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Short communication

Sardinian Boraginaceae are new potential sources of gamma-linolenic acid

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ABSTRACT

The aim of this work was to establish the richness in γ-linolenic acid (GLA, 18:3n6) and stearidonic acid (SDA, 18:4n3) of several Sardinian Boraginaceae species. To this end, seeds of selected species were collected from their natural habitats and analysed. The highest GLA contents were found in the seed oils of two endemic *Borago* taxa, i.e. *B. morisiana* (24.4 and 24.6% GLA of total fatty acids for samples from San Pietro Island and Sardinia Island, respectively), and 22.9% GLA for *B. pygmaea*. Both *Borago* species contained more GLA than *B. officinalis* collected in the same ecosystems. SDA was found in significant amounts in *Echium plantagineum* seed oil from the Lattias Mountains (15% SDA of total fatty acids). It is notable that both *Borago* GLA-rich species are under threat of extinction, thus revealing the importance of the preservation of the natural Sardinian ecosystems for endangered species and human health.

1. Introduction

γ-Linolenic acid (GLA, *all-cis*-6,9,12-octadecatrienoic acid, 18:3n6) and stearidonic acid (SDA, *all-cis*-6,9,12,15-octadecatetraenoic acid, 18:4n3) are polyunsaturated fatty acids (PUFAs) belonging to the n – 6 and n – 3 series, respectively. Both FAs are produced in the body from their metabolic precursors linoleic acid (LA, *all-cis*-9,12-octadecadienoic acid, 18:2n6) and α-linolenic acid (ALA, *all-cis*-9,12,15-octadecatrienoic acid, 18:3n3), respectively, through the action of the enzyme Δ6-desaturase. GLA is further metabolized to dihomo-γ-linolenic acid (DGLA, *all-cis*-8,11,14-eicosatrienoic acid, 20:3n6), which undergoes oxidative metabolism by cyclooxygenases and lipoxygenases to produce anti-inflammatory eicosanoids (series-1 prostaglandins and series-3 leukotrienes) that are hormone-like bioactive compounds involved in the regulation of various physiological mechanisms in animals and humans (Guil-Guerrero, 2007; Horrobin, 1992; Meesapyodsuk & Qiu, 2012). In addition, GLA and its metabolites also affect the expression of various genes, having a significant role in immune functions and apoptosis (Kapoor & Huang, 2006), and several studies indicate that GLA possesses anti-cancer properties, including inhibition of cell proliferation and induction of apoptosis (Menéndez et al., 2001; Xu & Qian, 2015). Furthermore, recent works have attributed prominent health benefits to dietary supplementation with GLA, such as improved blood lipid profile and skin perspiration, showing promising effects in the treatment of dermatitis, skin hyperproliferation and osteoporosis among other syndromes (Kawamura et al., 2011; Tasset-Cuevas et al., 2013; Tso, Caldwell, Lee, Boivin, & DeMichele, 2012). Consequently, given its widely reported beneficial physiological actions, GLA is used increasingly in the cosmetic (Griinari & Bruheim, 2013) and food industries (Flider, 2005).

SDA is elongated and desaturated to eicosapentaenoic acid (EPA, *all-cis*-5,8,11,14,17, 20:5n3), which is the precursor of anti-inflammatory eicosanoids (series-3 prostaglandins and series-5 leukotrienes), and docosahexaenoic acid (DHA, *all-cis*-4,7,10,13,16,19, 22:6n3), which is required for the maintenance of normal brain function in adults (Guil-Guerrero, 2007). There is great interest in SDA-rich oils because the biosynthesis of EPA starting from SDA is much more efficient than from ALA (Guil-Guerrero, 2007). Both GLA and SDA sources are scarce in nature. Despite some microbiological cultures, GLA is primarily obtained

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from three plants: borage (*Borago officinalis* L., 21–23% GLA of total FAs); evening primrose (*Oenothera biennis* L., 9–12% GLA of total FAs); and blackcurrant (*Ribes nigrum* L., 15–20% GLA of total FAs) (Guil-Guerrero, García-Maroto, & Giménez-Giménez, 2001; Gunstone, 1992). The percentage of SDA in oils is low, with only *Buglossoides arvensis* (>14% SDA of total FAs) (Guil-Guerrero et al., 2001) containing significant amounts and, because of this, the oil from this species has been patented (Hebard, Coupland, Boughton, & Surette, 2008). The intake of oils rich in GLA and SDA reduces the amount of oil needed to achieve any health benefits compared to oils with lower amounts of these PUFAs as well as caloric intake. Furthermore, GLA and SDA purification processes can be carried out more easily when starting with GLA- or SDA-rich oils. Thus, the search for such oils is on-going. Boraginaceae species have been surveyed for seed oils with a high content of both GLA and SDA (Guil-Guerrero, 2007), and several endemic Boraginaceae species have been identified as potential new sources of GLA and SDA (Guil-Guerrero, Gómez-Mercado, Ramos-Bueno, Rincón-Cervera, & Venegas-Venegas, 2014; Guil-Guerrero, Rincón-Cervera, Gómez-Mercado, Ramos-Bueno, & Venegas-Venegas, 2013).

Interestingly, some of the best species, in terms of GLA and SDA contents, are being cultivated as SDA sources, e.g. *Echium plantagineum* and *Buglossoides arvensis*. If GLA is the target PUFA, *Borago officinalis* is still the most suitable option. However, some *Borago* species, occurring mainly in Tyrrhenian islands (Mediterranean Basin), remain unanalysed. These places are considered centres of relictual endemism of Boraginaceae and a source of some endemic *Borago* species (Médail & Quézel, 1999).

This paper focuses on the characterization of FA profiles of several Sardinian Boraginaceae species, which have remained unanalysed until now. Among them, two *Borago* species showed higher GLA contents than either wild or farmed *B. officinalis*.

2. Experimental procedures

2.1. Sample collection

Seeds were collected from their natural habitats (Table 1). Upon arriving at the laboratory, after cleaning and labelling, seeds were placed in a glass desiccator until analysis.

2.2. Oil extraction and transesterification and fatty acid analyses

Seeds were ground with the aid of a mortar, and then 150–200 mg were taken for further analysis. Extraction and trans-esterification were simultaneous, and FA analyses and quality control were carried out according to previous reports (Guil-Guerrero et al., 2013, 2014). Briefly, FA methyl esters (FAMEs) were analysed using a Focus GC (Thermo Electron, Cambridge, UK) equipped with a flame ionization detector (FID) and an Omegawax 250 capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness; Supelco, Bellefonte, USA). Peaks were identified by retention times obtained for known FAME standards (PUFAs No. 1, 47033; methyl γ-linolenate 98.5% purity, L6503; and methyl stearidonate 97% purity, 43959 FLUKA) from Sigma, (St. Louis, USA), while FA contents were estimated by using methyl pentadecanoate (15:0; 99.5% purity; 76560 Fluka) from Sigma as internal standard.

FA compositions (FA% of total FAs area), expressed as mean value obtained from three different samples analysed in triplicate, are given in Table 2. Standard deviations (SD) were routinely less than 5% of means.

Table 1
Data on collection of Sardinian Boraginaceae species.

Species	Sample location	Herbarium code	Geographical coordinates	Collection date
Tribe Boragineae				
Anchusa capellii Moris	Cagliari: Esterzili. Sta. Vittoria mountain	HUAL 25607	N 39.759 E 9.304	07/04/2014
Anchusa crispa Viv. subsp. *maritima* (Vals.) Selvi & Bigazzi	Olvia-Tempio. Isola Rossa	HUAL 25609	N 41.015 E 8.887	05/12/2013
Anchusa crispa Viv. subsp. *maritima* (Vals.) Selvi & Bigazzi	Olvia-Tempio. Pirotto Li Fratti. Bahía delle Mimose	HUAL 25612	N 40.945 E 8.826	05/12/2013
A. formosa Selvi, Bigazzi & Bacchetta	Cagliari: Uta. Arcosu mountain	–	N 39.185 E 5.854	06/20/2013
Borago morisiana Bigazzi *et* Ricceri	Isola S. Pietro. Carbonia Iglesias: Carloforte. Calavinagra	HUAL 25639	N 39.163 E 8.242	07/03/2014
Borago morisiana Bigazzi *et* Ricceri	Oristano: Laconi. Tanca de Cuccuru	HUAL 25965	N 39.874 E 9.091	07/04/2014
Borago officinalis L.	Cagliari. Near Lattias mountain.	HUAL 25637	N 39.144 E 8.861	05/09/2013
Borago officinalis L.	Medio Campidano: Gonnosfanadiga. Linas mountain	HUAL 25633	N 39.435 E 8.624	05/12/2013
Borago pygmaea (DC.) Chater & Greuter	Ogliastra: Gairo. Montarbu di Seui, Girolamo river	HUAL 25608	N 39.839 E 9.455	07/04/2014
Tribe Echieae				
Echium anchusoides Bacchetta, Brullo & Selvi	Medio Campidano: Gonnosfanadiga. Linas mountain, near Punta Cammedda	HUAL 25603	N 39.436 E 8.638	07/06/2014
Echium italicum L.	Cagliari: Donori	HUAL 25601	N 39.451 E 9.155	07/07/2014
Echium plantagineum L.	Oristano: Assolo	HUAL 25602	N 39.818 E 8.916	07/06/2014
Echium plantagineum L.	Cagliari. Near Lattias mountains	HUAL 25640	N 39.148 E 8.860	05/09/2013
Echium sabulicolum Pomel	Carbonia-Iglesias. Buggerru: Costa Verde (Near Cala Domestica)	HUAL 25636	N 39.364 E 8.398	05/10/2013
Tribe Cynoglosseae				
Cynoglossum barbaricinum Arrigoni & Selvi	Nuoro: Orgosolo. Monte Novo San Giovanni	HUAL 25604	N 40.117 E 9.415	07/05/2014
Cynoglossum officinale L.	Cagliari. Near Lattias mountain	HUAL 25634	N 39.148 E 8.860	05/10/2013
Tribe Eritrichieae				
Myosotis arvensis (L.) Hill	Carbonia-Iglesias. Buggerru: Costa Verde (Near Cala Domestica)	HUAL 25635	N 39.364 E 8.398	05/10/2013
Myosotis soleirolii Godr.	Ogliastra: Villagrande Strisaili. Bruncu Spina	HUAL 25606	N 40.016 E 9.301	07/05/2014

Table 2
Fatty acid profiles of seeds from Sardinian Boraginaceae.

Species[b]	Fatty acids (FA% of total FAs)[a]																	FA amount (g/100 g seed)
	12:0	14:0	16:0	16:1n7	18:0	18:1n9	18:1n7	18:2n6	18:3n6	18:3n3	18:4n3	20:0	20:1n9	22:0	22:1n9	24:0	24:1n9	
Tribe Boragineae																		
07 *Anchusa capellii*	–	–	9.1	–	3.0	30.0	–	22.2	12.0	12.4	4.0	–	4.4	0.3	2.3	0.1	0.4	24.2
09 *Anchusa crispa* ssp. *maritima*	–	–	9.2	–	2.6	26.5	–	23.7	12.3	12.9	3.6	0.3	4.1	0.2	4.0	0.2	0.5	16.9
12 *Anchusa crispa* ssp. *maritima*	–	–	10.9	–	4.7	25.3	–	23.3	11.7	13.2	3.3	0.3	3.5	0.3	3.1	–	0.4	18.6
- *Anchusa formosa*	–	–	9.4	–	2.4	25.0	–	21.4	14.0	15.3	5.1	0.4	4.8	–	2.0	–	0.4	24.8
39 *Borago morisiana*	0.2	0.8	13.2	0.2	5.9	16.6	0.3	33.0	23.4	1.1	0.8	0.5	3.1	–	–	–	1.1	24.2
65 *Borago morisiana*	0.3	1.0	11.6	0.28	4.6	14.9	0.5	34.1	24.6	1.4	1.0	0.1	2.5	0.3	1.9	–	1.15	15.9
37 *Borago officinalis*	–	–	10.0	0.2	8.7	22.0	–	31.5	19.2	0.4	0.3	0.5	3.6	0.3	1.8	0.1	1.3	27.9
33 *Borago officinalis*	–	–	10.9	–	8.7	22.7	–	31.4	16.2	0.9	0.7	0.6	4.1	0.4	2.2	–	1.3	35.1
08 *Borago pygmaea*	–	–	14.0	–	6.7	20.0	–	27.4	22.9	1.3	1.2	0.5	2.8	0.3	1.9	0.2	0.8	21.9
Tribe Echieae																		
03 *Echium anchusoides*	–	–	8.4	–	3.9	16.6	–	19.5	8.8	32.7	9.4	–	0.6	0.3	–	–	–	25.4
01 *Echium italicum*	–	–	8.5	–	3.9	13.9	–	13.4	5.6	43.4	10.4	0	0.4	–	0.3	–	0.2	16.6
02 *Echium plantagineum*	2.9	–	27.9	–	4.5	8.3	–	26.2	7.4	3.9	15.0	3.7	–	–	0.3	–	0.2	2.05
40 *Echium plantagineum*	–	–	9.6	–	3.2	16.8	–	21.1	9.3	30.9	8.3	–	0.7	–	–	–	–	18.8
36 *Echium sabulicolum*	–	–	8.9	–	3.7	11.6	–	18.8	9.5	35.7	11.3	–	0.6	–	–	–	–	18.0
Tribe Cynoglosseae																		
04 *Cynoglossum barbaricinum*	–	–	17.4	–	3.5	42.0	–	4.3	1.0	17.1	4.1	0.8	4.1	0.4	4.0	–	1.5	4.0
34 *Cynoglossum officinale*	–	1.1	9.4	–	1.6	49.2	–	2.2	0.5	13.6	3.7	1.1	5.9	1.2	7.4	0.5	2.7	3.3
Tribe Eritrichieae																		
35 *Myosotis arvensis*	–	–	10.9	–	2.7	25.1	–	27.5	6.1	12.0	8.3	0.6	3.5	0.4	2.2		0.9	26.4
06 *Myosotis soleirolii*	–	–	11.9	–	4.2	35.0	–	27.4	4.4	5.7	6.4	1.0	4.3	–	–	–	–	25.5

[a] SD was routinely less than 5% of means (n = 3).
[b] The two numbers preceding each species are the last two digits of the herbarium code shown in Table 1.

3. Results and discussion

Basic data for the collected samples are shown in Table 1, while the FA profiles of seed oils are reported in Table 2, grouped by tribes. This is because the FA profiles of seed oils from Boraginaceae species have taxonomic significance due to the differential activities of the enzyme Δ6-desaturase, which preferentially desatures LA to GLA, but also ALA to SDA, and determines PUFA profiles in Boraginaceae oils (García-Maroto, Mañas-Fernández, Garrido-Cárdenas, & López Alonso, 2006). Boragineae are characterized by high percentages of LA and GLA; Echieae by high percentages of n-3 PUFAs, i.e. ALA and SDA; Cynoglosseae by high oleic acid (OA, 18:1n9) percentages; and Eritrichieae by similar percentages of OA and LA. All these observations are in agreement with previous findings (Guil-Guerrero et al., 2001, 2013, 2014; Velasco & Goffman, 1999; Özcan, 2008).

The highest GLA percentage was found in the seeds of the two endemic *Borago* surveyed, i.e. *B. morisiana* (24.4 and 24.6% GLA for San Pietro Island and Sardinia Island respectively), and *B. pygmaea* (22.9% GLA). When comparing the amounts of total FAs/100 g seeds in samples of different origin, *B. morisiana* from San Pietro Island contained 24.2 g FAs/100 g seeds, while only 15.9 g FAs/100 g seeds were found in the same species from Sardinia Island. This could be attributed to differences in environmental conditions, although in both cases the plant was found on the margins of streams; San Pietro samples were however near the sea while those from Sardinia Island were in the mountains. Differences in temperature, humidity, soil composition and other abiotic factors could influence composition, although genetic differences between the two analysed populations might also contribute, despite the closeness of their FA profiles otherwise.

Seeds from other two *B. officinalis* populations were collected containing 19.2 and 16.2% GLA, respectively (Table 2). Thus, in the wild, the two endemic *Borago* taxa analysed surpassed the GLA% found in *B. officinalis*. However, after careful selection, *B. officinalis* seed oil can reach 21–23% GLA (Guil-Guerrero et al., 2001; Gunstone, 1992). Potentially, the two endemic *Borago* species analysed in this work might benefit from using the same techniques to achieve much higher GLA percentages than *B. officinalis*. Other species with high GLA percentages were the four *Anchusa* species surveyed (11.7–14.0% GLA of total FAs).

Echium plantagineum seed oil from the Lattias Mountains contained the highest SDA percentage (15% SDA of total FAs) followed by *E. sabulicolum* and *E. italicum* (11.3 and 10.4% SDA of total FAs respectively). Surprisingly, this *E. plantagineum* sample was very different from others samples collected in Assolo, (8.3% SDA) with also ten times more ALA. When comparing both FA profiles with figures reported for the same species (Guil-Guerrero, 2007), the sample from Assolo agreed with previous reports. Thus, the sample from the Lattias Mountains seems to constitute a different chemotype. Although both samples showed morphological characteristics compatible with *E. plantagineum*, they presented some morphological differences in habit and indument.

Considering the whole seed weight, total FA amount ranged between 4% in *Cynoglossum barbaricinum* and 35.4% in *B. officinalis* from the Lattias Mountains. Based on the FA percentage contained in the seeds of *B. morisiana* from San Pietro Island (24.2% of total seed weight) and the GLA percentage (24.4%), ca. 6 g GLA/100 g seeds would be expected in this species. *E. anchusoides* seeds produced the highest SDA amount (2.4 g SDA/100 g seeds). Considering that the oil content could be easily modified by agronomic practices or culture lines selection (Berti et al., 2007), GLA percentage or the amount of oil produced might be significantly increased. However, an increase in oil content does not necessarily lead to an increased GLA amount, so careful crop selection considering both factors would be necessary. Further studies to assess which climatic areas would be appropriate to grow these species are also required.

It is notable that *B. morisiana*, which was the best species for GLA production, is under threat of extinction; both populations, which are the only ones known, show a steady reduction in numbers of individuals (Bacchetta, Fenu, & Mattana, 2008). Thus, environmental authorities should take measures to preserve and improve their natural habitats: results from this work clearly point out towards potential uses as agronomic resources of some wild endangered species, as sources of beneficial FAs, to enhance the economic development of the areas where they grow.

In conclusion, both endemic *Borago* taxa from Sardinia analysed in this work contained more GLA than either farmed or wild *B. officinalis*. On this basis, these plants might be considered as suitable candidates for cultivation and selection with an interesting potential in terms of agronomic aptitudes and/or GLA percentage. Other target actions for research and development of new GLA-producing taxa could be hybridization and further selection between these species or between any of them with cultured borage as well as application of genetic engineering tools for Δ-6 desaturase gene transfer from these plants to other Boraginaceae species in order to increase the GLA percentage in their seed oils.

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