution was cooled at room temperature for 45 min. The extracts were passed through a filter paper and the filtrates were collected by high-deionized water in a 20 ml of polyethylene bottles and kept at 4 °C in laboratory for ICP-AES analysis. Each sample was analyzed in triplicate. Phosphorus (P) and nitrogen (N) content were determined by vanadomolybdate method (Chapman and Pratt, 1961) and modified Kjeldahl method (Kacar and İnal, 2008) while K, Ca, Mg, S, P, Fe, Zn, Mo, Mn and Cu were ascertained by ICP-OES.

For all analytical works, distilled-deionized water was used. All the glassware and polyethylene bottles were attentively leached with 2–4% HCl and rinsed through deionized water for three times. Merck standards (R1 and R2 groups) were used as analytical reagent grade chemicals.

2.4. Preparation of leaf extracts

The air-dried and finely powdered leaves of leaf samples (5 g) were stirred with 100 ml of methanol for 30 min, respectively. Extraction was carried out using maceration at room temperature for 24 h followed by filtration through Whatman No. 4 filter paper. The extracts were then concentrated in vacuo at 40 °C using a Rotary Evaporator. Then the extracts were preserved in sealed vials at 4 °C until further analysis.

2.5. Determination of total phenolic content

Total phenolic content was determined according to the Folin–Ciocalteu reagent method (Singleton et al., 1999). The amount of total phenol was calculated as mg/g (Gallic Acid Equivalents) from calibration curve of Gallic acid standard solution (R^2 = 0.9993). An aliquot of each sample (0.1 ml) was diluted to 1 ml with distilled water. Briefly, 0.5 ml of Folin–Ciocalteu reagent (1:1 v/v) and 1.5 ml of 20% (w/v) sodium carbonate were added to the diluted sample solution, and the mixture was then vortexed and allowed to stand for 2 h at room temperature for color development. The volume was completed to 10 ml with distilled water and their absorbance was measured at 765 nm (Evolution 201 UV–visible Spectrophotometer). The total phenolic content was expressed as