

Water relations and drought-induced embolism in olive (*Olea europaea*) varieties ‘Meski’ and ‘Chemlali’ during severe drought

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Summary We examined the effects of drought on the water relations, osmotic adjustment and xylem vulnerability to embolism of olive (*Olea europaea* L.) varieties, ‘Meski’ and ‘Chemlali’. Two-year-old self-rooted cuttings growing in sand-filled pots in a greenhouse were subjected to water stress by withholding water for 60 days. Water relations and gas exchange measurements showed that ‘Chemlali’ was more drought resistant than ‘Meski’ and had a greater capacity for osmotic adjustment through solute accumulation. However, when water stress was acute, the effect of osmoregulation on leaf cell turgor was largely counteracted by xylem cavitation. Cavitation vulnerability curves showed that both varieties were highly resistant to embolism formation. The xylem water potential inducing 50% loss of stem conductivity approached -7 MPa in ‘Meski’ and only slightly less in ‘Chemlali’. Although the difference between varieties in susceptibility to xylem embolism was small, it appears to account in large part for the difference between them in the ability to tolerate severe drought.

Keywords: hydraulic conductivity, osmotic adjustment, resistance, response variability, water deficit, xylem cavitation.

Introduction

Drought is the most frequent cause of plant stress (Boyer 1982). Plants subjected to drought regulate tissue water status by means of a range of morphological, anatomical and physiological adaptations that control water loss and enhance water uptake. In addition, plants have means to protect the integrity of cell macromolecules and membranes enabling them to sustain tissue desiccation without metabolic inactivation.

Osmotic adjustment is one such mechanism of adaptation to drought (Hsiao et al. 1976, Morgan 1984, Chaves et al. 2003). Plant cells synthesize and accumulate solutes, thereby promoting water uptake and increasing turgor. Several studies have demonstrated osmoregulation in olive (*Olea europaea* L.)

(Rieger 1995, Chartzoulakis et al. 1999, Xiloyannis et al. 1999, Dichio et al. 2003, 2005, Ennajeh et al. 2006).

Olive leaves can tolerate leaf water potentials below -8 MPa (Xiloyannis et al. 1988, Moriana et al. 2003), in part, through adaptations that quench photons absorbed in excess of those used in photosynthesis. Furthermore, xylem water columns of olive trees are able to withstand the high tensions that develop during a severe drought (Connor 2005), without embolization (Sperry and Tyree 1988, Cochard et al. 1992, 1994).

A detailed knowledge of mechanisms of adaptation to drought may provide new insights into factors controlling plant productivity and survival in drought-prone regions (Brodribb et al. 2002, Nardini and Salleo 2005). Studies on vulnerability to cavitation have been made on several sclerophyllous species of the Mediterranean region (Lo Gullo and Salleo 1988, Lo Gullo et al. 1998, Martínez-Vilalta et al. 2002), but not on cultivated olive.

In this study, we investigated the relative contributions of osmotic adjustment and resistance to xylem cavitation to drought tolerance of the olive varieties, ‘Chemlali’ and ‘Meski’, which differ in drought tolerance (Ennajeh et al. 2006).

Materials and methods

Plant material and experimental design

Two-year-old self-rooted cuttings of olive cultivars ‘Chemlali’ and ‘Meski’ were transplanted to 10-l pots filled with a mixture of sandy soil (85% coarse- to fine-grained sand, 15% silt) and vermiculite (4:1; v/v). Plants of the two cultivars were 120–150 cm high and had comparable leaf areas. The pots were covered with plastic film and aluminum foil to reduce evaporation from the soil surface and to minimize solar heating.

Plants were kept in a greenhouse, covered with a shade net, at the Faculty of Sciences of Gabès (southern Tunisia, 33°50' N, 10°5' E). Greenhouse temperature was 25–32 °C

and photosynthetic photon flux (PPF) was about 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Trees were watered weekly, alternately, with tap water or a complete nutrient solution. After 1 month, water was withheld for 60 days for 45 trees of each variety.

At 4-day intervals during the drought treatment, water relations, gas exchange and hydraulic conductivity were measured on three plants of each variety, selected for similarity in pre-dawn leaf water potential (Ψ_{pd}). Soil water potential (Ψ_{soil}) in the pots in the drought treatment was about -2.5 MPa at the end of the experiment.

Plant water relations

Plant water status was evaluated by measuring Ψ_{pd} and leaf relative water content (RWC; $\text{RWC} = (\text{FM} - \text{DM})100/(\text{TM} - \text{DM})$), where FM is fresh mass, DM is dry mass and TM is the turgid mass of the leaf. Olive leaves were weighed immediately on harvest to determine FM. Turgid mass was determined after leaves had been immersed for 12 h in distilled water. After the determination of TM, leaves were oven-dried at $80 \text{ }^\circ\text{C}$ for 48 h and DM determined.

Xylem water potential (Ψ_{xylem}) was measured by the covered leaf technique. The terminal part of the branch was enclosed in an aluminum bag for at least 2 h before measurement, to prevent leaf water loss and thus allow leaf and xylem water potentials at the base of the bag to equilibrate. We measured Ψ_{xylem} on 1-year-old leafy twigs because the petioles were too short to allow measurements with the Scholander pressure chamber (The PMS instrument Company, Albany, OR) on individual leaves.

Osmotic potentials (Ψ_s) were measured on the samples used to measure Ψ_{pd} . Plant tissue samples were stored at $-80 \text{ }^\circ\text{C}$ for 12 h. Frozen tissues were equilibrated at $20 \text{ }^\circ\text{C}$ for 15 min, then cell contents were collected by vacuum extraction (Bollard 1953) and Ψ_s was measured in a 10- μl aliquot with a thermocouple psychrometer (Wescor C-52 sample chambers and HR-33T Dew Point Microvoltmeter, Wescor, UT). Turgor potential (Ψ_p) was calculated as: $\Psi_p = \Psi_{\text{pd}} - \Psi_s$.

Osmotic potential due to net solute accumulation (Ψ_{sa}) was calculated by the method of Wright et al. (1997) as: $\Psi_{\text{sa}} = \Psi_s - [(\Psi_{\text{st}}(\text{RWC}_t))/\text{RWC}]$, where Ψ_s and RWC are osmotic potential and relative water content at a measured Ψ_{pd} value, and Ψ_{st} and RWC_t are the osmotic potential and a relative water content, respectively, at full turgor ($\Psi_{\text{pd}} = 0$), with $(\Psi_{\text{st}}(\text{RWC}_t))/\text{RWC}$ being the osmotic potential due to passive solute concentration with water loss. Values of Ψ_{st} were derived from the linear regression equation describing the relationship between Ψ_s and Ψ_{pd} in each cultivar during the drought. Similarly, the RWC_t values for each variety were derived from the relationship between RWC and Ψ_{pd} . Osmoregulation was expressed as the change in Ψ_{sa} .

Soil water status

Soil water potential was measured with a thermocouple psychrometer (PST-55-15-SF, Wescor; connected to HR-33T) placed at 20-cm depth.

Gas exchange measurements

Gas exchange was measured under saturating light between 0900 and 1000 h at air temperatures between 20 and $30 \text{ }^\circ\text{C}$ with an LCpro+ portable photosynthesis system (ADC, BioScientific, Hoddesdon, U.K.). Four measurements were made on each of three leaves per tree. Three trees per variety were selected for measurement on each measurement occasion.

Xylem embolism and vulnerability curves

Xylem embolism was quantified by measuring the percentage loss of hydraulic conductivity (PLC) at several values of Ψ_{xylem} . Vulnerability curves (VCs) were constructed by plotting PLC values against Ψ_{xylem} . The value of PLC, due to air blockage, is an indirect estimate of the percentage of cavitated vessels (Cochard et al. 2000). For hydraulic conductivity measurements, branches were excised under water to eliminate air embolisms formed at the time of sample collection. Samples were immediately placed in black plastic bags to prevent excessive transpirational water loss during transport to the laboratory. Hydraulic conductivity (K) was measured as described by Sperry et al. (1988).

The technique involves measuring the hydraulic conductance of 3-cm-long branch segments before (K initial, K_i) and after (K maximum, K_m) water refilling. Branch segments were excised under water and their cut ends recut with a sharp razor blade. One of the cut ends was then attached to the hydraulic apparatus. Flow rates were measured with an analytical balance ($\pm 0.1 \text{ mg}$) interfaced to a computer. The measurement solution was a 10 mM KCl, and the delivery pressure was about 5 kPa. The solution flowed from a beaker sitting on the balance through the branch segment, which was kept under water during the measurement. We measured K_i at low pressure (5 kPa). To measure K_m , air obstructing stem xylem vessels was removed by applying a series of 10-s hydraulic pressure flushes (0.2 MPa) until measured values of K_m remained constant between flushes. We calculated PLC as: $\text{PLC} = (1 - K_i/K_m)100$.

Statistical analysis

The effect of drought on measured variables was evaluated by analysis of variance (ANOVA) for a randomized complete block design. All measurements were replicated at least three times. Where applicable, means were separated by LSD.

Results

Plant water relations

A plot of Ψ_{pd} as a function of Ψ_{soil} showed that the varieties had similarly high Ψ_{pd} when values of Ψ_{soil} were moderate (Figure 1). However, as the soil dried, 'Chemlali' had a more negative Ψ_{pd} than 'Meski'.

As shown in Figure 2A, there was a strong positive correlation between Ψ_{pd} and Ψ_s ($r = 0.88$, $P < 0.01$). At high Ψ_{pd} , Ψ_s

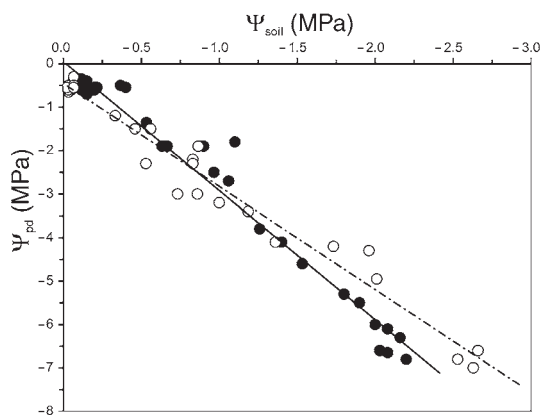


Figure 1. Relationship between predawn leaf water potential (Ψ_{pd}) and soil water potential (Ψ_{soil}) in olive varieties 'Chemlali' (●) and 'Meski' (○) subjected to drought.

was less negative and had comparable values in both varieties; however, when Ψ_{pd} decreased, Ψ_s of 'Chemlali' became more negative than that of 'Meski' for the same value of Ψ_{pd} . This difference between varieties in the relationship between Ψ_{pd} and Ψ_s when Ψ_{pd} was low, may explain the difference in Ψ_p between 'Meski' and 'Chemlali' (Figure 2B). The Ψ_p of 'Chemlali' stayed positive even at a Ψ_{pd} of -7 MPa, indicating efficient active osmotic adjustment, whereas we recorded neg-

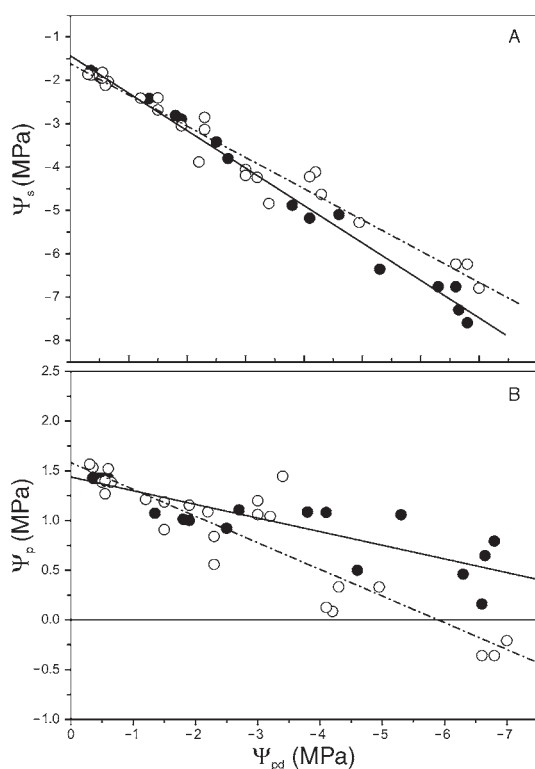


Figure 2. Changes in (A) osmotic potential (Ψ_s) and (B) turgor potential (Ψ_p) as a function of predawn leaf water potential (Ψ_{pd}) in olive varieties 'Chemlali' (●) and 'Meski' (○) subjected to drought.

ative values of Ψ_p for 'Meski' starting at a Ψ_{pd} of about -6 MPa.

Figure 3 illustrates the development of Ψ_{sa} , as calculated by Wright et al. (1997). The Ψ_{sa} decreased with increasing water stress, and the reduction in Ψ_{sa} was more severe for 'Chemlali' than for 'Meski', indicating that 'Chemlali' has a higher capacity for active osmotic adjustment.

Gas exchange

For both varieties, a plot of net photosynthetic rate (A) as a function of Ψ_{pd} (Figure 4A) showed that A was high when water stress was moderate but decreased as water stress intensified. However, for the same Ψ_{soil} , 'Chemlali' had higher A than 'Meski'. The effects of Ψ_{pd} on stomatal conductance (g_s) and transpiration (E) rate showed similar patterns for both varieties (Figures 4B and 4C). Stomatal conductance and E were high when water stress was moderate, and decreased gradually as water stress intensified. However, 'Meski' maintained slightly higher g_s and E than 'Chemlali' throughout the period of severe drought.

Xylem embolism

For both varieties, the xylem vulnerability curves showed that PLC was low during moderate water stress (Figure 5). When water stress became severe, PLC increased but more so in 'Meski' than in 'Chemlali' at comparable Ψ_{xylem} values. The Ψ_{xylem} inducing a PLC of 50% was about -7 MPa in 'Meski' and slightly more negative than this value in 'Chemlali', indicating that 'Meski' is more vulnerable to embolism than 'Chemalali'.

Discussion

Olive is renowned for its drought resistance. Nevertheless, differences in drought resistance among varieties exist. For instance, we previously showed that 'Chemlali' is more resistant to drought than 'Meski' (Ennajeh et al. 2006). In the present

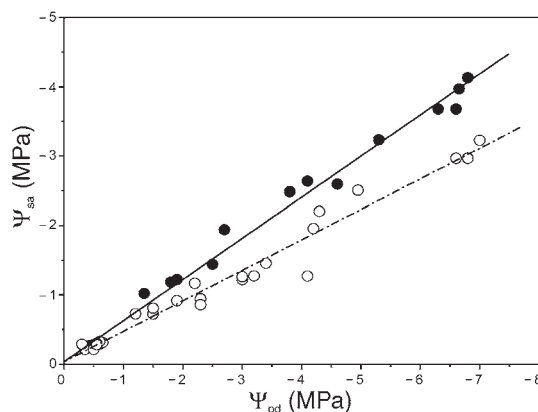


Figure 3. Relationship between osmotic potential due to net solute accumulation (Ψ_{sa}) and predawn leaf water potential (Ψ_{pd}) in olive varieties 'Chemlali' (●) and 'Meski' (○) subjected to drought.

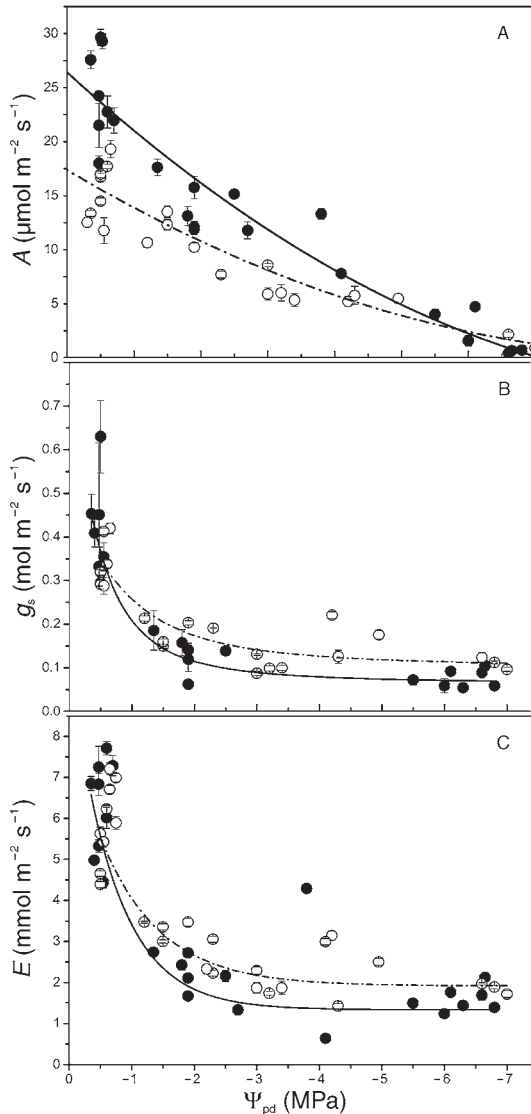


Figure 4. Effects of predawn leaf water potential (Ψ_{pd}) on (A) net photosynthetic rate (A), (B) stomatal conductance (g_s) and (C) transpiration (E) in olive varieties 'Chemlali' (●) and 'Meski' (○) subjected to drought. Each value represents the mean of 12 measurements. Vertical bars = \pm SE.

study, 'Chemlali' trees maintained a higher Ψ_p and A at a given Ψ_{pd} than 'Meski' throughout the experiment (Figures 2B and 4A), thus confirming its superior drought resistance to that of 'Meski'.

Loss of turgor can compromise plant metabolism and growth (Morgan 1992, Bray 1997), particularly photosynthesis (Lawlor and Fock 1975, Chaves et al. 2003). However, we found that 'Chemlali' leaves maintained substantial photosynthetic activity below the point of turgor loss at water potential values of -6 MPa. Similar results have been reported by Xiloyannis et al. (1988) and Angelopoulos et al. (1996) for the olive variety 'Coratina'.

Several studies have shown that olive leaves have effective

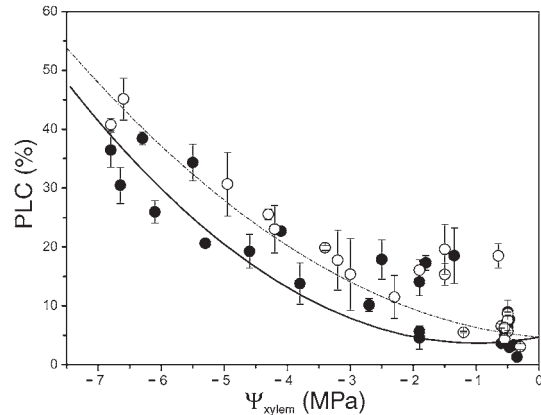


Figure 5. Xylem cavitation vulnerability curves for olive varieties 'Chemlali' (●) and 'Meski' (○), represented as the percentage loss of conductivity (PLC), as a function of decreasing xylem water potential (Ψ_{xylem}). Each value represents the mean of five measurements. Vertical bars = \pm SE.

stomatal control of transpiration in response to leaf water status and atmospheric humidity (Loreto and Sharkey 1990, Fernández et al. 1997, Moriana et al. 2002). Similarly, we found that both 'Chemlali' and 'Meski' showed a gradual decrease in transpiration rate and stomatal conductance with decreasing Ψ_{soil} ; however, the response was earlier and more marked in 'Chemlali' (lower E and g_s) than in 'Meski', implying a more efficient stomatal response to drought.

Beside stomatal responses, osmoregulation is one of the most effective adaptations to drought in plants (Hsiao et al. 1976, Morgan 1984). As reported elsewhere (Rieger 1995, Chartzoulakis et al. 1999, Xiloyannis et al. 1999, Dichio et al. 2003, 2005), we found that, in response to drought, olive is capable of high osmotic adjustment leading to a high water potential gradient between leaves and soil. This was especially true for 'Chemlali' which reached lower Ψ_s values than 'Meski' as a result of greater solute accumulation (Figure 3).

During drought, the value of osmotic adjustment may be negated by cavitation in xylem conduits (Sperry and Tyree 1990, Cochard et al. 1992, Cruziat et al. 2001), resulting in loss of xylem hydraulic conductivity (Sperry and Tyree 1988, Cochard et al. 1992, Sobrado 1997). We found that 'Chemlali' and 'Meski' were highly resistant to xylem embolism compared with many other plants (Hacke et al. 2000, Brodribb et al. 2003, Domec and Gartner 2003). For example, to reach a PLC of 50%, Ψ_{xylem} in both varieties had to be lower than -6 MPa (Figure 5), corresponding to a Ψ_{soil} below -2 MPa. Nevertheless, analysis of the vulnerability curves revealed that 'Meski' was more vulnerable to embolism than 'Chemlali'.

Stomatal closure and osmoregulation—the two principal mechanisms that maintain cell turgor during drought—are sometimes compromised by loss of xylem hydraulic conductivity caused by xylem cavitation. Jones (1998) demonstrated that stomata respond to hydraulic signals. The leaf water potentials triggering stomatal closure are correlated with the xy-

lem water potentials inducing xylem cavitation (Hubbard et al. 2001, Nardini et al. 2001, Cochard et al. 2002). Xylem cavitation and stomatal closure may be linked through the effects of leaf turgor on guard cells. This would occur if xylem cavitation leads to a drop in leaf water potential such that cells begin to lose turgor, triggering stomatal closure.

To investigate the role that stomata may have in limiting xylem cavitation, we plotted g_s and PLC data as functions of Ψ_{xylem} (Figure 6). It appears that, for both varieties, stomata were nearly closed before appreciable embolism formation occurred (PLC < 10%), suggesting that stomata participate in the control of incipient xylem cavitation in olive trees. Similar results have been reported in maize (Cochard 2002) and rubber (*Hevea brasiliensis* Muell. Arg.) (Sangsing et al. 2004). However, stomatal closure was earlier and more marked in 'Chemlali' than in 'Meski', perhaps making 'Chemlali' better able to prevent further embolism formation.

The ability of olive trees to transfer water from tissues to the xylem sap, both when well-watered and when subject to drought, results in a greater lowering of water potential compared with that observed in most other tree species (Xiloyannis et al. 1988). However, to determine whether this mechanism contributes to the drought resistance of olive trees, we need to quantify the solutes in xylem sap.

Another drought adaptation of olive trees is the ability of the leaves to release about 60% of the water stored in their tissues at full turgor to transpiration (Tombesi et al. 1986), which contributes to the transpiration requirements as water stress in-

creases to extreme values of -7.0 MPa.

Olive trees are able to slow the onset of water stress (avoidance) by controlling transpiration and by water uptake from a root system that exploits a large soil volume. Most important, however, is the ability of olive to sustain tissue desiccation while maintaining sufficient metabolic activity for survival (Levitt 1980, Connor 2005). The superior drought tolerance of 'Chemlali' compared with 'Meski' can be explained by (1) efficient osmotic adjustment that creates a high soil water potential gradient that maintains water uptake and (2) a conducting system that is less vulnerable to embolism than that of 'Meski'.

It appears that the capacity of 'Chemlali' to sustain highly negative water potentials before considerable cavitation occurs, plays a larger role in its resistance to drought than osmotic adjustment. The role of osmotic adjustment in maintaining turgor is reduced under severe drought, because of xylem cavitation. Rieger (1995) found that, in olive trees, a 2.5-fold reduction in root hydraulic conductivity induces a linear decrease in the turgor acquired by osmotic adjustment. Thus, the efficiency of the water-transport system largely accounts for the variability in drought resistance among olive varieties.

In conclusion, under severe drought, the difference in drought resistance between 'Chemlali' and 'Meski' can be explained by the difference between them in susceptibility to xylem embolism. Measurement of resistance to embolism may, therefore, provide an effective tool for selecting olive varieties suitable for the drought-prone south Mediterranean region where severe droughts are common and irrigation is not always possible.

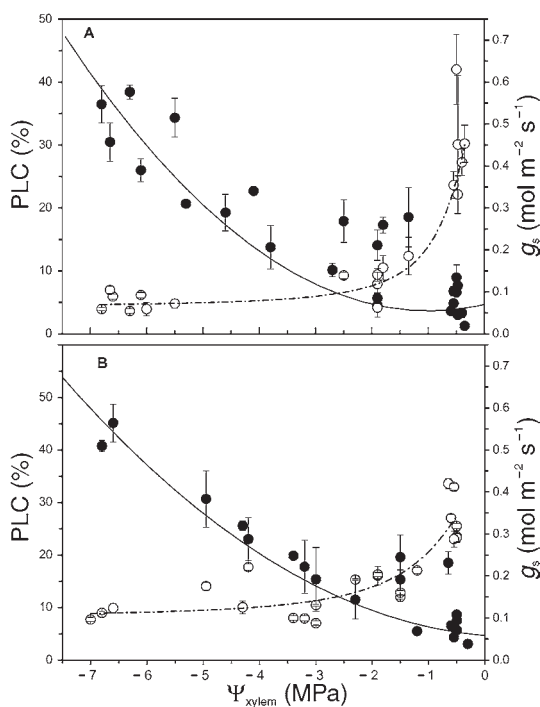


Figure 6. Leaf stomatal conductance (g_s ; ○) versus xylem water potential (Ψ_{xylem}) superimposed on vulnerability curves represented as the percentage loss of conductivity (PLC; ●) for olive varieties (A) 'Chemlali' and (B) 'Meski'.

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