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Borago Officinalis L. Foliar Fatty Acids

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Abstract: Fatty Acid (FA) composition of rosette and stalk leaves from *Borago officinalis* L. collected at intervals over the growing season from two different regions in Tunisia was studied. Total Fatty Acids (TFA) amount was higher in Semmech than Amdoun for both rosette and stalk leaves. In both regions, TFA amount was maximal in December, minimal at the beginning of November for rosette. In stalk leaves, TFA amount was higher at the end of March and lower in May. The predominant FA in rosette and stalk leaves were γ-linolenic, stearidonic, palmitic (C16:0), linoleic (LA, C18:2 ω6) and γ-linolenic acids while palmitoleic, oleic, stearic and arachidic acids were the minor ones.

Key words: *Borago officinalis*, borage, fatty acid, rosette leaves, stalk leaves

Introduction

Borage (*Borago officinalis* L.) is a member of the Boraginaceae family and native to some parts of the Mediterranean region (Baubaire and Simon, 1987). This plant represents valuable source of a wide spectrum of complex lipids with different potential applications. Mediterranean Boraginaceae family has been the object of various studies and it has been proposed as potential renewable source of unsaturated and uncommon FA (Guil-Guerrero *et al*., 2003). Currently, a great deal of attention has been given to the *Borago officinalis* L. seed oil.

Gómez and Martínez de la Ossa (2002) found that the seeds are rich in γ-linolenic acid (GLA, C18:3 ω6) whose percentage ranged from 21 to 23% on TFA as reported by Guil-Guerrero *et al*. (2003). Hamrouni *et al*. (2002) showed that oil yield of borage seed was 30-35% by weight, of which 17-25% is GLA. Thus, this species was considered as the best crop source of GLA (Gunstone, 1992).

Borage seed oil extracted by supercritical carbon dioxide showed that linoleic (LA, C18:2 ω6), γ-linolenic and oleic (C18:1 ω9) acids were the predominating Unsaturated Fatty Acids (UFA) while palmitic acid (C16:0) was the main Saturated Fatty Acid (SFA) (Gómez and Martínez de la Ossa, 2002). FA profile in leaves was characterized by the prevalence of two polyunsaturated fatty acids (PUFA): α-linolenic acid (ALA, C183 ω3) followed by stearidonic acid (SDA, C18:4 ω3) (Sayanova *et al*., 1999a, b; Griffiths *et al*., 1996). They constituted essential FA in leaves and are precursors of n-3 PUFA (Yamazaki *et al*., 1992).

GLA have potential nutritional, pharmaceutical and industrial applications. It is involved in membrane structure in various cells and in the biosynthesis of very long-chain PUFA, both in humans and animals (Gunstone, 1992). Dietary supplementation with GLA reduces the risks of heart disease, diabetes, eczema, arthritis, multiples sclerosis and cyclical mastalgia (Horrobin, 1997). As a result, there is considerable interest in the large-scale production of GLA in oil seed crops. More recently, genetic manipulation of *Brassica juncea* resulted in production of high oil quality with an appreciable percentage of GLA which accounted for 40% of total seed FA (Hong *et al*., 2002).

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In this study, FA composition of Tunisian borage leaves from Semmech and Amdoun regions was described.

Materials and Methods

Plant Material

Borago officinalis L. rosette and stalk leaves were collected from two different regions from Tunisia: Amdoun (Northwest) and Semmech (Northeast) during growth cycle. Rosette leaves were sampled from the beginning of November (2004) to the end of January (2005) and from the beginning of February to the end of May 2005 for stalk leaves.

Total Lipids Extraction

Total lipids (TL) from rosette and stalk leaves were extracted by the modified method of Bligh and Dyer (1959). One gram of fresh leaves was fixed by boiling water in order to deactivate the phospholipases (Benson and Strickland, 1960; Douce, 1964). Then, the sample was ground in a mortar in a mixture of solvents: chloroform/methanol (2:1, v/v).

Fatty Acid Methylation

Fatty acids were converted to Fatty Acids Methyl Esters (FAMEs) by using sodium methylate according to the method described by Cecchi *et al*. (1985). For FA quantification, a known quantity of methyl heptadecanoate (17:0) used as internal standard was added during methylation.

Chromatographic Analysis

Fatty acids methyl esters (FAMEs) from each sample were analysed by GC, using a Hewlett-Packard 6890 gas chromatograph (Palo Alto, CA) equipped with a flame-ionization detector (FID) and an Electronic Pressure Control (EPC) injector. A polyethylene glycol fused silica capillary column (HP-Innowax: 30 m×0.25 mm ID, 0.25 μm film thickness) purchased from Agilent (Wilmington, DE) was used. The flow of the carrier gas (N_2) was 1.6 mL min^{-1}. The split ratio in the injector was 60:1. The detector and injector temperatures were set at 275 and 250°C, respectively. The initial oven temperature was held at 150°C for 1 min^{-1}, increased at a rate of 15°C min^{-1} to 200°C and then held there for 3 min and finally ramped at 2°C min^{-1} to 242°C. Fatty acid (FA) were identified by comparison of their retention times with those of pure reference standards.

Statistical Analysis

Fatty acid composition is expressed as weight percentages of TFA and also as means±SD of three determinations. The t-test and p-value of <0.05 to check for significant differences between means at different sampling date were used. All analyses were performed by the statistical package KyPlot v 2.0 beta 15.

Results and Discussion

Total fatty acids (TFA) amounts of analysed samples are given in Table 1. In both rosette and stalk leaves, TFA amounts from Semmech were significantly ($p<0.05$) higher than Amdoun ones. On the other hand, rosette TFA amount exhibited a wide variability, ranging from 22.96±1.76 in the beginning of November to 33.38±0.81 mg g^{-1} DW in December and from 5.03±0.07 to 21.38±0.81 mg g^{-1} DW respectively in Semmech and Amdoun samples.

In both regions, the TFA amount in stalk leaves was higher at the end of March (21.33±1.56 and 31.75±2.08 mg g^{-1} DMW in Amdoun and Semmech, respectively) and decreased significantly ($p<0.05$)

Table 1: Total fatty acids amounts (mg g^{-1} DW) in both rosette and stalk leaves of *Borago officinalis* L. from two different Tunisian regions

	Semmech	Amdoun
Rosette leaves		
November 2004: Beginning	22.96±1.76	5.03±0.07
November 2004: Middle	26.7±1.15	16.58±0.68 ***
November 2004: End	31.48±0.61	17.49±0.84
December 2004	33.38±1.83	20.38±0.81 *
January 2005	20.32±0.38 ***	6.04±0.2 ***
Stalk leaves		
February 2005	18.25±0.96	12.48±1.2
March 2005: Beginning	18.71±0.71	14.57±0.9
March 2005: End	31.75±2.08 ***	14.97±0.7
April 2005	14.84±2.03 ***	21.33±1.56
May 2005	8.02±0.46 *	15.51±0.6

*** Values are significant at $p<0.001$, *Values are significant at $p<0.05$

Table 2: Seasonal variation in fatty acid composition of borage rosette and stalk leaves from two Tunisian localities (Semmech and Amdoun)

	Total fatty acids (%)									
	Rosette leaves					Stalk leaves				
	November 2004: beginning	November 2004: middle	November 2004: end	December 2004: middle	January 2005: beginning	February 2005:	March 2005: beginning	March 2004: end	April 2004:	May 2005:
Semmech										
C16:0	16.5±1.3	15.6±1.1	12.8±1.4	13.3±0.5	14.2±1.3	19.0±0.1	16.0±1.2	17.6±1.4	14.0±0.8	20.6±2.3
cis C16:1	3.1±2.9	0.8±0.5	0.7±0.2	0.6±0.2	-	-	-	-	-	-
trans C16:	14.4±0.6	1.7±0.6	2.1±0.02	2.8±0.6	1.7±0.3	2.0±2.1	1.1±1.2	1.4±0.2	1.7±0.9	3.8±3.4
C18:0	2.0±0.2	2.1±0.2	2.5±0.7	1.8±0.1	1.3±0.4	0.5±0.5	0.7±1.58	1.6±2.1	1.7±3.3	2.5±1.6
C18:1 ω9	4.4±0.6	6.3±2.8	7.1±0.9	3.3±1.0	1.7±1.2	10.1±0.7	8.5±1.5	7.6±1.6	2.8±2.1	3.2±2.4
C18:2 ω6	15.6±1	13.7±1	14.2±1.7	10.5±1.0	9.3±1.2	13.6±4.1	11.1±1.5	8.2±1.7	5.5±2.5	5.0±3.9
C18:3 ω6	12.8±1.9	10.9±1.6	8.8±0.1	4.9±1.8	5.6±0.05	7.4±2.7	10.9±3.6	8.0±1.9	5.0±3.4	6.9±0.6
C18:3 ω3	23.8±1.7	26.3±0.7	28.4±1.6	36.4±0.1	36.8±1.2	26.8±2.4	30.2±1.9	32±2.7	41.2±36	26.8±2.4
C18:4 ω3	16.8±1.9	18.2±0.7	19.4±0.1	22.2±1.6	18.4±0.2	17.3±2.6	19.5±3.1	20.4±1.5	25.9±24	18.6±2.8
C20:0	1.7±1.4	4.2±1.3	3.4±0.8	4.5±0.6	7.9±0.5	2.1±1.6	2.7±1.8	3.1±3.1	3.8±1.5	10.5±2.9
Amdoun										
C16:0	13.9±0.2	14.5±0.7	16.9±2	14.3±1.2	11.8±2.2	21.2±3.9	21.2±2.3	16.6±1.4	17.9±2.1	20.6±3.5
cis C16:1	1.5±0.1	0.1±0.01	0.8±0.1	0.8±0.6	0.9±1.1	-	-	-	-	-
trans C16:	12.9±0.04	1.3±0.3	0.9±0.1	1.5±0.6	3.7±1.1	0.2±0.02	1.7±0.1	1.7±0.31	.6±0.8	1.6±0.4
C18:0	2.6±0.1	2.1±0.3	2.5±0.1	2.5±0.8	2.3±0.9	2.9±0.18	1.8±0.33	2.3±0.2	1.6±0.02	1.1±0.2
C18:1 ω9	6.5±1.2	3.3±1.1	2.6±0.4	2.1±0.15	2.1±0.9	4.2±0.5	3.3±0.4	5.3±0.6	3.5±0.2	-
C18:2 ω6	11.4±0.5	11.5±1.3	11.6±0.9	11.1±1.3	8.6±1.4	14.1±2.4	9.5±3.2	9.2±0.8	8.1±0.6	12.5±2.1
C18:3 ω6	8.0±1.6	9.9±1.1	11.3±1.1	8.0±1.4	5.9±0.3	17.5±2.6	10.6±3.1	6.6±1.5	3.4±4.3	7.4±2.4
C18:3 ω3	26.0±1.2	28.8±2.2	29.3±1.1	33.6±0.8	33.1±4.8	22.2±2.6	30.1±3.1	34.2±1.5	35.6±4.3	30.3±2.4
C18:4 ω3	19.5±0.2	21.5±1.9	19.8±1.6	20.9±0.7	19.0±2.2	14.2±0.8	18.5±1.3	20.5±2.6	20.6±1.3	17.8±2.4
C20:0	6.8±0.5	3.6±0.6	4.3±1.8	5.1±0.5	11.3±3.9	3.3±1.6	3.4±1.8	2.6±2.3	6.4±0.9	8.3±0.4

to reach minimum in May (13.48±1.01 and 8.02±0.46 mg g^{-1} DW in Amdoun and Semmech, respectively). The observed decrease in May was attributed to senescence and loss of membrane lipids (Sewon and Tyystjarvi, 1993).

The amounts of TFA found in leaves were in great contrast with those reported for the seeds of *Borago officinalis* L. (309.7 mg g^{-1} DW) and for other Boraginaceae species (225.2 mg g^{-1} DW in *Anchura azuraea*, 191.6 mg g^{-1} DW in *Asperugo procumbers*, 152.6 mg g^{-1} DW in *Echium vulgare*) (Guil-Guerrero *et al*., 2001a). In contrast, our values were in agreement with those reported for other plant leaves as *Chenopodium album*, *Verbena officinalis*, *Portulaca oleracea*, *Crithmum maritimum* (Guil-Guerrero and Rodríguez-García, 1999).

The FA composition is presented in Table 2. The predominant FA in rosette leaves from Semmech and Amdoun were C18:3 ω3, C18:4 ω3, C16:0, C18:2 ω6 and C18:3 ω6 while C18:3 ω3 was the major FA. The minor fatty acids were palmitoleic (C16:1), oleic (C18:1), stearic (C18:0) and arachidic (C 20:0) acids. The percentages evolution of these fatty acids varied greatly during three sampling months as shown in Table 2.

Concerning stalk leaves, the same FA detected in both regions differed by their percentages. This could be explained by photosynthetic rate or/and environmental parameters such as soil, photoperiod, temperature, etc.

Nevertheless, it is important to mention that *cis*-C16:1 was not detected in stalk leaves. Moreover, the prevalence of C18:3 ω3, C18:4 ω3, C18:2 ω6 and GLA in our samples suggested important enzymatic activities and particularly those of desaturases using 18 C fatty acids as substrates stimulation, etc. In addition to the usual ALA, appreciable percentages of unusual FA such as GLA and SDA were previously reported in borage (Uzzan, 1986; Whipkey *et al*., 1998). Although GLA is present in some plant sources as *Oenothera biennis* (Pina *et al*., 1984) and *Ribes nigrum* (Lercker *et al*., 1988), GLA was also detected in some species belonging to the Cariophyllaceae family as *Cerastium arvense* L. and *Minuartia laricifolia* subsp. *ophiolitica* Pignatti (Guil-Guerrero *et al*., 2004). In this case, GLA percentage of total saponifiable oil has been used as a taxonomic marker (Guil-Guerrero *et al*., 2000, 2001a, b).

The obtained results were in good agreement with those of Guil-Guerrero *et al*. (2003) who found nearly similar results for the seed oils of four Boraginaceae species, except for C16:1 and C 20: 1 ω9. In contrast, borage seed oil extracted either by supercritical carbon dioxide or soxhlet (16 h) using hexane as organic solvent showed high percentages of C18:2 ω6, GLA, C18:1 ω9 and C16:0 (Gómez and Martínez de la Ossa, 2002; Phylactos *et al*., 1994; Wretensjö *et al*., 1990).

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