

Parvaiz Ahmad · M.N.V. Prasad *Editors*

Abiotic Stress Responses in Plants

Metabolism, Productivity
and Sustainability

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Preface

Climate constrained world represents an ideal scenario of abiotic stresses in which there has been a change in the statistical distribution of weather (temperature, soil moisture, salinity, ecohydrology, soil fertility, emission of greenhouse gases, etc.) over periods of time that range from decades to centuries to millions of years. Plants do respond to these changes in the process of acclimation and acquiring tolerance – morphologically, structurally, physiologically, biochemical and molecular mechanisms.

Abiotic stress cause changes in soil–plant–atmosphere continuum which is responsible for reduced yield in several of the major crops in different parts of the world. Therefore, the subject of abiotic stress response in plants – metabolism, productivity and sustainability is gaining considerable significance in the contemporary world.

This is a collective and companion volume to our previous edition *Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change*. This volume deals with an array topics in the broad area of abiotic stress responses in plants focusing “*metabolism, productivity and sustainability*” by selecting some of the widely investigated themes. Chapter 1: Abiotic stress responses in plants – present and future. Chapter 2: Abiotic stress-induced morphological and anatomical changes in plants. Chapter 3: Abiotic stress responses in plants – metabolism to productivity. Chapter 4: Approaches to increasing salt tolerance in crop plants. Chapter 5: Understanding and exploiting the impact of drought stress on plant physiology. Chapter 6: Sustainable fruit production in Mediterranean orchards subjected to drought stress. Chapter 7: Drought stress-induced reactive oxygen species and antioxidants in plants. Chapter 8: Role of glutathione reductase in plant abiotic stress. Chapter 9: Flavonoids as antioxidants in plants under abiotic stresses. Chapter 10: Proteomic markers for oxidative stress – new tools for reactive oxygen species and photosynthesis research. Chapter 11: Environmental stress and role of arbuscular mycorrhizal symbiosis. Chapter 12: Effects of exogenous application of 5-aminolevulinic acid (ALA) in crop plants. Chapter 13: Abiotic stress and role of salicylic acid in plants. Chapter 14: Trehalose and abiotic stress tolerance. Chapter 15: Uptake of mineral elements during abiotic stress. Chapter 16: Effect of micronutrient deficiencies on plants stress responses. Chapter 17: Stress-induced flowering. Chapter 18: Postharvest stress treatments in fruits and vegetables. Chapter 19: Abscisic acid signalling in plants. Chapter 20: Plant tolerance and fatty acid profile in

responses to heavy metals. Chapter 21: Cadmium accumulation and subcellular distribution in plants and their relevance to the trophic transfer of Cd. Chapter 22: The role of soil organic matter in trace element bioavailability and toxicity. Chapter 23: Oxidative stress and phytoremediation. Chapter 24: Phytoremediation of low levels of heavy metals using duckweed (*Lemna minor*). We fervently believe that this volume will provide good information and understanding of abiotic stress tolerance in plants.

We are extremely thankful to all the contributors for comprehensive and cogent reviews which ultimately resulted in the present form. We are pleased to place on record the superb and skillful job of Amna Ahmad, Andy Kwan and the rest of the technical team at the production unit for publishing this work in record time.

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Parvaiz Ahmad
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Sustainable Fruit Production in Mediterranean Orchards Subjected to Drought Stress

6

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and Cristos Xiloyannis

Abstract

Drought stress is the main cause of reduced fruit tree growth and productivity in Mediterranean semi-arid regions and causes a complex of responses at molecular, cellular, physiological and developmental level. In particular, the response of fruit trees to water scarcity is a species- and cultivar-dependent and a series of studies have been carried out to clarify and deepen the mechanisms of their adaptation, avoidance, resistance or tolerance against drought. Considering that 16% of the total cultivable land of the Mediterranean area is occupied by fruit crops, the choice of an appropriate and rational irrigation management is of key importance. Furthermore, plant water status in an orchard is related to many biotic and abiotic factors, such as the amount of light intercepted, plant densities and canopy architecture, which play a key role in determining orchard productivity and fruit quality. The recent research on the physiology of fruit trees and on soil chemical and biological fertility in fruit orchards have revealed that sustainable and innovative soil management systems, with a particular emphasis on water management (e.g., sustained deficit irrigation, regulated deficit irrigation and partial root-zone drying), can determine an optimal plant nutritional equilibrium, avoid nutrients accumulation and leaching risks, improve irrigation efficiency and prevent soil erosion and root asphyxia. The application, optimization and innovation of sustainable agricultural techniques with a low negative environmental impact allow to recover or increase the normal levels of total fertility in fruit agro-ecosystems, so

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maintaining top yields of high quality. On this basis, the aim of this work is to give an detailed information on drought stress in fruit trees.

Keywords

Drought stress • *Prunus* species • Olive tree • *Citrus* species • Mediterranean fruit species

1 Introduction

Plants are sessile organisms and their only alternative to a rapidly changing environment is a fast adaptation to abiotic and biotic stresses. This concept is particularly valid for the productive, physiological and biochemical responses against water deficit (Chaves et al. 2003; Foyer et al. 2005; Morales et al. 2006). In particular, trees carry on the same processes as other seed plants, but their larger size, slower maturation and much longer life accentuate their susceptibility to drought stress in comparison to smaller plants having a shorter life span (Pallardy 2008).

Drought stress is the main cause of reduced fruit tree growth and productivity in Mediterranean semi-arid regions and causes a complex of responses at molecular, cellular, physiological and developmental level. In Mediterranean ecosystems, in which the summer months are characterized by lack of precipitation, elevated temperatures and high irradiance levels, fruit tree species are subjected to a continuous and severe water deficit. Under these adverse environmental conditions, photoinhibition, photooxidation and photorespiration occur, which in turn can negatively affect fruit yield and quality.

Fruit orchards constitute an integral and significant part of the Mediterranean environment and culture, and their ecological importance has only recently been acknowledged. Considering that 16% of the total cultivable land of the Mediterranean area is occupied by fruit orchards (Olesen and Bindi 2002), it is easy to understand that the study of the response of fruit trees to drought stress is of key importance for the agriculture and the economy of the Mediterranean countries. The plant water status in an orchard is

related to many biotic and abiotic factors, such as the amount of light intercepted, plant densities and canopy architecture, which play a key role in determining orchard productivity and fruit quality (Grossman and DeJong 1998).

In the Mediterranean basin, fruit trees are often grown in marginal and unfertile lands with low levels of soil organic matter, and orchards are often subjected to soil degradation (Celano et al. 2002; Xiloyannis et al. 2005). Adoption of agricultural systems utilizing non-sustainable techniques, such as frequent and intensive cultivation, zero organic matter inputs (e.g., from cover crops and pruning residues), and use of excessive amounts of water and chemical fertilizers, can further worsen the situation. While such non-sustainable practices are still common among growers, there is evidence that suitable agricultural management practices, such as minimum tillage or no tillage, recycling of the carbon sources internal to the fruit grove (pruning material, spontaneous or/and seeded cover crops, compost amendments) and adequate irrigation, fertilization and pruning, are recommended to maintain an optimal water status in plants, save and use rationally irrigation water, restore soil organic matter, reduce erosion process and environmental pollution and increase the CO₂ sequestration processes from the atmosphere into the soil (Lal 2004; Dichio et al. 2007; Sofu et al. 2010a, b). Furthermore, the adoption of environmentally sustainable agricultural techniques in productive orchards has positive effects on soil microbiota, enhancing soil fertility, plant growth and fruit yields and quality by increasing nutrients availability and turnover in the soil (Kushwaha et al. 2000; Gruhn et al. 2000; Jagadamma et al. 2008; Sofu et al. 2010a, b). Finally, a sustainable orchard management is of particular importance

in Mediterranean climates, where soil mineralisation rate is high (Kimmins 1997).

The aim of this work is to give an up-to-date overview of these studies, that will be herein discussed in detail for each tree species. We decided to include the most important and typical productive fruit species of the Mediterranean basin.

2 The Genus *Prunus*

The genus *Prunus* (family Rosaceae) comprises more than 400 species adapted to temperate areas and cultivated in Europe. In particular, stone fruit crops, such as peach (*Prunus persica* L.), plum (*P. cerasifera* L. and *P. domestica* L.), almond (*P. dulcis* L.), apricot (*P. armeniaca* L.) and cherry tree (*P. avium* L.), are typical and economically important and mainly localized in Mediterranean regions. Productive stone fruit trees are usually grafted plants with a lower part, the rootstock and an upper grafted part, which is the genotype of the commercial variety. Rootstocks are important for agronomic purposes, as they have a different genetic background compared to the commercial varieties and can be used to confer various traits such as drought stress resistance.

A better understanding of the effects of water deficit on *Prunus* species has a primary importance for improved management practices (Girona et al. 2005b), breeding programmes (Rieger et al. 2003) and for predicting fruit growth and quality (Torrecillas et al. 1996; Besset et al. 2001; Esparza et al. 2001; Girona et al. 2002). During periods of water deficit, species of the genus *Prunus* show significant decrease in gas exchange (Ruiz-Sánchez et al. 2000a; Besset et al. 2001; Klein et al. 2001). The decrease of soil humidity, together with high values of vapour pressure deficit (VPD), causes reduction in leaf water potential (LWP), carbon assimilation and transpiration in different species of *Prunus* (Rieger and Duemmel 1992; Rieger 1995; Berman and DeJong 1996; Alar on et al. 2000; Besset et al. 2001; Esparza et al. 2001; Klein et al. 2001; Rieger et al. 2003; Matos et al. 2004; Romero et al. 2004c; Gomes-Laranjo et al. 2006; Intrigliolo and Castel 2006; Dichio et al. 2007; Godini et al.

2008; Egea et al. 2010). Some studies also highlighted the activation of antioxidant defenses as a strategy to face drought-dependent oxidative stress in this genus (Scebba et al. 2001; Sofo et al. 2005; Sorkheh et al. 2011).

2.1 Peach and Apricot

The peach tree (*Prunus persica* L.) and the apricot tree (*Prunus armeniaca* L.) are two of the most common and economically important species of the Mediterranean basin (Grossman and DeJong 1998; Alar on et al. 2000; Besset et al. 2001; Girona et al. 2002). The drought tolerance of peach and apricot is mainly based on stomatal control (Arndt et al. 2000) and morphological characteristics (Rieger et al. 2003), together with some degree of osmotic adjustment (Alar on et al. 2000). Work in these two species covered subjects from the physiological processes adopted to regulate water status under drought conditions (Ruiz-Sánchez et al. 2000a; Rieger et al. 2003) to the biochemistry underlying plant response to water deficits and oxidative stress (Arndt and Wanek 2002; Sofo et al. 2005). Peach and apricot trees are highly sensitive to drought stress at particular phenological stages, such as flowering and fruiting, and during stem extension and fruit growth (Berman and DeJong 1997; Xiloyannis et al. 2005). Considering the sensitivity of these two species to water deficit, several authors highlighted the importance of considering wetting patterns, soil depth and root exploration in peach and apricot irrigation management (Ruiz-Sánchez et al. 2000a; Girona et al. 2002). Keeping in view an efficient use of water in peach and apricot orchards, it is of key importance to consider the type of training system and plant architecture, and particularly the distribution of light in the various parts of the canopy, as well as the system of irrigation and its management (Xiloyannis et al. 2010).

Among the indicators used for monitoring water status of peach and apricot trees, two of the most reliable were indicated by Alar on et al. (2000) and Arndt and Wanek (2002). The formers used foliar carbon isotope composition ($\delta^{13}\text{C}$)

measured in leaves of peach under water deficit as a tracer to study whole plant carbon allocation patterns. In fact, it is known that foliar carbon isotope discrimination decreases in water-deficit situations as discrimination by the photosynthetic primary carboxylation reaction decreases. On the other side, Arndt and Wanek (2002) used sap flow measured with a heat-pulse technique as an indicator of transpiration and the water status of young apricot plants. They observed that when apricot trees are drought-stressed, measures of sap flow slightly underestimate actual transpiration, confirming an increasing hydraulic resistance under drought conditions. In apricot (cv. "Búlida"), a preconditioning treatment using a substantial reduction in the irrigation water (25% of crop evapotranspiration, ET_c) promotes a better drought-hardening of the plants due to a greater osmotic adjustment (0.77 MPa) that prevents severe plant dehydration and leaf abscission (Ruiz-Sánchez et al. 2000a). This treatment may be valuable for young apricot plants in the nursery stage in order to improve their subsequent resistance to drought.

Regarding the relationship between drought and fruit yield, Berman and DeJong (1996) demonstrated that in well-watered peach plants (cv. "Elegant Lady"), tree water status is independent of crop load, whereas in trees receiving reduced irrigation, the degree of drought stress increased with increasing crop load. The same authors found that drought stress induces fruit fresh weight reductions at all crop loads, whereas fruit dry weight is not reduced by drought stress in trees having light to moderate crop loads. These results suggest that the degree of drought stress imposed did not affect the dry weight sink strength of peach fruit. On the other hand, drought-stressed trees with heavy crop loads had significantly reduced fruit dry weights, which were likely due to carbohydrate source limitations resulting from large crop carbon demands and drought stress limitations on photosynthesis. Crisosto et al. (1994) observed a higher density of trichomes and a continuous and much thicker cuticle on peaches (cv. "O'Henry") from the deficit and optimum irrigation treatments than from the excess irrigation treatment, indicating that a

well-designed irrigation management can improve fruit quality and extend shelf life. In peach trees, there is a direct correlation between water availability and carbohydrate synthesis (Girona et al. 2002), and between photosynthetic rate and types of carbohydrates synthesised (Escobar-Gutiérrez et al. 1998). During fruit growth, high photosynthetic rates are necessary for growth requirements of peach (Besset et al. 2001). Sorbitol and sucrose are the two main photosynthetic carbohydrates of peach plants and their function depends on the organ of utilization and its developmental stage (Lo Bianco et al. 2000). In well-watered peach plants, these sugars are translocated from their sources, mainly mature leaves, and then absorbed by sink organs, such as shoot apices (Lo Bianco et al. 2000), developing fruits (Grossman and DeJong 1998) and buds during dormancy release (Marquat et al. 1998). Under drought stress, sucrose metabolism is only marginally reduced, whereas sorbitol accumulates in sinks and sources, contributing up to 80% to osmotic adjustment (Lo Bianco et al. 2000).

Regulated deficit irrigation (RDI), the practice of reducing applied water at selected phenological stages less sensitive to water deficit, was successfully applied to both peach and apricot (Ruiz-Sánchez et al. 2000b; Girona et al. 2005a; Dichio et al. 2007). The application of RDI, based on imposing plant drought stress in a controlled manner, is a feasible water-saving practice for Mediterranean arid areas. Moreover, RDI extended over a long period lead to adaptation of peach tree to dry conditions due to a better extraction of water from deeper soil. The success of RDI strongly depends on the appropriate use of localized irrigation techniques, which allows the control of soil water content (SWC) and plant water status. Moreover, an efficient use of irrigation water is particularly important for improving water uptake by root system. In peach, the application of RDI during the early stages of fruit growth until the end of shoot growth slightly influences fruit size and number (Boland et al. 2000) and a water deficit treatment during the final stage of rapid fruit growth causes decreases in fruit size but also significant increases in total fruit soluble solids (Crisosto et al. 1994; Besset

et al. 2001; Naor et al. 2001). Thus, peach quality and taste can be considered as being improved by a water deficit applied in this phenological phase.

On the other hand, withholding irrigation applied after harvest reduces vegetative growth of early-maturing peach trees (Johnson et al. 1992; Ghrab et al. 1998; Girona et al. 2005a) and can improve fruit quality (Gelly et al. 2004; Dichio et al. 2007). It is important to note that RDI, though applied during the post-harvest stage, has to be performed avoiding high levels of drought stress, which could negatively influence the accumulation of reserve carbohydrates, flower development and thus, indirectly, crop yield of the following year (Xiloyannis et al. 2005). Dichio et al. (2007) evaluated the effects of RDI applied in the post-harvest stage of mature peach plants (cv. “Springcrest”) trained to transverse Y in an experimental field located in Southern Italy. These authors confirmed the possibility to reduce the irrigation water by applying RDI during phenological stages less sensitive to water deficit without negatively affecting peach growth and yield. In their experiment, from bud break to harvest, irrigation was carried out by applying 100% ET_c , while from harvest to early autumn, plants were separated into three groups and subjected to different irrigation treatments (100%, 57% and 34% ET_c). RDI determined the reduction in the growth of waterspouts and lateral shoots but did not influence the growth of fruiting shoots. No significant reductions in crop yield and quality were observed in the 57% ET_c treatment, whereas about 1,100, 1,800 and 2,500 $m^3 ha^{-1}$ of water were saved in the first, the second and the third year, respectively. In the second year of the trial, the use of RDI in the post-harvest stage determined carbohydrate and nitrogen (N) accumulation in roots, branches, shoots and floral buds. Therefore, the results of Dichio et al. (2007) demonstrate that, under scarce water supply conditions, a clear benefit for both vegetative growth, and carbon and N allocation of peach trees can be obtained through the use of RDI during the post-harvest stage. In another study, Ruiz-Sánchez et al. (2000b) evaluated the response of apricot trees (cv. “Búlida”) to RDI under Mediterranean climate. The authors applied an RDI treatment

irrigated at 100% ET_c during the critical periods (second rapid fruit growth period and 2 months after harvest) and with a reduction of 40% ET_c during the other periods. An average water saving of 34% was achieved in the fourth year RDI treatment and apricot quality was not modified by RDI treatment. Furthermore, when irrigation water saving was around 25%, the yield obtained was similar to that of the control treatment. It is noteworthy that apricot fruit growth showed few differences between the control and the RDI treatment during the deficit irrigation period, but an accelerated rate of growth was noted when irrigation was increased to 100% ET_c . In conclusion, the satisfactory yield obtained with RDI in Mediterranean peach and apricot orchards suggests to adopt it for early ripening cultivars grown in semi-arid areas with limited water resources in order to improve irrigation efficiency and save water while maintaining top yields of high quality.

With the adoption of a sustainable management under semi-arid climatic conditions (an example is reported in Fig. 6.1), peach and apricot yield can be enhanced up to 25–30%, and the amounts of water and of N, P, K and soil carbon inputs annually incorporated into the soil increase significantly if compared to a non-sustainable orchard (Xiloyannis et al. 2010). Among the sustainable practices, cover crops are of key importance, as in Mediterranean apricot groves the use of mixture of herbaceous species with a high biomass production, such as *Vicia faba/Avena sativa*, can produce approximately 1.0 $ton ha^{-1}$ of humus, with clear benefits for soil fertility (Celano et al. 2002). Moreover, in rain-fed conditions, it is beneficial to sow cover crops in autumn and sow them just before spring in order to avoid water and nutrient competition (Xiloyannis et al. 2005). Sustainable practices also have positive effects on soil microbiota, that influences soil fertility and plant growth by regulating nutrient availability and increasing their turnover (Kushwaha et al. 2000; Borken et al. 2002; Widmer et al. 2006; Govaerts et al. 2008). A molecular approach was often used to reveal qualitative changes in the structure of soil bacterial and fungal communities in various Mediterranean agro-ecosystems

SUSTAINABLE MANAGEMENT	CONVENTIONAL MANAGEMENT
	
<ul style="list-style-type: none"> • Minimum tillage and cover crops (30 kg ha⁻¹ of <i>Trifolium subterraneum</i> seeds and spontaneous grass) 	<ul style="list-style-type: none"> • Conventional tillage (strong and deep soil plowing)
<ul style="list-style-type: none"> • Guided fertilization (fertigation based on plant nutrient demand evaluated by leaf mineral analyses and on soil measured nitrogen levels) • Compost amendment (15 t ha⁻¹ fresh weight) • Incorporation of cover crop and pruning residues into the soil (light harrowing at a depth of 10 cm carried out in Autumn) 	<ul style="list-style-type: none"> • Chemical fertilization (100 kg N, 10 kg P, 20 kg K ha⁻¹) without considering soil nutrient levels and plant nutrient requirements • Removal of pruning residues from the field
<ul style="list-style-type: none"> • Guided drip irrigation based on crop evapotranspiration (3 drip emitters per plant along the tree lines with a capacity of 4 L h⁻¹ each) 	<ul style="list-style-type: none"> • Empirical irrigation (using excessive amounts of water, without considering soil moisture and crop evapotranspiration)
<ul style="list-style-type: none"> • Pruning aimed to vegetative-productive equilibrium of plants (winter pruning based on the selection of shoots with a high number of floral buds and on a better light interception in the canopy) 	<ul style="list-style-type: none"> • Empirical pruning

Fig. 6.1 Comparison between a sustainable and a conventional management of a peach orchard (*Prunus persica* (L.) Batsch Nectarine, cv. “Supercrimson” grafted on GF677) located in Southern Italy, under a semi-arid

climate with an average annual rainfall of 525 mm. Peach trees were trained to vase (500 plants ha⁻¹) with a north-south row orientation (data from Sofo et al. 2010a)

(Bending et al. 2002; Marschner et al. 2003) but little is known on the molecular and metabolic aspects of soil microbial community at orchard level. One of the few researches on this subject was carried out by Sofo et al. (2010a), that examined the short-time effects (after 4 years) of two different management (sustainable and non-sustainable) systems on microbial genetic, functional and metabolic diversity of a Mediterranean peach orchard (cv. “Supercrimson”), evaluated by a combination of culture-dependent and culture-independent techniques. They revealed qualitative and quantitative changes of soil microbial communities (different electrophoretic patterns of bacterial 16S ribosomal and fungal 18S ribosomal RNA genes and higher indexes of microbiological diversity) in response to a sustainable soil management.

2.2 Almond

Almond (*Prunus dulcis* L.) is the most important tree nut produced on a global basis, and its limited gene pool limits the cultivation to specific areas with Mediterranean climate (Sorkheh et al. 2011). This species is one of the oldest tree nut crops, and today represents the largest production of any commercial tree nut product. The response to water deficit of almond trees is a well-documented process (Esparza et al. 2001, 2010; Klein et al. 2001; Gomes-Laranjo et al.; 2006, Rouhi et al. 2007; Egea et al. 2010). The results obtained on the agronomic response of almond trees to different deficit irrigation strategies demonstrate the prevalence of direct and strong links between the intensity of the water restriction and the response of several parameters related to tree growth, yield and water status (Rouhi et al. 2007; Egea et al. 2010). Besides predawn or midday LWP, midday stem water potential (SWP) and midday leaf stomatal conductance, a series of indicators of plant water status were applied on drought-stressed almond trees. The results obtained by Nortés et al. (2005) indicate that both maximum daily trunk shrinkage and trunk growth rate in almond are sensitive to drought stress and that the second

is the most useful parameter for quantifying water deficit intensity and duration.

As regard to fruit production, in almond trees (cv. “Nonpareil”) an average loss in yield of 7.7 kg tree⁻¹ occurs in response to each 1 MPa decrease in stem water potential (SWP) below -1.2 MPa, if a severe irrigation deprivation is carried out during the harvest period of the previous year (Esparza et al. 2001). This yield loss is likely due to the decrease in the number of fruiting positions per tree, even though the authors did not observe effects of irrigation deficit on the percentage of spurs that flowered or set fruit during subsequent years. In another study, Esparza et al. 2001 confirmed that a severe drought stress during the harvest period in almond causes a reduction in non-structural carbohydrates content but not in N content per tree, so limiting vegetative growth in the following year and impacting subsequent fruit-bearing capacity rather than directly affecting flowering, fruit set or fruit growth. Differences in N-allocation patterns between fruiting and non-fruiting shoots were recently observed by Nortés et al. (2009) in drought-stressed almond plant (50% ET_c during the entire growing season), if compared to fully watered plants (100% ET_c). They found that in the 50% ET_c treatment, a high N status is maintained in the leaves of fruit-bearing shoots, to the detriment of N resources allocated to vegetative shoots.

The studies on RDI applied in Mediterranean almond orchards and aimed to improve fruit yield and quality are numerous (Romero et al. 2004a, b, c; Girona et al. 2005b; Egea et al. 2010). It was found that an RDI of 20% ET_c applied during the pre-harvest period (kernel-filling stage) does not cause reduction in kernel yield and size in almond (cv. “Cartagenera”) and improves water-use efficiency, but only if predawn LWP is maintained above a threshold value of -2.0 MPa (Romero et al. 2004c). In the same cultivar, Romero et al. (2004a, b) indicated that a severe RDI (20% ET_c) during the kernel-filling stage, and a recovery at 75% ET_c during the post-harvest phase allows to save 220–273 mm yr⁻¹ irrigation water without negatively affecting plant growth and fruiting.

Related almond species, interspecific crosses and spontaneous interspecific hybrids demonstrate a greater resistance to abiotic and biotic stresses and so represent valuable germplasm sources for rootstock breeding, especially under non-irrigated conditions (Browicz and Zohary 1996). The wide adaptation of the related wild almond species indicate their potential as sources for resistance to drought stress as well as modified tree and nut traits. On this basis, the selection for drought resistance in almond rootstock material and for the increase of quality of cultivated almond production under stress conditions is particularly important. Among the different wild almond varieties, Rouhi et al. (2007) studied the intrinsic water use efficiency, defined as the ratio of assimilation rate over stomatal conductance, in cultivated and wild almond species, finding that *P. dulcis* is the species most tolerant to drought, *P. scoparia* tries to avoid drought, and *P. lycioides* has an intermediate behavior and this latter can have potential for use as rootstock for commercial almond production. As wild almond species can be very important for rootstock selection, in a recent paper, Sorkheh et al. (2011) examined the changes of antioxidant enzyme activities and the level of some antioxidant compounds involved in the ascorbate-glutathione cycle in drought-stressed plants of eight wild almond species from different geographical points of Iran. The authors found that after 70 days without irrigation, mean pre-dawn LWP in all the species fell from 0.32 to -2.30 MPa and marked decreases in CO_2 uptake and transpiration occurred. The activities of the antioxidant enzymes involved in the ascorbate-glutathione cycle increased in relation to the severity of drought stress in all the wild species studied. Furthermore, the levels in total ascorbate and glutathione and H_2O_2 were directly related to the increase of drought stress. The up-regulation of the activities of some antioxidant compounds during drought stress is an immediate and efficacious response to scavenge the excess of activated oxygen species (AOS), such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (HO^\cdot) and singlet oxygen ($^1\text{O}_2$), and it was also observed in other fruit tree species, such as apricot (Scebba et al. 2001), olive (Sofa et al. 2004a) and plum rootstocks (Sofa et al. 2005).

As seen for olive (Sofa et al. 2004b), the role of proline during drought stress is particularly important for the osmotic homeostasis of the plants. The results of a forthcoming paper on wild almond (Sorkheh et al. 2011) highlight that the cell membrane damage is a direct consequence of oxidative stress by H_2O_2 and that the application of exogenous proline can alleviate these detrimental effects. Thus, it is possible to recommend exogenous proline treatment of wild species of almond in order to increase their antioxidant defenses when subjected to drought.

2.3 Plum and Cherry

Many plum genotypes are used as rootstock for almost all other *Prunus* species and, among them, Myrobalan plum (*Prunus cerasifera* L.) clones often show positive agronomic features for resistance to pathogens and abiotic stresses (Lecouls et al. 2004; Intrigliolo and Castel 2006). In the Mediterranean regions, drought is the main limiting factor for plum growth (Rato et al. 2008). Sofa et al. (2005) studied the effects of water deficit on photosynthetic performance and on the components of the ascorbate-glutathione cycle in four interspecific plum hybrids, used as rootstocks, hypothesizing that an excess of reducing power, with the consequent increase in H_2O_2 and other AOS concentration, causes the up-regulation of some antioxidant enzymes during a drought period. Their results showed that the activities of antioxidant enzymes and the levels of the molecules involved in the ascorbate-glutathione cycle (antioxidant enzymes, total ascorbate and glutathione and H_2O_2) increased in all the hybrids examined in parallel to the severity of drought stress. After 70 days of water shortage, mean pre-dawn LWP of all the hybrids fell from -0.34 to -3.30 MPa and marked decreases in net photosynthesis and transpiration occurred. All these physiological and biochemical responses could limit cellular damage caused by AOS during periods of water deficit. On the basis of these results, it appears that the ability of *Prunus* hybrids to regulate the enzymatic antioxidant system during drought stress can be an important attribute linked to drought tolerance. As regard to

yield susceptibility to reduced irrigation in plum (*P. salicina*, cv. “Black Gold”), Intrigliolo and Castel (2006) pointed out that an RDI applied from pit hardening to harvest reduces fruit weight by 10–21%, indicating that phase III of fruit growth is a phenological period highly sensitive to water deficits in this species.

As in plum, water relations and photosynthesis of sweet cherry (*P. avium* L.) grown in Mediterranean environments are mainly influenced by the rootstock genotype, and the regulation of fruit quality is mainly dependent on the cultivar genotype (Gonçalbes et al. 2005; Godini et al. 2008). An interesting and wide comparison among cherry rootstocks subjected to non-irrigated conditions in Southern Italy highlighted the satisfactory performance of “SL 64” and the promising performance by dwarfing “Weiroot® 158” and semi-dwarfing “MaxMa 14” under water-limited growing conditions. An optimal water and nutrient management is of primary importance for cherry trees grown under semi-arid conditions, and drip fertigation is a valid irrigation system for this species (Nielsen et al. 2005). Furthermore, water scarcity could determine a higher percentage of double fruits as well as a phytohormonal disequilibrium, with consequent losses of commercial product (Engin and Ünal 2008).

3 The Olive Tree

The genus *Olea* encloses more than 30 species belonging to the family *Oleaceae*. The cultivated species, *Olea europaea* L., is a very important crop in the world and occupies an area of about 9 Mha (FAOSTAT, 2010) with more than 2,600 cultivars, many of which may be ecotypes (Therios 2009). Olive is an evergreen and long-lived species cultivated between 30° and 45° of latitude but it finds the optimal conditions for its growth and productive expression in Mediterranean countries. In such area, climate is characterized by winter rains, which fall in a short cold season, and a dry and hot summer period.

Olive is cultivated for its edible fruits subjected to different processing technologies to obtain table olives or oil, both characterized by

high nutritional and organoleptic values. Such products are among the basic aliments of the so-called Mediterranean diet and show interesting properties for human health preservation (Visioli et al. 1999, 2006). The olive tree products (oil, leaves) can be usefully employed in the herboristic and cosmetic sectors. A promising perspective could be the recovery from olive mill wastewater (OMW) of phenolic compounds, such as hydroxytyrosol, with high added value due to their powerful antioxidant and potential beneficial properties for human health and effective antimicrobial activity. These compounds could be used as integrators in food, pharmaceutical and cosmetic products or as a natural pesticide against a variety of seed infections (Allouche et al. 2004; Yanguí et al. 2009). In addition, the recovery process could partially solve the long-standing problem of OMW disposal. OMW, whose worldwide production is around 30 Mm³ yr⁻¹, has high polluting effects for the environment, especially when its disposal is carried out using unsuitable procedures (Allouche et al. 2004; Celano et al. 2010).

3.1 Olive Responses to Drought

Olive tree shows a great capacity to tolerate the long summer water shortage by means of numerous strategic devices aimed to control water losses and increase water uptake from soil. Besides the anatomical and morphological features of leaves (small size, high specific leaf weight, thick and waxy cuticle, hairy leaf surfaces, high stomatal density), typical adaptations of drought-tolerant plants, olive presents specialized physiological and biochemical mechanisms.

Under severe drought stress, olive tree significantly lowers water content and water potentials of its tissue establishing a high potential gradient between leaves and roots (predawn LWP values of -7.0 MPa and -3.5 MPa, respectively) which allows the root system to utilize water up to soil water potential of -2.5 MPa. Such value is well below the permanent wilting point, measured at -1.5 MPa for most of the fruit species. Under such conditions, and especially in soil characterized by a good water storage capacity, olive plants

have access to a greater and readily available soil water (between field capacity and -2.5 MPa), so withstanding long drought period (Xiloyannis et al. 2003). In olive tree, stomata progressively reduce their activity starting from predawn LWP below -0.9 MPa, and they can remain open up to -7.0 MPa (Xiloyannis et al. 1999). A progressive closure of stomata as predawn LWP decreased was observed in other fruit tree species but their stomatal closure was reached at values of predawn LWP ranging from -1.5 to -2.5 MPa (Lakso 1979; Castel and Fereres 1982). Under stressful conditions, olive tissues are able to transpire large amounts of water, accumulated during the afternoon and night, ensuring a certain level of leaf functionality. As a matter of fact, olive leaves can give up to transpiration about 60% of the water stored in their tissues contributing to the demands of transpiration as stress increases up to extreme values (Xiloyannis et al. 1999). At predawn LWP of -6.0 MPa, olive maintains a certain transpirative and photosynthetic activity (around 10% and 20%, respectively, of that of well-watered plants), that allows the plants to produce assimilates and accumulate them in the various organs. Particularly, long-term soil water deficit reduces in young olive trees the development of the above-ground organs with respect to the under-ground part (roots and stump), so raising the under/above-ground ratio. The effect is particularly marked in leaf area, that is significantly reduced under rain-fed conditions (47% lesser than irrigated plants at the seventh year from planting) (Dichio et al. 2002). Such reduction of canopy size limits the water demand for transpiration.

Another strategy adopted by the olive tree to overcome water deficit is osmotic adjustment which consists in either active synthesis and accumulation of osmotically active compounds (carbohydrates, some aminoacids, organic and inorganic acids, cations and anions) within cells (active osmotic adjustment) or loss of water from plant cells, with the consequent increase in osmolyte concentration (passive osmotic adjustment) (Xiloyannis et al. 1999; Cataldi et al. 2000; Sofu et al. 2004b; Dichio et al. 2009). This physiological process is measured by the variation in osmotic potential within plant tissues (Dichio et al. 2007). A higher concentration of osmolytes

(particularly mannitol, glucose and proline) facilitates water diffusion in cells and maintains the turgor of plant tissues essential for plant physiological activity. The maintenance of cell turgor in roots also avoids or delays the separation of these organs from the soil. Under drought conditions, olive trees activate metabolic processes to produce substances that increase cell tissue rigidity, likely by regulating some enzymes involved in lignin biosynthesis such as peroxidases (Sofu et al. 2004b). This mechanism results in an increase in elastic modulus (ϵ) as cell walls become more rigid or thicker. Higher ϵ values produces a faster turgor loss of cells for a given percentage of dehydration. An increase of cell tissue rigidity together with low values of Ψ_p , due to active and passive osmotic adjustment, can be responsible for the observed high gradients of water potential between leaves and soil, and thus can facilitate water extraction from the soil.

In olive trees, the activities of some antioxidant enzymes significantly increase in leaves and roots of drought-stressed plants (Sofu et al. 2004a). These enzymes limit the cellular damages caused by AOS, so allowing the plant to maintain a photosynthetic efficiency also under severe drought conditions (Xiloyannis et al. 2003). Significant increases of lipoxygenase (LOX) activity and malondialdehyde (MDA) content, two markers of oxidative stress, were also found during the progressive increment of drought stress in both leaf and root tissues of olive plants (Sofu et al. 2004a, b), so suggesting that water deficit is associated with the oxidation of membrane lipids. In olive plants under drought stress, the damage of photosynthetic apparatus, and the resulting decrease in photosynthetic efficiency, occurs particularly by means of the light-dependent inactivation of the photosystem II (photoinhibition) and the oxidation of chloroplastic pigments (photo-oxidation) (Angelopoulos et al. 1996; Sofu et al. 2009). Despite these damages, olive tree is able to recover its water status faster (5 days) than other fruit tree species even if it shows a slow recovery of photosynthesis and transpiration (Angelopoulos et al. 1996).

Finally, olive tree can respond to short period stress by regulating the activity and the expression of its root water channels (aquaporins)

(Tataranni 2009). As the adverse conditions continue, root suberification occurs, so avoiding dehydration. In fact, an increase of suberification process was observed in root cell walls at exodermis and endodermis level. Under such conditions, root activity recovery is preceded by the emergence of root primordia (Tataranni 2009).

3.2 Effects of Irrigation Management on Productivity, and Fruit and Oil Quality

Generally, irrigation raises significantly the vegetative growth of olive tree and its productive response. This leads to early bearing, steady and satisfactory yields, and improvement of fruit features. In addition, as the productive tree performances are not influenced by moderate levels of drought stress, a reduced irrigation is recommended in arid and semi-arid areas to save water. Deficit irrigation strategies in olive orchards can be applied following different approaches (Feres and Soriano 2007).

Sustained deficit irrigation (SDI) distributes a reduced water volume, as percentage of ET_c , throughout the whole irrigation season. Many studies, carried out under diverse pedo-climatic conditions, compared irrigation regimes based on different levels of ET_c restitution and their influence on fruit and oil quality of different olive cultivars. Patumi et al. (2002), Magliulo et al. (2003), d'Andria et al. (2004), Grattan et al. (2006), Berenguer et al. (2006) and Dabbou et al. (2010) found that a restitution ranging from 66 to 75% of ET_c is enough to obtain good yields similar to those harvested from fully irrigated trees. However, phenolic compounds in oils significantly decreased passing from the lowest to the highest irrigation levels. Although reduction in polyphenol content modified slightly sensory properties of oils decreasing their bitterness and pungency, it did not compromise oil storage capacity. Stefanoudaki et al. (2009) referred about a contradictory effect of irrigation which decreased contents of both undesirable (pungent and bitter attributes) and favourable sensory qualities (intense green notes). As a matter of fact, irrigation could be managed to meet consumer's

particular needs. Patumi et al. (1999), Tovar et al. (2001) and Tovar et al. (2002) studied the effect of several irrigation treatments on L-phenylalanine ammonia-lyase activity (PAL) in developing fruits. PAL is the key enzyme in phenolic biosynthesis and a high PAL activity is associated with the accumulation of anthocyanins and other phenolic compounds in tissues of several fruit species (Weaver and Herrmann 1997; Ryan et al. 2002). PAL activity and phenolic level decreased during fruit development and were influenced by irrigation, being lowered as the water supplied increased.

Regulated deficit irrigation (RDI), firstly proposed by Chalmers et al. (1981), reduces water supplies during specific periods characterized by a less plant sensibility to water stress with minimal effects on yield. While water deficit can reduce fruit and oil yields due to the effect on flowering, fruit set and oil accumulation phases, many researchers agree in identifying pit hardening, generally occurring in midsummer, as the less sensitive phenological stage of olive tree (Lavee and Wodner 1991; Goldhamer 1999; "Moriania et al. 2003; Orgaz and Feres 2004; Iniesta et al. 2009). On the other hand, in environments characterized by good spring rainfall and deep soil profiles, irrigation applied from the beginning of pit hardening to early fruit veraison could control tree vigour while maintaining crop yield and oil quality (Gómez-Rico et al. 2006; Tognetti et al. 2006, 2007; d'Andria et al. 2009).

Partial root-zone drying (PRD) is an irrigation strategy aimed to maintain in a drying state at least half of the tree root system while the other half is kept under wet soil conditions. Such technique is based on the existence of a chemical signal between root and shoot which determines plant responses to soil drought stress limiting shoot and leaf growth. Particularly, under mild soil drought stress, abscisic acid (ABA), moving in the xylem from the roots, reaches the epigeal parts of the tree, where it regulates stomatal movement and shoot meristem activity. The alternation of wet and dry conditions in the soil is a requirement to allow roots to produce ABA. Generally, a PRD cycle lasts 10–15 days, depending on soil type and other factors such as rainfall and temperature (Davies et al. 2000; Stoll et al. 2000; Stikic et al. 2003;

Sepaskhah and Ahmadi (2010). Wahbi et al. (2005) reported that PRD strategies slightly reduced yield (15–20%) and increased plant water use efficiency of 60–70%. Aganchich et al. (2008) showed that PRD irrigation of “Picholine marocaine” plants, besides water saving (50%), positively affects both fruit biometric parameters and oil production (highest oil content, precocious fruit ripeness), and causes increases in total polyphenol. Instead, Fernández et al. (2006), comparing PRD and RDI treatments (50% ET_c), did not find significant improvement of the physiological parameters measured. Such findings led the authors to advise against the use of PRD because of its high cost and difficulty in management.

Moriana and Orgaz (2003) proposed an irrigation scheduling adapted to the typical alternate bearing habit of the olive which supplies water only in “on” years. Although this approach was successfully tested in pistachio plants (Stevenson and Shackel 1998), the authors expressed some doubts on the viability of such program for olive. As a matter of fact, an exceptional severe drought during the rainfed “off” year, able to completely deplete water in the soil profile, could have an important impact in flowering and fruit set of the following “on” year resulting in very low yields. On the other hand, Palese et al. (2010) reported that after a rainfed “off” year, olive trees continuously non-irrigated showed a great capacity of recovery, which led to a vegetative activity and productive response similar to those of the irrigated plants. This is due to a complete replenishment of soil water reserve following autumn–winter rains. As reported by Martín-Vertedor et al. (2011), the application of SDI during “off” year could be advisable when a lower water consumption occurred. Therefore, the optimal irrigation amount could be determined each year, according to crop load levels.

3.3 Strategies for Rainwater Capture and Storage Under Rainfed Conditions

In traditional olive cultivation areas of Mediterranean Basin, rainfall is the only source of water for the olive tree. Therefore, under rainfed

conditions, strategies aimed to improve the recharge of rainwater in soils by using specific soil management techniques, or to capture rainwater in collection systems (i.e. Tunisian “jes-sour”, hand-made stone terraces, basin at farm and hydrographic level), are recommended (de Graaff and Eppink 1999; Fleskens et al. 2005; Tubeileh et al. 2009).

Among the soil management techniques, mechanical tillage is still the most common in Mediterranean olive orchards, where it is performed also as a dry farming technique with the aim of reducing soil evaporation by interrupting water capillary rise and increasing soil surface roughness (Ozpinar and Cay 2006). Furthermore, tillage should improve infiltration and percolation into soil of rainfall water but such effects often occur only for a short period of time immediately after the machine passage (Pastor et al. 2000). Unfortunately, continuous tillage may result in the degradation of soil structure which can significantly reduce water infiltration rate causing runoff, erosion processes and fertility loss (Abid and Lal 2008). These degradation mechanisms are quickened by the high air temperatures that induce an intense microbial biomass activity and the mineralization of the labile fraction of organic matter, the most active in the soil. A significant loss of organic matter leads to a further deterioration of soil hydraulic properties directly involved in the recharge and storage of rainfall into the soil (Lipecki and Berbeć 1997; Strudley et al. 2008).

Autumn–winter cover crops, spontaneous or sown, can represent an alternative to tillage in rainfed olive orchards showing a beneficial effect in intercepting raindrops, reducing runoff, facilitating and speeding infiltration of excess surface water into the deepest soil layers even thanks to the channels left by their dense death root network (Pastor et al. 2000; Pardini et al. 2002; Hernández et al. 2005; Durán-Zuazo et al. 2009; Palese et al. 2009a). A study carried out by means of a non-invasive geophysical techniques (electrical resistivity imaging, ERI) revealed that a cover cropped mature olive orchard was more efficient to intercept and store rainwater than tilled grove, resulting in a significant water reserve at the deepest soil layers (>1.0 m),

convenient for the root system of rainfed olive trees in the driest months (Celano et al. 2011). On the other hand, cover crops show very high hydric consumptions from the soil (from 200 up to 350 mm per year) and so they could compete with olive trees for water, especially when annual rainfall is less than 500 mm (Bellini 1983; Pardini et al. 2002). Therefore, it is fundamental to choose the most opportune date for cover crops suppression (by mechanical or chemical means), avoiding the overlapping between weed growth and some critical phases for the olive productive performance such as flowering and fruit set (Orgaz and Fereres 2004).

The improvement of soil water holding capacity can be reached also by means of techniques aimed to increase and/or preserve soil carbon content. Pruned material represents an important source of dry matter internal to the olive orchard and characterised by high content of lignin, low nitrogen level ($C/N > 25$) and slow decomposition process (Celano et al. 2003). Once cut and buried in the soil, pruning material is able, in the long period, to build up soil organic matter which, in turn, improve soil hydraulic features (Pastor et al. 2000; Hernández et al. 2005). The recycle of polygenic organic material inside the olive orchard (spontaneous cover crops + pruned material), offering mixed organic substrates, strongly affects the activity of soil microbial communities which show a higher complexity and diversity at genetic, functional and metabolic levels (Sofo et al. 2010b).

3.4 Use of Non-conventional Water Sources for Irrigation

Olive trees are widely diffused in arid and semi-arid environments where water shortage and competition among the different water consumption sectors are relevant problems. For this reason, the use of low quality water for irrigation (e.g., saline water or municipal wastewater) could represent a realistic way to overcome the scarcity of “conventional” water assigning it especially for human consumption. In addition, an increase of the irrigated olive-grown area could lead to improved farmers’ income, with a general benefit to the local rural economy.

In the Mediterranean regions, large amounts of saline water (with an electrical conductivity, $EC > 2.0 \text{ dS m}^{-1}$) are available for irrigation. Among Mediterranean fruit tree species, olive tree is moderately salt tolerant (Ayers and Westcot 1976; FAO 1985; Rugini and Fedeli 1990), and it shows a different tolerance behaviour depending on cultivars, salt concentration (EC from 5.0 to 13.7 dS m^{-1} , the latter identified as the tolerance limit) and salt type dissolved in the irrigation water (Rugini and Fedeli 1990; Chartzoulakis 2005). Salt tolerance in olive cultivars is basically related to salt-exclusion mechanisms occurring within roots, which prevent salt translocation rather than salt absorption by keeping Na^+ and Cl^- at root level and limit the accumulation of such ions into actively growing shoots. Furthermore, Ca^{2+} has a main role in regulating the selectivity of the ionic absorption, decreasing Na^+ uptake and its transport to the shoot and reducing toxic effects of Na^+ on integrity of the plasmatic membrane in root cells (Benlloch et al. 1991; Tattini et al. 1995; Melgar et al. 2009). As a matter of fact, the increase of Ca/Na ratio by adding Ca^{2+} to irrigation water has been recommended to mitigate the detrimental effects of salinity stress (Rinaldelli and Mancuso 1996; Melgar et al. 2009). The correction of water irrigation, together with the use of drip irrigation and the choice of a tolerant cultivar, can be useful tools for an appropriate employment of saline water. As reported by Melgar et al. (2009), the long-term irrigation of mature olive trees of cv. “Picual”, a salt-tolerant cultivar, with saline water (EC up to 10.0 dS m^{-1}) did not affect growth and yield, and no salt accumulation was found in the upper 30 cm soil layer thanks to the ion leaching linked to the rain season (annual precipitation of 702 mm). On the other hand, irrigation with saline water could be harmful in low rainfall areas (less than 250 mm). Under such conditions, it is essential to plan a proper soil leaching management (Wiesman et al. 2004).

Another alternative water resource is reclaimed urban wastewater. Olive trees can lend themselves to irrigation with this low quality water because their fruits are usually harvested 1 month, or more, after the last water application (according to the variety and its maturation time), and

they are eaten after processing (to obtain oil or table olives). Such conditions decrease risk of fruit microbial contamination. Furthermore, the use of microirrigation system avoids the contact among wastewater, fruits and leaves allowing the production of safe high-value olive yields and avoiding health risk for the farm workers and the consumers (Palese et al. 2006, 2009b; Bedbabis et al. 2009). A sustainable orchard management (Fig. 6.2) coupled with an intense water absorption by the roots of olive trees and cover crops active in the wetted soil volume, excluded water logging by runoff and percolation to deeper soil layers avoiding aquifer pollution by faecal bacteria (Palese et al. 2009b). From an agronomic point of view, wastewater is rich of mineral elements (particularly P, N and K) and organic matter, both important for yield and vegetative development of olive trees and soil fertility, and often eliminated during the sewage treatment (Ramirez-Fuentes et al. 2002; Yadav et al. 2002; Tarchouna Gharbi et al. 2010a). The reduction of the treatment level decreases fertilization costs and pollution and the price of the treated water allowing, in economic terms, its sustainable reuse (Lopez et al. 2006; Palese et al. 2009b). Nutrients supplied by wastewater should be taken into account in preparing the annual fertilization plan (Palese et al. 2008). On the other hand, reclaimed urban wastewater can be an important source of both salts and potentially toxic metals. Although urban wastewater usually shows a low concentration of heavy metals, long-term irrigation can increase their concentration into soils even if not at critical values (Ramirez-Fuentes et al. 2002; Yadav et al. 2002; Tarchouna Gharbi et al. 2010b; Klay et al. 2010). Therefore, a systematic monitoring of metal content in wastewater, soil and plant is recommended to avoid hazardous situations for populations and environment.

4 The Genus *Citrus*

An efficient water management for *Citrus* spp. trees in any cropping situation requires accurate quantitative information on water use. Interpretation of the water relations of most *Citrus*

spp. cultivars is difficult due to the occurrence of stomatal oscillations whose origin is not well known and which cause sampling problems in irrigation management (Dzikiti et al. 2006, 2008; Wright 2008).

Vegetative growth, and particularly leaf development and stem diameter, of orange trees (*Citrus sinensis* (L.) Osbeck) is particularly susceptible to water scarcity (Dzikiti et al. 2006; Aiyelaagbe and Orodele 2007), and plants respond to drought by changes in gas exchange, phytohormonal balance and polyamine contents (Wang and Liu 2009). Moreover, internal water storage contributed significantly to the daily total leaf transpiration in the species (Dzikiti et al. 2006, 2008). García Petillo et al. (2004) compared the effects of different irrigation volumes on “Washington Navel” orange yields during a 5-year period (0, 50%, 100% and 150% ET_c). To apply these treatments, one irrigation drip line per tree row, with drippers, of 2, 4 and 6 Lh^{-1} capacity, separated 1 m apart were used for the 50%, 100% and 150% ET_c treatments. Another treatment received the same amount of water as 100% ET_c , but with two drip lines spaced 1 m apart per tree row and 2 Lh^{-1} drippers and showed significant increases in total fruit yield and fruit size if compared to 100% ET_c . The application of the PRD irrigation method (50 and 100% ET_c) to orange trees was evaluated over two growing seasons by Dzikiti et al. (2008b). The authors found that stomatal conductance in the PRD treatments was lower than in the control fully-watered treatment but no significant changes in average fruit yield were found between the two PRD treatments and the control plants. Regarding RDI, it has been demonstrated that the irrigation cut-off during the final fruit growth and maturity process (phase III) in orange (cv. “Lane late” grafted on “Carrizo” citrange) reduces midday SWP, does not reduce fruit yield and increases total soluble solids and titrable acidity, without altering fruit quality and the final maturity index (Pérez-Pérez et al. 2009). On the contrary, García-Tejero et al. (2010) found that RDI applied during the flowering and early fruit-growth phases (cv. “Navelina” grafted onto “Carrizo” citrange), both yield and fruit quality (in terms of total soluble solids and titrable acidity) were negatively affected.



SUSTAINABLE MANAGEMENT	CONVENTIONAL MANAGEMENT
	
<ul style="list-style-type: none"> • No tillage - Spontaneous weeds and grasses mowed at least twice a year • Pruning material cut and left on the ground as mulch 	<ul style="list-style-type: none"> • Conventional tillage (milling at 10 cm soil depth) performed 2-3 times per year in order to keep the soil bare
<ul style="list-style-type: none"> • Guided fertilization: fertigation based on a nutrient balance approach which takes into account nutrient input (by wastewater), output (by yield), and recycling/immobilisation in the grove system (by pruned material, senescent leaves, cover crops) 	<ul style="list-style-type: none"> • Mineral fertilization carried out empirically once per year by using granular product applied to the soil
<ul style="list-style-type: none"> • Guided drip irrigation with treated municipal wastewater based on crop evapotranspiration calculated according to FAO equation: $ET_c = K_r \times K_c \times ET_o$ (K_r = reduction coefficient; K_c = crop coefficient; ET_o = potential evapotranspiration) - (6 self-compensating drippers per tree delivering 8 L h⁻¹) 	<ul style="list-style-type: none"> • No irrigation
<ul style="list-style-type: none"> • Light winter pruning performed each year in order to reach vegetative-reproductive balance of trees 	<ul style="list-style-type: none"> • Heavy pruning carried out every two years - Pruned residues burned out of the olive grove

Fig. 6.2 Comparison between a sustainable and a conventional management of a mature olive orchard (*Olea europaea* L., cv. “Maiatica”) located in Southern Italy,

under a semi-arid climate with an average annual rainfall of 561 mm. Olive trees were vase trained (156 plants ha⁻¹) (data from Palese et al. 2008, 2009b)

Other *Citrus* species showed responses against drought stress similar to those found in orange. Huang et al. (2000) examined the growth changes generated by mild drought stress on potted tangerine trees (*Citrus reticulata* Blanco, cv. “Zhuju”) during early juice sac expansion stage. They observed that fruit growth was inhibited by drought stress but a greater water uptake was caused by a lower water potential in fruits of stressed plants, likely due to a higher water loss from fruit to transpiring leaves during water shortage and some active adaptive physiological responses (osmotic adjustment and cell wall loosening) of fruit to this stress. In satsuma mandarin trees (*Citrus unshiu* Marc.), a positive relationship between flower-bud induction and the level of endogenous plant hormones was found as a result of the application of mild (predawn LWP=from -0.5 to -1.0 MPa) and moderate drought stress (predawn LWP=from -1.5 to -2.0 MPa) (Yoshita and Takahara 2004). The results indicate that gibberellin levels were enhanced by severe drought stress, higher in the leaves from the branches that produce fewer flowers during flower-bud induction periods, whereas the levels of indole-3-acetic acid were higher in the leaves from the branches that produced more flowers during the season when flower-buds develop. As in *C. sinensis*, the measurement of maximum daily trunk shrinkage is a suitable and reliable indicator of the level water deficit reached by plants of other *Citrus* species, such as lemon (*C. limon* (L.) Burm. fil.) and sour orange (*C. aurantium* L.) (Ortuno et al. 2009).

5 Other Mediterranean Fruit Species

5.1 Pomegranate

Pomegranate trees (*Punica granata* L.) are considered as a crop with a high level of tolerance to soil water deficit. Pomegranate cultivation is mainly confined to the tropics and subtropics and it grows well in arid and semi-arid climates, but it is now widely cultivated in Mediterranean (Hepaksoy et al. 2009). In Spain, for example, its culture is concentrated in the south east, where fresh water available for agriculture is very scarce.

The water relations of field grown pomegranate trees grown under different drip irrigation regimes were recently investigated by Intrigliolo et al. (2011). These authors observed that during spring and autumn, midday SWP was not significantly different between irrigation treatments while there were considerable differences in leaf photosynthesis and stomatal conductance, suggesting a near-isohydric behaviour of pomegranate trees. This means that plants control gas exchange such that daytime water content is almost unaffected by soil water deficits, and that other mechanisms (e.g., ABA production and signaling) can be responsible for the regulation of plant water status. There is little knowledge about the response of pomegranate to drought, and in general to abiotic stresses. In one of the few researches, Bhantana and Lazarovitch (2010) studied the evapotranspiration, crop coefficient and growth of two young pomegranate varieties under salt stress, confirming that this species exhibits a high tolerance under adverse environmental conditions. If compared to other irrigation techniques, drip irrigation is the best way to increase fruit yield and plant growth of pomegranate, as its root system is particularly inhibited by water stagnation, whereas fruit yield is not significantly influenced by the level of irrigation (Sulochanamma et al. 2005). Furthermore, irrigation of pomegranate trees is very important, as fruit splitting and cracking can occur, unless they are regularly irrigated. Excess watering or excessive rain during the maturation period may also cause similar damage to the fruits (Hepaksoy et al. 2009). Finally, vitamin C, reducing sugar and total sugar content were observed in fruit of drought-stressed plants (Lawand et al. 1992).

5.2 Pistachio

Pistachio is a crop indigenous to western and central Asia but its cultivation has spread to the Mediterranean region, which has become its second most important centre of diversity after Iran. The importance of *Pistacia* spp. is not limited to this product alone: the tree's great tolerance to drought stress and their ability to thrive in poor soil conditions make them particularly suitable

for forestry programmes on marginal lands, where they can also represent a source of additional income for local farmers (Padulosi et al. 1998; Sedaghat 2008). Pistachio cultivation requires the use of rootstock because grafting is the only form of vegetative propagation, thus the choice of the most effective rootstocks plays a key role, as they determine the physiological and biochemical responses of the plants to drought (Ranjbarfordoei et al. 2000, 2002; Gijón et al. 2010). The extreme drought resistance of *Pistacia* spp. enables farmers in arid and semi arid lands to grow this nut without irrigation (Kaska 2002). Despite the economic importance of edible pistachio (*Pistacia vera* L.), very little information is available on its nutrient requirements and water needs. Potassium (K) fertilization is found to be effective in increasing leaf K status, nut yield and quality in this species, and K uptake occurs mainly during the nut fill period (Zeng et al. 1998). Tajabadipour et al. (2006), studied the effects of three irrigation frequency and five K levels on the plant water relations and growth of three pistachio cultivars (“Badami”, “Ghazvini” and “Sarakhs”), founding that the dry weights of leaves, stems and roots significantly decreased with increasing irrigation intervals, whereas K application had no significant effect on LWP, osmotic potential and turgor potential. From a molecular point of view, Yakubov et al. (2005) observed the accumulation of dehydrin-like proteins both in the inflorescence bud and in the bark of young pistachio stems, suggesting that they may have a role in drought and cold tolerances, as well as serving as storage proteins. An irrigation experiment involving pistachio (*P. vera*, cv. “Kerman”, on *P. terebinthus* rootstocks) was performed by Gijón et al. (2009) over a 4-year period to determine the effect of RDI (at 65% and 50% of control irrigation) on nut yield and quality. The growth season was divided into three phenological stages: stage I – from sprouting until the end of rapid nut growth; stage II – from maximum nut size until the beginning of kernel growth; and stage III – from the beginning of kernel growth until harvest. The plants subjected to RDI were only significantly stressed during stage II, showing midday LWP of around -1.4 MPa. The application of RDI resulted in smaller nut

diameter and lower total yield. Moreover, trees subjected to RDI had a total yield and percentage of split nuts similar to those of the controls, and did not show the normal alternate bearing pattern of this tree crop. The authors concluded that this rootstock-scion combination presents a high degree of drought-resistance and could be efficiently applied in pistachio cultivation.

5.3 Prickly Pear

Opuntia, also known as “nopales” or “paddle cactus”, is a genus in the family Cactaceae. The most common culinary species belonging to this genus is the Indian fig *Opuntia ficus-indica* (L.) Miller, commonly known as “prickly pear”. This species is native to Mexico but it is also found in southern Europe and northern Africa, where it contributes, like olive tree, to the typical Mediterranean landscape. The prickly pear tree is able to store high water amounts in its succulent organs and it has a very wide (even though not deep) root system with dense and rapidly regenerating root hairs, that allow plants to efficiently use extremely low rainfall (Mulas and Mulas 2004). Prickly pear has a CAM photosynthesis and thus maintains the stomata of mature cladodes open only in the night, but, under extremely severe water deficits, the stomata remain closed all day long and so the plants use to photosynthesize only the CO_2 deriving from the respiration (Nieddu et al. 1997). Pimienta-Barrios et al. (2000) evaluated the effects of seasonal variation in temperature, irradiation and soil moisture content on the photosynthetic rates of prickly pear. They demonstrated that this species is strongly adapted to arid climates and that stem photosynthesis by cladodes (stem modified for photosynthesis that looks like leaves) allows plants to fix carbon to be used during the periods when soil water content is very low. Drought significantly affects cladode morphology and inhibits new cladode production, as these latter have a C_4 -photosynthesis and open the stomata during the day, with consequent water losses (Nieddu et al. 1997). Furthermore, cladodes can reach temperatures 15°C higher than the environmental ones, maintaining their enzymatic activities up to 60°C .

The fruit yield of prickly pear is quite low, likely due to limiting environmental factor (low water amounts, soils with low levels of organic matter), but a fertilization up to 160 kg ha⁻¹ determines a yield increase and a high fruit quality (Mulas and Mulas 2004). Mulas and D'hallewin (1997) estimated that fruit yield in irrigated plants is at least two folds higher than that of un-watered plants, due to higher fruit number per cladode and not to increases in fruit weight. On the other hand, irrigated plants presented an increase in fruit peel thickness, which reduced the juice percentage, and in seed weight (Mulas and D'hallewin 1997). Snyman (2006) aimed at quantifying the effects of drought stress on the growth of tap roots, side roots and rain roots of the species *Opuntia ficus-indica* (L.) (cv. "Morado", with green cladodes) and *O. robusta* Wendl. (cv. "Monterey", with blue cladodes), both having edible fruits. They planted 1-year-old cladodes in root boxes and pots in a greenhouse. Placing the cladodes flat on the soil, more areoles came in contact with the soil, and each areole complex formed on average three roots. From the analysis of the growth of tap roots, side roots and rain roots and from the data on root size and density, *O. robusta* appeared to be less sensitive to drought than *O. ficus-indica*.

5.4 Loquat

Loquat (*Eriobotrya japonica* Lindl.), also called Japanese medlar or Japanese plum, is a subtropical evergreen tree crop indigenous to southeastern China but very well adapted to mild-winter areas of the Mediterranean basin (Hueso and Cuevas 2008). Drought stresses causes significant decreases in leaf expansion rate, area and photosynthetic pigments and in stomata size, and increases in stomata density (Luo et al. 2007). Both deficit irrigation during the entire season and post-harvest RDI from mid-May through the end of August (reduction of 20% water needs, with water savings established around 1,450 m³ ha⁻¹ yr⁻¹) were successfully applied in this species (Hueso and Cuevas 2008). It was observed that post-harvest RDI usually advances

full bloom 10–20 days, allowing to obtain a more precocious and valuable yield, whereas the effects of continuous deficit irrigation is less noticeable (Hueso and Cuevas 2008; Fernández et al. 2010). On the contrary, yield and fruit quality are not affected for the different deficit irrigation strategies. The optimal month for the application of post-harvest RDI in loquat seem to be July, due to the positive effects on the advancement of bloom and harvest date and its harmlessness for flower development, even though every RDI applied in the period June–August (with a water reduction up to 75%) does not influence negative fruit set, size and yield (Cuevas et al. 2007).

6 Conclusions and Future Perspectives

A new approach in fruit orchard management is imposed by the environmental emergencies that are marking this recent period (e.g., soil degradation as a result of erosion and desertification, water shortage, greenhouse effect). In semi-arid Mediterranean lands, the adoption of agricultural systems by means of conventional, non-sustainable techniques causes the reduction of soil organic matter, groundwater contamination, soil deficiency of mineral elements (in particular phosphorus and nitrogen), alkalization/salinization and nutritional imbalances in plants. On the other hand, the recent researches on the physiology of fruit trees and on soil chemical and biological fertility in fruit orchards have revealed that sustainable and innovative soil management systems, with a particular emphasis on irrigation, allow to obtain an optimal plant nutritional equilibrium, avoid nutrients accumulation and leaching risks, improve irrigation efficiency and prevent soil erosion and root asphyxia. As highlighted in this chapter, the definition of appropriate irrigation techniques (e.g., SDI, RDI, PRD) and soil management in Mediterranean fruit orchards are indispensable requisites for preserving soil quality, positively affecting soil microbial activity and fertility and maintaining top yields of high quality. Considering the scientific, practical and socio-economical importance of

these topics, and the increasing environmental emergencies related to water scarcity, a conspicuous number of studies is expected in the next years. At the moment, it is clear that the application, optimization, innovation of sustainable agricultural techniques with a low negative environmental impact can allow to recover or increase the normal levels of total fertility in agro-ecosystems, with positive effects on both soil and yield quality.

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