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REGULAR ARTICLE

Arbuscular mycorrhizal fungi and potassium bicarbonate enhance the foliar content of the vinblastine alkaloid in *Catharanthus roseus*

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Abstract Vinca (*Catharanthus roseus* (L.) G. Don.) is an important medicinal plant species from which antineoplastic alkaloids such as vinblastine are extracted. However, neither abiotic stress nor inoculation of arbuscular mycorrhizal fungi (AMF) has been evaluated on the accumulation of vinca alkaloids under controlled conditions. This study evaluated the effects of AMF and/or abiotic stress induced by the application of potassium bicarbonate ($KHCO_3$) and/or sodium chloride (NaCl) on plant growth, and on total content of phenolic compounds (TCPC), total antioxidant activity (TAOX), and total content of vinblastine alkaloid in leaves of vinca. TCPC, TAOX, and vinblastine were measured via spectrophotometric methods. After 75 days under greenhouse conditions, either the AMF inoculation without abiotic stress or the application of $KHCO_3$ (2.5 and 7.5 mM) resulted in significantly ($P \leq 0.001$) enhanced plant growth, TCPC, TAOX, and total content of vinblastine. The application of NaCl significantly diminished plant growth, but did not stimulate the content of vinblastine. The combined application of NaCl and $KHCO_3$ significantly decreased AMF-colonization in roots. The sole inoculation of AMF or the single application of 7.5 mM $KHCO_3$ induced the accumulation of vinblastine in leaves of vinca.

Keywords Glomus · $KHCO_3$ · Plant stress · Salinity · Total antioxidant activity · Vinca

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Introduction

Secondary metabolites are important compounds that apparently do not play a significant role in main physiological processes in plant life cycles, but their synthesis has a significant contribution in specific plant–environment interactions (Treutter 2006; Neumann et al. 2009). Plants are often a source of secondary metabolites that have multiple applications such as pharmaceuticals, pesticides, dyes, flavorings, and fragrances, among other uses. However, the synthesis of these compounds is usually in low concentrations and is also spatially and timely regulated in relation to the type of plant tissue (cells, organs, etc.), and to its phenology and environmental conditions (Trejo and Rodríguez 2007).

Either biotic or abiotic stress causes significant physiological and biochemical responses in plants like signaling recognition, changes on intercellular fluxes of ions (for instance, Ca^{2+}), accumulation of reactive oxygen species, protein phosphorylation, and gene transcription involved in the synthesis of ABA, ethylene, jasmonate, salicylic acid, etc. The physiological activation of those processes leads to successive metabolic changes that result in the accumulation of specific metabolites that favor plant adaptation to stressful conditions (Malenčić et al. 2004; Ryabushkina 2005).

Plant secondary metabolites are also important for human health. For instance, phenolic compounds protect against oxidative burst, and also have therapeutic effects for atherosclerosis. Alkaloids are the most diverse group among secondary metabolites, which have been identified in plants that belong to botanical families such as Apocynaceae, Loganiaceae, and Rubiaceae (Kato et al. 2002; Cragg et al. 2010; Mesia et al. 2010; Verpoorte et al. 2010).

Vinca (*Catharanthus rosesus* (L.) G. Don.) is an important plant species that produces secondary metobolites such as alkaloids (vincristine, vinblastine, vinorelbine and vinflunine) which have anticancer properties. Due to these important pharmaceutical alkaloids, *C. roseus* is one of the most extensively studied medicinal plant species (Himes 1991; Kruczynski and Hill 2001; Sottomayor and Ros-Barcelo 2006; Rabbani-Chadegani et al. 2009; Risinger et al. 2009).

Arbuscular mycorrhizal fungi (AMF) are ubiquitous microorganisms that form a beneficial symbiosis with more than 80% of extant terrestrial plant species (Dickson 2004; Harrison 2005). The benefits of AMF are usually related to improved plant growth and nutrition, plant adaptation to stressful environments, and more recently, to enhanced accumulation of secondary metabolites in medicinal plants. For instance, Kapoor et al. (2004) showed that *Glomus fasciculatum* and *Gl. macrocarpum* on *Foeniculum vulgare* Mill., significantly enhanced the accumulation of essential oil. Moreover, Morone-Fortunato and Avato (2008) indicated that leaves of *Origanum vulgare* had more abundant oil-secretory glands due to the inoculation of *Gl. viscosum*. The beneficial effects of AMF on vinca are related to the adaptation and stress alleviation induced by the application of alkaline irrigation water (Cartmill et al. 2008), but no significant effects were observed under salinity conditions (Cartmill et al., unpublished data). Nevertheless, Cartmill et al. (2008) have shown that either AMF inoculation or potassium bicarbonate application to vinca plants resulted in increased total antioxidant activity in leaves, but no significant effects were obtained on plant growth. However, neither the effects of abiotic stress induced by the application of bicarbonates or salinity nor the effects of AMF on the accumulation of antineoplastic alkaloids such as vinblastine, have yet been reported. Thus, the purpose of this study was to evaluate the effects of AMF-inoculation or the application of potassium bicarbonate and salinity as inducers of abiotic stress on growth, total antioxidant activity, total phenolic compounds, and on the leaf content of the alkaloid vinblastine of vinca plants.

Materials and methods

Plant and fungal material, and experiment establishment

Seven-week-old seedlings of *C. roseus* (Plantulas de Tetela S.R.L.C.V., Cuernavaca, Morelos, Mexico) were transplanted to pots filled with pasteurized substrate (peat moss:perlite, 1:1 v/v), and established under greenhouse conditions. Maximum and minimum temperature and relative humidity were monitored by a datalogger, model 150. The average maximum and minimum relative humidity were 82.9±7.0 and 25.8± 11.1, respectively, while the average temperature was

35.44±5.4 and 13.7±1.6°C (maximum and minimum, respectively).

At transplanting time, half the seedlings were inoculated with 10 g of an arbuscular mycorrhizal inoculum (AMF-plants) previously isolated from the medicinal plant *Rumex mexicanus* Meisn. (De la Rosa-Mera et al., unpublished data). This inoculum consisted of soil from trap cultures (220 spores in 50 g^{-1} soil) and root fragments (40% colonization). Spores of AMF corresponded to an unidentified *Glomus* species. The remaining seedlings were kept without mycorrhizal inoculation (non-AMF plants) as controls.

Two weeks after AMF inoculation, either AMF-plants or non-AMF-plants were irrigated with 200 mL^{-1} of a water solution with either potassium bicarbonate ($KHCO_3$) or sodium chloride (NaCl) as inducers of abiotic stress on plants, every 3 days. Two doses of $KHCO_3$ (2.5 or 7.7 mM), one dose of NaCl (40 mM), and the combination or each $KHCO_3$ concentration with the NaCl, were applied to the plants. The doses of either $KHCO_3$ or NaCl were chosen based on previous experiments with vinca (Cartmill et al. 2008, and unpublished data). In addition, plants without abiotic stress were considered as controls. No fertilization treatment was applied to the plants along experimentation.

Plant growth parameters

After 75 days of the application of abiotic stresses, plants were harvested to evaluate the number of leaves, the leaf area (Leaf area meter, model LI-3100), and the total dry matter by dissecting and drying leaves, stems, and roots at 70°C for 72 h.

Total content of phenolic compounds and total antioxidant activity

The total content of phenolic compounds (TCPC) and the total antioxidant activity (TAOX) in leaf tissue were also determined after 75 days. Phenolics were evaluated by the Folin-Ciocalteu reagent assay utilizing chlorogenic acid as a standard curve (Singleton and Rossi 1965; Soong and Barlow 2004). In brief, 0.100 g of leaf fresh tissue was macerated in a chilled mortar with 3 mL of 80% methanol. Extracts were centrifuged for 15 min at 15,000*g*. Aliquots (30 μL) of the extracts were reacted with 90 μL of Na_2CO_3 and 150 μL of Folin-Ciocalteau reagent in a 96-well microplate. After 30 min, the absorbance was measured at 725 nm using a spectrophotometer (Synergy 2; Biotek® Instruments). Results were expressed as milligrams of chlorogenic acid equivalents per plant.

Total antioxidant activity was determined by the 1,1-diphenyl-2-picryldrazyl (DPPH) radical decoloration assay (Matthäus 2002). Briefly, 15 μL of leaf extracts obtained for phenolic determinations were reacted with 250 μL of DPPH-solution in 96-well microplates. Initial absorbance (515 nm) readings were taken and then microplates were incubated for 15 min to take a final absorbance reading using a spectrophotometer (Synergy 2; Biotech® Instruments). Antioxidant activity was calculated by applying known aliquots of Trolox (antioxidant compound) to known concentrations of DPPH solution. Results were expressed as micromoles of Trolox equivalents per plant.

Content of the vinblastine alkaloid

The content of the vinblastine alkaloid in leaf tissue was determinate by the TPT procedure (Nagaraja et al. 2002). Briefly, leaf extracts (0.100 g in 3 mL of 80% methanol) were obtained and immediately centrifuged at 15,000*g* for 15 min. The reaction mixture consisted of mixing 30 μL of the extract added with 46 μL of an Fe-III solution (0.01 mol dm^{-3}) and 184 μL of 2,4, 6-tris (2-pyridyl)-1,3,5, triazine (0.2%) in microtubes, and incubated at 100°C for 5 min. Samples were cooled and diluted with 80% methanol (1:25 v/v). Afterwards, 200 μL of each sample were set in 96-well microplates, and absorbance readings were taken at 590 nm using a spectrophotometer (Synergy 2; Biotech® Instruments). Results were expressed as micrograms of vinblastine per plant. A standard curve (5–100 μg mL^{-1}) of vinblastine sulfate salt ($C_{46}H_{58}N_4O_9{\bullet}H_2SO_4$; Sigma-Aldrich) in 80% methanol was utilized for estimating the concentration of the vinblastine in the samples.

Arbuscular mycorrhizal colonization assessment

Root mycorrhizal colonization was estimated by the clearing and staining root procedure (Phillips and Hayman 1970). Briefly, roots were harvested and gently washed with tap water, and exposed to 10% KOH solution at 121°C for 15 min, and immediately washed with tap water to eliminate KOH residues.

Roots were submerged in a 10% H_2O_2 solution for 15 min and also washed, and then a 10% HCl solution was applied for 15 min to acidify the roots. Once acidified, roots were immersed in 0.05% tripan blue dye in a lactoglycerol solution (lactic acid, glycerin, and water, 1:1:1 v/v) at 121°C for 15 min. The stained roots were set on glass slides (25 root fragments per slide), and observed under light microscopy at ×40 for estimating the frequency of the presence of AMF-structures (hyphae, vesicles, and arbuscules) in the cortical cells for each root fragment. AMF-colonization was expressed as a percentage.

Experimental design and statistical analysis

A 2 x 6 factorial experiment was set in a completely randomized design, which included 12 treatments (AMF-inoculation factor: AMF and non-AMF plants; and abiotic stress factor: control, 2.5 mM of $KHCO_3$, 7.5 mM of $KHCO_3$, 40 mM of NaCl; 2.5 mM $KHCO_3$+40 mM NaCl, and 7.5 mM $KHCO_3$+ 40 mM NaCl). Each treatment had 10 replicates. Four replicates were harvested for determining the plant growth parameters, and the remaining six replicates were utilized for TCPC, TAOX, vinblastine content and mycorrhizal colonization determinations. Data were analyzed via two-way analysis of variance and the mean comparison test of Tukey (α=0.05), by using the SAS program for windows (SAS Institute 2002).

Results

Plant growth parameters

The number of leaves, leaf area, and the leaf dry weight (DW) showed significant (P<0.001) effects due to AMF-inoculation, application of abiotic stress, and the interaction AMFxStress. The AMF inoculation significantly decreased the number of leaves, and leaf DW when compared to non-AMF plants (Table 1). The application of abiotic stress resulted on significant (P<0.001) effects, in which plants exposed to either 2.5 or 7.5 mM $KHCO_3$ had greater number of leaves and leaf DW, when compared to controls or to plants with 40 mM NaCl (Table 1). Non-AMF plants with the application of 2.5 or 7.5 mM $KHCO_3$ showed the highest number of leaves and leaf DW than did AMF-plants with the combination of NaCl with 2.5 or 7.5 mM of $KHCO_3$ (Table 1).

The total DW of plants was not significantly enhanced by the AMF inoculation (Fig. 1). In

Table 1 Effects of arbuscular mycorrhizal fungi (AMF), and the application of potassium bicarbonate ($KHCO_3$) or sodium chloride (NaCl) on the number of leaves, leaf area, and leaf dry weight of *Catharanthus roseus*, after 75 days

Mycorrhizal condition	Treatments of abiotic stress	Number of leaves	Leaf area (cm^2)	Leaf dry weight (g)
Non-AMF	Control	50.7 cde	205.8 cd	0.50 cde
	40 mM NaCl	40.0 ef	185.4 cd	0.40 ef
	2.5 mM $KHCO_3$	72.0 a	322.3 a	0.83 a
	7.5 mM $KHCO_3$	64.5 ab	304.7 ab	0.78 ab
	40 mM NaCl+2.5 mM $KHCO_3$	58.2 bc	233.6 bc	0.61 bcd
	40 mM NaCl+7.5 mM $KHCO_3$	57.7 bcd	240.6 abc	0.61 bcd
AMF	Control	58.7 bc	220.1 bcd	0.62 bc
	NaCl (40 mM)	46.0 def	167.2 cde	0.42 def
	$KHCO_3$ (2.5 mM)	51.7 cde	175.0 cde	0.49 cde
	$KHCO_3$ (7.5 mM)	57.5 bcd	207.0 cd	0.58 cde
	40 mM NaCl+2.5 mM $KHCO_3$	35.2 f	98.5 e	0.28 f
	40 mM NaCl+7.5 mM $KHCO_3$	43.0 ef	147.7 de	0.42 ef
Significance:		0.001	0.001	0.001
AMF		0.001	0.001	0.001
Stress		0.001	0.001	0.001
AMF × stress		0.001	0.001	0.001

Means followed by the same letters within columns are not significantly different (Tukey α=0.05); n=4

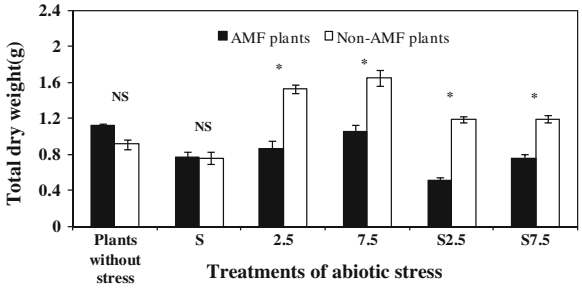

Fig. 1 Effects of arbuscular mycorrhizal fungi (AMF), and the application of potassium bicarbonate ($KHCO_3$) or sodium chloride (NaCl) on the total dry weight of *Catharanthus roseus*, after 75 days; *n*=4; means±standard error. *S* 40 mM NaCl, *2.5* 2.5 mM $KHCO_3$, *7.5* 7.5 mM $KHCO_3$, *S+ 2.5* 2.5 mM $KHCO_3$+40 mM NaCl, *S+ 7.5* 7.5 mM $KHCO_3$+40 mM NaCl. * Significant differences (Tukey, α=0.05) between AMF plants and non-AMF plants; *NS* not significant

contrast, the application of 2.5 or 7.5 mM $KHCO_3$ to non-AMF plants showed significant increases (66.7 and 77.8%, respectively) on total DW in comparison to its control (Fig. 1).

Total content of phenolic compounds and total antioxidant activity

AMF-plants without stress had significantly ($P \leq 0.001$) higher TCPC in comparison to non-AMF plants (Fig. 2). In contrast, non-AMF-plants with the application of 7.5 mM of $KHCO_3$ had 25% more TCPC when compared to non-AMF-plants without stress (Fig. 2). The combined application of 2.5 mM $KHCO_3$ and 40 mM NaCl to AMF-plants resulted in low TCPC (Fig. 2).

The TAOX of AMF-plants without stress was significantly higher (>90%) than non-AMF-plants (Fig. 3). At 7.5 mM $KHCO_3$, non-AMF-plants had 111.8% greater TAOX than AMF-plants. In contrast, the lower TAOX was achieved in AMF-plants with the combined application of 2.5 mM $KHCO_3$ and 40 mM NaCl (Fig. 3). There were no significant differences on the TAOX between AMF-plants without stress and non-AMF-plants with 7.5 mM $KHCO_3$ (Fig. 3).

Total content of the vinblastine alkaloid

AMF-plants without stress had significantly higher concentration and total content of vinblastine (7.7 μg g^{-1} and 48.6 μg $plant^{-1}$, respectively) when compared to non-AMF-plants (Fig. 4a, b). Regardless AMF-inoculation, plants with 7.5 mM $KHCO_3$ had greater total content (>111%) of vinblastine in comparison to those plants without the application of stress ($KHCO_3$ or salinity) (Fig. 4b). Salinity did not stimulate either the concentration or total content of vinblastine in leaves of vinca.

Arbuscular mycorrhizal colonization

The mycorrhizal colonization showed significant differences ($P \leq 0.001$) among treatments (Fig. 5). Either $KHCO_3$ or NaCl application resulted in

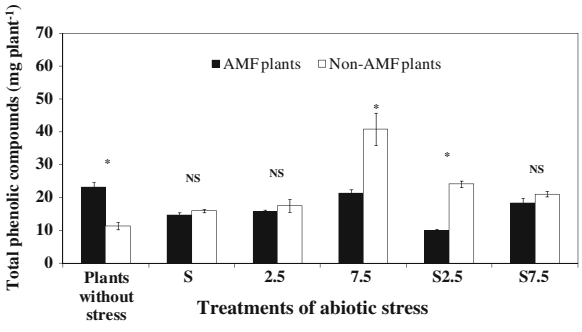

Fig. 2 Effects of arbuscular mycorrhizal fungi (AMF), and the application of potassium bicarbonate ($KHCO_3$) or sodium chloride (NaCl) on the total antioxidant activity in leaves of *Catharanthus roseus*, after 75 days; *n*=6; means±standard error. *S* 40 mM NaCl, *2.5* 2.5 mM $KHCO_3$, *7.5* 7.5 mM $KHCO_3$, *S+ 2.5* 2.5 mM $KHCO_3$+40 mM NaCl, *S+ 7.5* 7.5 mM $KHCO_3$+ 40 mM NaCl. *Significant differences (Tukey, α=0.05) between AMF plants and non-AMF plants; *NS* not significant

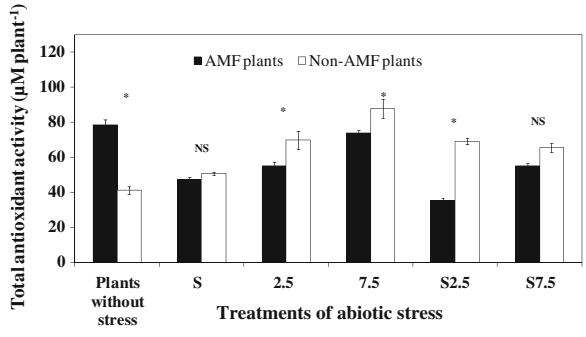

Fig. 3 Effects of arbuscular mycorrhizal fungi (AMF), and the application of potassium bicarbonate ($KHCO_3$) or sodium chloride (NaCl) on the total content of phenolic compounds in leaves of *Catharanthus roseus*, after 75 days; *n*=6; means± standard error. *S* 40 mM NaCl, *2.5* 2.5 mM $KHCO_3$, *7.5* 7.5 mM $KHCO_3$, *S+ 2.5* 2.5 mM $KHCO_3$+40 mM NaCl, *S+ 7.5* 7.5 mM $KHCO_3$+40 mM NaCl. *Significant differences (Tukey, α=0.05) between AMF plants and non-AMF plants. *NS* not significant

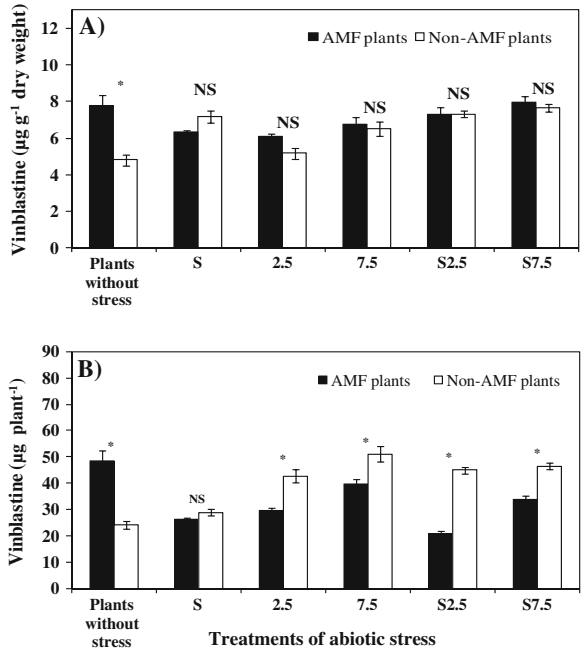

Fig. 4 Effects of arbuscular mycorrhizal fungi (AMF), and the application of potassium bicarbonate ($KHCO_3$) or sodium chloride (NaCl) on the foliar concentration and total content of vinblastine in *Catharanthus roseus*, after 75 days. n=6; means± standard error. *S* 40 mM NaCl, *2.5* 2.5 mM $KHCO_3$, *7.5* 7.5 mM $KHCO_3$, *S+ 2.5* 2.5 mM $KHCO_3$+40 mM NaCl, *S+ 7.5* 7.5 mM $KHCO_3$+40 mM NaCl. *Significant differences (Tukey, α=0.05) between AMF plants and non-AMF plants. *NS* not significant

diminished AMF-colonization in the root system of *C. roseus*. The colonization percentage estimated in plants with the application of 7.5 mM $KHCO_3$ was 35% (Fig. 5), while the lowest colonization was

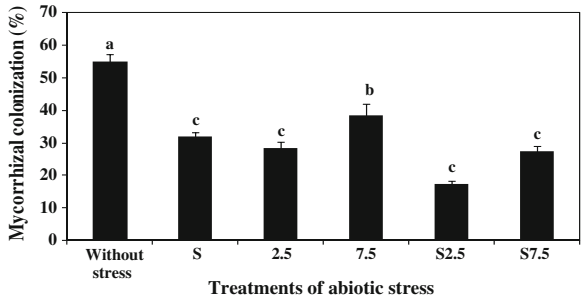

Fig. 5 Effects of the application of potassium bicarbonate ($KHCO_3$) or sodium chloride (NaCl) on the arbuscular mycorrhizal colonization in roots of *Catharanthus roseus*, after 75 days, n=6; means±standard error. *S* 40 mM NaCl, *2.5* 2.5 mM $KHCO_3$, *7.5* 7.5 mM $KHCO_3$, *S+ 2.5* 2.5 mM $KHCO_3$+40 mM NaCl, *S+ 7.5* 7.5 mM $KHCO_3$+40 mM NaCl. *Different *letters above bars* indicate significant differences (Tukey, α=0.05) among treatments of abiotic stress

obtained at plants with the combined application of 40 mM NaCl and 2.5 mM $KHCO_3$ that showed 69% less colonization when compared to AMF-plants without stress application (Fig. 5). Mycorrhizal colonization was not detected at all treatments without AMF-inoculation.

Discussion

AMF-inoculation did not enhance the growth of vinca plants which is contrary to the reported benefits of AMF on the growth of several horticultural plants (Greipsson and El-Mayas 2002; Robertson et al. 2007). Our results concur with those findings reported by Cartmill et al. (2008) in which AMF inoculation had no significant effects on the growth of vinca plants without stress application.

The application of 40 mM NaCl resulted in growth reductions of AMF-plants or non-AMF-plants. The negative effects of NaCl on plant growth and some physiological responses have been reported for plant species such *Citrus* and *Phragmites australis* (Asaeda et al. 2003; Anjum 2008). Moreover, AMF-plants with the combined application of NaCl with $KHCO_3$ had lower growth than non-AMF-plants. This effect may be explained in part due to the C-demand that mycorrhizal symbiosis represents for its host, thus resulting in impaired plant growth especially under stressful conditions. In contrast, the beneficial effects of $KHCO_3$ on plant growth have been reported for vinca and *Rosa multiflora* when this chemical compound was applied at concentrations lower than 2.5 mM (Cartmill et al. 2007, 2008).

Although the total DW of AMF-plants without application of abiotic stress did not show significant differences when compared to non-AMF-plants, its total plant DW was 31% higher than non-AMF-plants (Fig. 1). The latter agrees with the plant biomass promotion achieved by some mycorrhizal plants including *Curculigo orchioides* Gaertn., which has anticancer properties (Eon et al. 1994; Kahiluoto et al. 2000; Monzón and Azcón 2001; Giri et al. 2007; Sharma et al. 2008).

AMF inoculation in plants without stress resulted in twofold TCPC. This result indicates that AMF have a significant effect on the stimulation of phenolic compounds as it has been reported for some AMF-plants (Charitha and Reddy 2002; Carlsen et al.

2008). The phenolic compounds such as flavonoids are important plant secondary metabolites whose chemical structure has several OH-radicals and excellent properties such as iron quelators that confer their antioxidant activity (Woo et al. 2005; Nagahashi and Douds 2005; Hanen et al. 2008). The content of phenolic compounds are also influenced by abiotic stressful conditions that limit plant growth (Rivero et al. 2001; Juszczuk et al. 2004; Chung et al. 2006; Yuan et al. 2010), which agrees with the stimulation on the TCPC obtained by the application of 7.5 mM $KHCO_3$ in this study.

There are some reports about the effect of AMF on enhancing the content of phenolic compounds in plants. For instance, the initial establishment of AMF species on the root systems is a process mediated by the release of phenolic compounds such as flavonoids (Woo et al. 2005; Nagahashi and Douds 2005). Moreover, the mycorrhizal colonization is reported as a modifier of plant defense mechanisms like the synthesis of phytoalexins that counteract the damage caused by plant pathogens (Guenoune et al. 2001; Ozgonen and Erkilic 2007; Pozo and Azcón-Aguilar 2007).

The beneficial effect of AMF inoculation on antioxidant compounds (rosmaric and cafeic acid) has been reported on plants of *Ocimum basilicum* (Toussaint et al. 2007), indicating that AMF play a significant role on increasing plant antioxidants as observed in the present study. Plants with a high antioxidant activity may be considered as a therapeutically component addressed to alleviate several pathologies such as cardiovascular diseases and Alzheimer, ischemia, coronary heart disease (CHD), atherosclerosis or cancer (Winkelman 1986; Duarte-Silva et al. 2000; Grassmann et al. 2002; Nikolaeva et al. 2007). Thus, either AMF inoculation or the application of abiotic stress may be utilized as inducers of both antioxidant activities and alkaloids in vinca.

Regardless of AMF-inoculation, NaCl resulted in low TAOX , and this negative effect may be explained in part due to alterations of osmotic adjustments in plant cells and then an induction of oxidative stress by the accumulation of reactive oxygen species (ROS) that cause cell damage (Khan et al. 2002; Sairam et al. 2005; Pang and Wang 2008). Although salinity may result in an activation of physiological mechanisms of plant cells for detoxifying the accumulation of ROS to alleviate the toxic effects of salinity (Sairam et al. 2005; Pang and Wang 2008), vinca plants are considered highly tolerant to salinity by which the null effect observed on TOAX may be explained.

The present study shows that the mycorrhizal condition in plants allows the accumulation of secondary metabolites as previously indicated for some plant species (Khaosaad et al. 2006; Kapoor et al. 2007). Secondary metabolites in plants are the result of several stressful conditions (biotic or abiotic) that stimulate their synthesis which play significant role on plant growth and adaptation to those adverse conditions (Ryabushkina 2005; Neumann et al. 2009). For medicinal plants, it has been demonstrated that AMF may induce the synthesis of secondary metabolites. For instance, Sailo and Bagyaraj (2005) showed that AMF-plants of *Coleus forskohlii* enhanced their content of forskolin (diterpene) which is an important plant compound for the treatment of heart diseases, glaucoma, asthma, and certain types of cancer. In the same manner, the inoculation of *Glomus macrocarpum* in plants of *Anethum graveolens* increased the concentration of essential oil up to 90% (Kapoor et al. 2002).

The latter information supports our hypothesis about the benefits of AMF on inducing greater synthesis and accumulation of one important alkaloid (vinblastine) in *Catharanthus roseus*. In addition, our results show that the sole application of $KHCO_3$ may increase the content of vinblastine.

With regard to the application of abiotic stress, it has been reported that salinity conditions significantly increase the content of essential oils in medicinal plants such as *Coriandrum sativum* L. (Neffati and Marzouk 2008). However, in the present study, the individual application of 40 mM NaCl or its combination with $KHCO_3$ did not significantly enhance the accumulation of vinblastine in vinca. In general, the induction of salinity in plants showed similar contents of this alkaloid when compared to non-AMF-plants without stress (Fig. 4b).

There is a special and increasing interest in using plants as an alternate medicine. Although plants are considered as a significant source of several chemical compounds (secondary metabolites), they accumulate them in low concentrations since the synthesis of those compounds is regulated by the interaction of different factors.

The production of vinblastine from *C. roseus* is much in demand due to this plant species represents a promising source of compounds with pharmaceutical interest (Aslam et al. 2009; Pereira et al. 2010). Based

on our results, it is feasible to consider either the inoculation of AMF or the application of $KHCO_3$ on *C. roseus* in order to enhance the accumulation of this alkaloid. In spite of this plant species being considered highly tolerant to salinity, NaCl application did not enhance the accumulation of vinblastine in leaves when compared to AMF-plants without stress.

Our results also show that salinity had a stronger inhibition on mycorrhizal colonization than the application of $KHCO_3$. The latter chemical compound seems to have a less detrimental effect on mycorrhizal colonization; however, the application of both doses of $KHCO_3$ caused inhibitory effects on AMF colonization. The same findings have been reported for *Rosa multiflora* and *C. roseus* following the application of increased concentrations of $KHCO_3$ (Cartmill et al. 2007, 2008). This negative effect may in part explain the incompatibility between $KHCO_3$ and the inoculation of AMF for improving not only the growth but also for stimulating the TCPC, TAOX activity, and the total content of vinblastine in vinca.

With regard to the negative effects on mycorrhizal colonization due to salinity stress, it has been well demonstrated that excessive levels of salts in soils also significantly inhibit mycorrhizal colonization, although some fungal species are more tolerant to these conditions (Ruíz-Lozano and Azcón 2000; Cantrell and Linderman 2001; Brundrett and Abbott 2002; Giri et al. 2007; Sheng et al. 2008).

Conclusions

The sole AMF inoculation without the application of any abiotic stress (NaCl or $KHCO_3$) significantly enhanced (>100%) the content of vinblastine in *Catharanthus roseus*. Nevertheless, the individual application of 7.5 mM $KHCO_3$ significantly increased the content of this alkaloid (>111%), the total content of phenolic compounds and the total antioxidant activity. The application of 40 mM NaCl did not exert significant effects on the content of vinblastine. The combined application of $KHCO_3$ and NaCl resulted in significant inhibition on the total content of vinblastine, and on the mycorrhizal colonization.

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