Effects of Fertilization, Arbuscular Mycorrhiza, and Salinity on Growth, Yield, and Bioactive Compounds of Two *Aloe* Species

Mariateresa Cardarelli

Agricultural Research Council–Research Centre for the Soil-Plant System, 00184 Rome, Italy

Youssef Rouphael

Department of Crop Production, Faculty of Agricultural Engineering and Veterinary Medicine, Lebanese University, Dekwaneh-El Maten, Beirut, Lebanon

Elvira Rea

Agricultural Research Council–Research Centre for the Soil-Plant System, 00184 Rome, Italy

Luigi Lucini and Marco Pellizzoni

Institute of Environmental and Agricultural Chemistry, Università Cattolica del Sacro Cuore, via Emilia Parmense 84, 29122 Piacenza, Italy

Giuseppe Colla¹

Department of Agriculture, Forestry, Nature and Energy, University of Tuscia, via San Camillo De Lellis snc, 01100 Viterbo, Italy

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Abstract. Three greenhouse experiments were carried out to compare the responses of *Aloe arborescens* and *Aloe barbadensis* with organic fertilization (standard or reduced fertilization level), arbuscular mycorrhiza [with AM (+AM) or without AM (–AM)], and salinity (1 or 80 mM NaCl) in terms of plant growth, leaf yield, mineral composition, and nutraceutical value. In all experiments, the yield of fresh leaves was significantly higher by 320%, 252%, and 72%, respectively, in *A. barbadensis* in comparison with *A. arborescens*. Doubling the fertilizer dose, plant growth parameters increased, but the bioactive compounds were negatively affected. The highest antioxidant activity was recorded with *A. barbadensis* using both fertilization regimes, whereas the highest values of anthraquinones aloin were observed in *A. barbadensis* using a reduced fertilization regime and when plants were inoculated with AM fungi. β -polysaccharide concentration was significantly higher in *A. barbadensis* in comparison with *A. arborescens* and was increased by 33% when plants were inoculated with AM fungi. In both *Aloe* species, increasing the salinity decreased the leaf fresh weight and total dry biomass but increased the aloin and β -polysaccharides content by 66% and 21%, respectively. The results suggest that cultural practices such as organic fertilization, inoculation with AM fungi, and irrigation with saline water can represent effective tools to achieve a more favorable phytochemical profile.

Aloes are xerophytes in the *Aloeaceae* in the Liliales that are cultivated for ornamental, medicinal, vegetable, and cosmetic purposes

in Africa, North America, Europe, and South-east Asia (Tawaray et al., 2007). Approximately 500 species have been described in the genus *Aloe*, ranging from diminutive shrubs to large tree-like forms and it is represented in several biodiversity hotspots (Zapata et al., 2013). Basically, all the *Aloe* species have similar constituents; however, *Aloe barbadensis* Miller (often called *Aloe vera* L.) and *Aloe arborescens* Miller are the most extensively cultivated in the world (Liao et al., 2006).

The chemical composition of the leaf gel is very complex, composed mainly of polysaccharides and soluble sugars followed by proteins, many of which are enzymes, amino acids, vitamins, and anthraquinones (Liu et al.,

2007). It has been shown that polysaccharides derived from *A. barbadensis* enhance immunity activity and exert antioxidant effects (Zhanhai et al., 2009) and most of the activity has been attributed to β -polysaccharides (Ramachandra and Srinivasa Rao, 2008). Anthraquinones are the second class of bioactive metabolites, including C-glucosyl derivatives such as barbaloin (10-glucopyranosyl-1,8-dihydroxy-3-hydroxymethyl-9–10H-anthracenone), a mixture of the two diastereoisomers aloin A and B as well as glucose-free compounds such as aloe-emodin (Fanali et al., 2010). Anthraquinones were reported to have cathartic effects, anti-inflammatory effects in vivo as well as antibacterial, antiviral, and anticancer effects (Park et al., 2009; Pellizzoni et al., 2012).

Manipulation of target compounds in plants such as phytochemicals and antioxidants by the management of the mineral nutrition and salinity has been recognized as a research area attracting applied scientists interest (Fallov et al., 2009a, 2009b; Fanasca et al., 2006a, 2006b). Proper management of the fertilization and the use of saline water can provide an effective tool to improve the target compounds in plants such as phytochemicals and antioxidants (Rouphael et al., 2012a). At present, most research focused on the effects of mineral fertilizers on field production of aloe (Wang, 2007), whereas there is a lack of information on the use of organic fertilizers as a nutrient source for aloe despite the growing interest of organically production of aloe.

Arbuscular mycorrhizal fungi are widespread microorganisms able to establish a symbiotic association with the roots of most terrestrial plants. Host plants have an improved ability for nutrient uptake and tolerance to biotic and abiotic stresses (Cardarelli et al., 2010; Colla et al., 2008; Rouphael et al., 2010a). AM fungi can also induce changes in the accumulation of secondary metabolites, e.g., phenols, in host plant roots (Devi and Reddy, 2002; Rojas-Andrade et al., 2003; Yao et al., 2003). Many plant species in Liliales, to which Aloes belong, are known to be mycorrhizal-dependent (Tawaray, 2003). However, little is known about the effect of AM fungi colonization on the accumulation of active phytochemicals in medicinal plants.

At present, most research (Mogbeli et al., 2012; Mota-Fernandez et al., 2011; Tawaray et al., 2007; Toussaint et al., 2007; Wang, 2007; Zheng et al., 2009) about the effect of cultural practices (e.g., fertilization, salt stress, AM colonization) on the growth and yield focuses on *A. barbadensis*, whereas there have been no reports about other *Aloe* species such as *Aloe arborescens*. Besides, most of the literature information focused on field production of aloe, whereas the soilless culture of aloe plants under greenhouse conditions has received little attention.

Starting from these considerations, the aim of this study was to compare the responses of young potted *Aloe barbadensis* and *Aloe arborescens* plants at the nursery level with fertilization rate (Expt. 1), AM colonization of roots (Expt. 2), and salinity (Expt. 3). To this end, plant growth, leaf yield, chlorophyll

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¹To whom reprint requests should be addressed; e-mail giucolla@unitus.it.

and carotenoids assay, mineral composition, antioxidant activity, β -polysaccharide, and aloin and aloe-emodin concentrations were analyzed.

Materials and Methods

Plant material, growth conditions, treatments, and arbuscular mycorrhizal inoculation. Three soilless greenhouse experiments were carried out at the experimental farm of Tuscia University, central Italy (lat. 42°25'N, long. 12°08'E, altitude. 310 m above sea level): Expt. 1 (fertilization regime experiment) from 10 May 2010 to 2 Sept. 2010; Expt. 2 (arbuscular mycorrhiza experiment) from 2 May 2010 to 31 Aug. 2010; and Expt. 3 (salinity experiment) from 2 May 2010 to 31 Aug. 2010. In all experiments, plants were grown under natural light conditions. The greenhouse was maintained at daily temperatures between 16 and 32 °C. In all experiments, rooted cuttings of *Aloe barbadensis* and *Aloe arborescens* were obtained from a commercial nursery (Torsanlorenzo, Ardea, Italy). The rooted cuttings had three or four roots (≈ 3 cm long) and three or four leaves. Before transplanting, the roots of cutting were soaked 1 h in water, surface-sterilized by shaking for 5 min in 5% NaClO, and thoroughly rinsed twice in sterilized, distilled water. In all experiments, the rooted cuttings were transplanted into pots (17 cm diameter, 16 cm height) containing 3.6 L of a mixture coconut fiber:pumice (particle size of 2 to 5 mm in diameter) in a 3:1 volume ratio. The pots were placed on 18 cm wide and 5-m long troughs with 30 cm between pots and 30 cm between troughs, giving a plant density of 11 plants per square meter.

In Expt. 1, a randomized complete-block design with three replicates (12 plants per experimental unit) was used to compare two organic fertilization regimes (standard and reduced fertilization). The organic fertilization treatment consisted of preplant and postplant applications. Preplant fertilization was carried out by mixing 4 (reduced fertilization) and 8 g·L⁻¹ (standard fertilization) of a guano-based fertilizer containing 60.0 g·kg⁻¹ nitrogen (N), 65.5 g·kg⁻¹ phosphorus (P), 24.9 g·kg⁻¹ potassium (K), 12.0 g·kg⁻¹ magnesium (Mg), 7.1 g·kg⁻¹ calcium (Ca), and trace elements (Guanito; Italtollina S.p.A., Rivoli Veronese, Verona, Italy) with the substrate before transplanting, whereas the postplant fertilization was based on the application of organic based-fluid fertilizers (Trainer and Myr line; Italtollina S.p.A.) through a subirrigation system (fertigation). The nutrient solution used in the standard fertilization regime had the following composition: 120 mg·L⁻¹ N, 100 mg·L⁻¹ K, 72 mg·L⁻¹ Ca, 30 mg·L⁻¹ Mg, and trace elements. The nutrient solution in the reduced fertilization regime had 50% of the macronutrient and micronutrient concentrations of the standard nutrient solution. All fertilizers used were allowed in organic farming according to EC Reg. 834/2007.

In Expt. 2, a randomized complete-block design with three replicates (12 plants per

experimental unit) was used to compare the performance of *A. barbadensis* and *A. arborescens* under two mycorrhizal treatments (with AM or without AM). Part of the nutrients was applied before transplanting by mixing 4 g·L⁻¹ of a guano-based fertilizer (Guanito; Italtollina S.p.A.). Three weeks after transplanting, plants were fertigated with organic based-fluid fertilizers (Trainer and Myr line; Italtollina S.p.A.) giving 60 mg·L⁻¹ N, 50 mg·L⁻¹ K, 36 mg·L⁻¹ Ca, 15 mg·L⁻¹ Mg, 23 mg·L⁻¹ sodium (Na), 35 mg·L⁻¹ chlorine (Cl), and trace elements. Before planting, half of the pots were inoculated with *Glomus intraradices* and *Glomus mosseae* (Aegis Sym Clay; Italtollina S.p.A.) by mixing 1 m³ of the substrate with 15 L of inoculum mixture containing 95% of clay minerals as granular carriers, root fragments, and spores (50 spores/g). The calcined clay (particle size average 5 mm) was an attapulgite used as substrate for propagation of AM fungi (Plenchette et al., 1996). The commercial inoculum was originally cultured in leek roots (*Allium porrum* L.) as reported by Calvet et al. (2001).

In Expt. 3, a randomized complete-block design with three replicates (12 plants per experimental unit) was used to compare the performance of *A. barbadensis* and *A. arborescens* under two nutrient solutions (non-salt control or saline solution). The control treatment consisted of the organic nutrient solution used in Expt. 2, whereas the saline nutrient solution had the same basic composition plus an additional 4.582 g·L⁻¹ of NaCl (79 mM). The pH of the nutrient solution for all treatments was 5.5 $\pm$ 0.3.

In all experiments, the nutrient solution was pumped from independent tanks (one tank of 30 L per experimental unit) through a subirrigation system. In the subirrigation system, the nutrient solution was pumped at the elevated end of the benches and allowed to run slowly down the trough past the pots, and the excess was drained back to the tank for later recirculation. Irrigation scheduling was performed using electronic low-tension tensiometers (LT Irrrometer) in which control irrigation was based on substrate matric potential (Norrie et al., 1994). Tensiometers have been placed at about the midpoint of the pots (≈ 8 cm depth). In each treatment, three tensiometers were installed, and they were located in different pots to provide a representative reading of the moisture tension. Tensiometers were connected to an electronic programmer that controlled the beginning (-5 kPa) and the end of irrigation (-1 kPa), which correspond to high and low tension set points for the major part of media (Kiehl et al., 1992).

Plant growth and substrate measurements. At final harvest, at the end of each experiment (115, 121, and 121 d after transplanting in Expts. 1, 2, and 3, respectively), 10 plants per plot were separated into stems, leaves, and roots, and their tissues were dried in a forced-air oven at 80 °C for 72 h for biomass determination. Height (H) was determined as the distance from the surface of the medium to the top of the plant. The number of leaves per

plant and the off-shoot number coming from the roots of the main shoot were also recorded.

In all experiments, and after the plants were harvested, the growth medium was sampled for electrical conductivity (EC) and pH measurement. The substrate from 10 pots from each experimental unit was used. The EC and pH of the water extract were obtained using the 1:2 (growth medium:deionized water, v/v) method by adding 80 cc of deionized water to a sample of 40 cc. Growth medium and water were well mixed for 30 min and then the mixture was filtered and the solids discarded (Rouphael and Colla, 2005, 2009; Rouphael et al., 2008). The EC and pH of the filtered extracts were then measured using a conductivity meter (EC 214; Hanna Instruments) and with a pH meter (HI-9023; Hanna Instruments), respectively.

Arbuscular mycorrhizal-fungi root colonization. At the end of Expt. 2, the root colonization by AM fungi was determined on the same plants sampled for shoot and root measurements. Root samples were cleared with 10% KOH, stained with 0.05% trypan blue in lactophenol as described by Phillips and Hayman (1970), and microscopically examined for AM fungi colonization by determining percentage of root segments containing arbuscules + vesicles using a grid-line intercept method (Giovannetti and Mosse, 1980).

Mineral analysis. In all experiments (1, 2, and 3), dried plant tissues (leaves, stems, and roots) were ground separately in a Wiley mill to pass through a 20-mesh screen and 0.5 g of the dried tissues was analyzed for the contents of the following: N, P, K, Ca, Mg, Na, iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), and boron (B). The N concentration in plant tissues was determined after mineralization with sulfuric acid by "Kjeldahl method" (Bremner, 1965), whereas P, K, Ca, Mg, Na, Fe, Cu, Zn, Mn, and B concentrations were determined by dry-ashing at 400 °C for 24 h, dissolving the ash in 1:25 HCl, and assaying the solution obtained using an inductively coupled plasma emission spectrophotometer (ICP Iris; Thermo Optek, Milano, Italy) (Karla, 1998). In Expt. 3, chloride was determined by titration with AgNO₃ in the presence of K₂CrO₄ (Eaton et al., 1995).

Sampling procedure of leaves. Three samples of fresh leaves per replicate were harvested after 114, 120, and 120 d in Expts. 1, 2, and 3, respectively, to determine the chlorophyll and carotenoids content, aloin and aloe-emodin, β -polysaccharides, and DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical scavenging activity.

Chlorophyll and carotenoid assay. Chlorophyll and carotenoids were extracted by grinding the leaf tissue with a mortar and pestle using ammoniacal acetone. The resulting extracts were centrifuged at 3000 $\times$ g for 3 min. The total chlorophyll and carotenoid contents were determined by ultraviolet-Vis spectrophotometry (Beckman DU-50 spectrophotometer; Beckman Instruments, Inc., Fullerton, CA). The absorbance of the solution was measured at 470, 647, and 664 nm.

Formulae and extinction coefficients used for the determination of leaf pigments (total chlorophyll and carotenoids) were described by Lichtenthaler and Wellburn (1983). The content of the total chlorophyll and carotenoids was expressed in microgram per gram of fresh weight.

Aloin and aloe-emodin analysis. For aloin and aloe-emodin determination, leaves from each sample were transversally sliced and then randomly split into two subsamples; one was thoroughly homogenized and used to analyze aloin and aloe-emodin, whereas the outer green rind was removed from the second leaf and the remaining inner parenchyma was homogenized to determine β -polysaccharides. The anthraquinones aloin (as a sum of the two stereoisomers aloin A and aloin B) and aloe-emodin were determined in each sample by liquid chromatography followed by tandem mass spectrometry with electrospray ionization source (LC-ESI-MS/MS) in the negative mode and quantified by the external standard method. A 1200 series liquid chromatograph system, equipped with quaternary pump and electrospray ionization system, coupled to a G6410A triple quadrupole mass spectrometer detector (all from Agilent Technologies, Santa Clara, CA) was used. The plant material (2 g) was extracted by Ultra-Turrax in an 8 + 4 mL of ethyl acetate/methanol mixture (9:1 by volume) after adding 4 mL of a 20% NaCl aqueous solution. After centrifugation (313 g for 15 min), the extract was diluted with methanol, filtered through a 0.45- μ m membrane, and then analyzed by reversed phase LC-MS/MS using a Zorbax Eclipse plus C18 column (100 $\times$ 3.0 mm, 3.5 μ m) from Agilent Technologies. The solvent system used water (solvent A) and acetonitrile (solvent B) at a flow rate of 0.3 mL $\cdot$ min $^{-1}$; the gradient was designed to decrease solvent A from 35% at 0 min to 20% at 2.5 min. The injection volume was 10 μ L and the drying gas was N at 5 L $\cdot$ min $^{-1}$.

Data handling was performed by the MassHunter software under Multiple Reaction Monitoring acquisition: aloin transition was from m/z 417 [M-H] $^{-}$ to 297 (collision energy 15 V), whereas aloe-emodin transition was from m/z 269 [M-H] $^{-}$ to 240 (collision energy 25 V) (Pellizzoni et al., 2012).

Analysis of β -polysaccharides. The β -polysaccharide fraction was determined colorimetrically at 540 nm, after reaction with the Congo red dye, on the basis of the work described by Eberendu (Eberendu et al., 2005). Colorimetric measurements were done using a Perkin Elmer lambda 12 ultraviolet/VIS spectrometer. Aloe pulp (4 g) was extracted in 10 mL of double-distilled water on a horizontal shaker for 2 h; 500 μ L of 1.5% KOH was added to the solution and then 2 mL of Congo red solution (obtained diluting 50 times a saturated aqueous solution) was also added. The solution was left for 1 h and then analyzed colorimetrically at λ = 540 nm. Semiquantitative determination of the solutions was carried out using a pure β -glucan standard, and therefore results were expressed as β -glucan equivalents. Three measurements

on each of three extracts were performed per sample.

Assay of DPPH radical scavenging activity. All leaf samples were lyophilized in vacuum for 1 d and then ground into a fine powder for further use. Fifty milliliters of 80% ethanol (v/v) was added to 1 g of lyophilized powder in a round flask, the sample was sonicated for 15 min, and then filtered onto GF/A filters (Whatman, Buckinghamshire, U.K.). The residue was washed twice with 10 mL of ethanol (96%). The filtrates were combined in a pre-weighed flask and concentrated to dryness at 50 $^{\circ}$ C by rotary evaporation. The mass of solids in the different extracts was finally recorded and the flasks were stored at -18° C for further use. The dehydrated extracts were redissolved in 80% ethanol (v/v) to a final concentration of 100 mg $\cdot$ L $^{-1}$ of solid immediately before the antioxidant activity assay.

The stable radical DPPH $\cdot$ was used to investigate the radical scavenging activity of each solution. A total of 2 mL of these solutions was placed in a cuvette and 2 mL of a 1.0×10^{-4} mol $\cdot$ L $^{-1}$ daily prepared ethanol solution of DPPH $\cdot$ was added. Absorbance measurements were carried out at 517 nm using a Perkin Elmer (Lambda 12 Spectrometer, Ontario, Canada) spectrophotometer. The decrease in absorbance started immediately after addition of the radical and was determined continuously at 15-min intervals until the absorbance reached a steady state. All the determinations were performed in duplicate. The inhibition of the DPPH $\cdot$ radical by the samples was calculated according to Yen and Duh (1994); the antioxidant activity coefficients (AAC) were calculated as follows:

$$AAC = 100 * (A - \text{sample}^{eq} - A - \text{blank}^{eq}) / (A - \text{blank}^{t0} - A - \text{blank}^{eq})$$

where A-sample and A-blank were the absorbance of the sample and the blank (80% ethanol) at the beginning (t0) and at the steady state, respectively.

Statistical analysis. All data were statistically analyzed by analysis of variance using the SPSS software package (SPSS 10 for Windows, 2001; SPSS Inc., Chicago, IL).

Duncan's multiple range test was performed at $P = 0.05$ on each of the significant variables measured.

Results

Expt. 1: Fertilization experiment. The growth medium EC at the end of the cultural cycle was significantly ($P < 0.01$) affected by the fertilization regime (data not shown) with the highest values recorded with standard (avg. 1.28 dS $\cdot$ m $^{-1}$) than with reduced fertilization regime (avg. 0.66 dS $\cdot$ m $^{-1}$), whereas no difference among treatments was observed for the growth medium pH (avg. 5.5).

Irrespective of the fertilization level, the weight of fresh leaves and the total dry biomass were 320% and 164% higher, respectively, in *A. barbadensis* in comparison with *A. arborescens*, whereas an opposite trend was observed for the plant height, number of leaves, and shoots per plant (Table 1). The plant growth parameters increased in both *Aloe* species by doubling the fertilizer dose. The highest number of leaves, leaf fresh weight, and the total dry biomass were observed with standard fertilization [avg. 13.6, 573.2, and 22.7 g dry weight (DW), respectively], whereas the lowest values were recorded in reduced fertilization (avg. 12.2, 424.4, and 17.7 g DW, respectively).

The N, K, Ca, Mg, Na, Mn, and Zn concentrations in leaf tissue were significantly affected by the fertilization level with the higher values recorded with standard fertilization than with the reduced fertilization (Table 2). Moreover, when averaged over fertilization level, the concentrations of N, P, K, Ca, and Mg were significantly higher by 32.0%, 9.3%, 7.9%, 27.6%, and 22.4%, respectively, in *A. arborescens* leaf tissue in comparison with *A. barbadensis* (Table 2).

The total chlorophyll content was influenced by *Aloe* species ($P < 0.05$) and fertilization level ($P < 0.01$) with no *Aloe* species $\times$ fertilization interaction (data not shown). Chlorophyll content was higher in *A. barbadensis* [avg. 337.9 mg $\cdot$ kg $^{-1}$ fresh weight (FW)] than *A. arborescens* (avg. 241.1 mg $\cdot$ kg $^{-1}$ FW) and in plants from reduced fertilization (321.1 mg $\cdot$ kg $^{-1}$ FW) compared with standard fertilization

Table 1. Effect of fertilization level on plant height, leaf and offshoot number, leaf fresh weight and dry biomass production, and partitioning in two *Aloe* species.

Aloe species	Fertilization level	Plant				Dry biomass			
		Ht (cm)	Leaves (number)	Offshoots (number)	Leaf fresh wt (g)	Leaves (g)	Stem (g)	Root (g)	Total (g)
<i>Aloe arborescens</i>	Reduced ^z	45.7	13.3	2.1	176.9	7.5	1.1	1.4	10.0
	Standard	49.7	14.3	1.9	206.6	8.8	1.4	1.9	12.1
	Mean	47.7	13.8	2.0	191.7	8.2	1.3	1.7	11.1
<i>Aloe barbadensis</i>	Reduced	34.2	11.2	0.0	672.0	21.4	0.1	3.9	25.4
	Standard	32.5	13.0	0.0	939.8	28.1	0.3	4.9	33.3
	Mean	33.3	12.1	0.0	805.9	24.7	0.2	4.4	29.4
Significance ^y									
Aloe species (A)		***	*	**	***	***	***	***	***
Fertilization (F)		NS	*	NS	**	*	NS	*	*
A $\times$ F		NS	NS	NS	NS	NS	NS	NS	NS

^zReduced fertilization level corresponded to half of the nutrient levels applied in standard fertilization treatment.

^yNS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

(257.6 mg·kg⁻¹ FW). *A. barbadensis* leaves had higher carotenoid concentration (48.1 µg·g⁻¹ FW) than that of *A. arborescens* (37.8 mg·kg⁻¹ FW).

Aloe-emodin was not detected in both *Aloe* species, whereas aloin was detected in all samples and its highest content was measured in *A. barbadensis* using reduced fertilization. The higher antioxidant activity was recorded with *A. barbadensis* under both fertilization regimes. When averaged over fertilization levels, the β-polysaccharides concentration was 48.2% higher in leaf tissue of *A. barbadensis* in comparison with *A. arborescens* (Table 3).

Expt. 2: Arbuscular mycorrhizal colonization experiment. AM had no effect on growth medium EC (avg. 0.61 dS·m⁻¹) and pH (avg. 6.1). No AM fungi colonization was recorded in roots of control plants at the end of the trial. However, when plants were inoculated with *G. intraradices* and *G. mosseae*, colonization was higher in *A. arborescens* (30%) than *A. barbadensis* (12%).

Irrespective of mycorrhizal treatment, the leaf FW and total dry biomass were 252% and 110% higher, respectively, in *A. barbadensis* in comparison with *A. arborescens*, whereas an opposite trend was observed for the number of leaves per plant (Table 4).

The concentrations of P, K, Ca, Mg, Na, Fe, and Zn were significantly affected by *Aloe* species × mycorrhizal treatment interaction with the higher values recorded in +AM *A. arborescens* plants (Table 5). Moreover, when averaged over mycorrhizal treatment, the higher leaf N and Mn concentrations were observed in *A. arborescens*.

The total chlorophyll content was only affected ($P < 0.05$) by mycorrhizal treatment (data not shown) with the higher values observed in +AM (331.7 mg·kg⁻¹ FW) rather than -AM plants (308.0 mg·kg⁻¹ FW), whereas no significant difference among treatments was reported on carotenoids content (47.8 mg·kg⁻¹ FW).

Again, aloe-emodin was not detected in any sample. No difference among treatments was observed for the antioxidant activity (avg. AAC 38.9). The highest values of aloin were observed in mycorrhized *A. barbadensis*. Finally, β-polysaccharides concentration was higher by 45.7% in *A. barbadensis* in comparison with *A. arborescens*, whereas it increased by 33% when plants were inoculated with *G. intraradices* and *G. mosseae* (Table 6).

Expt. 3: Salinity experiment. High salinity treatment resulted in higher EC in the growth medium (8.93 dS·m⁻¹) in comparison with low salinity treatment (0.75 dS·m⁻¹), whereas no significant difference among treatments was observed for the growth medium pH (avg. 6.1).

The highest number of shoots per plant and leaf FW was recorded in *A. arborescens* and *A. barbadensis*, respectively, under non-saline treatment (Table 7). Moreover, under saline treatment, the leaf FW reduction in comparison with the control was lower in *A. arborescens* compared with *A. barbadensis*

Table 2. Effect of fertilization level on mineral composition of leaves in two *Aloe* species.

<i>Aloe</i> species	Fertilization level	Major elements (g·kg ⁻¹ DW)						Trace elements (mg·kg ⁻¹ DW)				
		N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu	B
<i>Aloe arborescens</i>	Reduced ^z	19.5	4.6	34.3	17.4	6.5	16.9	16.0	97.1	19.7	3.9	17.4
	Standard	22.6	4.9	36.6	21.4	7.7	18.7	17.9	123.3	20.8	3.7	13.8
	Mean	21.0	4.7	35.4	19.4	7.1	17.8	17.0	110.2	20.3	3.8	15.6
<i>Aloe barbadensis</i>	Reduced	14.7	4.2	31.1	12.8	5.3	9.9	18.0	84.9	14.7	2.7	12.4
	Standard	17.0	4.4	34.4	17.7	6.4	10.7	12.2	96.9	16.5	2.7	12.5
	Mean	15.9	4.3	32.8	15.2	5.8	10.3	15.1	90.9	15.6	2.7	12.4
Significance ^y												
<i>Aloe</i> species (A)		**	*	**	***	***	***	NS	*	***	*	*
Fertilization (F)		**	NS	**	***	***	*	NS	*	*	NS	NS
A × F		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

^zReduced fertilization level corresponded to half of the nutrient levels applied in standard fertilization treatment.

^yNS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

DW = dry weight.

Table 3. Effect of fertilization level on antioxidant activity coefficient (AAC), aloins, and β-polysaccharides in two *Aloe* species.

<i>Aloe</i> species	Fertilization level	AAC	Aloins (mg·kg ⁻¹ FW)	β-polysaccharides (mg·kg ⁻¹ FW)
<i>Aloe arborescens</i>	Reduced ^z	10.2 c	301.6 c	698.8
	Standard	9.0 d	294.6 c	717.5
	Mean	9.6	298.1	708.1
<i>Aloe barbadensis</i>	Reduced	36.1 a	666.7 a	1077.9
	Standard	14.9 b	485.5 b	1021.7
	Mean	25.5	576.1	1049.8
Significance ^y				
<i>Aloe</i> species (A)		***	***	***
Fertilization (F)		***	***	**
A × F		*	***	NS

^zReduced fertilization level corresponded to half of the nutrient levels applied in standard fertilization treatment.

^yNS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple.

FW = fresh weight.

Table 4. Effect of arbuscular mycorrhizal (AM) fungi on plant height, leaf and offshoot number, leaf fresh weight and dry biomass production, and partitioning in two *Aloe* species.

		Plant				Dry biomass			
		Ht (cm)	Leaves (number)	Offshoots (number)	Leaf fresh wt (g)	Leaves (g)	Stem (g)	Root (g)	Total (g)
<i>Aloe</i> species	AM								
<i>Aloe arborescens</i>	−AM ^z	36.0	14.0	0.8	206.8	7.7	2.5	1.4	11.6
	+AM	37.3	14.2	1.2	201.3	7.6	2.6	1.3	11.5
	Mean	36.7	14.1	1.0	204.1	7.7	2.5	1.3	11.5
<i>Aloe barbadensis</i>	−AM	47.2	12.0	0.8	734.9	21.6	0.2	3.0	24.8
	+AM	47.0	11.5	0.2	703.8	20.6	0.2	2.8	23.6
	Mean	47.1	11.8	0.5	719.3	21.1	0.2	2.9	24.2
Significance ^y									
<i>Aloe</i> species (A)		NS	***	NS	***	***	***	**	***
Mycorrhizae (M)		NS	NS	NS	NS	NS	NS	NS	NS
A × M		NS	NS	NS	NS	NS	NS	NS	NS

^z+AM, -AM mycorrhizal and non-mycorrhizal plants, respectively.

^yNS, **, *** Nonsignificant or significant at $P \leq 0.01$ and 0.001, respectively.

(Table 7). Irrespective of salinity, *A. arborescens* plants were heavier with more leaves than *A. barbadensis*. When averaged over *Aloe* species, increasing the nutrient solution salinity from 1 to 80 mM NaCl decreased the plant height, the number of leaves and shoots per plant, the leaf FW, and the total dry biomass by 22.1%, 9.5%, 75.0%, 51.5%, and 34.7%, respectively.

The highest concentrations of N, P, K, Mg, Zn, and B were recorded in the non-salinized treatment compared with plants treated with NaCl, whereas an opposite trend was observed for Na and Cl concentration in leaves (Table 8).

The tissue concentrations of Zn and B declined as the external NaCl concentration increased. Finally, Mn concentration in leaves was lower in *A. barbadensis* compared with *A. arborescens* (Table 8).

Chlorophyll content was higher in *A. barbadensis* (avg. 356.2 mg·kg⁻¹ FW) than *A. arborescens* (avg. 282.3 mg·kg⁻¹ FW) and with non-saline treatment (335.2 mg·kg⁻¹ FW) compared with saline treatment (303.4 mg·kg⁻¹ FW). The total carotenoid contents were higher with *A. barbadensis* (51.2 mg·kg⁻¹ FW) in comparison with *A. arborescens* (41.1 mg·kg⁻¹ FW).

As previously observed, aloe-emodin was not detected in both *Aloe* species. The highest

Table 5. Effect of arbuscular mycorrhizal (AM) fungi on mineral composition of leaves in two *Aloe* species.

<i>Aloe</i> species	AM	Major elements (g·kg ⁻¹ DW)						Trace elements (mg·kg ⁻¹ DW)				
		N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu	B
<i>Aloe arborescens</i>	–AM ^z	17.0	8.9 b	22.7 b	16.0 b	4.6 b	19.1 b	9.2 b	84.3	15.6 b	2.4	15.1
	+AM	18.5	9.8 a	27.6 a	18.5 a	5.3 a	24.1 a	11.3 a	103.6	19.3 a	4.3	16.5
	Mean	17.8	9.3	25.15	17.2	4.9	21.6	10.2	93.9	17.5	3.3	15.8
<i>Aloe barbadensis</i>	–AM	13.6	8.0 c	22.0 b	15.5 b	4.1 c	16.3 c	9.5 b	39.6	13.0 c	2.7	15.8
	+AM	13.7	8.3 bc	24.3 b	15.3 b	4.0 c	16.6 c	10.2 ab	84.8	13.7 c	2.3	17.1
	Mean	13.7	8.1	23.2	15.4	4.1	16.5	9.8	62.2	13.3	2.5	16.5
Significance ^y												
<i>Aloe</i> species (A)		**	*	***	***	***	***	NS	**	*	NS	NS
Mycorrhizae (M)		NS	***	***	**	***	*	*	**	*	NS	**
A × M		NS	**	*	***	***	*	**	NS	*	NS	NS

^z+AM, –AM mycorrhizal and non-mycorrhizal plants, respectively.^yNS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple.

DW = dry weight.

Table 6. Effects of arbuscular mycorrhizal (AM) fungi on antioxidant activity coefficient (AAC), aloins, and β -polysaccharides in two *Aloe* species.

<i>Aloe</i> species	AM	AAC	Aloins (mg·kg ⁻¹ FW)	β -polysaccharides (mg·kg ⁻¹ FW)
<i>Aloe arborescens</i>	–AM ^z	36.2	189.4 d	580.0
	+AM	48.3	273.8 c	768.5
	Mean	42.3	231.6	674.3
<i>Aloe barbadensis</i>	–AM	34.1	480.0 b	842.5
	+AM	36.9	678.8 a	1122.7
	Mean	35.5	579.4	982.6
Significance ^y				
<i>Aloe</i> species (A)		NS	***	**
Mycorrhizae (M)		NS	***	**
A × M		NS	*	NS

^z+AM, –AM mycorrhizal and non-mycorrhizal plants, respectively.^yNS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple.

FW = fresh weight.

Table 7. Effect of salinity level on plant height, leaf and offshoot number, leaf fresh weight and dry biomass production, and partitioning in two *Aloe* species.

<i>Aloe</i> species	Salinity (mm NaCl)	Plant				Dry biomass			
		Ht (cm)	Leaves (number)	Offshoots (number)	Leaf fresh wt (g)	Leaves (g)	Stem (g)	Root (g)	Total (g)
<i>Aloe arborescens</i>	1	49.0	17.3	2.8 a	357.9 b	13.2 b	5.0	4.1	22.3
	80	39.1	16.2	0.7 b	248.2 c	11.5 c	3.6	2.4	17.5
	Mean	44.1	16.8	1.8	303.1	12.4	4.3	3.2	19.9
<i>Aloe barbadensis</i>	1	45.8	12.0	0.0 c	751.6 a	23.1 a	0.3	3.0	26.4
	80	34.7	10.3	0.0 c	289.9 c	12.6 bc	0.1	1.6	14.3
	Mean	40.3	11.2	0.0	520.7	17.8	0.2	2.3	20.3
Significance ^z									
<i>Aloe</i> species (A)		*	***	**	***	**	***	NS	NS
Salinity (S)		***	**	*	***	***	**	**	**
A × S		NS	NS	*	**	*	NS	NS	NS

^zNS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple.

values of antioxidant activity were observed in *A. barbadensis* at both 1 and 80 mm of NaCl. When averaged over *Aloe* species, increasing the nutrient solution salinity from 1 to 80 mm NaCl increased the aloin and the β -polysaccharides concentration by 66.4% and 21.4%, respectively. Finally, β -polysaccharides concentration was significantly higher by 105.8% in *A. barbadensis* in comparison with *A. arborescens*.

Discussion

In all three experiments, marked differences were observed in plant growth, leaf mineral composition, and bioactive compounds

of the two *Aloe* species. In Expt. 1, *A. barbadensis* showed higher leaf weight and total biomass (avg. 805.9 g/plant and 29.4 g/plant, respectively) than *A. arborescens* (avg. 191.7 g/plant and 11.1 g/plant, respectively) (Table 1). Similarly, in the AM colonization experiment (Expt. 2) and salinity experiment (Expt. 3) the *A. barbadensis* showed higher leaf yield (avg. 719.3 g/plant and 751.6 g/plant, respectively) than those observed in *A. arborescens* (avg. 204.1 g/plant and 357.9 g/plant, respectively) (Table 4). Among the macroelements studied, K was the mineral with a higher concentration in the two *Aloe* species with a mean concentration of 34.1 g·kg⁻¹ DW followed by N with a mean concentration

of 18.4 g·kg⁻¹ DW (Table 2). Among the microminerals studied here, Mn was the most abundant element in both *Aloe* species with a mean concentration of 100.5 mg·kg⁻¹ DW compared with other microelements (Table 2). Similarly to Expt. 1, K and Mn, recorded in Expts. 2 and 3, were the macro- and micro-mineral with the highest concentration in the two *Aloe* species with a mean concentration of 24.1 g·kg⁻¹ and 78.0 mg·kg⁻¹ DW, respectively, in Expt. 2 (Table 5) and 17.3 g·kg⁻¹ and 83.7 mg·kg⁻¹ DW, respectively, in Expt. 3 (Table 8).

Pharmaceutical, medicinal, and cosmetics properties of *Aloe* leaves are linked to their phytochemical profile, which includes high levels of aloin and β -polysaccharides. One of the main biologically constituents of *Aloe* extracts is aloin, which is found in nature as a mixture of two diastereoisomers, aloin A (10R) and aloin B (10S). Anthraquinones and β -polysaccharides are generally used as key components for the quality control of this plant and its derivatives (Zapata et al., 2013). Most of the literature about aloin content has been reported for the most studied species, that is *A. barbadensis*, for which concentrations between 0.3 and 15 mg/100 g were found (Bozzi et al., 2007; Miranda et al., 2009). In Expt. 1, aloin concentration was strongly affected by *Aloe* species and fertilization regime with the highest values recorded in *A. barbadensis* under reduced fertilization regime (666.7 mg·kg⁻¹ FW). Similar to aloin, the *A. barbadensis* showed higher β -polysaccharides concentration than those recorded in *A. arborescens* (Table 3).

It is known that the concentration of phytochemicals varies as a function of many factors such as genetic material, environment, biotic and abiotic stresses, and cultural practices (e.g., fertilization) (Rouphael et al., 2010b, 2012a). In Expt. 1, doubling the fertilizer dose from 50% to 100% increased plant growth but negatively affected the leaf bioactive compounds, in particular in *A. barbadensis*, by decreasing the aloin and the β -polysaccharide concentration. The reduction in phytochemical content in leaves of *Aloe* with the increase of the fertilization dose suggests that the secondary metabolism of plant was promoted by low nutrient availability as observed by Rouphael et al. (2012b)

Table 8. Effect of salinity level on mineral composition of leaves in two *Aloe* species.

<i>Aloe</i> species	Salinity (mm NaCl)	Major elements (g·kg ⁻¹ DW)							Trace elements (mg·kg ⁻¹ DW)				
		N	P	K	Ca	Mg	Na	Cl	Fe	Mn	Zn	Cu	B
<i>Aloe arborescens</i>	1	20.4	9.5	20.8 a	17.7	4.8	12.5	23.4	13.1	104.5	15.9	1.7	16.6
	80	18.5	6.6	15.5 b	15.3	3.0	39.5	46.4	11.2	102.4	11.0	1.2	11.0
	Mean	19.4	8.0	18.1	16.5	3.9	26.0	34.9	12.2	103.5	13.5	1.4	13.8
<i>Aloe barbadensis</i>	1	15.5	3.8	20.7 a	12.3	3.1	10.9	21.6	10.8	74.9	13.1	1.2	15.5
	80	13.9	2.3	12.5 c	10.8	1.9	32.2	37.5	10.1	53.1	9.1	1.1	12.5
	Mean	14.7	3.1	16.6	11.5	2.5	21.6	29.6	10.4	64.0	11.1	1.1	14.0
Significance ^z													
<i>Aloe</i> species (A)		**	***	*	*	***	***	*	NS	**	NS	NS	NS
Salinity (S)		***	***	***	NS	***	***	**	NS	NS	*	NS	**
A × S		NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS

^zNS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple.
DW = dry weight.

on artichoke grown in a floating system under different nutrient solution concentrations.

Many plant species in the Liliales, to which Aloes belong, are known to form AM colonization (Harley and Harley, 1987). The effect of AM colonization was less pronounced than the effect of *Aloe* species on plant growth parameters because no significant differences among treatments (+AM and -AM) were observed (Table 4). Our findings are in contrast with the findings of Tawaraya et al. (2007), who reported that total length of leaves and total number of leaves of *A. barbadensis* were higher in inoculated plants (with *Glomus clarum* or *Gigaspora decipiens*) than in uninoculated plants. Explanations for this disagreement could be the different environments where the plants were grown and to the different types of AM fungi used. In the current study, *Aloe* species were inoculated with *G. intraradices* and *G. mosseae*, whereas in the former study, *A. barbadensis* were inoculated by *G. clarum* or *G. decipiens*.

The major elements composition of leaves, in particular P, K, Ca, Mg, Fe, and Zn, were higher in +AM than -AM plants, especially in *A. arborescens* (Table 5). Differences in leaf mineral composition between the two *Aloe* species could be ascribed to differences in mycorrhizal responsiveness and in percentage root colonization between the two species. In fact, the percentage root colonization was significantly higher in *A. arborescens* (30%) than in *A. barbadensis* (12%).

The colonization of the roots with *G. intraradices* and *G. mosseae* had a positive effect on the production of aloin and β -polysaccharides concentration in both *Aloe* species with a more pronounced effect recorded in *A. barbadensis* (Table 6). These results are consistent with the findings of Mota-Fernandez et al. (2011) who observed that the concentration of nutraceutical compounds (flavonols and flavanones) increased when *A. barbadensis* plantlets were inoculated with both AM fungi (*G. claroideum* or *G. fasciculatum*) with the largest increase found in *G. fasciculatum*.

A. barbadensis, as a xerophyte, can be planted in saline soil and irrigated with saline water. The osmotic adjustment of *A. barbadensis*

Table 9. Effect of salinity level on antioxidant activity coefficient (AAC), aloins, and β -polysaccharides in two *Aloe* species.

<i>Aloe</i> species	Salinity (mm NaCl)	AAC	Aloins (mg·kg ⁻¹ FW)	β -polysaccharides (mg·kg ⁻¹ FW)
<i>Aloe arborescens</i>	1	19.2 c	370.0	885.6
	80	29.0 b	548.8	1054.3
	Mean	24.1	459.4	969.9
<i>Aloe barbadensis</i>	1	35.7 a	291.0	1793.9
	80	36.1 a	551.3	2199.4
	Mean	36.0	421.1	1996.7
Significance ^z				
<i>Aloe</i> species (A)		***	NS	***
Salinity (S)		NS	***	*
A × S		*	NS	NS

^zNS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple.
FW = fresh weight.

through the accumulation of inorganic ions in plant tissues plays a great role in its salt resistance (Jin et al., 2007). In Expt. 3, salt stress had a negative influence on crop growth parameters and yield in both *Aloe* species (Table 7) as has also been reported in other studies on *A. barbadensis* (Jin et al., 2007; Moghbeli et al., 2012; Zheng et al., 2009). Moreover, with 80 mm of NaCl, the percentage of leaf weight reduction in comparison with control was significantly lower in the *A. arborescens* (-31%) than *A. barbadensis* plants (-61%) suggesting *A. arborescens* was more tolerant than *A. barbadensis*. Potassium is an essential element for living cells and an absolute requirement for many cellular functions such as osmotic regulation, protein synthesis, and enzyme activation (Peng et al., 2004). Salt-resistant plants usually show a stronger capacity to maintain uptake of K⁺ (Koyro, 2000; Wang et al., 2007). In Expt. 3, the maintenance of higher K concentration in leaves of *A. arborescens* compared with *A. barbadensis* may have been one of the factors of their lowest leaf weight reduction under saline conditions.

In general, high salinity resulted in slower growth and lower yield but, in many cases, improves their quality as observed in plants grown in both soil and soilless culture (Francois and Maas, 1994). Increasing the nutrient solution salinity from 1 to 80 mm NaCl improved the bioactive compounds of leaves by increasing the antioxidant activity coefficient, aloin, and β -polysaccharide concentration,

with the highest values of higher AAC and β -polysaccharides recorded in *A. barbadensis* (Table 9). A similar positive effect of salinity on phytochemicals was also found in leaves of other horticultural crops (Colla et al., 2013).

Conclusions

The results of the experiments showed that *A. barbadensis* had higher yield and aloin and β -polysaccharide concentrations than *A. arborescens*. Doubling fertilizer dose from 50% to 100% increased plant growth (leaf FW and total dry biomass) but negatively affected the leaf bioactive compounds in both *Aloe* species by decreasing the AAC, aloin, and β -polysaccharide concentrations. Furthermore, AM fungi had a positive effect on the production of aloin and β -polysaccharides. The results also indicate that increasing salinity in the nutrient solution decreased plant growth but improved the bioactive compounds in leaves by increasing their nutraceutical value.

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