Mycorrhiza (2012) 22:347–359
DOI 10.1007/s00572-011-0408-9

ORIGINAL PAPER

Water deficit improved the capacity of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of antioxidant compounds in lettuce leaves

Marouane Baslam · Nieves Goicoechea

Received: 15 March 2011 / Accepted: 22 August 2011 / Published online: 6 September 2011

Abstract Lettuce, a major food crop within the European Union and the most used for the so-called 'Fourth Range' of vegetables, can associate with arbuscular mycorrhizal fungi (AMF). Mycorrhizal symbiosis can stimulate the synthesis of secondary metabolites, which may increase plant tolerance to stresses and enhance the accumulation of antioxidant compounds potentially beneficial to human health. Our objectives were to assess (1) if the application of a commercial formulation of AMF benefited growth of lettuce under different types and degrees of water deficits; (2) if water restrictions affected the nutritional quality of lettuce; and (3) if AMF improved the quality of lettuce when plants grew under reduced irrigation. Two cultivars of lettuce consumed as salads, Batavia Rubia Munguía and Maravilla de Verano, were used in the study. Four different water regimes were applied to both non-mycorrhizal and mycorrhizal plants: optimal irrigation (field capacity [FC]), a water regime equivalent to 2/3 of FC, a water regime equivalent to 1/2 of FC and a cyclic drought (CD). Results showed that mycorrhizal symbiosis improved the accumulation of antioxidant compounds, mainly carotenoids and anthocyanins, and to a lesser extent chlorophylls and phenolics, in leaves of lettuce. These enhancements were higher under water deficit than under optimal irrigation. Moreover, shoot biomass in mycorrhizal lettuces subjected to 2/3 of FC were similar to those of non-mycorrhizal plants cultivated under well-watered conditions. In addition, lettuces subjected to 2/3 FC had similar leaf RWC than their respective well-watered controls, regardless of mycorrhizal inoculation. Therefore, results suggest that mycorrhizal symbiosis can improve quality of lettuce and may allow restrict irrigation without reducing production.

Keywords Anthocyanins · Carotenoids · Drought · *Lactuca sativa* · Mycorrhizal symbiosis · Phenolic compounds

Introduction

Lettuce (*Lactuca sativa* L.) is a major food crop within the European Union. According to FAOSTAT (FAO Statistics Division) 2011, the production quantity of lettuce and chicory in Spain, France, Germany and Greece were 1,000,000, 430,000, 320,000 and 80,000 tons, respectively, in 2009. Lettuce is the most used food crop for the called 'Fourth Range' of vegetables. The term originally meant fresh, cleaned, possibly chopped and mixed vegetables ready to be seasoned and eaten (Borghi 2003). These vegetables are widely accepted by consumers because they are easy to prepare for eating.

Batavia Rubia Munguía (*L. sativa* L. var. Capitata) and Maravilla de Verano (*L. sativa* L. var. Capitata) are two types of lettuce extensively cultivated in greenhouses, highly commercialized and very appreciated to be consumed in salads in Spain. Both cultivars are characterized for an excellent shelf life that allows maintenance of their crispness from the time they are harvested until the time they are consumed. Batavia Rubia Munguía has yellow-green leaves, with very ruffled borders and a consistent, crisp texture. It develops a round, dense head. Maravilla de Verano has leaves with green color and red pigmentation especially in the borders of the most ruffled leaves. It develops good size, firm head.

M. Baslam · N. Goicoechea (✉)
Dpto. Biología Vegetal, Sección Biología Vegetal
(Unidad Asociada al CSIC, EEAD, Zaragoza e ICVV, Logroño),
Facultades de Ciencias y Farmacia, Universidad de Navarra,
Irunlarrea 1,
31008 Pamplona, Spain
e-mail: niegoi@unav.es

Cultivation of lettuce requires frequent irrigation for better growth and development because this crop plant lacks for a deep root system. Although growing lettuce in dry soil conditions promotes bolting, soil moisture contents ranging from 50% to 75% of field capacity (FC) allow lettuce plants to produce similar biomass than plants under plentiful water regime (Gallardo et al. 1996; Tsabedze and Wahome 2010). These studies, however, do not show any data concerning the effect of suboptimal irrigation on the nutritional quality of lettuce, and other studies dealing with the loss of lettuce quality under conditions that favor the wilting phenomenon (Aguëro et al. 2011) are focused on the postharvest period of this crop vegetable.

Mycorrhizal fungi colonize the roots of over 90% of plant species to the mutual benefit of both plant host and fungus (Harley and Smith 1983). The most common are the arbuscular mycorrhizas (AM), which are formed by the majority of crop and horticultural plants, including lettuce. This symbiosis can increase drought resistance of plants by several mechanisms. In the association of lettuce with AM, fungal hyphae can contribute to water uptake (Ruiz-Lozano and Azcón 1995), being *Glomus intraradices* and *G. mosseae* some of the most effective AMF in taking up soil water (Marulanda et al. 2003). In addition, the establishment of this mutualistic association involves a continuous cellular and molecular dialogue between the mycorrhizal fungus and the host plant (Bonfante-Fasolo 1984; Balestrini and Lanfranco 2006) that includes the activation of the antioxidant (Garmendia et al. 2004), phenylpropanoid (Azcón-Aguilar et al. 2002) or carotenoid metabolic pathways (Strack and Fester 2006). It is becoming evident that the AM symbiosis can stimulate the synthesis of secondary metabolites, which can both increase plant tolerance to abiotic and biotic stresses and enhance the accumulation of antioxidant compounds potentially beneficial to human health in plant tissues (Seeram 2008), including greenhouse-grown lettuces (Baslam et al. 2011).

Natural phenolic compounds are secondary metabolites and a major class of antioxidants in vegetables (You et al. 2011), with anthocyanins being the most important group of water-soluble pigments in plants and regarded as important components in human nutrition due to their antioxidant capacities (Stintzing and Carle 2004). In addition, anthocyanins exhibited anti-carcinogenic effects in several cell cultures systems (You et al. 2011). While caffeic acid derivatives have been mainly found in green varieties of lettuce, anthocyanins have been detected in higher quantities in red varieties (Llorach et al. 2008). Carotenoids have also beneficial effects on human health. β-carotene has been shown to be inversely related to the risk of cardiovascular diseases and certain cancers whereas lutein to the disorders related to the eye (Calvo 2005). In addition, β-carotene can be converted to vitamin A, a compound with known efficacy in preventing some diseases (Rao and Rao 2007).

The main objectives of this work were (1) to test if the application of a mycorrhizal commercial inoculum benefited growth of lettuce under different types and degrees of water deficit; (2) to assess to what extent water deficits affected the nutritional quality of lettuce before harvesting (paying special attention to the levels of non-structural sugars, proteins and antioxidant compounds); and (3) to study if the application of the formulation of arbuscular mycorrhizal fungi (AMF) favored the quality of lettuce when plants grew under restricted irrigation.

As the discussion and conclusions are mainly focused on the nutritional quality of two types of lettuce consumed as salads, results have been expressed and discussed on a fresh basis.

Materials and methods

Biological material and experimental design

Batavia Rubia Munguía (*L. sativa* L. var. Capitata) and Maravilla de Verano (*L. sativa* L. var. Capitata) were the two types of lettuce chosen for this study. Seeds of Batavia Rubia Munguía and Maravilla de Verano were surface sterilized by 10% bleach for 10 min and sown (on September 18) in a mixture of peat and sand (1:1, v:v). When seedlings had two to three fully developed leaves, they were transferred (on October 15) to 1.5-l pots (one plant per pot, 48 pots with Batavia Rubia Munguía and 48 pots with Maravilla de Verano thus making a total of 96 pots) filled with a mixture of vermiculite/sand/peat (2.5:2.5:1, v/v/v). Peat was previously sterilized at 100°C for 1 h on 3 consecutive days. At transplanting, 24 pots with Batavia Rubia Munguía and 24 pots with Maravilla de Verano were inoculated with the commercial inoculum AEGIS Endo Gránulo (Atens, Tarragona, Spain). The commercial inoculum (CI) was a mixture of *G. intraradices* (Schenck and Smith) and *G. mosseae* (Nicol. and Gerd.) Gerd. and Trappe that contained around 100 spores and other infective propagules per gram of product. A total of 9.5 g of the commercial mycorrhizal formulation was added to each pot. Other 24 plants of each cultivar of lettuce were not inoculated and kept as non-mycorrhizal controls.

Different irrigation regimes were also imposed at transplanting. Twelve non-mycorrhizal (six pots for each plant cultivar) and 12 mycorrhizal plants (six pots for each plant cultivar) were always watered at FC and kept as well-watered treatments. After transplanting and until the final harvest, 12 non-mycorrhizal (six pots for each plant cultivar) and 12 mycorrhizal plants (six pots for each plant cultivar) were grown under a soil water content equivalent to 2/3 of FC (2/3 FC). Other 12 non-mycorrhizal (six pots for each plant cultivar) and 12 mycorrhizal (six pots for each plant cultivar) plants were grown under a soil water content equivalent to 1/2 of FC (1/2 FC). All the aforementioned plants were fertilized once a week with 300 ml of Hewitt's nutrient solution (Hewitt 1952) with some modifications (Marulanda et al. 2003). In order to achieve FC, well-watered plants also received 600 ml of distilled water per week. Plants subjected to 2/3 or 1/2 FC received (in addition to 300 ml of modified Hewitt's solution) 300 or 150 ml of distilled water per week, respectively. Finally, 2 weeks after transplanting, other 12 non-mycorrhizal (six pots for each plant cultivar) and 12 mycorrhizal (six pots for each plant cultivar) plants (which had previously grown under optimal irrigation) were subjected to cyclic water deficit. Cyclic drought (CD) consisted of withholding irrigation for 1 week and then watering pots with 300 ml nutrient solution in order to avoid an additional nutrient starvation. Each plant was subjected to a total of 5 cycles of drought. All lettuce plants were harvested 7 weeks after transplanting, when the active growth had not still finished because both varieties of lettuces are consumed as salads.

Lettuce plants were grown in a greenhouse at 25/15°C day/night temperatures and 50/85% day/night relative humidity (RH), and received natural daylight supplemented with irradiation from fluorescent lamps (Sylvania DECOR 183, Professional-58 W, Erlangen, Germany) that provided a minimum photosynthetic photon flux (PPF) of around 300–400 μmol m^{-2} s^{-1} during a 14-h photoperiod.

Samples for analytical determinations were collected from both inner (internal zone) and outer (external zone) leaves of lettuces. Both zones were visually delimited and each one had a mean of approximately 15 leaves. The internal zone was quite close to the meristematic tip of the shoot and included light green leaves. The harvested inner leaves were placed midway between the center of the head and the outer portion. Outer leaves exhibited darker color and larger size than inner leaves and were not compact in the lettuce head.

Growth parameters

Dry matter (DM) was determined after drying the plant material at 80°C for 2 days.

Mycorrhizal analysis

Root samples of lettuce plants were cleared and stained as described by Phillips and Hayman (1970) and mycorrhizal colonization was determined by examining 1-cm root segments (n=45 per each type of lettuce and water regime) under the microscope. Results are expressed as percentage of infection (Hayman et al. 1976). The mycorrhizal efficiency index (MEI) was estimated according to Bagyaraj (1994): MEI = DM of inoculated plant − DM of non-inoculated plant × 100/DM of inoculated plant. Determination of MEI allows assessment of the growth improvement brought about by inoculation of plants with a mycorrhizal fungus.

Relative water content in leaves

Relative water content (RWC) was estimated by a modification of the method of Weatherley (1950) and calculated as $\mathrm{RWC} = 100 \times (\mathrm{FW} - \mathrm{DM})/(\mathrm{TW} - \mathrm{DM})$. FW and DM denote fresh weight and DM. Turgid weight (TW) was calculated after fully hydrating fresh leaves in darkness at 4°C for 24 h. Results were expressed as percentages.

Starch, total soluble sugars and total soluble proteins in leaves

Starch, total soluble sugars (TSS) and total soluble proteins were quantified in potassium phosphate buffer (KPB) (50 mM, pH=7.5) extracts of fresh leaves (1 g). These extracts were filtered through four cheese cloth layers and centrifuged at 38,720×g for 10 min at 4°C. The pellet was used for starch determination (Jarvis and Walker 1993). The supernatant was collected and stored at 4°C for TSS and protein determinations. TSS were analyzed with the anthrone reagent in a Spectronic 2000 (Bausch and Lomb, Rochester, NY) (Yemm and Willis 1954). Leaf soluble proteins were measured by the protein dye-binding method of Bradford (1976) using bovine serum albumin (BSA) as standard. Results were expressed as mg of starch, TSS or total soluble proteins per gram of FW. Outer and inner leaves were separately analyzed.

Chlorophylls and carotenoids

Contents of chlorophylls (chl a + chl b) and total carotenoids were determined according to Séstak et al. (1971). Samples of fresh outer or inner leaves (1 cm^2, approximately equivalent to 30 mg of FW in Batavia Rubia Munguía and to 40 mg of FW in Maravilla de Verano) were immersed in 5 ml of 96% ethanol at 80°C during 10 min to extract the pigments. The absorbance of extracts was measured at 470, 649, 665 and 750 nm using a Spectronic 2000 (Bausch and Lomb). Estimation of chl a and chl b and total carotenoids in the same extract solution was performed by using the extinction coefficients and equations determined by Lichtenthaler (1987). Results were expressed as mg of total chlorophylls (a + b) or carotenoids per gram of FW. Outer and inner leaves were analyzed separately.

Total phenolics and anthocyanins

Total phenolic compounds were extracted according to Chapuis-Lardy et al. (2002) with some modifications. Samples (0.5 g FW) were pulverized in liquid nitrogen, mixed with 20 ml of 80% methanol, and homogenized at room temperature for 1 min. After filtration, 0.5 ml of each sample were mixed with 10 ml distilled water. Total phenolic content was determined from aqueous solutions by spectrophotometric analysis at 760 nm with Folin–Ciocalteu reagent (Waterman and Mole 1994). Although it is not completely specific for phenolic compounds (e.g., it is affected by other constituents) and not all phenolic compounds exhibit the same level of activity in the assay (Kang and Saltveit 2002), the Folin–Ciocalteu method is commonly used to measure phenolic content (e.g., Bunning et al. 2010; You et al. 2011). Results were expressed as milligrams of gallic acid per gram of FW. Outer and inner leaves were separately analyzed.

Anthocyanins were analyzed according to Cevahir et al. (2004) with some modifications (Pietrini and Massacci 1998). Samples of 1 cm^2 of leaves were collected and homogenized in 1 ml acidified methanol (2.27 ml HCl 37% +97.73 ml methanol) and maintained at 4°C overnight in the dark to avoid degradation of chlorophylls. After adding 665 µl of distilled water, chlorophylls were separated with 1.6 ml of chloroform. Particulates were removed by centrifugation at 26,890×*g* for 10 min and the supernatant was passed through four cheese cloth layers. Total anthocyanins were determined by measuring A_{530} and A_{657} of the aqueous phase. The relative amount of anthocyanins was calculated as the optical density (OD) per gram of FW as described by Mancinelli (1984). Outer and inner leaves were analyzed separately.

Ascorbate

Ascorbate (ASC) and dehydroascorbate (DHA) content were assayed photometrically by reduction of 2,6-dichlorophenolindophenol (DCPIP) according to Leipner et al. (1997). Leaves (0.5 g FW) were homogenized in liquid nitrogen in presence of 1 g NaCl and extracted in 5 ml ice-cold 2% (w/v) metaphosphoric acid. The homogenate was filtered. An aliquot of 0.3 ml was mixed with 0.2 ml 45% (w/v) K_2HPO_4 and 0.1 ml 0.1% (w/v) homocysteine to reduce DHA to ASC and determine the total ASC pool (ASC + DHA). For the determination of ASC, the homocysteine solution was replaced by the same volume of water. After 15 min incubation at 25°C, 1 ml of citrate-phosphate buffer 2 M (pH 2–3) and 1 ml 0.003% (w/v) DCPIP were added. The absorbance at 524 nm was measured immediately using a spectrophotometer. The content of ASC was calculated by reference to a standard curve. The amount of DHA resulted from the subtraction of the total ASC pool (ASC + DHA) and ASC. ASC redox state was calculated as the ratio between ASC and DHA. Results were expressed as mg of total ASC, ASC or DHA or per gram of FW of either outer or inner leaves.

Statistical analysis

Analysis of variance was performed for each parameter. Means ± standard errors (SE) were calculated and, when the F ratio was significant ($P \leq 0.05$), a least difference (LSD) test was applied as available in the SPSS statistical package version 15.0 programs for Windows XP.

Results

Growth parameters

Shoot FW was very sensitive to water deficit and significantly decreased in both types of lettuce when irrigation was reduced regardless plants were inoculated or not with AMF (Table 1). However, shoot DM in both cultivars of lettuce, Batavia Rubia Munguía and Maravilla de Verano, achieved control values when plants grew under the water regime equivalent to 2/3 FC. In addition, mycorrhizal plants subjected to the irrigation regime equivalent to 2/3 FC achieved values of shoot DM comparable to their respective non-mycorrhizal controls under well-watered conditions, independently of the cultivar of lettuce. CD was the most detrimental type of drought for the development of lettuce even when plants were associated with AMF. Water regime determined biomass partitioning between shoots and roots, being the influence more evident in non-mycorrhizal than in mycorrhizal plants. In non-mycorrhizal Batavia Rubia Munguía subjected to either a water regime equivalent to 1/2 FC or CD, the largest amount of energy and resources were employed to produce aerial part, so that DM partitioning to shoots achieved, approximately 65% of total plant biomass. In non-mycorrhizal Maravilla de Verano, this effect was especially evident in plants that received irrigation equivalent to 2/3 FC. When plants were associated with AMF, biomass partitioning was similar under optimal irrigation and water deficit, regardless the type of lettuce, and reached values of around 60–65% in shoots and 35–45% in roots (data not shown).

Mycorrhizal analysis

Reduced irrigation favored the colonization of lettuce roots by AMF in both Batavia Rubia Munguía and Maravilla de Verano (Fig. 1a). However, the percentages of mycorrhizal

Table 1 Growth parameters of non-mycorrhizal (NM) and mycorrhizal (M) lettuce plants cv. Batavia Rubia Munguía and Maravilla de Verano grown under either field capacity (FC), water content equivalent to 2/3 of field capacity (2/3 FC), water content equivalent to 1/2 of field capacity (1/2 FC) or subjected to cyclic drought (CD)

		Shoot FW (g plant^{-1})	Root FW (g plant^{-1})	Shoot DM (g plant^{-1})	Root DM (g plant^{-1})
Batavia Rubia Munguía					
NM	FC	166.01 d	41.48 a	10.65 bc	10.67 a
	2/3 FC	122.98 fg	31.05 bc	8.93 cd	8.54 abc
	1/2 FC	94.23 ij	21.72 ef	7.33 de	5.63 abc
	CD	62.39 l	10.23 h	4.78 f	2.95 c
M	FC	180.30 c	31.66 bc	12.48 ab	9.21 ab
	2/3 FC	132.19 ef	27.37 cde	9.67 c	7.24 abc
	1/2 FC	112.75 gh	20.62 f	8.90 cd	6.38 abc
	CD	75.13 kl	10.40 g	5.00 f	2.61 c
Maravilla de Verano					
NM	FC	211.76 b	32.07 bc	12.24 b	10.84 a
	2/3 FC	170.27 cd	31.64 bc	10.30 bc	5.87 abc
	1/2 FC	115.15 g	22.01 ef	6.81 def	4.76 abc
	CD	82.17 jk	12.80 gh	5.58 ef	2.99 bc
M	FC	233.10 a	36.07 ab	13.56 a	9.80 a
	2/3 FC	173.08 cd	28.18 cd	12.28 ab	6.23 abc
	1/2 FC	145.32 ef	23.30 def	10.26 bc	6.59 abc
	CD	100.41 hi	18.92 fg	6.24 ef	5.67 abc

Values are means (n=6 plants). Within each parameter, values followed by the same letter are not significantly different ($P \leq 0.05$)

FW fresh weight, DM dry matter

infection in plants that underwent CD were lower than those observed in plants subjected to irrigation regimes equivalent to 2/3 FC and 1/2 FC. Values of MEI were higher in Batavia Rubia Munguía than in Maravilla de Verano under optimal irrigation (Fig. 1b). However, when plants were subjected to water regimes equivalent to 2/3 FC or 1/2 FC, MEI was greater in Maravilla de Verano than in Batavia Rubia Munguía. Moreover, MEI increased in Maravilla de Verano cultivated under water regimes equivalent to 2/3 FC and 1/2 FC compared with their respective well-watered controls. The imposition of CD had negative effect on MEI in Batavia Rubia Munguía and did not affect MEI in Maravilla de Verano.

Relative water content in leaves

RWC in outer leaves of non-mycorrhizal and mycorrhizal Batavia Rubia Munguía only decreased significantly when plants were subjected to 1/2 FC (Fig. 2). In contrast, RWC in inner leaves declined under both 1/2 FC and CD. In terms of leaf RWC, Maravilla de Verano was more resistant than Batavia Rubia Munguía to water deficit. Only when plants were subjected to CD, RWC decreased in both external and internal leaves. No differences were observed between non-mycorrhizal and mycorrhizal plants cv. Maravilla de Verano.

Starch, total soluble sugars and total soluble proteins in leaves

Mycorrhizal association increased starch concentrations in both outer and inner leaves of Batavia Rubia Munguía under optimal irrigation and when lettuces were grown under a water regime equivalent to 2/3 FC (Fig. 3a). Imposition of CD caused a decline in starch levels of both non-mycorrhizal and mycorrhizal plants. In contrast, both external and internal leaves of non-mycorrhizal Maravilla de Verano had greater levels of starch than leaves of mycorrhizal plants under plentiful irrigation. Reduced irrigation decreased starch contents in Maravilla de Verano, being this effect more significant in non-mycorrhizal than in mycorrhizal plants.

In Batavia Rubia Munguía, internal leaves accumulated greater amount of TSS than external leaves regardless irrigation regime and presence or absence of AMF in roots (Fig. 3b). Except for the higher quantity of TSS in inner leaves of mycorrhizal plants subjected to CD, leaves of non-mycorrhizal and mycorrhizal Batavia Rubia Munguía had similar levels of TSS. Every type and degree of water deficit caused decreases in the concentrations of TSS in external leaves of Maravilla de Verano when plants were not associated with AMF. In contrast, mycorrhizal Maravilla de Verano maintained similar

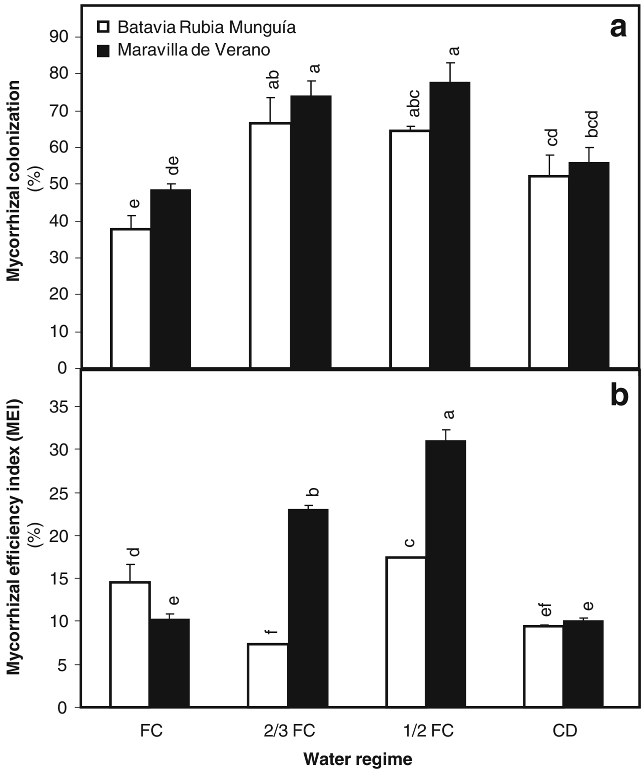


Fig. 1 Percentage of mycorrhizal colonization (%) (**a**) and mycorrhizal efficiency index (MEI) (%) (**b**) in lettuce plants cv. Batavia Rubia Munguía (*white histograms*) and Maravilla de Verano (*black histograms*) grown under either field capacity (FC), water content equivalent to 2/3 of field capacity (2/3 FC), water content equivalent to 1/2 of field capacity (1/2 FC) or subjected to cyclic drought (CD). Values are means ± SE (n=3 plants). Within each graph, histograms with the same letter indicate that values are not significantly different ($P\leq0.05$)

levels of TSS than well watered plants under water regimes equivalent to 2/3 FC or 1/2 FC.

The association of the two cultivars of lettuce with AMF favored the accumulation of total soluble proteins in inner leaves of plants cultivated under optimal irrigation (Fig. 3c). In non-mycorrhizal lettuces, the imposition of CD caused the strongest reductions in the levels of proteins, being decreases more evident in Maravilla de Verano than in Batavia Rubia Munguía. In contrast, and except for the reduced quantity of proteins in inner leaves of Batavia Rubia Munguía, water deficits only induced slight variations in the concentrations of proteins in leaves of mycorrhizal plants.

Chlorophylls and carotenoids

The association of plants with AMF enhanced the amount of total chlorophylls in inner leaves of Batavia Rubia Munguía and Maravilla de Verano regardless the water regime applied to lettuce plants (Fig. 4a). Levels of chlorophylls in external leaves of mycorrhizal Batavia Rubia Munguía were also higher than in non-mycorrhizal lettuces when plants were subjected to either an irrigation equivalent to 1/2 FC or to CD.

The presence of AM also induced the accumulation of total carotenoids in both outer and inner leaves of Batavia Rubia Munguía and Maravilla de Verano regardless the water regime applied (Fig. 4b). The only exception was the quantity of carotenoids in external leaves of Maravilla de Verano grown under the irrigation regime equivalent to 1/2 FC. When compared the amount of carotenoids in mycorrhizal plants we found that the application of a constant water deficit for 7 weeks (2/3 FC or 1/2 FC) to mycorrhizal plants produced an enhancement in the carotenoid quantities in external leaves of Batavia Rubia Munguía and internal leaves of Maravilla de Verano. In non-mycorrhizal plants a maintained water deficit (2/3 FC or 1/2 FC) only increased levels of carotenoids in the cultivar Maravilla de Verano.

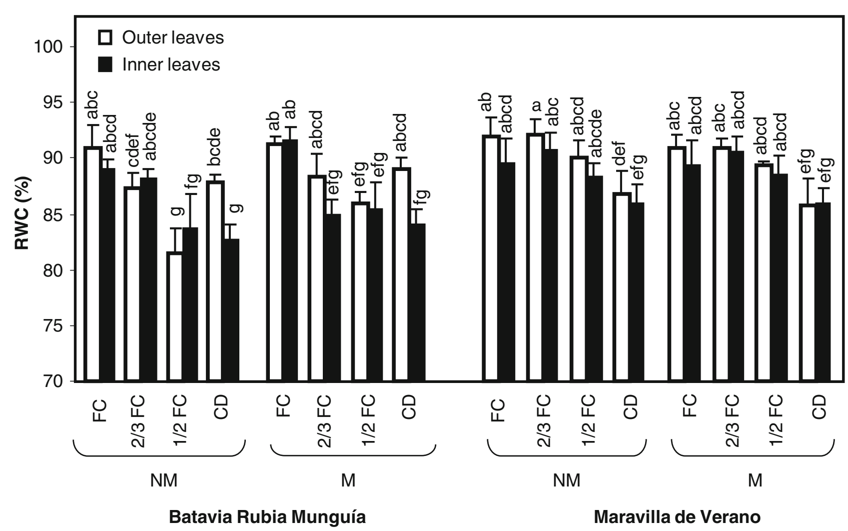


Fig. 2 Relative water content RWC (%) in outer (*white histograms*) and inner (*black histograms*) leaves of non-mycorrhizal (NM) or mycorrhizal (M) lettuce plants cv. Batavia Rubia Munguía and Maravilla de Verano grown under either field capacity (FC), water content equivalent to 2/3 of field capacity (2/3 FC), water content equivalent to 1/2 of field capacity (1/2 FC) or subjected to cyclic drought (CD). Values are means ± SE (n=6 plants). Within each graph, histograms with the same letter indicate that values are not significantly different ($P\leq0.05$)

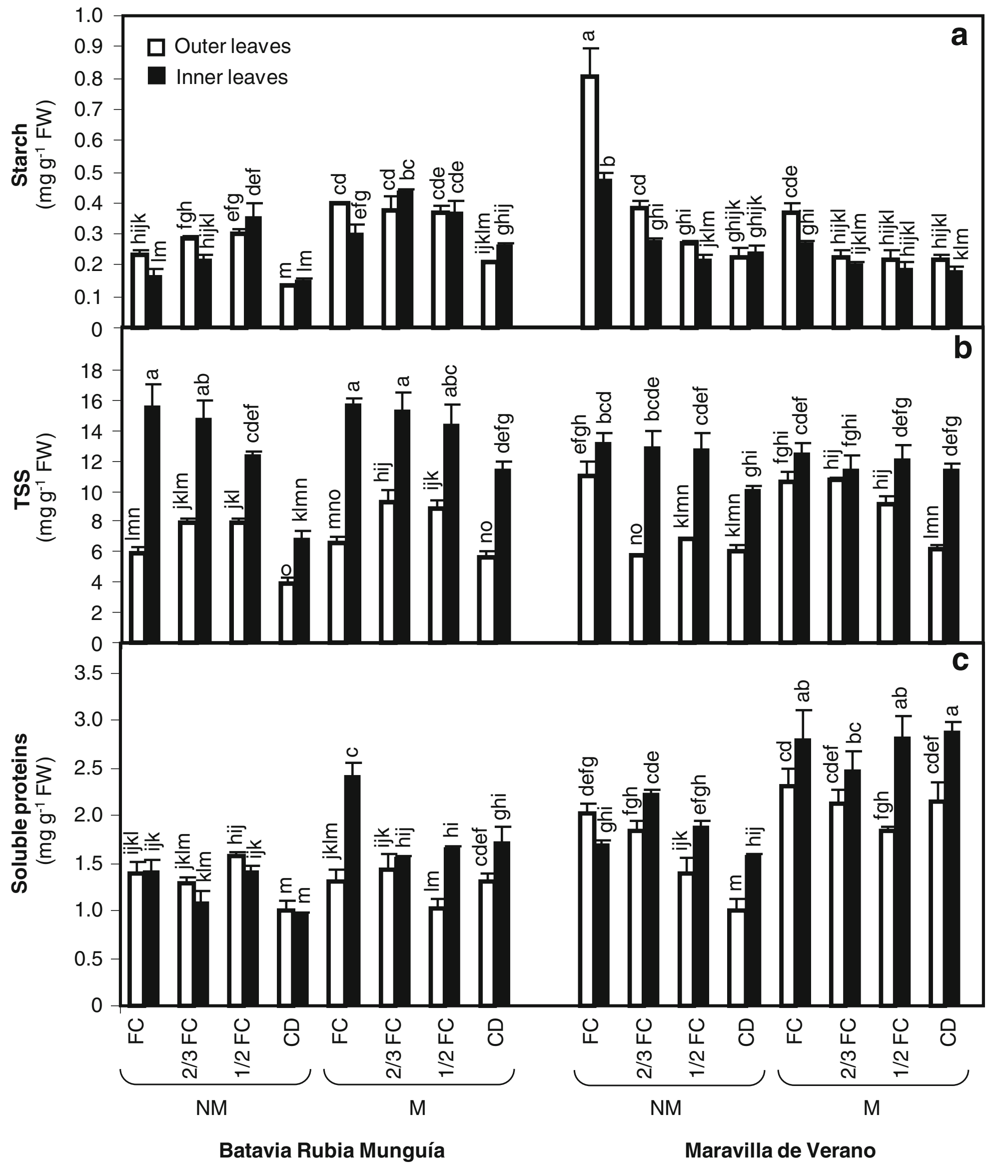


Fig. 3 Concentrations of starch (mg g^{-1} FW) (**a**), total soluble sugars (TSS) (mg g^{-1} FW) (**b**) and total soluble proteins (mg g^{-1} FW) (**c**) in outer (*white histograms*) and inner (*black histograms*) leaves of non-mycorrhizal (NM) or mycorrhizal (M) lettuce plants cv. Batavia Rubia Munguía and Maravilla de Verano grown under either field capacity (FC), water content equivalent to 2/3 of field capacity (2/3 FC), water content equivalent to 1/2 of field capacity (1/2 FC) or subjected to cyclic drought (CD). Values are means ± SE (n=3–6 plants). Within each graph, histograms with the same letter indicate that values are not significantly different ($P \leq 0.05$)

Total phenolics and anthocyanins

In non-mycorrhizal Batavia Rubia Munguía the concentrations of phenolic compounds slightly increased in external leaves when plants were subjected to either an irrigation regime equivalent to 1/2 FC or to CD (Fig. 5a). The association of Batavia Rubia Munguía with AMF significantly enhanced the accumulation of phenolics in external leaves under optimal irrigation and in internal leaves under CD. In contrast, neither water deficit nor mycorrhization significantly affected the contents of phenolic compounds in leaves of Maravilla de Verano.

Levels of anthocyanins in leaves were very sensitive to the presence of AMF colonizing roots of both varieties of lettuce, Batavia Rubia Munguía and Maravilla de Verano (Fig. 5b). In addition, restricted water regimes enhanced, in most cases, the accumulation of anthocyanins in mycorrhizal plants. In the case of Batavia Rubia Munguía, mycorrhizal plants always showed increased amounts of anthocyanins in inner leaves and the highest levels of these compounds were measured in the plants that had been subjected to either a water regime equivalent to 2/3 FC or to CD. In Maravilla de Verano, the greatest concentrations of anthocyanins were observed in outer leaves of plants associated with AMF that had been subjected to water regimes equivalent to 2/3 FC or 1/2 FC.

Ascorbate

The concentrations of total ascorbate (ASC + DHA) in leaves were quite similar in all plants regardless of the presence or absence of mycorrhizal symbiosis, cultivar of lettuce and irrigation regime (Fig. 6a). In contrast, the application of the mycorrhizal inoculum slightly decreased

Fig. 4 Concentrations of total chlorophylls ($a + b$) (mg g^{-1} FW) (**a**) and total carotenoids (mg g^{-1} FW) (**b**) in outer (*white histograms*) and inner (*black histograms*) leaves of non-mycorrhizal (NM) or mycorrhizal (M) lettuce plants cv. Batavia Rubia Munguía and Maravilla de Verano grown under either field capacity (FC), water content equivalent to 2/3 of field capacity (2/3 FC), water content equivalent to 1/2 of field capacity (1/2 FC) or subjected to cyclic drought (CD). Values are means ± SE (n=3 plants). Within each graph, histograms with the same letter indicate that values are not significantly different ($P \leq 0.05$)

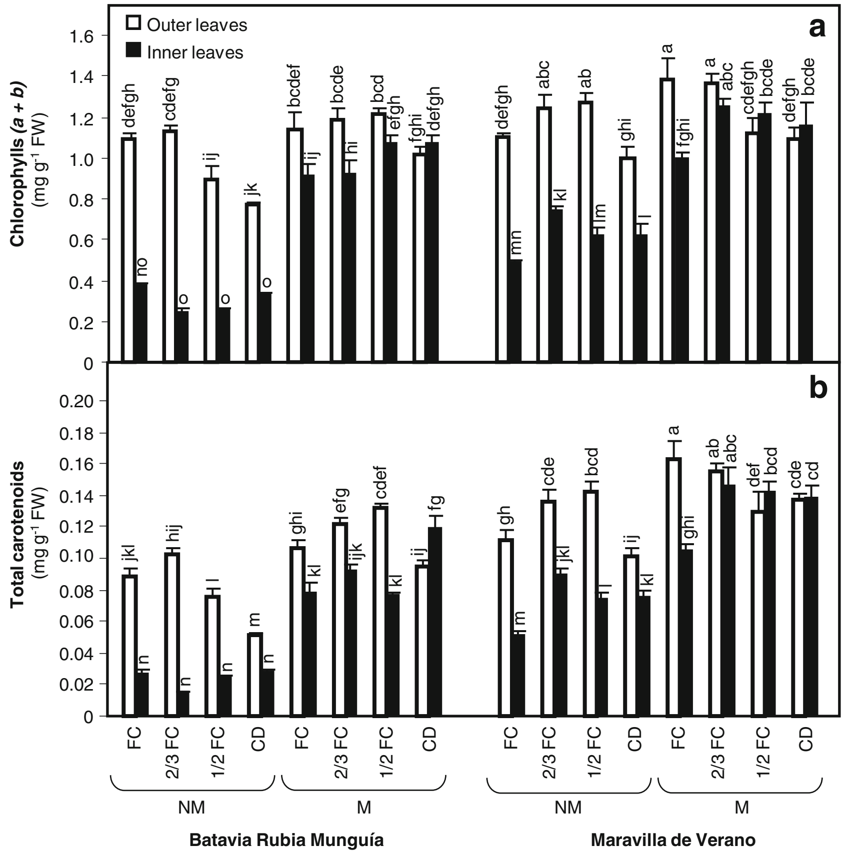
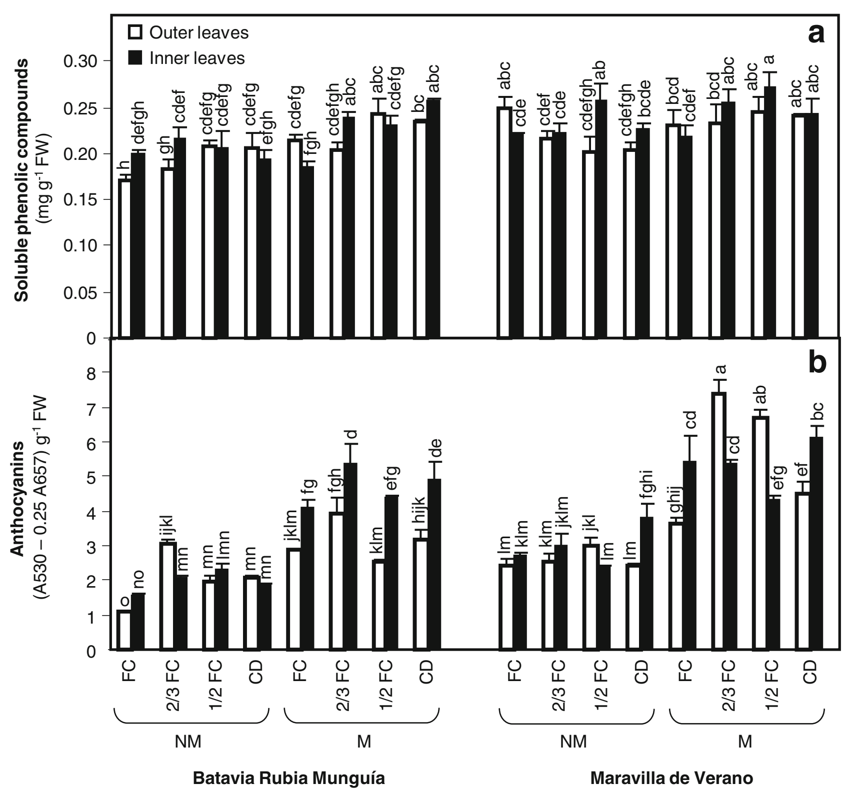


Fig. 5 Concentrations of total soluble phenolic compounds (mg g^{-1} FW) (**a**) and anthocyanins (**b**) in outer (*white histograms*) and inner (*black histograms*) leaves of non-mycorrhizal (NM) or mycorrhizal (M) lettuce plants cv. Batavia Rubia Munguía and Maravilla de Verano grown under either field capacity (FC), water content equivalent to 2/3 of field capacity (2/3 FC), water content equivalent to 1/2 of field capacity (1/2 FC) or subjected to cyclic drought (CD). Data on anthocyanins are expressed as optical density (OD) (A530 – 0.25 A657) g^{-1} FW. Values are means ± SE (n=3 plants). Within each graph, histograms with the same letter indicate that values are not significantly different ($P \leq 0.05$)



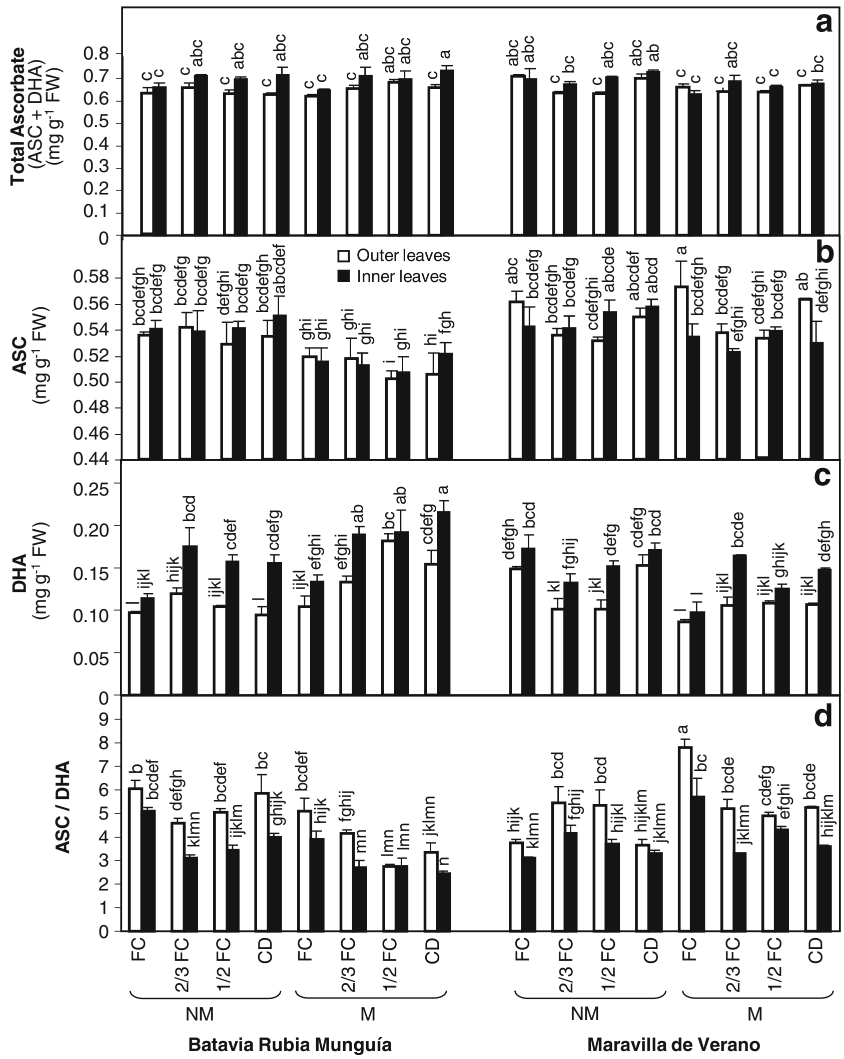


Fig. 6 Concentrations of total ascorbate pool (ASC + DHA) (mg g^{-1} FW) (**a**), reduced ascorbate (ASC) (mg g^{-1} FW) (**b**) and dehydroascorbate (DHA) (mg g^{-1} FW) (**c**) as well as redox state (ASC/DHA) (**d**) in outer (*white histograms*) and inner (*black histograms*) leaves of non-mycorrhizal (NM) or mycorrhizal (M) lettuce plants cv. Batavia Rubia Munguía and Maravilla de Verano grown under either field capacity (FC), water content equivalent to 2/3 of field capacity (2/3 FC), water content equivalent to 1/2 of field capacity (1/2 FC) or subjected to cyclic drought (CD). Values are means ± SE (n=3 plants). Within each graph, histograms with the same letter indicate that values are not significantly different ($P \leq 0.05$)

the amount of ASC in both outer and inner leaves of Batavia Rubia Munguía when compared with their respective non-mycorrhizal controls (Fig. 6b). The imposition of any water deficit resulted in higher quantities of DHA in inner than in outer leaves of the two varieties of lettuce plants independently they were or not associated with AMF (Fig. 6c). Redox state (ASC/DHA) was comparable in outer leaves of non-mycorrhizal and mycorrhizal Batavia Rubia Munguía under optimal irrigation (Fig. 6d). In contrast, mycorrhizal Maravilla de Verano had significantly higher redox state than non-mycorrhizal controls in both external and internal leaves under well-watered conditions. In Batavia Rubia Munguía, application of maintained drought (2/3 FC or 1/2 FC) decreased redox state in both non-mycorrhizal and mycorrhizal plants, being reductions more evident in inner than in outer leaves. In Maravilla de Verano the application of water regimes equivalent to either 2/3 FC or 1/2 FC only reduced redox state in mycorrhizal plants.

Discussion

In experimental designs that involve potted plants, as in our study, drought can affect mycorrhizal colonization increasing root colonization more often than decreasing it (Augé 2001). In our case, restricted irrigation enhanced the colonization of lettuce roots by AMF, especially when the reduction in water supply was maintained for several weeks. Chronic drought may promote more extensive mycorrhizal colonization mainly due to decreased P diffusion rates in the soil (Bolgiano et al. 1983). Maintenance of water restriction also benefited the efficiency of mycorrhizal symbiosis (MEI) in improving growth of Maravilla de Verano (both 2/3 FC and 1/2 FC) and Batavia Rubia Munguía (only 1/2 FC). Contrariwise, application of CD had no effect or slightly decreased MEI. In lettuce, it has been demonstrated that the nature of the water stress (severity and duration) can affect the mycorrhizal effect on the physiology (and, consequently, on the growth) of plants under drought conditions (Ruiz-Lozano et al. 1995).

One of the main features of lettuce quality is its high amount of water in tissues (Agüero et al. 2008). In our study, leaves of both non-mycorrhizal and mycorrhizal lettuce plants maintained control values of RWC when subjected to moderate water limitation (2/3 FC) and, in the case of Maravilla de Verano, also when applied a water regime equivalent to 1/2 FC. However, the quality and shelf life of this highly perishable vegetable are influenced not only by water content but also by water status in tissues (Barg et al. 2009). Consequently, RWC data themselves do not allow us predicting the evolution of vegetable quality after harvesting. However, there are two aspects that make us hypothesize that lettuce plants grown under either optimal irrigation or moderate drought (2/3 FC) in the present study would be able to maintain an adequate hydration that would favor their sensory quality after harvesting: (1) Plants were harvested at final stages of their vegetative growth. According to Barg et al. (2009), lettuces can retain high water status indices at final development stages. (2) With the exception of non-mycorrhizal Maravilla de Verano, the maintenance or slight increases in the amount of TSS accumulated in leaves when applied moderate water deficit (2/3 FC) could contribute to an osmotic adjustment than would allow retaining water inside cells. Nevertheless, more research is needed to prove our statement.

The increased concentrations of starch together with the similar quantities of TSS in leaves of mycorrhizal Batavia Rubia Munguía when compared with their respective non-mycorrhizal controls suggest an enhancement of the photosynthesis in plants associated with AMF (Sánchez-Díaz et al. 1990) under either optimal irrigation or moderate and maintained drought (2/3 FC). In contrast, the lower levels of starch and similar contents of TSS in leaves of well-watered mycorrhizal Maravilla de Verano in comparison with non-mycorrhizal plants may indicate a translocation of sugars from shoots to roots in this cultivar of lettuce to support both the maintenance of the fungal structures and the functionality of the symbiosis (Harley and Smith 1983). It is possible that the same type of AMF can follow distinct dynamics when establishing a functional symbiotic association with different varieties of lettuce. However, the association of Maravilla de Verano with AMF was especially beneficial for plants when they were undergoing restricted water supplies. In fact, only when associated with AMF, lettuce plants cv. Maravilla de Verano subjected to 2/3 FC or 1/2 FC had similar levels of TSS in both outer and inner leaves than their respective well-watered controls. Similarly, mycorrhizal Maravilla de Verano had comparable amounts of soluble proteins in inner leaves under optimal irrigation and when subjected to any water deficit. It has been found that mycorrhizal symbiosis may be more beneficial for host plants when plants are subjected to adverse conditions, such as drought (Goicoechea et al. 2004).

The association of plants with AMF significantly enhanced the concentrations of total chlorophylls in inner leaves of both varieties of lettuce, regardless water irrigation regime. The increased levels of chlorophylls presumably would have contributed to higher photosynthetic rates in mycorrhizal lettuce, thus benefiting both growth of host plants and the development and functionality of the symbiosis. Similarly, Zuccarini (2007) measured greater contents of chlorophylls in lettuce inoculated with a mixture of *Glomus mosseae*, *G. intraradices* and *G. coronatum* than in non-mycorrhizal controls. Since outer leaves of head lettuce are usually stripped off during harvest, accumulation of chlorophylls in inner leaves of mycorrhizal lettuce plants is very interesting from the nutritional point of view. In recent years, there has been a growing interest in natural and semi-synthetic chlorophyll derivatives, not only as food colorants, but as food supplements due to their potential effect against the development of several chronic diseases (Fernandes et al. 2007) and to their anti-inflammatory activity in vitro (Mulabagal et al. 2010).

Mycorrhizal symbiosis also favored the accumulation of total carotenoids in both outer and inner leaves of Batavia Rubia Munguía and Maravilla de Verano under either optimal irrigation or restricted water supplies compared with their respective non-mycorrhizal controls. Likewise, Krishna et al. (2005) measured increased concentrations of carotenoids in leaves of *Vitis vinifera* when associated with AMF. When compared mycorrhizal plants subjected to different types and degrees of water deficits, we found that prolonged water restrictions (2/3 FC and 1/2 FC) were the most effective in enhancing the concentrations of carotenoids in external leaves of Batavia Rubia Munguía. Any type of drought increased the quantities of carotenoids in inner leaves of Maravilla de Verano. As aforementioned, carotenoids are thought to be responsible for the beneficial properties of fruits and vegetables in preventing human diseases including cardiovascular diseases, cancer and other chronic diseases (Rao and Rao 2007).

In lettuce, two main classes of phenols and polyphenols have been identified: caffeic acid derivatives (Ke and Salveit 1988) and flavonols (Herrmann 1976). Caffeic acid derivatives seem to be the main phenolics in green varieties, while flavonols have been detected in higher quantities in red varieties. However, lettuce leaf tissue contains small amounts of phenolic compounds when plants are grown under nonstressful conditions (Tomás-Barberán et al. 1997). In our study, the imposition of water deficits to non-mycorrhizal plants only induced a slight accumulation of soluble phenolic compounds in the outer leaves of the cultivar Batavia Rubia Munguía. In contrast, Oh et al. (2009) found that the activity of the enzyme phenylalanine ammonia-lyase (PAL), involved in the biosynthesis of many phenolic compounds, increased in lettuce cv. Baronet exposed to water stress. Activation of phenylpropanoid pathway may be dependent on both the type and degree of water deficit applied and the genotype of lettuce. When associated with AMF, water restrictions increased the levels of these secondary metabolites in the inner leaves of Batavia Rubia Munguía and, in a lesser extent, in the internal leaves of Maravilla de Verano. Although the enhancement of phenolic content in tissues can be linearly correlated with the antioxidant capacity of lettuce (Kang and Saltveit 2002), the accumulation of phenolic compounds is usually associated with subsequent tissue browning, and browning of freshly cut lettuce reduces its quality and shelf life (López-Galvez et al. 1996). According to Kang and Saltveit (2002), the most adequate decision in order to combine healthy properties with high quality and shelf life of freshly cut lettuce would be to apply treatments (i.e., treatments with antioxidants and calcium solutions or exclusion of oxygen) that prevent browning by decreasing the oxidation of the accumulated phenolic compounds. Those treatments would produce a healthier product than anti-browning treatments that work by reducing the synthesis and accumulation of phenolics in tissues.

Anthocyanins are the most important group of water-soluble pigments in plants and may be induced by several environmental factors, including water stress (Chalker-Scott 1999). In non-mycorrhizal lettuce plants, we observed an increase in the concentrations of anthocyanins in leaves of Batavia Rubia Munguía subjected to any type and degree of drought. In non-mycorrhizal Maravilla de Verano, only the application of CD produced an enhancement in the levels of anthocyanins in inner leaves. On the other hand, the association of plants with AMF clearly enhanced the levels of anthocyanins in both varieties of lettuce, with increases especially significant in plants undergoing moderate and maintained water deficit (2/3 FC). Lee and Scagel (2009) also observed increases of around 35% in the concentrations of anthocyanins in leaves of *Ocimum basilicum* associated with *G. intraradices* in comparison with non-mycorrhizal controls in plants cultivated in greenhouse under optimal irrigation. Apart from the environmental significance of anthocyanins in plant stress responses, these pigments are regarded as important components in human nutrition due to their antioxidant capacities (Stintzing and Carle 2004). In addition, as explained in the 'Introduction' section, they have exhibited anti-carcinogenic effects in several cell cultures systems including cancer cells of the colon, endothelial, liver and leukemic (You et al. 2011).

Oh et al. (2009) concluded that the adaptation of lettuce plants to water stress was associated to the activation of the enzyme L-galactose dehydrogenase (L-GalDH) implied in the biosynthesis of ascorbic acid. In our study, however, any of the drought regimes applied had significant effects on the concentrations of the total ascorbate (ASC + DHA) pool in the two varieties of lettuce, regardless of whether or not they were associated with AMF. In contrast, the ratio between ASC and DHA changed in plants undergoing restricted irrigation, with changes being mainly dependent on both cultivar of lettuce and mycorrhizal symbiosis. Redox state can be defined as the ratio between reduced and oxidized molecules within a pool and the most abundant contributor to general redox metabolism in plant cells is ascorbate (Potters et al. 2010). In non-mycorrhizal Maravilla de Verano the relative abundance of ASC (high ASC/DHA) in plants subjected to maintained water deficits (2/3 FC or 1/2 FC) suggests that these redox metabolites cooperated in scavenging harmful concentrations of reactive oxygen species (ROS) in cells of leaves by fueling the ASC–glutathione cycle. However, as well as the 'big three' antioxidants — ascorbate (ASC), glutathione (GSH) and the pyridine nucleotides NADH and NADPH — plants contain many distinct redox-active compounds, including phenolics and carotenoids, that actively contribute to the global cellular redox state (Potters et al. 2010). Therefore, declines in the ratio ASC/DHA in non-mycorrhizal Batavia Rubia Munguía and, especially, in all mycorrhizal lettuce plants when subjected to different types and degrees of water deficits could be partially overcome by enhancements in the concentrations of phenolics and carotenoids. In any case, whereas at the outset of plant stress, ROS were only considered as damaging the cell components, the view nowadays has shifted to ROS being an integrative part of cell signaling metabolism modulated by the cellular redox state, leading to different responses related to programmed cell death, gene expression, plant defense or plant development (Potters et al. 2010).

In summary, the results of our study reinforce the idea that one of the services provided by AM symbiosis to the ecosystem is the bioregulation of plant development and increase in plant quality for human health due to the ability of AMF for modifying plant metabolism and physiology (Gianinazzi et al. 2010). The beneficial effect of AM symbiosis on the accumulation of potential antioxidants in leaves of lettuce (mainly carotenoids and anthocyanins and, in a lesser extent, chlorophylls and phenolics) was more evident under water deficit than under optimal irrigation. On the other hand, shoot biomass in mycorrhizal lettuce plants (Batavia Rubia Munguía and Maravilla de Verano) subjected to moderate and maintained water deficit (2/3 FC) were similar to those of non-mycorrhizal plants grown under well-watered conditions. In addition, lettuces subjected to 2/3 FC had similar leaf RWC than their respective well-watered controls. Taking together all these findings, we can conclude that mycorrhizal symbiosis not only can improve quality of lettuce but also may allow reducing irrigation without subsequent detrimental effects on lettuce production. However, it is important to note that the beneficial effect of AMF on the nutritional quality of lettuce may depend on AMF species or isolates, cultivars of lettuce and fertilization regime (see Gianinazzi et al. 2010 for a review). The possible presence of some microorganisms accompanying the mycorrhizal population could also contribute to enhance the beneficial effect of AMF on the nutritional quality of greenhouse-grown lettuces (Azcón-Aguilar and Barea 1997). Results obtained by Kohler et al. (2008) supported the potential use of a plant-growth-promoting rhizobacterium (PGPR) together with AMF as an inoculant to alleviate the oxidative damage of lettuce plants produced under water stress.

Acknowledgements Marouane Baslam is the recipient of a grant from Asociación de Amigos de la Universidad de Navarra (ADA). The authors are very grateful to Adriana Hernández, from Atens, Tarragona, Spain, for kindly providing the commercial inoculum.

References

Agüero MV, Barg MV, Yommi A, Camelo A, Roura SI (2008) Postharvest changes in water status and chlorophyll content of lettuce (*Lactuca sativa* L.) and their relationship with overall visual quality. J Food Sci 73:47–55

Agüero MV, Ponce AG, Moreira MR, Roura SI (2011) Lettuce quality loss under conditions that favor the wilting phenomenon. Postharv Biol Technol 59:124–131

Augé RM (2001) Water relations, drought and vesicular–arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42

Azcón-Aguilar C, Barea JM (1997) Applying mycorrhiza biotechnology to horticulture: significance and potentials. Sci Hortic 68:1–24

Azcón-Aguilar C, Jaizme-Vega MC, Calvet C (2002) The contribution of arbuscular mycorrhizal fungi for bioremediation. In: Gianinazzi S, Schüepp H, Barea JM, Haselwandter K (eds) Mycorrhizal technology in agriculture. From Genes to Bioproducts. Birkhäuser Verlag, Berlin, pp 187–197

Bagyaraj DJ (1994) Vesicular–arbuscular mycorrhiza: application in agriculture. In: Norris JR, Read DJ, Varma AK (eds) Techniques for the study of mycorrhiza. Academic, London, pp 819–833

Balestrini R, Lanfranco L (2006) Fungal and plant gene expression in arbuscular mycorrhizal symbiosis. Mycorrhiza 16:509–524

Barg M, Agüero MV, Yommi A, Roura SI (2009) Evolution of plant water status indices during butterhead lettuce growth and its impact on post-storage quality. J Sci Food Agric 89:422–429

Baslam M, Garmendia I, Goicoechea N (2011) Arbuscular mycorrhizal fungi (AMF) improved growth and nutritional quality of greenhouse-grown lettuce. J Agric Food Chem 59:5504–5515

Bolgiano NC, Safir GR, Warnacke DD (1983) Mycorrhizal infection and growth of onion in the field in relation to phosphorus and water availability. J Am Soc Hortic Sci 108:819–825

Bonfante-Fasolo P (1984) Anatomy and morphology of VA mycorrhizae. In: Powell CL, Bagyaraj DJ (eds) VA mycorrhiza. CRC, Boca Raton, pp 35–46

Borghi S (2003) Special: IV range (vegetables). Colture Protette 32:21–43

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Bunning ML, Kendall PA, Stone MB, Stonaker FH, Stushnoff C (2010) Effects of seasonal variation on sensory properties and total phenolic content of 5 lettuce cultivars. J Food Sci 75:156–161

Calvo MM (2005) Lutein: a valuable ingredient of fruit and vegetables. Crit Rev Food Sci 45:671–696

Cevahir G, Yentür S, Yazgan M, Ünal M, Yilmazer N (2004) Peroxidase activity in relation to anthocyanin and chlorophyll content in juvenile and adult leaves of “mini-star” *Gazania splendens*. Pak J Bot 36:603–609

Chalker-Scott L (1999) Environmental significance of anthocyanins in plant stress responses. Photochem Photobiol 70:1–9

Chapuis-Lardy L, Contour-Ansel D, Bernhard-Reversat F (2002) High performance liquid chromatography of water-soluble phenolics in leaf litter of three *Eucalyptus* hybrids (Congo). Plant Sci 163:217–222

Fernandes TM, Gomes BB, Lanfer-Marquez UM (2007) Apparent absorption of chlorophyll from spinach in an assay with dogs. Innov Food Sci Emerg 8:426–432

Gallardo M, Jackson LE, Schullback K, Snyder R, Thompson R, Wyland L (1996) Production and water use in lettuces under variable water supply. Irrig Sci 16:125–137

Garmendia I, Goicoechea N, Aguirreolea J (2004) Antioxidant metabolism in asymptomatic leaves of *Verticillium*-infected pepper associated with an arbuscular mycorrhizal fungus. J Phytopathol 152:593–599

Gianinazzi S, Gollotte A, Binet M-N, van Tuinen D, Redecker D, Wipf D (2010) Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza 20:519–530

Goicoechea N, Merino S, Sánchez-Díaz M (2004) Contribution of arbuscular mycorrhizal fungi (AMF) to the adaptations exhibited by the deciduous shrub *Anthyllis cytisoides* under water deficit. Physiol Plant 122:453–464

Harley JL, Smith SE (1983) Mycorrhizal symbiosis. Academic, London

Hayman DS, Barea JM, Azcón R (1976) Vesicular–arbuscular mycorrhiza in southern Spain: its distribution in crops growing in soil of different fertility. Phytopathol Mediterr 15:1–6

Herrmann K (1976) Flavanols and flavones in food plants: a review. Int J Food Sci Technol 11:433–448

Hewitt EJ (1952) Sand and water culture methods used in the study of plant nutrition. Tech Commun no. 22, Famham Royal. Commonwealth Agriculture Bureau, Bucks, UK

Jarvis CE, Walker JRL (1993) Simultaneous, rapid, spectrophotometric determination of total starch, amylose and amylopectin. J Sci Food Agric 63:53–57

Kang H-M, Saltveit KE (2002) Antioxidant capacity of lettuce leaf tissues increases after wounding. J Agric Food Chem 50:7536–7541

Ke D, Salveit ME Jr (1988) Plant hormone interaction and phenolic metabolism in the regulation of russet spotting in iceberg lettuce. Plant Physiol 88:1136–1140

Kohler J, Hernández JA, Caravaca F, Roldán A (2008) Plant-growth promoting rhizobacteria and arbuscular mycorrhizal fungi modify alleviation biochemical mechanisms in water-stressed plants. Funct Plant Biol 35:141–151

Krishna H, Singh SK, Sharma RR, Khawale RN, Grover M, Patel VB (2005) Biochemical changes in micropropagated grape (*Vitis vinifera* L.) plantlets due to arbuscular–mycorrhizal fungi (AMF) inoculation during ex vitro acclimatization. Sci Hortic 106:554–567

Lee J, Scagel CF (2009) Chicoric acid found in basil (*Ocinum basilicum* L.) leaves. Food Chem 115:650–656

Leipner J, Fracheboud Y, Stamp P (1997) Acclimation by suboptimal temperature diminishes photooxidative damage in maize leaves. Plant Cell Environ 20:366–372

Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In: Colowick SP, Kaplan NO (eds) Methods in enzymology. Academic, San Diego, pp 350–382

Llorach R, Martínez-Sánchez A, Tomás-Barberán FA, Gil MI, Ferreres F (2008) Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. Food Chem 108:1028–1038

López-Galvez G, Saltveit ME, Cantwell MI (1996) Wound-induced phenylalanine ammonia lyase activity: factors affecting its induction and correlation with the quality of minimally processed lettuce. Postharv Biol Technol 9:223–233

Mancinelli AL (1984) Photoregulation of anthocyanin synthesis: VIII. Effect of light pretreatments. Plant Physiol 75:447–453

Marulanda A, Azcón R, Ruiz-Lozano JM (2003) Contribution of six arbuscular mycorrhizal isolates to water uptake by *Lactuca sativa* plants under drought stress. Physiol Plant 119:523–533

Mulabagal V, Ngouajio M, Nair A, Zhang Y, Gottumukkala AL, Nair MG (2010) *In vitro* evaluation of red and green lettuce (*Lactuca sativa*) for functional food properties. Food Chem 118:300–306

Oh M-M, Trick HN, Rajashekar CB (2009) Secondary metabolism and antioxidants are involved in environmental adaptation and stress tolerance in lettuce. J Plant Physiol 166:180–191

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–161

Pietrini F, Massacci A (1998) Leaf anthocyanin content changes in *Zea mays* L. grown at low temperature: significance for the relationship between the quantum yield of PS II and the apparent quantum yield of CO_2 assimilation. Photosynth Res 58:213–219

Potters G, Horemans N, Jansen MAK (2010) The cellular redox state in plant stress biology – a charging concept. Plant Physiol Biochem 48:292–300

Rao AV, Rao LG (2007) Carotenoids and human health. Pharmacol Res 55:207–216

Ruiz-Lozano JM, Azcón R (1995) Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status. Physiol Plant 95:472–478

Ruiz-Lozano JM, Gómez M, Azcón R (1995) Influence of different *Glomus* species on the time-course of physiological plant responses of lettuce to progressive drought stress periods. Plant Sci 110:37–44

Sánchez-Díaz M, Pardo M, Antolín MC, Peña J, Aguirreolea J (1990) Effect of water stress on photosynthetic activity in the *Medicago–Rhizobium–Glomus* symbiosis. Plant Sci 71:215–221

Seeram NP (2008) Berry fruits: compositional elements, biochemical activities, and the impact of their intake on human health, performance, and disease. J Agric Food Chem 56:627–629

Séstak Z, Càtsky J, Jarvis P (1971) Plant photosynthetic production. Manual of methods. Dr Junk, The Hague

Stintzing FC, Carle R (2004) Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. Trends Food Sci Technol 15:19–38

Strack D, Fester T (2006) Isoprenoid metabolism and plastid reorganization in arbuscular mycorrhizal roots. New Phytol 172:22–34

Tomás-Barberán FA, Loaiza-Velarde J, Bonfanti A, Saltveit ME (1997) Early wound- and ethylene-induced changes in phenylpropanoid metabolism in harvested lettuce. J Am Soc Hortic Sci 122:399–404

Tsabedze MW, Wahome PK (2010) Influence of different irrigation regimes on production of lettuce (*Lactuca sativa* L.). Am–Euras J Agric Environ Sci 8:233–238

Waterman PT, Mole S (1994) Analysis of phenolic plant metabolites. Blackwell Scientific, London

Weatherley PE (1950) Studies in the water relations of the cotton plant. I. The field measurements of water deficits in leaves. New Phytol 49:81–87

Yemm E, Willis AJ (1954) The estimation of carbohydrates in plant extracts by anthrone. Biochem J 57:508–514

You Q, Wang B, Chen F, Huang Z, Wang X, Luo PG (2011) Comparison of anthocyanins and phenolics in organically and conventionally grown blueberries in selected cultivars. Food Chem 125:201–208

Zuccarini P (2007) Mycorrhizal infection ameliorates chlorophyll content and nutrient uptake of lettuce exposed to saline irrigation. Plant Soil Environ 53:283–289