Physiological and Biochemical Remarks on Environmental Stress in Olive (Olea europaea L.)

C. Vitagliano and L. Sebastiani
Scuola Superiore "S. Anna", di Studi Universitari e di Perfezionamento Pisa, Italy

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Abstract

Olive (Olea europaea L.) is an evergreen tree traditionally cultivated in the Mediterranean basin where plants undergo, to some degree, stress due to unfavourable environmental conditions. Water deficit, freezing, salinity and air pollution are a few of the stress factors restricting growth, so that olive productivity at the end of the growing season expresses only a fraction of the plant’s genetic potential. Understanding the physiological and biochemical processes that enable olive adaptation and acclimation, as well as the mechanisms of stress injury, is therefore of relevant importance. However, the studies on the physiology and biochemistry of stress resistance and on the role of gene expression and protein synthesis in olive are almost in an initial phase when compared with the progress achieved for other cultivated plants.

In this paper, the physiological and biochemical studies performed on olive plants exposed to the main environmental stresses will be analysed and discussed.

INTRODUCTION

Olive (Olea europaea L.) is an evergreen tree traditionally cultivated in the Mediterranean basin where several environmental constraints are limiting factors for its productivity.

Water deficit and salinity stresses are probably the main restrictions for olive cultivation in the Mediterranean climate, though olive behaves as an intermediate drought- and salt-tolerant species when compared with other temperate fruit trees.

Low (freezing) temperature becomes a stressful factor when olive cultivation areas approach climatic limits, as usually happens in the northern and central Italian regions and also in some southern zones of Europe. In these areas, plant survival can be seriously compromised when temperature approaches -10°C.

In the last decades, human activities have increased the number of environmental constraints that cultivated plants have to cope with and atmospheric pollution is probably the main one. Significant changes in the composition of atmosphere have been observed for several gases, such as for tropospheric ozone (O₃) and carbon dioxide (CO₂). These changes have opened exciting areas for environmental studies and several articles have been published on this subject. However, the effects of O₃, and CO₂ on olive physiology and biochemistry, especially when considering long-term realistic experiments, are still almost unexplored.

Despite the relevant importance of olive resistance to environmental stress, the studies in this field are still limited when compared to the progress achieved in other cultivated species.

In this paper, the physiological and biochemical studies performed on olive plants exposed to environmental stress will be analysed and discussed.

WATER DEFICIT

In Mediterranean climates water deficit is probably one of the main restrictions for olive cultivation, though olive behaves as an intermediate drought-tolerant species when compared with other temperate fruit trees. Olive leaves can, in fact, tolerate very low water potential (up to -6÷8 MPa) and lose almost 40% of tissue water, while maintaining a full rehydration capacity (Rhizopoulou et al. 1991). These adaptations enable olive
plants to establish a high water potential between leaves and roots and consequently to extract soil water up to –2.5 Mpa.

Drought stress determines growth inhibition and slows down photosynthetic activity (Bongi and Palliotti 1994). However, olive plants still maintain a slight net assimilation (10% of well-water plants) rate at very low (–6.0 MPa) pre-dawn leaf water potential (Xiloyannis et al. 1999) and prevent excessive water loss by stomata closure (Fernandez et al. 1997; Giorio et al. 1999). Studies on the diurnal course of leaf water potential and gas exchange parameters in olive plants subjected to different levels of water deficit showed that the net photosynthetic rates and stomatal conductance reached maximum values early in the morning both in well-watered and in stressed plants, but declined more and faster in plants exposed to stress conditions. Moreover, the inactivation of photosynthetic activity during severe drought stress affected the non-stomatal component of photosynthesis and perhaps induced a light-dependent inactivation of the primary photochemistry associated with photosystem II (PSII) (Angelopoulos et al. 1996).

The maintenance of a slight photosynthetic activity during drought stress enables olive plants to continue the production of assimilates and their accumulation in the root system, determining a higher root-leaf ratio in comparison with well-watered plants (Xiloyannis et al. 1999). These adaptations result in a better defence of the plants to drought conditions (Celano et al. 1999). Leaf activities and root contact with the soil particles depend on cell turgor, which can be regulated through active and passive osmotic adjustments. In water-stressed olive plants, mannitol and glucose have an important role in the active osmotic adjustments of leaves together with organic acids, such as malic and citric, while mineral elements are not involved (Xiloyannis et al. 1999).

Rewatering of drought stressed olive plants induced a period of leaf activity inertia. This effect was probably dependent on hormone balance and conductivity of the xylem system. Studies on the short-term water use dynamics in olive trees after rewatering by heat-pulse measurements of the sap flux (Moreno et al. 1996) showed differences between regularly irrigated and non-irrigated trees. Following irrigation, the regularly irrigated plants maintained, for 3 days after irrigation, a transpiration rate of 1.65 mm³ mm⁻² d⁻¹. Subsequent to this phase, the rate of water use declined and transpiration fell. The sap flow in the near-surface root dropped concomitantly. In non-irrigated plants, irrigation lifted the transpiration rate to only 1.12 mm³ mm⁻² d⁻¹ and leaf water potential did not recover because of plant inability to refill cavitated vessels. These data showed that, even after rewatering, olive behaves as a parsimonious and cautious consumer of soil water.

Further studies on the physiology and biochemistry of olive plants during water deficit and rewatering are important to better understand the cellular and molecular mechanisms involved in olive plant resistance.

SALINITY

Accumulation of salts in cultivated soils is becoming an extensive problem in those areas subject to irrigation with low quality water. In coastal areas of the Mediterranean basin, olive plants can experience temporary salinity stress during the dry period of the growing season when irrigation is used to overcome severe drought stress.

Olive is considered medium tolerant to salinity stress (Rugini and Fedeli 1990), but salinity resistance is a cultivar dependent character. Tattini et al. (1992) highlighted large differences in salt tolerance between Frantoio and Leccino cultivars. Furthermore, Marin et al. (1995) analysed the tolerance of 26 olive cultivars to excess (100 mM) NaCl in the growing medium and showed a broad genotypic variability to salt stress as indicated by the wide changes in shoot relative growth rate of salt-treated plants (from 16 to 70%).

The physiological and biochemical responses of olive to salinity stress are quite complex (Gucci and Tattini 1997). Visible growth inhibition was observed (Tattini et al. 1997) in plants exposed to alternating periods of salinity stress (50, 100, and 200 mM
NaCl) and relief cycles. Olive underwent growth recovery once salinization was relieved, but the rate of recovery was dependant on the salt level to which plants were exposed. Relief from low (50 mM) or intermediate (100 mM) NaCl treatments enabled a full recovery, which was not obtained in olive plants subjected to more severe NaCl stress (200 mM). Higher photosynthetic rate and stomatal conductance during relief from 50 and 100 mM NaCl treatments in comparison to the control were presumably responsible for the ready recovery of growth.

Plants exposed to salinity stress showed a significant reduction in net CO2 assimilation and stomatal conductance (Bongi and Loreto 1989). Assimilation declines were mainly a consequence of stomatal conductance reductions, but at high (200 mM) NaCl concentrations marked effects on carboxylation efficiency and CO2 compensation point were also observed. Nevertheless, the effects of salinity stress on gas exchange parameters do not correlate with the cultivar tolerance to salt. Net CO2 assimilation and stomatal conductance decreased, in fact, more in the salt tolerant cultivar Frantoio than in the salt sensitive one (Leccino) (Tattini et al. 1997). Better correlation was instead found when gas exchange values were recorded during relief.

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Significant reductions in water relation parameters, such as pre-dawn water potential ($\Psi_w$), osmotic potential ($\Psi_o$) and relative water content (RWC), were observed in Frantoio and Leccino plants exposed to NaCl (100 and 200 mM) (Gucci et al. 1997). The salt-induced decreases in $\Psi_w$ occurred with a decrease in $\Psi_o$, consequently maintaining the turgor potential ($\Psi_t$) in salinized plants at values comparable to or higher than in the control plants.

Olive leaves accumulated both glucose and mannitol during the period of salinity stress (Tattini et al. 1996). Moreover, Gucci et al. (1998) showed that salinity stress (100 mM NaCl) induced major changes in partitioning of photosynthetic carbon in fully-expanded leaves of Frantoio. In particular, mannitol partitioning increased, while glucose and sucrose partitioning decreased. However, osmotic adjustments in salt stressed olive plants were primarily achieved by accumulation of inorganic ions, despite the osmotic contribution of soluble carbohydrates, and they mainly reflected the different exclusion capacities for Na$^+$ and Cl$^-$, which the cultivars naturally possess. Thus salt tolerance in olive seems to be mainly achieved by means of a salt exclusion/retention mechanisms at the root level, which are capable of avoiding Na$^+$ and Cl$^-$ accumulation in actively-growing shoots (Tattini and Gucci 1999).

In the near future, studies on the biochemistry and genetics of these exclusion/retention mechanisms will be necessary to discover the molecular basis of olive salt tolerance and will probably provide a tool for improving olive resistance against this stress.

**LOW (FREEZING) TEMPERATURES**

Temperatures below zero (freezing temperatures) limit the distribution and the productivity of many plant species in temperate areas. In olive, freezing temperatures become stressful when the cultivation area of this sub-tropical species approaches the climatic limits which usually occur in the northern and central Italian regions and also in some southern zones of Europe. In these areas, temperature drops can approach -10ºC in occasionally severe winter and olive survival can be seriously compromised. Furthermore, significant productivity declines can arise also from relatively higher (-7ºC) freezing temperatures when leaf drop and twig desiccation can be induced.

Several field and laboratory experiments have been performed with the aim to identify and classify frost resistant olive genotypes. Guerriero et al. (1988) identified some Leccino clones with varying frost tolerance in field experiments. Subsequently, La Porta et al. (1994) analysed through ionic leakage technique these clones in comparison with the frost sensitive Moraiolo cultivar and confirmed a higher frost resistance for some of them. Bartolozzi and Fontanazza (1999) used visible symptoms, ionic leakage and differential thermal analysis to evaluate the frost resistance of twelve olive cultivars and selections. Differential thermal analysis did not allow any discrimination among the
cultivars studied, whereas ionic leakage results indicated Bouteillan and Nostrale di Rigali cultivars as the more tolerant to freezing, while Borsciona seem to be the less tolerant one. Moreover, visible symptoms confirmed ionic leakage results in Bouteillan. More recently, Mancuso (2000) applied electrical resistant changes to evaluate the freezing resistance of different organs in four olive cultivars and suggested this experimental procedure as a quick, easy and non-destructive tool.

Although olive cultivars have the genetic potential to tolerate freezing, the degree of resistance in the existing genotypes is not enough to ensure an effective protection against severe frost events. To improve olive freezing resistance, the physiological and biochemical mechanisms involved in olive cold acclimation must be studied (Bartolini et al. 1994; Bartolini et al. 1999; Bartolozzi et al. 1999).

Genetically competent plants (perennial and winter annual) acquire freezing tolerance through a process known as cold acclimation that involves a number of biochemical, physiological and genetic changes (Hallgren and Oquist, 1990). Cold acclimation begins in autumn when atmospheric temperatures are sub-optimal and the photoperiod becomes short. Sub-lethal freezing temperatures later in the acclimation process may induce a second phase in the acclimation process leading to maximal expression of freezing tolerance. Biochemical changes, such as soluble sugar, sugar alcohol, Krebs cycle acid and fatty acid have been recently followed in olive plants during cold acclimation and freezing stress (Bartolozzi et al. 1999), but simple relationships between such biochemical changes and frost resistance were not found.

Freezing causes injury symptoms in cellular membranes, including increased microviscosity, formation of gel phase domains, degradation of phospholipids, and accumulation of free fatty acids (McKersie, 1991). All these symptoms can be simulated in vitro on isolated membranes treated with superoxide radical. Furthermore, membranes from cold acclimated plants are more tolerant to activated oxygen species than the membranes from non-acclimated plants and also the antioxidant pool is often depleted after a freezing stress. These observations led to the hypothesis that these common injury symptoms are the consequence of oxidative stress and that oxidative stress tolerance is an important component of freezing tolerance, which could be improved by the manipulation of the antioxidant defence system. Following this approach, attempts to apply the existing antioxidant enzymes protocols in olive were performed (Scebba and Sebastiani 2000; Sebastiani et al. 2002). Nevertheless, further studies on the physiology and biochemistry of freezing resistance and on the role of gene expression and protein synthesis in olive plants during cold acclimation and freezing recovery are now necessary.

ATMOSPHERIC POLLUTANTS

Atmospheric pollution is a direct and undesirable effect of human activities. Air pollutants are those gases which should not be present at all in the clean atmosphere or which are normally present in it, but reach quantities that impair crop production. Visible symptoms of air pollutant injury on plants are not easily observable and can be monitored only in particularly experimental conditions (sensitive cultivars, controlled fumigation,...). For these reasons the effects of air pollutants on plants may be very subtle and the effects on crop productivity may be very slow and may escape control.

Since tropospheric O₃, and CO₂ are widely recognised to be the most diffused air pollutants we will focus on the effects of these chemical species on olive metabolism.

Ozone

Ozone is one of the major atmospheric pollutants in the Mediterranean basin, where significant O₃ concentrations (70-100 ppb) have been detected for several consecutive months in large areas, including remote sites from pollution sources. Several biochemical and physiological processes have been observed to be negatively affected in plants exposed to O₃ and the degree of inhibition was dependent on duration of exposure, pollutant concentrations and species or cultivar tested (Matyssek et al. 1998).

In olive, the first O₃ studies have been performed on Frantoio and Moraiolo
cultivars exposed to short-term sub-symptomatic O₃ (150 ppb as single O₃ pulse) levels (Minnocci et al. 1995). In such experiments, visible injury does not develop, while significant reductions in stomatal apertures and conductance were observed during O₃ fumigation and olive plants seem to use such mechanisms to avoid O₃ penetration into the internal leaf spaces. Genotypic differences in O₃ sensitivity were also observed. Indeed Moraiolo cultivar showed more noticeable O₃ effects than Frantoio. Subsequent long-term (4 months) fumigation trials with realistic (100 ppb) O₃ levels demonstrated that visible injury symptoms started to appear after 100 days as localised necrotic spots only in Moraiolo plants. Heavy reductions in transpiring stomatal surface and gas exchange parameters were observed both in Frantoio and Moraiolo cultivars (Vitagliano et al. 1999; Minnocci et al. 1999). To understand the possible acclimation strategy, further physiological and biochemical studies were performed on Frantoio and Moraiolo plants subjected to long-term (18 months) O₃ (100 and 50 ppb) treatments. Results confirmed the different physiological and biochemical behaviour of the two cultivars studied (Sebastiani et al. 2002) and the lower capability of Moraiolo leaves to avoid O₃ uptake during fumigation (Minnocci et al. 1995; 1999).

All these data suggested that in the Mediterranean area, where olive plants are widely cultivated and O₃ is present for several months of the year, this pollutant might significantly interfere with olive productivity.

**Carbon Dioxide**

Atmospheric concentrations of CO₂ ([CO₂]) currently rise at a rate of 1-2 µmol mol⁻¹ year⁻¹ and are expected to reach 650-700 µmol mol⁻¹ by the year 2075. Increasing global [CO₂] will affect agriculture production, since [CO₂] enrichment has the potential to alter biomass production and partitioning, photoassimilation, carbohydrate accumulation, secondary metabolism, water relations and many other ecophysiological processes.

Woody plants seem to respond more strongly to elevated [CO₂] than herbaceous plants and show morphological and physiological responses to elevated [CO₂] which vary not only among species, but also among genotypes (Tognetti et al. 1999).

Olive responses to increasing [CO₂] has been studied only recently using Moraiolo and Frantoio cultivars grown in conditions close to their natural environments in a free-air CO₂ enrichment (FACE) facility (Tognetti et al. 2001; Sebastiani et al. 2002). From these experiments it was observed that exposure to elevated [CO₂] enhanced rates of net photosynthesis and decreased stomatal conductance led to greater water use efficiency, while the ratio of intercellular to atmospheric [CO₂] did not differ among leaves grown at ambient [CO₂] or enriched [CO₂]. Cultivar differences in response to elevated [CO₂] were not strong in five-year-old olive plants though the two cultivars differed in intrinsic gas exchange and stomatal density (Tognetti et al. 2001). Indeed, in one-year-old olive plants photosynthetic acclimation was evident for the cultivar Frantoio, which showed strong reductions in leaf area and in leaf and stem biomass (Sebastiani et al. 2002). These first reports on olive response to elevated [CO₂] proves that this field of investigation is important and deserves further study.

**CONCLUDING REMARKS**

In the last decades several research activities on olive physiology and biochemistry during environmental stresses have been performed. Moreover, new fields of research, such as those on environmental pollution, have revealed new and interesting areas of investigation. These studies pointed out some of the physiological mechanisms involved in olive resistance to environmental injuries and highlighted wide genotypic variability. However, more emphasis will be indispensable to clarify the cellular and molecular basis of resistance and this approach will probably provide the tools for improving olive resistance to environmental stress. In the future, it will be important to examine thoroughly and understand the relationships existing between different stresses. Climatic changes, precipitation decrease and salinity problems at the Mediterranean
latitudes will, in fact, expose olive plants to several and concurrent environmental constraints.

**Literature Cited**


