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Assessing olive diversity for root traits associated with WUE

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SUMMARY – A plant's root system is a highly organized structure that not only serves as anchorage but also in the acquisition of water. As water resources are becoming limited water-use efficient genotypes with greater access to, and efficient exploitation of, sub-soil water are sought. However, measuring root traits to assess diversity within crops, is tedious work since this process involves studying large numbers of samples. To measure these root traits we used imaging techniques and quantitative modelling. The results show that there are indications of a relationship between root traits measured and stable carbon isotope discrimination (Δ), which is a surrogate for water use efficiency (WUE).

Key words: root system, olive, image analysis, water-use-efficiency

RESUME – *"Evaluer la diversité de l'olivier à travers les caractères du système racinaire liés à l'utilisation efficiente de l'eau". Le système racinaire des plantes est hautement structuré, sa fonction ne se limite pas à l'ancrage mais aussi à l'acquisition de l'eau. Avec le manque de ressources en eau il y a une tendance à employer des génotypes qui utilisent l'eau d'une façon plus efficiente. Cependant ces génotypes doivent également posséder un système racinaire plus performant avec des caractères permettant d'exploiter le sol d'une façon plus efficiente. Malgré leur importance, ces caractères ne sont pas souvent pris en compte dans la sélection. Ce travail vise donc à quantifier certains caractères du système racinaire chez l'olivier en utilisant l'analyse d'image. Les résultats préliminaires indiquent qu'il y a une relation entre ces caractères et le paramètre discrimination isotopique de carbone (Δ) qui est un substitut de l'utilisation efficiente de l'eau (WUE) chez les plantes.*

Mots-clés : *Système racinaire, olive, analyse d'image, utilisation efficiente de l'eau.*

Introduction

The olive tree (*Olea europaea* L.) is an important crop in terms of both its commercial value and the role it plays in the rural economy of the Mediterranean region, which is home to millions of olive producers. It is expected that its area of cultivation will increase mainly in the countries located in the south and east of the Mediterranean basin (Bari, 2007). If this trend is to continue, further increases will be constrained by harsh environmental conditions, in particular water scarcity. Recent estimates suggest that climate change will account for about 20% of the increase in global water scarcity (UNESCO/WWAP, 2003). An increase in magnitude and frequency of drought cycles has been reported in the Mediterranean region over the last two decades. As a result of climate change, the regions facing water-scarcity now will probably become drier (Kirby, 2004). Enhancing olive productivity through water-use efficiency is critical and more considering that agriculture accounts for more than two thirds of human water withdrawals in the Mediterranean and new sectors like tourism, urbanization and industry are increasingly competing for water resources (Gleick, 2002; Araus, 2004).

One of the options for managing water scarcity is to use water-use efficient genotypes among crops. Efficient genotypes, however, will require greater access to, and efficient exploitation of, sub-soil water (Paveley, 2000). Genetic variation between genotypes with respect to water-use efficiency (WUE) parameters (e.g. gas exchange and carbon isotope discrimination) and in root architecture/structure has been reported by a number of authors (Weyhrich *et al.*, 1995; Novello and de Palma, 1997; Pennington, 1999; Gaudillere *et al.*, 2002; Araus *et al.*, 2002; Grossnickle *et al.*, 2005).

Because a plant's root system is a highly organized structure that not only serves as anchorage but also in the acquisition of water and nutrients, Lynch (1995) suggested the consideration of the relationship between the architecture of root structure and its function, and eventually the relationship between root architecture and plant productivity under water scarcity. Recently Gregory (2006) suggested also to consider root architecture as a trait for selecting crops with a more efficient-use of water and nutrients. On the overall, however, in comparison to shoots, root traits have been frequently ignored due to methodological problems for selecting crop genotypes better adapted to drought, through changes in WUE and perhaps water-use.

Measuring variation in root system architecture/structure is a tedious and time consuming work since this process involves studying large numbers of samples and thus this may hinder assessing plant diversity (plant genetic resources). Hence, new rapid techniques for measurement of root system activities are highly desirable. The present study aims to test rapid screening techniques to assess diversity for root traits and defines those traits that could be associated with water-use efficiency. To capture and measure the root traits of olive tree cuttings, we used imaging and quantitative modelling based on fractal geometry (Bari *et al.*, 2004). The use of image analyses to extract root parameters has been proven to be of potential use in accurately estimating the different parameters and simulating the root system (Lynch *et al.*, 1997). To identify the root traits that might be associated with WUE the root data was contrasted with carbon isotope discrimination (Δ) data.

Material and methods

A set of 24 olive cultivars planted since 2000 at the Bioversity/ICARDA arboretum in Syria, were investigated to determine both root architectural parameters and water-use efficiency based on carbon isotope values. The root parameters were measured on 4-month old olive cuttings rooted under mist for a period of 4 months under a temperature of around 25ºC and 80% of RH. Twelve pencil-long, pencil-thick cuttings were taken from each cultivar. The lower leaves were removed from the cuttings to approximately two thirds of the way along each cutting. Prior to planting, all cuttings were dipped into indole butyric acid (4000 ppm) to induce root development. The cuttings were then planted in plastic boxes of 10x18 cm in size that contain perlite as substratum. Watering was carried out using an automatic system with a sensor to trigger misting.

The root parameters (Table 1) were measured on cuttings based on quantitative modelling and imaging techniques. Algorithms were developed using Matlab computing language (The MathWorks, Inc. Boston, MA, USA) to estimate these parameters to capture root length (L_t), root surface (S_t), root nodes (N_n), root tips (N_t), root abundance (K) and root branching measured by the adjusted fractal dimension (D_{bb}). The branching structure of a root system is the fundamental characteristic of a root system functioning (Sievers *et al.*, 2002).

Table 1. Root parameters used to capture root features

Variable	Description
D_{bb} (D)	Fractal dimension (box counting values adjusted to the bounding box)
K (log)	Abundance of the root system
L_t	Total root of the root system
N_n	Number of nodes
N_t	Number of tips
S_t	Root total surface
C	Rooting capacity (%)

For carbon isotope discrimination samples of leaves from trees of these root cuttings were dried and finely grounded. Carbon isotope composition ($\delta^{13}C$) was measured by mass spectrometry at Isotope Services, Inc. (Los Alamos, NM, USA). Precision of analyses were 0.1‰. The Δ value of each sample of olive leaf was then calculated from the $\delta^{13}C$ values of the sample and the air, where δ_a is the carbon isotopic composition of the source air (~ = -0.8‰) and δ_{tr} is the carbon isotopic composition of the olive tree sampled (Saurer *et al.*, 2004). Values were expressed in ‰. Total carbon content from the same samples was also measured using an elemental analyzer and values expressed in %.

To investigate the relationship between the different root parameters and Δ a regression analysis was performed. Of interest is the correlation of the fractal dimension, which is a surrogate of root branching (Masi and Maranville, 1998), with Δ, which is a surrogate for WUE. The relationship between the capacity to root, which is commonly used in olives and Δ, was also investigated.

Results and discussion

The cultivars differ significantly for both Δ and total carbon content with values ranging from 26.80 to 30.24‰ with an average of 27.55±0.75‰ for Δ and from 45.50 to 51.48% with an average of 48.05±1.21% for the carbon ratio. The cultivars differed also significantly in terms of their root parameters in particular the root branching based on fractal geometry D_{bb} (adjusted to the bounding box). Intriguingly, the results show that some of these root architectural parameters correlated negatively and significantly with Δ ($P \leq 0.05$) including D_{bb} and can thus be of potential use to assess diversity for roots traits associated with water-use efficiency (Table 2).

Table 2. Correlation between the different parameters and Delta

Variable	R^2	*P*	Significance
D_{bb}	0.480	0.020	*
K (log)	0.435	0.038	*
L_t (log)	0.432	0.040	*
N_n (log)	0.358	0.093	ns
N_t (log)	0.420	0.046	*
S_t (log)	0.435	0.038	*
C	0.220	0.314	ns

*Significant correlation at $P \leq 0.05$; ns: non-significant.

The high fractal dimension indicates high branching, which is also an indication of an increase in root system robustness. The genotypes having root systems with high fractal dimension values possess roots with high intricacy and are likely to sustain harsh conditions (Masi and Maranville, 1998). Using fractals Masi and Maranville (1998) reported that sorghum (*Sorghum bicolor*) genotypes of African origin were more highly branched, with deeper roots, than US-derived genotypes. The effect of drought on the root system architecture of wild pistachio (*Pistacia lentiscus*) has also been measured using the fractal dimension, revealing that higher fractal dimension values indicate a higher density of roots (Green *et al.*, 2001).

The variation in root traits may be more important in later stages of the plant. Nevertheless small differences at early stages, such as those of olive cuttings, may also be important as they could translate to large differences in a developed plant. In dry areas such as that are prevailing in the Mediterranean region where olive is commonly grown, early triggering of a water-use strategy such as early root branching in plants may lead to a more efficient use of water (Masle, 1999; Caruso *et al.*, 2005). Moreover these root traits when measured in cuttings may be also important as indicators for a better plant establishment and faster growth during the early stages of an olive orchard.

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