



Characterization of quaternary ammonium compounds in *Flourensia* xerophytic communities and response to UV-B radiation



L.A. Piazza^{a,*}, D. López^a, M.P. Silva^a, M.J. López Rivilli^a, J.J. Cantero^{b,c}, G.M. Tourn^{a,d}, A.L. Scopel^{a,e,**}

^a Estación de Biología Sierras, Facultad de Agronomía-Sede Punilla, Universidad de Buenos Aires, Casilda S/N, Huerta Grande, 5174, Córdoba, Argentina

^b Departamento Biología Agrícola, Facultad de Agronomía y Veterinaria, Universidad de Río Cuarto, Río Cuarto, 5800 Córdoba, Argentina

^c Instituto Multidisciplinario de Biología Vegetal (CONICET-UNC), 5000, Córdoba, Argentina

^d Cátedra de Botánica Agrícola, Facultad de Agronomía, Universidad de Buenos Aires, Argentina

^e Investigaciones en Biociencias Agrícolas y Ambientales (INBA), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

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ABSTRACT

As part of ongoing studies aimed at characterizing molecular components involved in the ecophysiological adaptations of native xerophytic plants from central Argentina, we demonstrated the presence of compatible solutes in *Flourensia campestris* (FC) and *Flourensia oolepis* (FO), specifically glycine betaine (GB) through TLC, LC, ¹H NMR and ¹³C-NMR. GB content (leaves: $38 \pm 7 \mu\text{mol g}^{-1}$ DW; adult plants > seedlings), and distribution (capitula > vegetative leaves > reproductive leaves > shoots > roots) were similar to other quaternary ammonium compound (QAC) accumulators. *Flourensia* seedlings from both species protected from UV-B exposure – a major abiotic stress in these natural environments – showed a significant increase of GB in the leaves ($p < 0.01$) and a significant decrease in the roots ($p < 0.05$). In FC and FO xerophytic shrub-dominated communities QACs were detected for the first time in 41% of co-occurring species ($N = 39$), 14 of 28 natives (50%) and 2 of 11 exotics (18%), being GB in natives only (57% of QAC accumulators). GB may be considered as a chemotaxonomical character for the genus *Flourensia*, since it was also detected in *Flourensia hirta*, *Flourensia niederleinii*, *Flourensia riparia*, *Flourensia fiebrigii*, *Flourensia macroligulata* and *Flourensia heterolepis*. Our controlled UV-B experiments, set up in the same natural environment where these species grow, clearly show that solar UV-B – and therefore oxidative stress – is involved in regulating GB contents and within-plant distribution in FC and FO seedlings. The findings in *Flourensia* co-occurring native species suggest that QACs accumulation may be considered as a community-specific ecophysiological trait in these xerophytic environments.

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1. Introduction

The genus *Flourensia* (Asteraceae) belongs to the subtribe Ecliptinae, tribe Heliantheae (Robinson, 1981), and comprises about 25 species of resinous shrubs that grow from southern United States to Argentina and Chile, twelve of which are present in Argentina (Funk et al., 2009). Two endemic species of the genus live in Central Argentina: *Flourensia campestris* Griseb (FC) and *Flourensia oolepis* S.F. Blake (FO) (Dillon, 1984). They are commonly known as “chilcas”, and have been traditionally used as aromatic, tinctorial and – specially their roots – as firewood (Barboza et al., 2009). FO and FC show distinct spatial distribution and are frequently found growing at high densities in almost

pure stands known as “chilcales” (Luti et al., 1979), in natural environments characterized by abiotic stress conditions (e.g., elevated UV-B, droughts, skeletal soils, low and high temperatures). We recently isolated a phytotoxic compound (–)-hamanasic acid A from FC and FO (Silva et al., 2012; López et al., 2013) that accumulates at high concentration on plant surfaces, which suggests its allelochemical potential in the adaptive strategies displayed by these species; the same compound was also characterized in other two from five South American *Flourensia* species (López et al., 2013).

The effects of stressful conditions on plants such as drought, salinity, high radiances, and high and low temperatures can have a major impact on plant growth and survival. In order to cope with these stresses plants have evolved an array of defense mechanisms which involve dynamic, complex cross-talk between different regulatory levels, including adjustment of metabolism and gene expression for physiological and morphological adaptations (Krasensky and Jonak, 2012). In response to osmotic and oxidative stress, many plant species accumulate significant amounts of small molecules (compatible solutes) including sugars, polyols, amino acids and quaternary ammonium compounds (QACs) such as glycine betaine, alanine betaine, proline betaine, choline

Abbreviations: FC, *Flourensia campestris*; FO, *Flourensia oolepis*; GB, glycine betaine; QAC, quaternary ammonium compound.

* Corresponding author. Tel.: +54 3548 426421.

** Correspondence to: A.L. Scopel, Estación de Biología Sierras, Facultad de Agronomía-Sede Punilla, Universidad de Buenos Aires, Casilda S/N, Huerta Grande, 5174 Córdoba, Argentina. Tel.: +54 3548 426421.

E-mail addresses: leonardoalbertopiazza@yahoo.com.ar (L.A. Piazza), scopel@agro.uba.ar (A.L. Scopel).

O-sulfate, hydroxyproline betaine and pipercolate betaine (Hanson and Wyse, 1982; Rhodes and Hanson, 1993; Carillo et al., 2008; Chen and Murata, 2011).

Glycine betaine (N,N,N-trimethylglycine), one of the best-studied QACs is synthesized through two steps from Cho oxidation by specific enzymes in plants, mammals, marine invertebrates, bacteria and hemophilic archaeobacteria (Rhodes and Hanson, 1993; Takabe et al., 2006; Chen and Murata, 2008). In plants, levels of GB vary considerably among species and organs, and they increase, translocate and/or show intracellular distributions when subjected to abiotic stress (Storey et al., 1977; Chen and Murata, 2011; Zhang et al., 2012). Results obtained from studies in natural GB-accumulating and transgenic plants (Robinson and Jones, 1986; Chen and Murata, 2002) show that GB enhances tolerance to abiotic stress mainly through stabilization of complex proteins and induction of ROS-scavenging enzyme genes. The protective effects exerted by GB on the photosynthetic machinery has been extensively investigated under the combined effects of light stress and other kinds of abiotic stresses, such as osmotic stress and chilling and freezing stress (Carillo et al., 2011; Chen and Murata, 2011). However, no studies have investigated the specific effect of UV-B radiation on GB levels and distribution within plants. As a major abiotic stress, UV-B (280–315 nm) has several effects on the physiology of terrestrial plants as a result of direct photochemical damage to key macromolecules such as proteins and nucleic acids, or as an indirect consequence of the increased production of ROS (Piri et al., 2011). The degree of damage caused by UV-B depends strongly on the efficiency of constitutive and UV-induced mechanisms of protection and repair, such as the accumulation of UV-absorbing sunscreens and the activation of antioxidant defenses (Mazza et al., 2000). Mohammed and Tarpley (2013) recently found that exogenous application of GB ameliorated the effects of UV-B irradiation resulting in increased rice yield. Based on these facts, it is reasonable to speculate that GB-accumulating plants growing under elevated UV-B radiation would have an additional strategy to tolerate this abiotic stress. At present there is no information regarding the presence of QACs as compatible solutes in the genus *Flourensia*, or their potential involvement in the tolerance to UV-B, a main abiotic stress in its natural environment. It could be hypothesized that plants coexisting in xerophytic environments may have evolved common traits that would allow them to endure and reproduce in these harsh conditions. In this sense, a generalized occurrence of QACs within a plant community would emphasize its ecophysiological role in the tolerance of plant species to abiotic stresses.

The present study was aimed at: 1) Assessing the presence, distribution and molecular characterization of QACs in *FC* and *FO* species; 2) Evaluating the effects of solar UV-B radiation on QACs content and within-plant distribution in *FC* and *FO* plants; 3) Exploring whether the accumulation of QACs may be a widespread adaptive strategy within *FC*- and *FO*-dominated communities; and 4) Assessing the presence of QACs in other species of the genus *Flourensia*.

QACs in general, and specifically GB and its precursor Cho were determined through a screening technique we devised, that involves a fast tissue extraction and TLC analysis. The methodology was conveniently validated for linearity, limits of detection and quantitation, precision, selectivity and accuracy. Molecular forms of detected QACs were studied through chromatographic isolation and spectroscopic identification (LC, ¹H NMR, ¹³C-NMR). Evidence of the accumulation of QACs in the plant material from UV-B experiments, was further studied through a combination of TLC and ¹H NMR studies.

2. Materials and methods

2.1. Plant material and study sites

Plant materials were collected in natural areas corresponding mainly to the Punilla Valley, Córdoba province, Argentina, and air dried plant organs were used for subsequent analysis. This hilly area (altitudes

between 700 and 1800 MASL) belongs to the northern outcrops of the Sierras Pampeanas. Soils are predominantly of litosolic characteristics (INTA Manfredi, 2006). Mean annual rainfall in the area is 550 mm, more than 90% of which fall between October and April, resulting in a pronounced dry season from May to September. Mean monthly temperature ranges between 16 and 17 °C with an annual amplitude of 14 to 15 °C. High and low temperatures, low relative humidity and high irradiances are prominent in the area. The study areas were located in shrub communities (total plant cover ~70–90%), dominated by the evergreen shrubs *F. campestris* Griseb. (*FC*) and *F. oolepis* S.F. Blake (*FO*), and plant material from these communities was collected in late spring (Nov) of 2009, 2010 and 2011.

For the initial assessment of QACs we used pooled samples of plant material collected at 2 different sites for each species: Pintos and El Dragón (*FC*), and Los Terrones and Los Cocos (*FO*), all four located in the Punilla Valley.

Plant material of co-dominant and conspicuous native species (Luti et al., 1979; Giorgis et al., 2011), as well as of the main exotic species invading these natural areas was also collected and analyzed.

2.1.1. *FC* and *FO* cohabiting species

2.1.1.1. *Native species.* Anacardiaceae: *Lithraea molleoides* (Vell.) Engl. “Molle”; Asteraceae: *Acanthostyles buniifolius* (Hook and Arn.) R.M. King. and H. Rob. “Chilca”, *Achyrocline satreoides* (Lam.) DC. “Marcela”, *Ambrosia tenuifolia* Spreng., *Baccharis articulata* (Lam.) Pers. “Carquejilla”, *Bidens pilosa* L. “Amor seco”, *Eupatorium viscidum* Hook. et Arn., *Grindelia cabreræ* Ariza, *Grindelia pulchella* Dunal, *Heterothalamus alienus* (Spreng.) Kuntze “Romerillo”, *Parthenium hysterophorus* L., *Viguiera tucumanensis* (Hook. et Arn.) Griseb. “Girasolillo”, *Vernonanthura nudiflora* (Less.) H. Rob. f. *nudiflora* “Falso alecrín”, *Thelesperma megapotamicum* (Spreng.) Kuntze “Te pampa”, *Xanthium spinosum* L. var. *spinosum* “cepa caballo”, *Zinnia peruviana* (L.) L. “Chinita del campo”; Euphorbiaceae: *Croton* Baill.; Fabaceae: *Acacia caven* (Mol.) Molina “Espinillo”, *Apurimacia dolichocarpa* (Gris.) Burkart, *Collaea argentina* Griseb. “Primavera”, *Sophora linearifolia* Griseb. “Sofora”; Rhamnaceae: *Colletia spinosissima* J. F. Gmel. “Barba del tigre”, *Condalia microphylla* Cav. “Piquillín”; Rosaceae: *Kageneckia lanceolata* Ruiz and Pav. “Durazno del campo”; Solanaceae: *Cestrum parqui* L' Hér. “Duraznillo negro”; Ulmaceae: *Celtis ehrenbergiana* (Klotzch) Liebm. “Tala”; Verbenaceae: *Aloysia gratissima* (Gillies and Hook. ex Hook.) Tronc. “Palo amarillo”; Zygophyllaceae: *Larrea divaricata* Cav. “Jarilla”.

2.1.1.2. *Exotic species.* Asteraceae: *Carduus acanthoides* L. “cardo”, *Xanthium cavanillesii* Schouw. “abrojo grande”; Fabaceae: *Robinia pseudoacacia* L. “acacia blanca” and *Gleditsia triacanthos* L. “acacia negra”; Meliaceae: *Melia azedarach* L. “paraíso”; Moraceae: *Morus alba* L.; Oleaceae: *Ligustrum lucidum* W.T. Aiton “ligustro” and *Ligustrum sinense* Lour. “ligustrina”; Pinaceae: *Pinus taeda* L.; Rosaceae: *Cotoneaster franchetii* Bois. “cotoneaster”; *Pyracantha atalantioides* (Hance) Stapf “crataegus”; Ulmaceae: *Ulmus pumila* L. “olmo”.

2.1.2. Other species of the genus *Flourensia*

Six additional South American *Flourensia* species were investigated and collected in their native habitats. *Flourensia hirta* S.F. Blake (*FH*, endemic), was collected in La Rioja province (1640 MASL), where it grows as a companion species in different bush-dominated communities (ca. 400 km northwest from Punilla Valley). *Flourensia niederleinii* S.F. Blake (*FN*, endemic) was also collected in La Rioja, on the slopes of Sierras de Velazco (1543 MASL), from almost pure stands. *Flourensia riparia* Griseb. (*FR*, endemic) and *Flourensia fiebrigii* S.F. Blake (*FF*) were collected in the province of Salta (ca. 900 km north from Punilla Valley), (1000 MASL and 3000 MASL, respectively), *Flourensia macroligulata* Seelgm. (*FM*) was collected in the province of Jujuy (ca. 1000 km north from Punilla Valley), and *Flourensia heterolepis* S.F. Blake (*FHe*) was

collected in the neighbor country of Bolivia, in Cochabamba province (ca. 1400 km north from Punilla Valley, 3050 MASL).

2.2. Screening technique

2.2.1. Identification of QACs in plant tissues

According to both, the expected QACs content in plants (Hanson et al., 1991; USDA, 2004) and the sensibility of the analysis, 5 g of dry grinded plant tissues were used for QACs identification. Samples were placed into 50 ml centrifuge tubes with 30 ml chloroform plus 10 ml methanol: distilled water (1:1, previously mixed). Tubes were sealed and vigorously shaken for 1 min and immediately centrifuged for 5 min at 3000 g. The clear upper layers (polar fractions) were separated and stored at -20°C until analysis. When necessary, 5 ml of methanol: distilled water was added. We followed Lerma et al. (1988) for the extraction methodology. When plant material was scarce, a micro-method, performed at 1:10 of that described, was suitable as well.

Owing to the high number of known QACs (Rhodes and Hanson, 1993), the present study was aimed at detecting QACs in general, and specifically GB and its precursor Cho. Standards of Cho chloride and GB hydrochloride were commercially available (Merck). The detection of QACs and specific identification of GB and Cho was performed through TLC on silica gel 60 (Merck, aluminum sheets, F₂₅₄) using two systems (Fig. 1). The first system was developed with methanol: ammonia 0.88 N (7.5:2.5; Solvent 1), as described by Rosenstein et al. (1999), leading to the separation of free Cho (Rf: 0.05) from GB (Rf: 0.55) (Fig. 1, A and C). The second one was developed with methanol: acetone:hydrochloric acid (9:1:0.05; Solvent 2) (Fig. 1, B and D), a variation of that originally described by McNeil et al. (1999), which allowed the separation of Cho from the origin of the chromatogram. QACs on TLC plates were visualized as spots after spraying with Dragendorff's reagent.

2.2.2. Method validation

A quantitative estimation of GB and Cho in plant samples was achieved by comparison of TLC spots with standard concentrations of 0.1, 0.3, 0.6, 1.2, 2.5 and 5.0 mg ml^{-1} (Fig. 1, A) by two independent

observers. These concentrations were selected from linearity experiments made from 0.01 to 15 mg ml^{-1} , the chosen range having the best regression analysis ($r = 0.89$). Samples with GB or Cho levels identified in the limits of the calibration curve were conveniently concentrated or diluted. System suitability testing of Dragendorff's stained TLC plates using a standard solution of 1.2 mg ml^{-1} was performed as already described (Askal et al., 2008). The precision of the proposed method as determined by replicate analysis ($N = 9$) was suitable for quality control analysis of QACs. Possible inter-day variation was avoided by using a standard solution with each group of samples. The sensibility for Cho and GB to Dragendorff's reagent was ca. 0.1 and 0.6 mg ml^{-1} (or mg g^{-1} DW in samples) respectively, corresponding to ca. 1.0 and 6 $\mu\text{mol g}^{-1}$ DW in samples, respectively. A counterstaining of Dragendorff's stained TLC plaques with I_2 vapors increased the sensibility of the assay by 2-fold (0.5 and 3 $\mu\text{mol g}^{-1}$ DW for Cho and GB, respectively) (Fig. 1, C and D). The achieved sensibility was at least 5 times higher than that reported in the original work of Storey and Wyn Jones (1975) which used different TLC solvents and supports.

In order to validate the specificity and accuracy of the methodology, plant species with known concentrations of QACs [soybean seeds (Cho) and spinach leaves (GB); USDA, 2004] were also processed (see Fig. 1, A–D). Further validation of the screening technique was obtained through the isolation (CC) and molecular characterization ($^1\text{H NMR}$) of QACs from FC and FO as detailed below. In UV-B experiments, each sample was analyzed and quantified through both TLC and $^1\text{H NMR}$, and thus additionally validated.

2.3. Isolation and molecular characterization of detected GB

In order to confirm the molecular form of GB identified by TLC, plant extracts were subjected to CC and spectral analysis. Polar fractions obtained from 10 g of dry leaves (as described above) were dried at 40°C with nitrogen flow, yielding a dark green residue (ca. 150 mg). The residue was suspended in 1.0 ml of Solvent 1 and fractionated by silica gel CC (1.0 cm i.d., 25 cm; 20 g silica gel 60 Merck (70–230 mesh)) eluted at a flow rate of 1 ml min^{-1} using the same solvent. The collected 1 min fractions were subjected to TLC using Solvent 1 and revealed

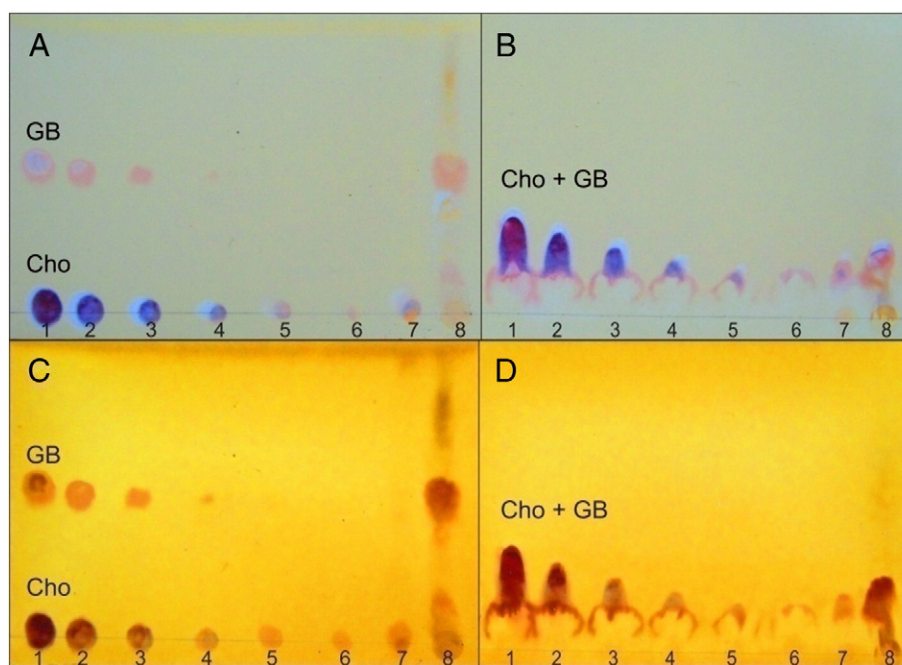


Fig. 1. TLC of standards Cho and GB. TLCs stained with Dragendorff's reagent (A and B) and counterstained with I_2 vapors (C and D), using Solvent 1 (A and C) or Solvent 2 (B and D). Lanes 1 to 6: Cho (choline) together with GB (glycine betaine) standards at same concentrations: 5, 2.5, 1.2, 0.6, 0.3 and 0.1 mg ml^{-1} , equivalent to 50, 25, 12, 6, 3 and 1.0 $\mu\text{mol g}^{-1}$ DW in plant extracts, respectively; lane 7: soybean seeds extract (Cho: 4.5 $\mu\text{mol g}^{-1}$ DW); lane 8: spinach extract (GB: 75 $\mu\text{mol g}^{-1}$ DW).

with Dragendorff's reagent. A duplicated TLC plate was visualized under UV₂₅₄ and stained with I₂ vapors in order to identify possible contaminations. Isolated GB-rich fractions (Fr. 46–60, ca. 15 mg) were pooled, dried and suspended in 1 ml of methanol; contaminants were removed using a 1 ml CC through washing the sample with pure methanol. In the plant materials tested (*FC* and *FO*), the procedure yielded ca. 0.05% (DW basis) of a colorless syrup containing an isolated compound that co-migrated with pure GB through TLC ($R_f = 0.55$, Solvent 1) stained with Dragendorff's reagent and/or with I₂ vapors.

The isolated QAC was subjected to ¹H NMR as previously described (Silva et al., 2012). Briefly, ¹H NMR (400 MHz), ¹³C-NMR (100 MHz), DEPT, HMBC and HSQC spectra were recorded at room temperature with Bruker AC 400 spectrometer. The spectra were recorded in DMSO-d₆ and the solvent signals (2.50 ppm for ¹H NMR and 39.52 ppm for ¹³C-NMR) were used as references (Gottlieb et al., 1997).

2.4. Assessment of QACs in plant extracts by ¹H NMR

¹H NMR spectra were obtained from plant materials prepared as described previously (Silva et al., 2012), and dissolving dried sample extracts in D₂O:MetHO-d₄ (1:1). A dominant singlet (peak) assignable to the authentic GB methyl groups [R-N(CH₃)₃] detected at 3.28 ppm was controlled in all the spectra and used for the assessment of changes in QACs composition. For quantification, the integration of the singlet versus a constant signal in all samples at 1.35 ppm was used, and results were expressed as a relative concentration of GB in each sample.

2.5. QACs in *FC* and *FO*

The concentration of QACs in *FC* and *FO* was evaluated in: a) different organs, b) leaves from vegetative vs. leaves from reproductive branches (hereafter vegetative and reproductive leaves, respectively), and c) seedlings as compared to adult plants.

2.6. UV-B radiation effects on *Flourensia* QACs

Controlled field experiments were carried out in order to test whether solar UV-B radiation induced QACs' changes in *Flourensia*. Seedlings were obtained from mature seeds collected from plants growing in natural areas. After acclimation, 25 seedlings of each species (*FC* and *FO*) were grown in the field under aluminum frames covered with plastic films that either had very high transmittance over the whole UV spectrum (+UV-B, near-ambient UV-B treatment: 0.02 mm thick polyethylene film, Rolopac, Buenos Aires) or selectively attenuated the UV-B component of sunlight (–UV-B, attenuated UV-B treatment: 0.1 mm thick clear polyester, Oeste Aislante, Buenos Aires). Both films have very high transmittance in the UV-A (>80%) and PAR (90%) regions of the spectrum (for spectral scans and details, see Ballaré et al., 1999 and Mazza et al., 2000). The biologically effective daily UV dose, calculated using the generalized plant action spectrum of Caldwell (1971) normalized at 300 nm, was 9.2–10 kJ m^{–2}. The natural UV-B dose during summer was 9.2 kJ m^{–2}. The filters were kept at a short distance (ca. 10 cm) from the upper canopy leaves and plants were watered as needed in order to maintain adequate water supply. Temperature was monitored throughout the experiment using an infrared thermometer; no consistent differences were found in soil or canopy temperature between UV-B treatments (data not shown). There were two sets of experiments during the summer of 2008–2009, with three filters for each experimental treatment. The height, stem base diameter and number of leaves of the seedlings were recorded throughout the experiments (3 months).

After harvest, seedlings were dried at 40 °C for 48 h. Due to the small size of the plants we used the micro-extraction method, and processed through TLC and ¹H NMR as described in Sections 2.2.1 and 2.4, respectively.

2.7. QACs in *Flourensia* community co-occurring species and in other taxa of the genus

A total of 39 co-occurring species from *Flourensia* dominate communities (28 natives, 11 exotic invaders; see Section 2.1.1) integrating *FC* and *FO* communities in the Punilla Valley, as well as six other South American *Flourensia* species (*FH*, *FN*, *FR*, *FF*, *FM* and *FHe*) with distant geographical distribution, were screened for QACs for the first time; the only exception being the known GB accumulator *Larrea tridentata*. All independent samples were processed in triplicate through the TLC screening method as described in Section 2.2.1.

2.8. Data analysis

Owing to the known variability of GB among plants, even within a same population, quantitative results were obtained from at least three to five independent samples. One way ANOVA or Mann–Whitney nonparametric test for the significance of the difference between the distributions of independent samples for ordinal data were applied.

3. Results and discussion

3.1. QACs in *FC* and *FO*

By means of chromatographic and spectral studies we demonstrated that both *Flourensia* species are natural GB accumulators. *FC* and *FO* revealed the presence of two QACs: a betaine and free Cho (Table 1). No other QACs could be detected in these species. The same betaine was found in all the organs of *FC* and *FO*, having $R_f = 0.55$ (Solvent 1), co-migrating with the betaine of spinach extracts (a natural GB accumulator) and confirmed later as GB through chromatographic isolation and spectroscopic analysis (Fig. 2). Despite the variability observed, *FO* generally showed higher contents of GB than *FC*, with values falling within those normally found in spinach (in the order of 50 μmol g^{–1} DW). Both species exhibited similar patterns of GB distribution among plant organs, the highest levels being found in capitula, followed by leaves, shoots, roots and seeds (Table 1). The levels and distribution of GB among the different plant organs in *FC* and *FO* coincided with those early reported for well-known GB natural accumulators (Hanson and Wyse, 1982). The high content found in the capitula was in agreement with previous reports in transgenic (Sulpice et al., 2003; Park et al., 2007) and natural GB accumulators (Bhuiyan et al., 2007). Genetic engineering of GB synthesis has proved to enhance the formation of flowers in salt-stressed *Arabidopsis* (Sulpice et al., 2003), protect seeds, vegetative organs, and flowers from chilling injury in tomato (Park et al., 2007), and increase the tolerance of plants to various kinds of stresses during germination and seedling growth (Chen and Murata, 2002; Ashraf, 2009). The high levels of GB found in reproductive organs of *Flourensia* should therefore be involved in protecting the development/formation of flowers and seeds against abiotic stresses, the specific effect remaining to be determined.

In natural GB accumulators, a large proportion of free Cho is diverted to the production of GB in response to stress. Since the early work of Hanson and Wyse (1982) most studies have shown that GB in flowers arises from Cho enzymatic oxidation in mature leaves which is afterwards translocated via phloem to the reproductive organs and roots (Mäkelä et al., 1996; Park et al., 2007). In contrast, the capitula of both *Flourensia* species also contained detectable levels of free Cho (Table 1). This finding, together with the fact that the biosynthetic enzymes of GB, choline monoxygenase (CMO) and betaine aldehyde dehydrogenase (BADH) were found in all the organs of a natural GB accumulator (Bhuiyan et al., 2007), would allow to speculate that GB concentration in the capitula of *Flourensia* may depend on both, what is translocated via phloem, and what is locally synthesized. As the reproductive stage is a critical one in commercial crops, there has been an increased interest in obtaining genetically-engineered plants containing

Table 1
GB and Cho contents in adult FC and FO plants and in seedlings under UV-B effects.

		GB ($\mu\text{mol g}^{-1}$ DW)					Cho ($\mu\text{mol g}^{-1}$ DW)		
		Seed	Root	Shoot	Leaf	Capit.	Seed	R,Sh,L	Capit.
FC	Adults	2 ± 1^a	4 ± 1^a	$14 \pm 5^{a,b}$	38 ± 7^b	$62 \pm 7^{b,c}$	–	–	4 ± 1
	Seedlings								
	+UV-B	NA	$2 \pm 1^{a*}$	$4 \pm 1^{a,b}$	$9 \pm 2^{b,c*}$	NA	NA	–	NA
	–UV-B	NA	$<0.5^a$	2 ± 1^b	38 ± 7^c	NA	NA	–	NA
FO	Adults	4 ± 1^a	9 ± 2^a	38 ± 7^b	$62 \pm 7^{b,c}$	$62 \pm 7^{b,c}$	<0.5	–	2 ± 1
	Seedlings								
	+UV-B	NA	$4 \pm 1^{a*}$	$9 \pm 2^{a,b}$	$18 \pm 4^{b,c*}$	NA	NA	–	NA
	–UV-B	NA	1 ± 0.5^a	7 ± 3^a	62 ± 7^b	NA	NA	–	NA

Values indicate means \pm se. Different letters (a, b, c), and (*), indicate significant differences among organs in a same species, and due to the effect of UV-B, respectively ($p < 0.05$). FO adults showed a tendency of higher GB and Cho contents (not significant) than FC. The GB contents in leaf of adults FC, and in shoot and leaf of adults FO were significantly higher than those in seedlings growing in natural areas or +UV-B ($p < 0.05$). GB values in seedlings harvested in natural areas were similar to +UV-B and therefore not shown. (–), not detected; NA, not applicable; Capit., capitula; R, root; Sh, shoot; L, leaf.

transgenes for GB production that would produce sufficient amounts of this compound to ameliorate stress effects; however results so far show limited success (Nuccio et al., 1998; Huang et al., 2000; Ashraf and Foolad, 2007). In this scenario, the study of the biochemical pathways present in FC and FO implicated in the synthesis of high levels of GB together with free Cho may have important biotechnological application, particularly where lack of the precursor Cho and/or the specific enzymes may pose serious limitations.

The content of GB in vegetative leaves was at least 30% higher than that found in reproductive leaves (data not shown). In vegetative branches, leaves producing GB persist on the plant for an extended period of time, sometimes even overwintering. Conversely, the reproductive leaves – typically 5 to 7 – which act as the main source of growth of flowers and seeds, senesce much sooner, almost concomitantly with seed filling (data not shown). Hence, the lower concentration of GB found in the leaves of reproductive branches could be the result of its continuous translocation toward reproductive organs (flowers, flower structures and seeds), as was also documented in other species (Cromwell and Rennie, 1953; Chen and Murata, 2011). In seedlings of FC and FO, harvested from natural areas or grown under +UV-B filters, the amount of GB in leaves and shoots was significantly lower than in adult plants growing together in the same natural environments (Table 1). As in adult plants, FO seedlings tended to show higher amounts of GB than FC seedlings, and higher levels of GB in leaves compared to shoots and roots (Table 1).

Although contrary to our expectations – since plants at the seedling stage are most vulnerable to abiotic stresses – our results coincide with the findings of Cromwell and Rennie (1953). These authors showed that in the GB accumulators *Beta vulgaris* and *Amaranthus caudatus*, GB levels increased throughout the vegetative period, reaching a maximum at the time of flowering. Our results and those of Cromwell and Rennie (1953) may support the notion that maximum GB production is attainable only when plants reach an advanced stage of development, which in turn may reflect the acclimation of plants to repetitive episodes of stress (Krasensky and Jonak, 2012). Further studies should be performed in order to test plant responses at different developmental stages, and caution should be posed when interpreting and extrapolating results derived from experiments carried out with seedlings only.

3.2. UV-B radiation effects on *Flourensia* QACs

Plant exposure to high doses of solar UV-B (+UV-B) reduced seedling height in 20% for FC and 25% for FO ($p < 0.05$, Silva et al., personal communication), findings which were similar to those already reported (Ballaré et al., 1999; Zavala and Ravetta, 2002; Piri et al., 2011). No changes were detected in stem base diameters (around 2–3 cm) or in the number of leaves (around 11–13 leaves).

UV-B radiation promoted significant GB organ-specific changes in both *Flourensia* species. The exposed (+UV-B) leaves of FC and FO, as expected, showed GB values very similar to those measured in seedlings

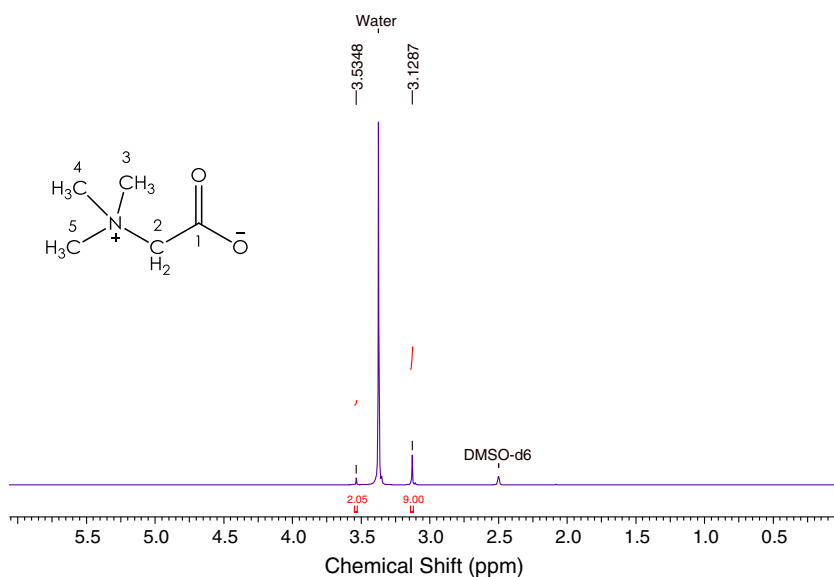


Fig. 2. ^1H NMR spectra of the isolated QAC (GB) from *Flourensia* leaves. ^1H NMR spectra and molecular characterization of isolated GB from FC previously detected through the purposed TLC screening technique.

of similar age growing in natural areas, and were therefore showed once (Table 1). Instead, GB values found in the leaves of seedlings protected from UV-B exposure (–UV-B) were significantly higher ($p < 0.01$), while those found in roots were significantly lower ($p < 0.05$, Table 1). In shoots of both species no significant changes could be observed between treatments. No other QACs were induced as a result of UV-B treatment as tested by TLC and ^1H NMR.

In a recent paper, Mohammed and Tarpley (2013) showed that exogenous application of GB to young rice plants growing under UV-B lamps at a dose equivalent to the one experienced by *Flourensia* in our experiments (10 kJ m^{-2}), increased leaf photosynthetic rate, pollen viability and leaf phenolic concentration (cf. to untreated plants), resulting in increased rice yield under UV-B stress conditions. Although these results demonstrate the beneficial effects of GB ameliorating the deleterious effects of UV-B, so far the effects of UV-B radiation in regulating endogenous levels of GB has never been addressed before.

Our results show, for the first time, that solar UV-B is indeed involved in regulating GB contents and within-plant distribution. From the growing evidence of the role of GB in protecting plants against oxidative stress, we anticipated that under solar UV-B seedlings would exhibit higher contents of GB. Contrary to our expectations, GB contents in leaves of +UV-B seedlings were 70% lower than those in –UV-B plants. Moreover, GB content in –UV-B seedling leaves attained values similar to those found in leaves of adult plants. As the decrease in the content of leaves GB in +UV-B experimental treatment was not accompanied by any detected increase in the content of free Cho, a possible effect of UV-B on the inhibition of CMO and BADH enzymes was primarily discarded. It can be hypothesized that the lower levels found in UV-B exposed leaves may be explained by a reduction of GB synthesis due to the prioritization of the (competitive, Kennedy pathway) production of phosphatidylcholine (PC). In this sense, Carillo et al. (2011) also found a decrease in GB levels in the leaves of *Triticum durum* exposed to high light stress ($900 \mu\text{E}$; no UV-B) relative to those that received lower doses of light; which they attributed to an inhibition of GB synthesis. Exposure of plant tissue to UV-B and high irradiances generates reactive oxygen species (ROS), and lipids are known to be a main target of H_2O_2 , producing lipid peroxidation (Allan and Fluhr, 1997; AH-Mackerness et al., 2001; Dawar et al., 1998; Bassman et al., 2001). PC is one of the most abundant phospholipids present in plant cell membranes, thus its peroxidation would trigger an increased rate of biosynthesis in order to replace this essential phospholipid and maintain cell membrane integrity and function. Lo et al. (2004) have demonstrated that sublethal doses of UV-B up-regulate a specific phospholipase, which preferentially deesterifies PC. Bavaro et al. (2007) found that the production of PC is enhanced under osmotic stress, which also generates H_2O_2 and produces lipid peroxidation as well.

Phosphoethanolamine (P-EA) is the common precursor of two alternative pathways leading to the production of either PC or Phosphocholine (PCho) and subsequent production of free Choline and GB. In addition, in plants it has been demonstrated that PCho-Choline is a reversible reaction and that PCho may also be driven to PC formation through two enzymatic reactions. Taking all these into consideration, the hypothesis that UV-B is involved in the regulation of GB production is plausible. Although this mechanism would be linked to the rate of PC peroxidation, from direct UV-B increased phospholipase activity, other direct effects on this biosynthetic pathway cannot be ruled out. Confirmation of this hypothesis deserves further investigation. Interestingly, GB system has been proved to be differentially affected by individual types of abiotic stress (Chen and Murata, 2011), a specific example of that giving by the report of Abdul Jaleel et al. (2007), where GB increases together with lipid peroxidation in response to salt stress.

Our results also showed that UV-B changed GB distribution within seedlings, inducing a 4-fold increase of GB in the roots as compared to those in –UV-B plants. Translocation of GB from the leaves to roots, as well as GB-activated biosynthetic related enzymes in the roots of

several natural accumulators has also been observed in plants subjected to various types of stresses (Hanson and Wyse, 1982; Bhuiyan et al., 2007; Hattori et al., 2009). The presence of compatible solutes in the roots would allow the maintenance of tissue water potential compatible with cell elongation and growth. The concentration of GB found in FC and FO roots, however, would not contribute substantially to lower the osmotic potential. In turn, increased GB content in the roots may play a critical role in preventing the accumulation of ROS in the cell walls, as proposed by Einset et al. (2008) in *Arabidopsis*. In a related work, Einset and Connolly (2009) were able to identify eleven 'GB-up-regulated' stress tolerance genes in *Arabidopsis*, hence, cross-talk between different stress related pathways cannot be ruled out.

Our controlled UV-B experiments, set up in the same natural environment where these species grow, clearly show that solar UV-B is involved in regulating GB contents and within-plant distribution in FC and FO seedlings.

3.3. QACs in *Flourensia* co-occurring native and exotic species

Plants growing in the same or similar environments may display similar functional and physiological traits. In this sense, the production of certain types of osmoprotectants could be regarded as a functional trait for plants growing in harsh environments. Although results derived from halophytic communities would support this notion (Stewart et al., 1979), community-level studies comprising xerophytic (non halophytic) environments are limited.

Our results show that in FC- and FO-dominated communities the accumulation of QACs may be a widespread adaptive strategy. By using the described TLC methodology we were able to detect the presence of QACs in the leaves of 41% of *Flourensia* (FC and FO) co-occurring species ($N = 39$, Table 2). Fifty percent of the native plants studied were found to accumulate QACs in their leaves, either as GB or other QACs, while only 18% of the exotics were determined to accumulate "other QACs". GB was the main QAC present and was detected in 8 of the 14 native natural QAC accumulators (57%), with concentrations ranging from 12 to $75 \mu\text{mol g}^{-1} \text{DW}$, while no exotic species accumulated GB (Table 2). These results are contrary to those reported by Ghayal and Dhumal (2011) for a xerophytic community in Pune (India) in which they found that QACs, and specifically GB, were more frequently found in exotics as compared to native plants. Free Cho was only found in the seeds of the exotic invader *G. triacanthos*, at concentrations of $8 \pm 2 \mu\text{mol g}^{-1} \text{DW}$ (Table 2), together with a high concentration of PC in the non polar fraction as well (data not shown). The remaining QAC accumulators exhibited other compounds that could not be identified as GB or Cho; the only exception being *A. tenuifolia* that accumulated both, GB and another QAC (Table 2).

The observation of the TLC plates after staining with Dragendorff's reagent counterstained with I_2 , or with I_2 alone, and under UV light, allowed a visual discrimination of the different compounds present in each species and a further comparison of the measured Rf's relative to free Cho and GB standards. This is exemplified in Fig. 3 for a selected number of species that encompass the diversity of QACs compounds found in this study, in which a couple of non QACs accumulating species were also included as reference. As shown in Fig. 3 we were able to detect at least nine different QACs species, although GB was by far the most abundant compound within QACs accumulators (53:47). Betaines have been shown to have taxonomic value for some families, such as Poaceae, Chenopodiaceae and Lamiaceae (Wyn Jones and Storey, 1981; Blunden et al., 1996); however the presence of QAC accumulators and non-accumulators within genera has also been reported (Rhodes et al., 1987; Chen et al., 2007). In the *Flourensia* communities studied no clear associations could be identified between QAC accumulators and particular taxonomic groups. However, it is interesting to note that GB was the only compound present in all native Asteraceae species found to be QAC accumulators (Table 2), except for *A. tenuifolia* in which another QAC entity was also detected (lane 6, Fig. 3-A). *V. tucumanensis*

Table 2
QACs in FC and FO co-occurring native and exotic species.

Species (Family)	QACs ($\mu\text{mol g}^{-1}$ DW)			Plant organs, habit
	Free Cho	GB	Other QACs	
Natives				
<i>Lithraea molleoides</i> (Anacardiaceae)	-	-	-	Leaves, W
<i>Acanthostyles buniifolium</i> (Asteraceae)	-	-	-	Leaves, shoots, WH
<i>Achyrocline satureioides</i> (Asteraceae)	-	-	-	Leaves, shoots, H
<i>Ambrosia tenuifolia</i> (Asteraceae)	-	62 ± 7^a	++	Leaves, H
<i>Baccharis articulata</i> (Asteraceae)	-	38 ± 7^b	-	Leaves, WH
<i>Bidens pilosa</i> (Asteraceae)	-	-	-	Leaves, H
<i>Eupatorium viscidum</i> (Asteraceae)	-	-	-	Leaves, H
<i>Grindelia cabreriae</i> (Asteraceae)	-	38 ± 7^b	-	Leaves, H
<i>Grindelia pulchella</i> (Asteraceae)	-	62 ± 7^a	-	Leaves, WH
<i>Heterothalamus alienus</i> (Asteraceae)	-	-	-	Leaves, WH
<i>Parthenium hysterophorus</i> (Asteraceae)	-	38 ± 7^b	-	Leaves, W
<i>Thelesperma megapotamicum</i> (Asteraceae)	-	-	-	Leaves, H
<i>Viguiera tucumanensis</i> (Asteraceae)	-	62 ± 7^a	-	Leaves, WH
<i>Vernonanthura nudiflora</i> (Asteraceae)	-	-	-	Leaves, WH
<i>Xanthium spinosum</i> (Asteraceae)	-	18 ± 4^b	-	Leaves, H
<i>Zinnia peruviana</i> (Asteraceae)	-	-	-	Leaves, H
<i>Croton lachnostachyus</i> (Euphorbiaceae)	-	-	++	Leaves, WH
<i>Acacia caven</i> (Fabaceae)	-	-	-	Leaves, seeds, W
<i>Apurimacia dolichocarpa</i> (Fabaceae)	-	-	+	Leaves, WH
<i>Collaea argentina</i> (Fabaceae)	-	-	+	Leaves, WH
<i>Sophora linearifolia</i> (Fabaceae)	-	-	+++	Leaves, WH
<i>Colletia spinosissima</i> (Rhamnaceae)	-	-	++	Leaves, W
<i>Condalia microphylla</i> (Rhamnaceae)	-	-	-	Leaves, W
<i>Kageneckia lanceolata</i> (Rosaceae)	-	-	-	Leaves, W
<i>Cestrum parqui</i> (Solanaceae)	-	-	++	Leaves, WH
<i>Celtis ehrenbergiana</i> (Ulmaceae)	-	-	-	Leaves, W
<i>Aloysia gratissima</i> (Verbenaceae)	-	-	-	Leaves, WH
<i>Larrea tridentata</i> (Zygophyllaceae)	-	38 ± 7^b	-	Leaves, W
Exotics				
<i>Carduus acanthoides</i> (Asteraceae)	-	-	+++	Leaves, H
<i>Xanthium cavanillesii</i> (Asteraceae)	-	-	+	Leaves, H
<i>Gleditsia triacanthos</i> (Fabaceae)	-	-	-	Leaves, W
	8 ± 2	-	-	Seeds
<i>Robinia pseudoacacia</i> (Fabaceae)	-	-	-	Leaves, W
<i>Melia azedarach</i> (Meliaceae)	-	-	-	Leaves, fruits, W
<i>Morus alba</i> (Moraceae)	-	-	-	Leaves, W
<i>Ligustrum lucidum</i> (Oleaceae)	-	-	-	Leaves, fruits, W
<i>Ligustrum sinense</i> (Oleaceae)	-	-	-	Leaves, W
<i>Cotoneaster franchetii</i> (Rosaceae)	-	-	-	Leaves, W
<i>Pyracantha atalantioides</i> (Rosaceae)	-	-	-	Leaves, fruits, W
<i>Ulmus pumila</i> (Ulmaceae)	-	-	-	Leaves, W

Data represent the mean of three independent samples estimated by the corresponding standard curve from TLC runs as described in M&M. Different letters (a, b) indicate significant differences ($p < 0.05$). (-), not detected (below 0.7 or 3 $\mu\text{mol g}^{-1}$ DW of Cho or GB, respectively); (-/+ , + , ++ , +++), traces, low, medium and high concentrations, respectively. Habit: W, woody; H, herbaceous; WH, woody herbs.

and *A. tenuifolia* (lanes 4 and 6, Fig. 3-A), and *G. pulchella* exhibited the highest values of GB ($62 \pm 7 \mu\text{mol g}^{-1}$ DW), followed by *B. articulata*, *G. cabreriae* and *P. hysterophorus* ($38 \pm 7 \mu\text{mol g}^{-1}$ DW), while *X. spinosum* fell in the lower range ($18 \pm 4 \mu\text{mol g}^{-1}$ DW) (Table 2). The other species shown to accumulate GB was *L. tridentata*, which belongs to Zygophyllaceae (Table 2).

Although no obvious pattern could be detected in the "other QACs" accumulating species, some of them shared Dragendorff's positive colored bands with very similar Rf values, that could be assignable to common QAC entities (Fig. 3-A). A similar QAC (Rf = 0.45) was found in four natives: *Colletia spinosissima* (Rhamnaceae, lane 5), *C. macrostachyus* (Euphorbiaceae, lane 10) and *C. argentina* and *A. dolichocarpa* (Fabaceae, lanes 12 and 13; respectively). The same compound was also revealed in these species under UV light (Fig. 3-B). Another entity with Rf = 0.25 was visible in two Asteraceae species (Fig. 3-A), the native *A. tenuifolia* (lane 6) and the exotic *Carduus acanthoides* (lane 11).

While most species accumulated just a single compound, others showed 2 to 5 different entities. The larger diversity of compounds was found in *S. linearifolia* with multiple red colored bands, which based on the Rfs obtained could be associated to 5 different compounds (Fig. 3-A, lane 9).

Fig. 3 also depicts the remarkable array of compounds that did not stain positive with Dragendorff's reagent, but could be detected either under UV light and/or when stained with I_2 alone (Fig. 3, cf. A, B and C, respectively). As exemplified in Fig. 3, for the 2 non-QAC accumulators included, almost no single band could be detected in the native *E. viscidum* (Asteraceae) (lane 7), while several bands were visible in the exotic *U. pumila* (Ulmaceae, lane 8) under both, UV and I_2 .

At the family level, studied Fabaceae have been typically characterized as QAC or non QAC accumulators (e.g., *Medicago sativa* or *Glycine max*, respectively; Nishimura et al., 2001). In the Fabaceae investigated from these communities, the three native woody-herbaceous species (*Collaea argentina*, *A. dolichocarpa* and *S. linearifolia*) were found to accumulate QACs, other than GB, while the woody species (1 native and 2 exotics) were non QAC accumulators. Our results from *Grindelia* spp. also support the notion that QACs distribution in nature is not necessarily genus-specific, the native *G. pulchella* and *G. cabreriae* in these communities exhibited high contents of GB, whereas in *Grindelia humilis* no QACs were reported (Rhodes et al., 1987).

Overall results strongly suggest that accumulation of QACs in these xerophytic plant communities may be an adaptive strategy to withstand abiotic stresses, enhancing plant survivorship under harsh environmental conditions. In this regard, previous studies have shown that GB

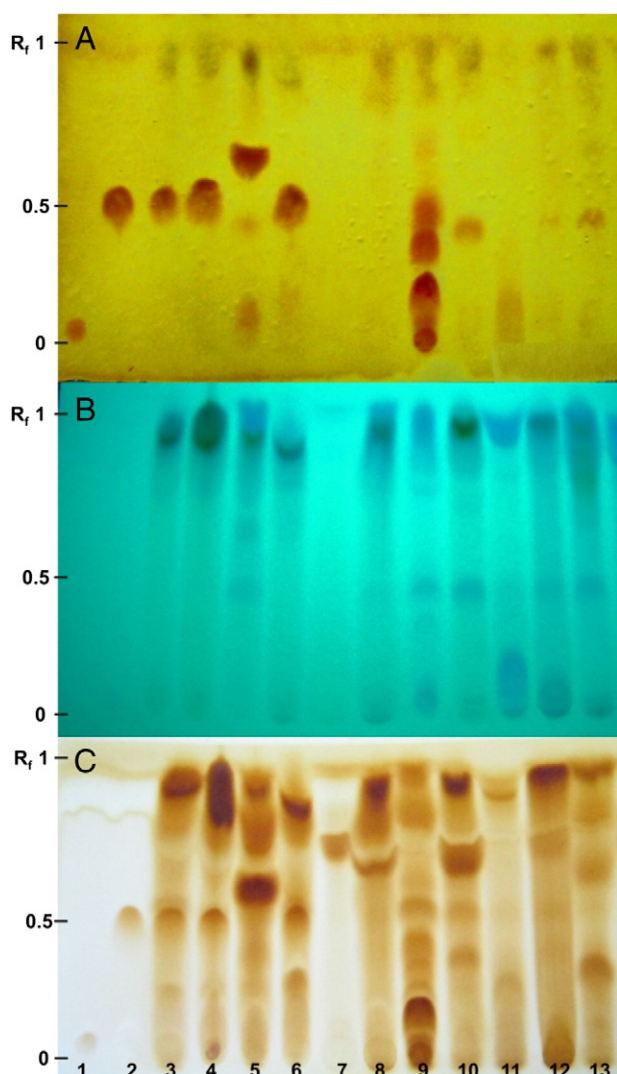


Fig. 3. QACs in *Flourensia* coexisting native and exotic species. TLC similar plates of standards and plant leaf extracts using Solvent 1. Cho and GB standards are specifically stained with Dragendorff's reagent counterstained with I_2 (Panel A), are not visualized through UV light (Panel B), and stained with I_2 (Panel C). (Lane) sample (R_f): (1) Cho (0.05), (2) GB (0.55), (3) FC, (4) native *Verbesina encelioides* with high concentration of GB only (0.55), (5) native *Colletia spinosissima* with three QACs (0.2, 0.45 and 0.65), (6) native *Ambrosia tenuifolia* with high GB levels and a detectable QAC (0.25), (7) native *Eupatorium viscidum* and (8) exotic *Ullmus pumila* with no QACs, (9) native *Sophora linearifolia* with 5 QACs (0.1, 0.3, 0.4, 0.5 and 0.7), (10) native *Croton lachnostachyus* with 1 QAC (0.45), (11) exotic *Carduus acanthoides* with 1 QAC (0.25), (12) native *Collaea argentina* and (13) native *Apurimacia dolichocarpa* with 1 QAC (0.45).

accumulators such as *Baccharis*, *Grindelia*, and *Larrea* species can grow and survive under severe abiotic stress (Ogle and Reynolds, 2002; Zavala and Ravetta, 2002; Medeiros and Pockmand, 2011).

3.4. QACs in other species of the genus *Flourensia*

All the South American species of *Flourensia* studied (*FH*, *FN*, *FR*, *FF*, *FM* and *FHe*), including *FC* and *FO* were found to be GB natural accumulators (Fig. 4-A); the highest content of GB in leaves was observed in *FR* ($90 \pm 9 \mu\text{mol g}^{-1}$ DW) and the lowest, in *FH* ($14 \pm 5 \mu\text{mol g}^{-1}$ DW). No other QACs were detected in the leaves of any of the *Flourensia* species tested. Using the TLC polarity system devised, GB was the only compound shared by all the species as revealed by UV light (not shown) or with I_2 (Fig. 4-B). The presence of GB in eight of the eighteen (44%) South American *Flourensia* species described (Dillon, 1984) suggests that this chemical character may be of taxonomic value for the genus.

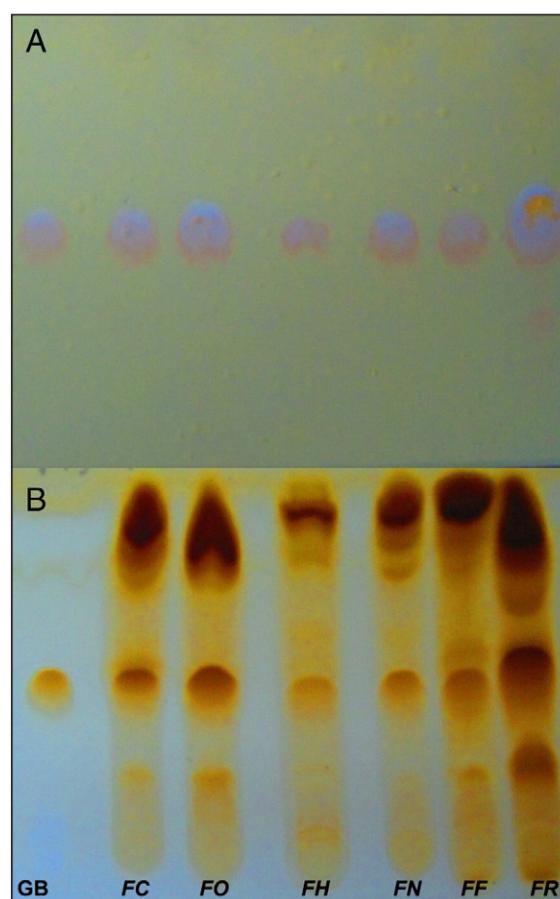


Fig. 4. QACs in species of *Flourensia* genus. TLC plates of *Flourensia* plant leaf extracts using Solvent 1, stained with Dragendorff's reagent (Panel A) or with I_2 (Panel B). [GB in $\mu\text{mol g}^{-1}$ DW, mean \pm se, $N = 5$]; different letters (a, b, c, d) indicate significant differences among species ($p < 0.05$). GB [2.5 mg ml^{-1} , equivalent to $25 \mu\text{mol g}^{-1}$ DW], FC [38 ± 7]^a, FO [62 ± 7]^b, FH [14 ± 5]^c, FN [35 ± 3]^a, FF [30 ± 5]^a and FR [90 ± 9]^d. Panel B shows that at this polarity range GB is the only shared compound in the genus *Flourensia*.

Dillon based the grouping of *Flourensia* species primarily on the comparison of exomorphic features and partial phytochemical data. In his phylogram, South American taxa are divided into three weakly differentiated lines; while our studied species are present in two of them. In a previous work, the bioactive sesquiterpene (–)-hamanasic acid A originally isolated from *FC*, failed to serve as a chemotaxonomic marker, as it was only detected in three (*FC*, *FO* and *FF*) of the six *Flourensia* species investigated (López et al., 2013). Again, it is interesting to note that no other shared compound could be detected among these species by means of different TLC polarity ranges as revealed through general methods (UV and I_2). This is in agreement with previous work for the genus in which despite some molecules and structural types such as prenylflavonoids and benzofuran derivatives (Bohlmann and Jakupovic, 1979; Bohlmann et al., 1984; Uriburu et al., 2004, 2007; Rios et al., 2013), together with similar surface deposited and volatile mono and sesquiterpenes (Silva et al., 2012; López et al., 2013; Urzúa et al., 2007; Priotti et al., 1997; Estell et al., 1994) are repeatedly found among some of the species, the presence of a single compound implying chemosystematic correlations could not be detected. As far as we reviewed, GB would represent the first chemotaxonomical character identified in the genus *Flourensia*.

Despite the fact that the genus has a wide range of distribution, all species typically grow in xeric environments characterized by multiple abiotic stressors, such as high irradiances and elevated solar UV-B, droughts, high temperature amplitude and poor soils. Hence, the presence of GB in all the species of *Flourensia* may have a putative role

in the genus, intimately related with the tolerance to abiotic stress, and with the ecophysiological adaptations of these species. Future phytochemical studies comprising the screening of GB in the vast majority of species within the genus will certainly provide new insights for a revision of species composition within lineages.

3.5. Concluding remarks

We have demonstrated the presence of GB in FC and FO, at concentrations and plant distributions similar to other QAC accumulators. Detectable levels of free Cho were found in capitula of *Flourensia* suggesting a local, putative GB synthesis. Overall results signal QACs (and GB) to be involved in the tolerance of *Flourensia* genus to oxidative stress, specifically to UV-B radiation. Our controlled UV-B experiments, set up in the same natural environment where these species grow, clearly show that solar UV-B is involved in regulating GB contents and within-plant distribution in FC and FO seedlings. Despite the consequence of this finding not being addressed in this work, a shift to higher contents of compatible solutes in the roots would play a critical role in preventing the accumulation of ROS in the cell walls.

In FC and FO communities QACs were detected and described for the first time in 41% of co-occurring species ($N = 39$), 14 of 28 natives (50%) and 2 of 11 exotics (18%), being GB in 57% of the native QAC accumulators. GB showed genus specificity since it was detected in all of the South American *Flourensia* species investigated (8 of 18; 44%), regardless of their geographic distribution: *F. hirta*, *F. nienderleinii*, *F. riparia*, *F. fiebrigii*, *F. macroligulata* and *F. heterolepis*; after which we propose GB as a chemotaxonomic marker for the genus *Flourensia*.

Overall results signal QACs (and GB) to be involved in the tolerance of *Flourensia* genus to abiotic stress, and specifically to UV-B radiation. In addition, the fact that QACs were found in 50% of the dominant native xerophytes studied strongly suggests that QACs accumulation may represent a community-level adaptation to abiotic stress in xeric environments. The application of the simple and fast screening technique we devised may facilitate and accelerate the acquisition of new data in multiple species and environments.

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References

Abdul Jaleel, C., Gopi, R., Sankar, B., Manivannan, P., Kishorekumar, A., Sridharan, R., Panneerselvam, R., 2007. Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. *South African Journal of Botany* 73, 190–195.

AH-Mackerness, S., John, C.F., Jordan, B., Thomas, B., 2001. Early signaling components in ultraviolet-B responses: distinct roles for different reactive oxygen species and nitric oxide. *FEBS Letters* 489, 237–242.

Allan, A.C., Fluhr, R., 1997. Two distinct sources of elicited reactive oxygen species in tobacco epidermal cells. *Plant Cell* 9, 1552–1572.

Ashraf, M., 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnology Advances* 27, 84–93.

Ashraf, M., Foolad, M.R., 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany* 59, 206–216.

Askal, H.F., Khedr, A.S., Darwish, I.A., Mahmoud, R.M., 2008. Quantitative thin-layer chromatographic method for determination of amantadine hydrochloride. *International Journal of Biomedical Sciences* 4 (2), 155–160.

Ballaré, C.L., Scopel, A.L., Mazza, C.A., 1999. Effects of solar UV-B radiation on terrestrial ecosystems: case studies from southern South America. In: Rozema, J. (Ed.), *Stratospheric Ozone Depletion: the Effects of Enhanced UV-B Radiation*. Backhuys, Leiden, pp. 293–311.

Barboza, G., Cantero, J.J., Núñez, C.O., Ariza Espinar, L., 2009. Medicinal plants: a general review and a phytochemical and ethnopharmacological screening of the native Argentine flora. *Kurtziana* 34, 7–365.

Bassman, J.H., Robberecht, R., Edwards, G.E., 2001. Effects of enhanced UV-B radiation on growth and gas exchange in *Populus deltoides* Bartr. *EX Marsh. International Journal of Plant Sciences* 162, 103–110.

Bavaro, L., Cattuelli, L., Depalo, N., Ventrella, A., Corcelli, A., Agostiano, A., 2007. Lipid content in higher plants under osmotic stress. *Bioelectrochemistry* 70, 12–17.

Bhuiyan, N.H., Hamada, A., Yamada, N., Rai, V., Hibino, T., Takabe, T., 2007. Regulation of betaine synthesis by precursor supply and choline monooxygenase expression in *Amaranthus tricolor*. *Journal of Experimental Botany* 58, 4203–4212.

Blunden, G., Yang, M.-H., Yuan, Z.-X., Smith, B.E., Patel, A., Cegarra, J.A., Mâthé, I., Janicsak, G., 1996. Betaine distribution in the Labiatae. *Biochemical Systematics and Ecology* 24, 71–81.

Bohlmann, F., Jakupovic, J., 1979. Neue sesquiterpene, triterpene, flavanone und andere aromatische Verbindungen aus *Flourensia heterolepis*. *Phytochemistry* 18, 1189–1194.

Bohlmann, F., Jakupovic, J., Schuster, A., King, R.M., Robinson, H., 1984. Eudesmanolides and cistic acid derivatives from *Flourensia macrophylla*. *Phytochemistry* 23, 1445–1448.

Caldwell, M.M., 1971. Solar ultraviolet radiation and the growth and development of higher plants. In: Giese, A.C. (Ed.), *Photophysiology*, vol. 6. Academic Press, New York, pp. 131–177.

Carillo, P., Mastrolonardo, G., Nacca, F., Parisi, D., Verlotto, A., Fuggi, A., 2008. Nitrogen metabolism in durum wheat under salinity: accumulation of proline and glycine betaine. *Functional Plant Biology* 35, 412–426.

Carillo, P., Parisi, D., Woodrow, P., Pontecorvo, G., Massaro, G., Annunziata, M.G., Fuggi, A., Sulpice, R., 2011. Salt-induced accumulation of glycine betaine is inhibited by high light in durum wheat. *Functional Plant Biology* 38, 139–150.

Chen, T.H.H., Murata, N., 2002. Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. *Current Opinion in Plant Biology* 5, 250–257.

Chen, T.H.H., Murata, N., 2008. Glycinebetaine: an effective protectant against abiotic stress in plants. *Trends in Plant Science* 13, 499–505.

Chen, T.H.H., Murata, N., 2011. Glycinebetaine protects plants against abiotic stress: mechanisms and biotechnological applications. *Plant, Cell and Environment* 34, 1–20.

Chen, Z., Cui, T.A., Zhou, M., Twomey, A., Naidu, B.P., Shabala, S., 2007. Compatible solute accumulation and stress-mitigating effects in barley genotypes contrasting in their salt tolerances. *Journal of Experimental Botany* 58, 4245–4255.

Cromwell, B.T., Rennie, S.D., 1953. The biosynthesis and metabolism of betaines in plants. The estimation and distribution of glycinebetaine (betaine) in *Beta vulgaris* L. and other plants. *Biochemical Journal* 55, 189–192.

Dawar, S., Vani, T., Singhal, G.S., 1998. Stimulation of antioxidant enzymes and lipid peroxidation by UV-B irradiation in thylakoid membrane of wheat. *Biologia Plantarum* 41, 65–73.

Dillon, M.O., 1984. A systematic study of *Flourensia* (Asteraceae, Heliantheae). *Fieldiana. Botany* 16, 1–67.

Einset, J., Connolly, E.L., 2009. Glycine betaine enhances extracellular processes blocking ROS signaling during stress. *Plant Signaling & Behavior* 4, 197–199.

Einset, J., Winge, P., Bones, A.M., Connolly, E.L., 2008. The FRO₂ ferric reductase is required for glycine betaine's effect on chilling tolerance in *Arabidopsis* roots. *Physiologia Plantarum* 134, 334–341.

Estell, R.E., Havstad, K.M., Fredrickson, E.L., Gardea Torresdey, J.L., 1994. Secondary chemistry of the leaf surface of *Flourensia cernua*. *Biochemical Systematics and Ecology* 22, 73–77.

Funk, V.A., Susanna, A., Steussy, T.F., Robinson, H.E., 2009. Classification of compositae. In: Funk, V.A., Susana, A., Steussy, T.F., Bayer, R.J. (Eds.), *Systematics, Evolution, and Biogeography of Compositae*. International Association for Plant Taxonomy (IAPT), Vienna, Austria, pp. 171–189.

Ghayal, N., Dhupal, K., 2011. Morphophysiological investigations in some dominant alien invasive weeds. In: Vasanthaian, H.K.N., Kambiranda, D. (Eds.), *Plants and Environment InTech open*, Croatia, pp. 15–48.

Giorgis, M.A., Cingolani, A.M., Chiarini, F., Chiappella, J., Barboza, G., Ariza Espinar, L., Morero, R., Gurvich, D.E., Tecco, P.A., Subils, R., Cabido, M., 2011. Composición florística del Bosque Chaqueño Serrano de la provincia de Córdoba, Argentina. *Kurtziana* 36, 9–43.

Gottlieb, H.E., Kotlyar, V., Nudelman, A., 1997. NMR chemical shift of common laboratory solvents as trace impurities. *Journal of Organic Chemistry* 62, 7512–7515.

Hanson, A.D., Wyse, R., 1982. Biosynthesis, translocation, and accumulation of betaine in sugar beet and its progenitors in relation to salinity. *Plant Physiology* 70, 1191–1198.

Hanson, A.D., Rathinasabapathi, B., Chamberlin, B., Gage, D.A., 1991. Comparative physiological evidence that beta-alanine betaine and choline-O-sulfate act as compatible osmolytes in halophytic *Limonium* species. *Plant Physiology* 97, 1199–1205.

Hattori, T., Mitsuya, S., Fujiwara, T., Jagendorf, A.T., Takabe, T., 2009. Tissue specificity of glycinebetaine synthesis in barley. *Plant Science* 176, 112–118.

Huang, J., Hirji, R., Adam, L., Rozwadowski, K.L., Hammerlindl, J.K., Keller, W.A., Selvaraj, G., 2000. Genetic engineering of glycinebetaine production toward enhancing stress tolerance in plants: metabolic limitations. *Plant Physiology* 122, 747–756.

INTA Manfredi (2006) Los Suelos, Nivel de Reconocimiento 1:500.000. In: Agencia Córdoba Ambiente S.E., Instituto Nacional de Tecnología Agropecuaria EEA Manfredi (Eds.), *Recursos Naturales de la Provincia de Córdoba, Serie C*. Accesible on Nov 2013 in www.inta.gov.ar.

Krasensky, J., Jonak, C., 2012. Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *Journal of Experimental Botany* 63, 1593–1608.

Lerma, C., Hanson, A.D., Rhodes, D., 1988. Oxygen-18 and deuterium labeling studies of choline oxidation by spinach and sugar beet. *Plant Physiology* 88, 695–702.

Lo, M., Taylor, C., Wang, L., Nowack, L., Wang, T.W., Thompson, J., 2004. Characterization of an ultraviolet B-induced lipase in *Arabidopsis*. *Plant Physiology* 135, 947–958.

- López, D., Piazza, L.A., Silva, M.P., López Rivilli, M.J., Cantero, J.J., Tourn, G.M., Scopel, A.L., 2013. Distribution of (–)-hamanasic acid A in South American species of *Flourensia* and phytotoxic effects of leaf aqueous extracts. *Natural Product Communications* 9, 341–345.
- Luti, R., Beltrán de Solís, M., Galera, F.M., Muller de Ferreira, N., Berzal, M., Nores, M., Herrera, M., Barrera, J.C., 1979. Vegetación. In: Vazquez, J., Miatelo, R., Roque, M. (Eds.), *Geografía Física de la Provincia de Córdoba*, Boldt, Buenos Aires, pp. 297–368.
- Mäkelä, P., Peltonen-Sainio, P., Jokinen, K., Pehu, E., Setälä, H., Hinkkanen, R., Somersalo, S., 1996. Uptake and translocation of foliar-applied glycinebetaine in crop plants. *Plant Science* 121, 221–230.
- Mazza, C.A., Boccacalandro, H.E., Giordano, C.V., Battista, D., Scopel, A.L., Ballaré, C.L., 2000. Functional significance and induction by solar radiation of ultraviolet-absorbing sunscreens in field-grown soybean crops. *Plant Physiology* 122, 117–125.
- McNeil, S.D., Nuccio, M.L., Hanson, A.D., 1999. Betaines and related osmoprotectants. Targets for metabolic engineering of stress resistance. *Plant Physiology* 120, 945–949.
- Medeiros, J.S., Pockmand, W.T., 2011. Drought increases freezing tolerance of both leaves and xylem of *Larrea tridentata*. *Plant, Cell & Environment* 34, 43–51.
- Mohammed, A.R., Tarpley, L., 2013. Effects of enhanced ultraviolet-B (UV-B) radiation and antioxidative-type plant growth regulators on rice (*Oryza sativa* L.). Leaf photosynthetic rate, photochemistry and physiology. *Journal of Agricultural Science* 5, 115–128.
- Nishimura, N., Zhang, J., Abo, M., Okubo, A., Yamazaki, S., 2001. Application of capillary electrophoresis to the simultaneous determination of betaines in plants. *Analytical Sciences* 17, 103–106.
- Nuccio, M.L., Russell, B.L., Nolte, K.D., Rathinasabapathi, B., Gage, D.A., Hanson, A.D., 1998. The endogenous choline supply limits glycine betaine synthesis in transgenic tobacco expressing choline monooxygenase. *The Plant Journal* 16, 487–496.
- Ogle, K., Reynolds, J.F., 2002. Desert dogma revisited: coupling of stomatal conductance and photosynthesis in the desert shrub, *Larrea tridentata*. *Plant, Cell and Environment* 25, 909–921.
- Park, E.-J., Jeknić, Z., Chen, T.H.H., Murata, N., 2007. The cod A transgene for glycinebetaine synthesis increases the size of flowers and fruits in tomato. *Plant Biotechnology Journal* 5, 422–430.
- Piri, E., Babaeian, M., Tavassoli, A., Esmaeilian, Y., 2011. Effects of UV irradiation on plants. *African Journal of Microbiology Research* 5, 1710–1716.
- Priotti, Z.E., Zygadlo, J.A., Ariza Espinar, L., 1997. Essential oils of *Flourensia olepis* S. L. Blake. *Journal of Essential Oil Research* 9, 345–347.
- Rhodes, D., Hanson, A.D., 1993. Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 44, 357–384.
- Rhodes, D., Rich, P.J., Myers, A.C., Reuter, C.C., Jamieson, G.C., 1987. Determination of betaines by fast atom bombardment mass spectrometry: identification of glycine betaine deficient genotypes of *Zea mays*. *Plant Physiology* 84, 781–788.
- Rios, M.Y., Estrada-Soto, S., Flores-Morales, V., Aguilar, M.Y., 2013. Chemical constituents from *Flourensia resinosa* S.F. Blake (Asteraceae). *Biochemical Systematics and Ecology* 51, 240–242.
- Robinson, H., 1981. A revision of the tribal and subtribal limits of the Heliantheae (Asteraceae). *Smithsonian Contributions to Botany* 51, 1–102.
- Robinson, S.P., Jones, G.P., 1986. Accumulation of glycinebetaine in chloroplasts provides osmotic adjustment during salt stress. *Australian Journal of Plant Physiology* 13, 659–668.
- Rosenstein, R., Futter-Bryniok, D., Götz, F., 1999. The choline-converting pathway in *Staphylococcus xylosum* C2A: genetic and physiological characterization. *Journal of Bacteriology* 181, 2273–2278.
- Silva, M.P., Piazza, L.A., López, D., López Rivilli, M.J., Turco, M.D., Cantero, J.J., Tourn, M.G., Scopel, A.L., 2012. Phytotoxic activity in *Flourensia campestris* and isolation of (–)-hamanasic acid A as its active principle compound. *Phytochemistry* 77, 140–148.
- Stewart, G.R., Larher, F., Ahmed, I., Lee, J.A., 1979. Nitrogen metabolism and salt-tolerance in higher plant halophytes. In: Jefferies, R.L., Davy, A.J. (Eds.), *Ecological Processes in Coastal Environments*. Blackwell Scientific Publications, Oxford, pp. 211–227.
- Storey, R., Wyn Jones, R.G., 1975. Betaine and choline levels in plants and their relationship to NaCl stress. *Plant Science Letters* 4, 161–168.
- Storey, R., Ahmad, N., Wyn Jones, R.G., 1977. Taxonomic and ecological aspects of the distribution of glycinebetaine and related compounds in plants. *Oecologia* 27, 319–332.
- Sulpice, R., Tsukaya, H., Nonaka, H., Mustardy, L., Chen, T.H.H., Murata, N., 2003. Enhanced formation of flowers in salt-stressed *Arabidopsis* after genetic engineering of the synthesis of glycine betaine. *The Plant Journal* 36, 165–176.
- Takabe, T., Rai, V., Hibino, T., 2006. Metabolic engineering of glycinebetaine. In: Rai, A.K., Takabe, T. (Eds.), *Abiotic Stress Tolerance in Plants: Toward the Improvement of Global Environment and Food*. Springer, Dordrecht, pp. 137–151.
- Uriburu, M.L., De la Fuente, J.R., Palermo, J., Gil, R.R., Sosa, V.E., 2004. Constituents of two *Flourensia* species. *Phytochemistry* 65, 2039–2043.
- Uriburu, M.L., Gil, R.R., Sosa, V.E., De la Fuente, J.R., 2007. Prenylflavonoids from *Flourensia fiebrigii*. *Phytochemistry* 68, 1295–1299.
- Urzúa, A., Santander, R., Echeverría, J., 2007. Analysis of surface and volatile compounds of flower heads of *Flourensia thurifera* (Mol) D.C. *Journal of the Chilean Chemical Society* 52, 1244–1245.
- USDA, 2004. Database for the choline content of common foods. Prepared by Howe CH, Williams JR, Holden JM, Zeisel SH, Mar MH Accessible on April 2012 in: <http://www.nal.usda.gov/fnic/foodcomp/Data/Choline/Choline.html>.
- Wyn Jones, R.G., Storey, R., 1981. Betaines. In: Paleg, L.G., Aspinall, D. (Eds.), *The Physiology and Biochemistry of Drought Resistance in Plants*. Academic Press, Sydney, pp. 171–204.
- Zavala, J.A., Ravetta, D.A., 2002. The effect of solar UV-B radiation on terpenes and biomass production in *Grindelia chiloensis* (Asteraceae), a woody perennial of Patagonia, Argentina. *Plant Ecology* 161, 185–191.
- Zhang, L., Gao, M., Hu, J., Zhang, X., Wang, K., Ashraf, M., 2012. Modulation role of abscisic acid (ABA) on growth, water relations and glycinebetaine metabolism in two maize (*Zea mays* L.) cultivars under drought stress. *International Journal of Molecular Sciences* 13, 3189–3202.