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Early water stress detection on olive trees (*Olea europaea* L. cvs 'chemlali' and 'Chetoui') using the leaf patch clamp pressure probe

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ABSTRACT

Plant water relations and their dependence on microclimate and soil moisture were studied during 45 days in young olive trees cvs Chemlali and Chétoui subjected to a drying period and rehydration. The recently developed leaf patch clamp pressure (LPCP) probe were used for monitoring turgor-dependent leaf patch pressures (*Pp*) and compared with some conventional methods to detect water stress. Results show that, in well-watered olive plants, diurnal Pp is highly linearly correlated with vapour pressure deficit (VPD). Pp night readings gradually increased with increasing soil drought and revealed the beginning of water stress. At the end of the drying period, when the soil water content reach values less than the wilting point, only Pp curves detect the water stress in the two olive cultivars before the others methods tested here (ex: relative water content, chlorophyll content, chlorophyll fluorescence and stomatal conductance).

Non-destructive, continuous leaf turgor pressure method showed promising potential for monitoring and detecting early water stress of young olive tree, in spite of sensibility to water stress, technological and data interpretation challenges requiring further attention.

1. Introduction

The olive tree (*Olea europea* L.) is one of the most widespread fruit trees in the Mediterranean countries, where it covers a surface area of about 9.5 million hectares. It grows in semi arid environments characterized by wet winter and dry summers. Since ancient times the olive tree is grown under rainfed conditions on marginal lands, due to its ability to adapt to limiting environmental conditions such as drought. However, soil water availability is the major constraint to olive productivity. In Tunisia, olive oil extends on all agricultural land and is currently representing 1.7 million hectares. There are two main olive oil cultivars, 'Chemlali' and 'Chetoui'. The 'Chemlali' accounts for 80% of the national olive oil production and is grown in central and southern Tunisia. 'Chetoui' cultivar is widespread in the north of the country, occurring in plains as well as in mountainous regions. Over 31% of the plantations are aged under 5 years, and that require a periodic irrigation to ensure their growth, especially during summer.

Water availability is an important factor affecting photosynthesis, growth and survival of plants, mainly in arid and semi-arid regions, such as the Mediterranean. In general, strategies of drought-avoidance or drought-tolerance are recognized for maintaining cellular turgor. The way in which plants respond to water deficits depends widely on their hydraulic architecture, which controls plant water relations, especially the balance between soil water uptake and transpirational water loss (Levitt, 1980).

Plant water relations can be investigated by measuring environmental parameters, e.g. atmospheric vapour pressure deficit (VPD) and soil moisture content, or more reliably by analysing physiological parameters which depend on plant water content (Remorini and Massai, 2003; Zweifel et al., 2007). One of the most standard techniques is the determination of leaf water potential using a pressure chamber (Scholander et al., 1965). However, this method is destructive, using detached leaves, and temporal and spatial variations limit sampling (O'Toole et al., 1984). Stomatal conductance, transpiration and leaf fluorescences parameters are commonly measured using porometry and gas exchange equipment and although these measurements can be carried out on intact leaves, they are disruptive and suffer from the same temporal and spatial resolution problems as leaf water potential

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measurements (Boussadia et al., 2008; Guerfel et al., 2009; Ben-Gal et al., 2010; Petridis et al., 2012). Thermal imaging using infra-red technology to measure leaf and canopy temperatures, as a surrogate for stomatal conductance, has been used for several decades, but recent advances in remote sensing and methods to normalise spatial and temporal variation has led to rapid adoption of thermal imaging in modern phenotyping programs (Jones et al., 2009; Munns et al., 2010; Ben-Gal et al., 2010; Reynolds, 2002). While thermal imaging has obvious advantages in scaling from leaves to whole fields, proponents of the technology have suggested that accompanying measurements of leaf water status, especially turgor, would provide the extra information needed to understand the effect of stomatal behavior on plant adaptation and growth rate (Munns et al., 2010). New magnetic leaf patch-clamp pressure probes (LPCP) potentially provide an alternative or complementary method of directly monitoring leaf hydration. ZIM-probes are non-invasive and continuously monitor the hydration status of leaves in real-time (Zimmermann et al., 2008, 2010). Because the devices communicate wirelessly to a remote controller connected by GSM to an Internet server, the data can be viewed online over the Internet at any time. The ZIM-probe technology uses miniature pressure sensors that are clamped to leaves via magnets, improving on the spring-loaded connection mechanism in the original prototype (Zimmermann et al., 2008). The magnets apply a constant clamp pressure to the leaf, so that the pressure sensors are able to detect relative changes in leaf turgidity. Turgor is related to the hydration status as cell and bulk leaf turgor pressure decline when leaves dehydrate during transpiration and in response to drought (Kramer and Boyer, 1995). The ZIM-probe technology has been used for monitoring water status of trees and irrigation scheduling of horticultural ⁎⁎crops (Ehrenberger et al., 2012; Rüger et al., 2010a; Zimmermann et al., 2010). The ZIM probe has been tested in olive by Ben-Gal et al. (2010), Fernández et al. (2011), Ehrenberger et al. (2012), Rodriguez-Dominguez et al. (2012) and Padilla-Díaz et al. (2016). Results show that this is a promising method to monitor water stress and to schedule irrigation.

For young olive groves a few studies have been investigated to monitor water stress and irrigation scheduling. Moriana and Fereres (2002) concluded that continuous monitoring of trunk diameter provides the most sensitive indicator for accurate, automated irrigation scheduling of young olive trees. In Tunisia, Masmoudi et al. (2011) reported the limitation to use sap flow as water stress indicator for young olive tree cv Chetoui.

This work was undertaken in order to evaluate the potential of leaf turgor pressure indices for detecting water deficits in young olive trees earlier than they would be detected by other measurements of tree water status, so that continuous and online monitoring leaf turgor pressure via internet could be used for irrigation scheduling in young olive trees (cvs. Chemlai and Chetoui) during the first five sensitive years of plantation.

2. Material and methods

2.1. Site description and experimental design

The experiment was carried out at the Olive Tree Institute of Sousse in Tunisia (35°49′34″N; 10°38′24″E). Two-years-old own-rooted olive trees (*Olea europaea* L. cv. 'Chemlali' and 'Chetoui') were transplanted in 20-L plastic pots containing freely drained light soil, with a pH of 7.6, a field capacity of 35% and permanent wilting point of 15%, and grown under greenhouse conditions. The two different cultivars were exposed to the same water regime during January of 2014. Ten plants of each cultivar were used as controls (well watered: WW) and irrigated every 2 days to maintain the soil water content close to field capacity. An additional ten plants from the two cultivars were stressed by withholding water during one month until the soil water content almost reached less than the wilting point. During the experiment all measurements were taken out during three states of water regime. State I: Well watered condition when the soil water moisture is around the field capacity (SM = 35%) at the beginning of the experiment, State II: Stressed condition when the soil moisture is less than the wilting point (SM = 10%) and State III: Rehydration by full irrigation and the soil moisture is at the saturated point (SM = 50%).

2.2. Measurements of microclimate and soil moisture

Relative humidity, ambient temperature and soil moisture were recorded in parallel to the ZIM-probe measurements using initially three sensors (ZIM-temperature probe, ZIM-relative humidity probe and soil moisture sensor, ZIM Plant Technology GmbH, Hennigsdorf, Germany).

2.3. Relative water content (RWC)

Five leaves per plant in a similar position were detached to determine their relative water content (RWC) with three replicate plantlets for each cultivar. After cutting, the petiole was immediately immersed in distilled water inside of a glass tube, which was immediately sealed. The tubes were then taken to the laboratory where the increased weight of the tubes was used to determine leaf fresh weight (FW). After 48 h in dim light, the leaves were weighed to obtain the turgid weight (TW). The dry weight (DW) was then measured after oven drying at 80 °C for 48 h, and RWC (%) was calculated as:

$$\mathrm{RWC} = 100 \times \left(\frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}}\right)$$

2.4. Stomatal resistance

Stomatal resistance (Rs, s cm^{-1}) measurements were made with an 'AP4 leaf porometer' (Delta-T Devices, UK) with the following specifications; air temperature (25 °C), relative humidity (60%) and a photosynthetic photon flux density (PPFD = 1500 mol m^{-2} s^{-1}). Measurements were carried out between 11:00 h and 12:00 h on five well-exposed leaves per cultivar in each treatment.

2.5. Chlorophyll a fluorescence parameters

Chlorophyll fluorescence emission from the upper surface of the leaves of intact plants was measured by a modulated fluorimeter (OS1-FL Modulated Fluorometer). The minimal (F0) and maximal fluorescence (Fm) emissions were assessed in leaves after 20 min of dark adaptation, using clips for leaves (Walz, model 2030-B) and the maximum quantum efficiency of PSII photochemistry was calculated as Fv/Fm = (Fm − F0)/Fm. This ratio of variable fluorescence to maximal fluorescence correlates with the number of functional PSII reaction centers, and dark-adapted values of Fv/Fm can be used to quantify photoinhibition (Maxwell and Johnson, 2000). Then, the leaves were continuously illuminated with a white actinic light, which was equivalent to the actual growth light of olive plants in order to measure Fs and Fm'(steady-state and maximal fluorescence level in light-adapted leaves, respectively).

Using fluorescence parameters determined in both light- and dark-adapted states, the following were calculated: the quantum yield of PSII electron transport or actual PSII efficiency (ΦPSII) was calculated as $\Delta F/Fm' = (Fm' - Fs)/Fm'$, which measures the proportion of the light absorbed by chlorophyll associated with