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ORIGINAL ARTICLE

Investigation of γ-Linolenic Acid and Stearidonic Acid Biosynthesis During a Life Cycle of *Borago officinalis* L.

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Abstract This study investigated the levels of γ-linolenic (GLA, 18:3n-6) and stearidonic acid (SDA, 18:4n-3) in various parts of the borage plant (*Borago officinalis* L.) to elucidate Δ6-desaturase activity. Lipid class and fatty acid (FA) composition during germination of the seeds and FA composition of various borage parts were determined as well as FA compositions of neutral lipids (NL), polar lipids (PL), glycolipids (GL) and monogalactosyl diacylglycerols (MGDG) of borage leaves. When seeds were germinated for 12 days in the dark then exposed to light for 8 h, an overall decrease in oil content was seen with a significant increase in PL from 0.2 to 39.1%. An increase in SDA (from 0.2 to 0.6 g/100 g oil) indicated minor FA synthesis during germination and therefore, Δ6-desaturase activity with the most marked increase after light exposure. The FA compositions of developing and mature seeds were similar, suggesting oil/FA synthesis takes place at the initial stage of seed development. Among all the borage parts, the leaves had the highest amounts of α-linolenic acid (ALA, 18:3n-3) (36.2%), indicating Δ15-desaturase activity, and SDA (25.2%), indicating Δ6-desaturase activity. In leaves, the GL and especially, MGDG fractions had the highest amounts of SDA (31.8 and 39.8%, respectively), indicating that Δ6-desaturase is most active in chloroplasts. Leaves and developing seeds appear to be the major sources of Δ6-desaturase in borage, associated with different organelles in the different tissues.

Keywords Lipid classes · Fatty acid composition · Monogalactosyl diacylglycerol · Borage · Germination · FA biosynthesis

Abbreviations

ALA	α-Linolenic acid
ANOVA	One-way analysis of variance
DAG	Diacylglycerol(s)
FA	Fatty acid(s)
FAME	Fatty acid(s) methyl ester(s)
FFA	Free fatty acid(s)
GL	Glycolipid(s)
GLA	γ-Linolenic acid
LA	Linoleic acid
LC	Long chain (C ≥ 20)
MGDG	Monogalactosyl diacylglycerol(s)
NL	Neutral lipid(s)
n-6	Omega 6
n-3	Omega 3
PL	Polar lipid(s)
PUFA	Polyunsaturated fatty acid(s)
SD	Standard deviation
SDA	Stearidonic acid
TAG	Triacylglycerol(s)

Introduction

Borage seed oil (*Borago officinalis* L.) is a good plant source of γ-linolenic acid (GLA, 18:3n-6) [1]. GLA is

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produced from linoleic acid (LA, 18:2n-6) catalyzed by the Δ6-desaturase enzyme. The Δ6-desaturase enzyme also produces stearidonic acid (SDA, 18:4n-3) from the precursor α-linolenic acid (ALA, 18:3n-3) [2]. GLA in general is not commonly found in seed oils. Only a few other species such as *Echium* [1], evening primrose [3] and black currant [1] seeds also contain products of the Δ6-desaturase enzyme (GLA and SDA, respectively). The formation of GLA and SDA is considered the rate-limiting step in the production of long-chain ($C > 20$) polyunsaturated fatty acids (LC-PUFA) in humans [4]. LC-PUFA, especially n-3 PUFA, are nutritionally vital for human health [5]. Understanding the activity and the possible extraction of Δ6-desaturase may enable in vitro modification of oils to produce enriched n-6 and n-3 lipids in enzyme reactors. Desaturases can be generally described as enzymes that are able to activate oxygen and use it as a reagent to produce an unsaturated double bond from saturated carbons [6]. Desaturases can be distinguished between the soluble ACP-desaturases found in plastids of plants that function in the biosynthetic pathway of fatty acids (FA), and membrane-bound acyl-lipid desaturases [7]. Extraction of these membrane-bound FA-modifying enzymes from developing borage seeds and spinach chloroplasts has been undertaken with limited success [8, 9].

In mature, dormant borage seeds, the high amount of GLA is not necessarily related to the actual presence of Δ6-desaturase. Lipids in oil seeds serve either as structural or as storage components [10]. During germination, the plant utilizes the stored triacylglycerol (TAG) as energy [11]. Structural components in the mature seeds, such as cell walls, develop during germination. This addition results in a higher phospholipid content in the germinating seed relative to the mature seed [12]. Germination is, therefore, a growth period that involves major changes in lipid class and FA composition and may be a period in which Δ6-desaturase is produced in order to modify the oil to meet the plant's requirements.

Different parts of a plant contain different lipid contents and different FA compositions, so it is to be expected that some of these parts could also be the potential sources of FA-modifying enzymes such as Δ6-desaturase. Borage leaves contain high levels of GLA (up to 6%) and SDA (up to 22%), depending on the maturity level of the leaf and the light intensity of the growth environment [13]. To the authors' knowledge, FA compositions of neutral lipids (NL), polar lipids (PL), glycolipids (GL) and monogalactosyl diacylglycerol (MGDG) of borage leaves have not been published. The membranes of chloroplasts contain high amounts of GL, and certain types of GL such as MGDG are produced within the envelope membranes [14]. MGDG is a proposed substrate of Δ6-desaturase [15]. Analysis of the borage leaves GL and MGDG FA composition might elicit information about the presence and activity of the Δ6-desaturase enzyme in borage leaves. Studies into the lipid content and FA composition of borage plant parts are limited. Del Río-Celestino et al. [16] reported oil content and FA composition of the edible parts of borage (seed fractions, petioles, leaves and stem used as "vegetables" in northern Spain), while Peiretti et al. [17] analyzed the oil content and FA composition of whole borage plants cultivated in Italy during their growth cycle. This study is the first that investigates FA compositions of a broad range of different borage plant parts within various stages of the plant's maturity including germination and seed development.

Oil synthesis and FA modification also occur during the development of borage seeds [18]. The lipid content in the seeds can reach up to 30% of the dry matter [12] and therefore, the seeds are the main location of lipid/FA synthesis and storage in the borage plant. After pollination, changes in the lipid and FA compositions occur through the various stages of seed development in *Echium vulgare* [19].

This study aimed to determine at which stages of seed germination and in which plant parts Δ6-desaturase is present and active in borage. Germination of the seeds was carried out for 13 days in complete darkness, with exposure to light on the last day, and the seedlings were sampled every second day for FA profiles and lipid class. Plants were grown in a green-house for approximately 10 weeks and the plant parts cotyledons, roots, stems, leaves, petals, carpels, the central flowers, carpels after flowering, developing seeds and mature seeds were also studied for oil and FA contents. Activity of the enzymes involved in the FA biosynthesis was evaluated by changes of the oil content in the dry matter, lipid classes by Chromarod/Iatroscan thin layer chromatography-FID, and FA composition by GC-FID of total oil and lipid fractions separated by solid phase extraction (SPE) column chromatography.

Materials and Methods

Borage Seed Growth and Sampling

Borage seeds were supplied by King Seeds Ltd, Katikati, New Zealand. For germination, seeds were surface sterilized in a 0.4% sodium hypochlorite (Jasol, Auckland, New Zealand) solution for 20 min, rinsed and soaked in water for 6 h. Germination took place on a wet layer of fine vermiculite (Crow Refractory Ltd, Auckland, New Zealand) at approximately 21 °C in complete darkness for 12 days. Sampling was carried out every second day. After day 12, seedlings were exposed to light for a period of 8 h and a final sample was taken at day 13.

Seeds were grown in a green-house until they were mature plants up to principal stage 5.1 and beyond

according to previously described growth parameters [20] for approximately 10 weeks. Cotyledons were sampled after seven days of seeding. After cyme development with the first flowers generated, samples of roots, stems and leaves were taken (approximately 8 weeks after seeding). In addition, from the unfertilized flowers, blue petals, carpels and central flowers were separated and sampled. From the fertilized flowers (within 1 week of fertilization), swollen ovaries were separated and sampled (further referred to as post bloom, PB). Approximately 10 weeks after seeding, developing seeds (green, full sized seeds) and mature seeds (black or dark brown seeds) were sampled. All samples were taken in triplicate from five to 20 different plants, frozen immediately in liquid nitrogen and stored at −80 °C for analysis.

Oil Extraction and Determination of Total Lipids

Samples were homogenized manually in liquid nitrogen and freeze dried at −20 °C and 1.03 mBar until constant weight was maintained. The dry matter in % was calculated from the weight of the homogenized samples before and after drying. This was conducted in duplicate for each triplicate sample.

Oil was extracted according to the modified Bligh and Dyer method [21]. To 500 mg freeze-dried sample, 2.25 mL isopropanol, 2.75 mL cyclohexane (Ajax- Finechem, Taren Point, NSW, Australia) and 3 mL MilliQ H_2O (resistance of 1.8×10^7 ohm/cm) were added. After 1 min sonication, 125 µL saturated NaCl (Scharlau Chemie S.A., Sentmenat, Spain) solution was added to prevent emulsification and break down any emulsion formed [22]. After centrifugation at 650 *g* for 5 min, the upper layer containing the oil was transferred to pre-weighed tubes. For the second extraction, 3 mL of a mixture containing isopropanol/cyclohexane (13:87; v/v) were included in the sample. After sonicating for 1 min, the samples were again centrifuged at 650 *g* for 5 min. The upper layer was transferred to the same weighed tubes and the second extraction step repeated. The solvent collected from the three extractions was then evaporated under nitrogen. Tubes were weighed and the total lipids calculated (% of the dry matter). Lipid extraction was carried out in duplicate for each triplicate sample.

Determination of Lipid Classes and Fatty Acid Composition

For determination of lipid class, two aliquots of each oil sample diluted in chloroform were spotted onto three preburned silica gel SIII Chromarods with a Drummond microdispenser. After conditioning in a $CaCl_2$ containing desiccator for 5 min, lipid classes were separated in hexane/diethyl ether/acetic acid (60:17:0.1; v/v/v) for 25 min [23]. Lipid classes were then analyzed with an Iatroscan MK 5 chromatography-flame ionization detector analyzer and quantified using Chromstar 4.10 software. A calibrated external standard consisting of TAG (tripalmitin from Sigma, St Louis, MO, USA), free fatty acids (FFA, palmitic acid from INC Biomedicals Inc., Aurora, OH, USA), diacylglycerols (DAG, 1,2-dipalmitoyl-rac-glycerol) and PL (L-α-phosphatidylcholine, both from Sigma, St Louis, MO, USA) was used for calculations. The hydrogen pressure of the Iatroscan was set at 160 mL min^{-1} with an air-flow rate of 2 L min^{-1}.

Lipid classes were separated using SPE Silica columns (GracePure™ Silica 1,000 mg/6 ml from Grace Davison Discovery Sciences, Baulkham Hills, NSW, Australia) by following the elution protocol according to Stern and Tietz [24] with a few modifications. The columns were first, conditioned with 17.5 mL chloroform before they were loaded with approximately 20 mg oil. NL were then eluted in the first fraction with 35 mL chloroform, GL in the second fraction with 30 mL chloroform/methanol (85:15; v/v) and PL in the third fraction with 17.5 mL methanol. The fractions were weighed after evaporation of the solvent and fatty acid (FA) composition of NL, GL and PL was determined. To further separate MGDG from the GL fraction, a new column was conditioned with 17.5 mL 0.5% ethanol in chloroform. Approximately 20 mg GL from the second fraction was placed on the column. Then, two fractions were eluted with 25 mL 0.5% ethanol in chloroform and 25 mL chloroform/acetone (1:1; vv), respectively. A new column was again conditioned with 17.5 mL 0.5% ethanol in chloroform. The second fraction from the latter separation was placed on the column and two more fractions were eluted with 12.5 mL 0.5% ethanol in chloroform and 25 mL chloroform/acetone (3:1; v/v). The last chloroform/acetone (3/1; v/v) elution contained merely MGDG which was verified by separation on a TLC plate in acetone/acetic acid/H_2O (100:2:1; v/v/v) as the mobile phase [25]. The weight of this fraction was established after solvent evaporation and subsequently, methylated for determination of FA composition. All solvents used for this study were analytical grade from Scharlau Chemie S.A., Sentmenat, Spain.

For determination of FA composition, two to three aliquots of each oil sample with a known concentration of an internal standard (13:0; Sigma, St. Louis, MO, USA) were methylated [26]. To 15 mg oil, 0.5 mL of a 0.5 M sodium methoxide solution was added and heated at 65 °C for 5 min. A further 1.5 mL of the methylating agent NH_4Cl/MeOH/sulfuric acid (2:60:3; w/v/v) was included and allowed to react for 3 min at 65 °C. The FA methyl esters (FAME) produced were extracted with hexane and injected into a Hewlett Packard Series II GC equipped with a FID and a DB-225 capillary column (15 m × 0.25 mm, film thickness: 0.25 µm, J & W Scientific, Folsom, CA, USA).

The carrier gas was helium with a pressure of 68.94 kPa and the make-up gas a nitrogen/air mixture with pressures of 137.88 and 206.82 kPa, respectively. The injection volume was 1 μL of sample, while the injection temperature was set at 230 °C. The detector temperature was 250 °C. The temperature profile of the GC oven was initially set at 50 °C; held for 1 min after injection and then ramped to a temperature of 175 °C. This was done at a rate of 25 °C per min without holding time. The final temperature of 225 °C was reached by an increase in temperature of 4 °C per min. This temperature was held for 5 min. Fatty acids were identified and quantified with an external 38 FAME standard (Supelco 37 Component FAME Mix, St Louis, MO, USA: 0.5 mg/mL + methyl stearidonate, Fluka analytical, Sigma- Aldrich, St. Gallen, Switzerland: 0.025 mg/mL). In addition, FA composition of some samples was confirmed by GC–MS run under similar conditions as described for the GC-FID.

Statistical Analysis

Data were reported as mean values, plus/minus their standard deviation (±SD). Means were compared by one-way analysis of variance (ANOVA), post hoc Tukey test with a significance level of $P < 0.05$. All percentages were transformed to arcsine values before analyzed with ANOVA. Statistical analysis was performed using SPSS® 16.0 software (SPSS, Chicago, IL, USA).

Results and Discussion

Water and Oil Content

During germination, the dry matter of the seedlings decreased significantly ($P < 0.05$) from 66.3% at day 0 to 13.6% at day 8 (Fig. 1). Between days 10 and 13, the dry matter reached its lowest level of ~10% and the changes were not significantly different ($P > 0.05$) in this period. A similar decreasing pattern was observed in the dry matter oil content (%). There was a significant ($P < 0.05$) decrease from day 4 to day 10 (from 28.9 to 8.7%), after which the oil content remained constant at ~7%. The oil content on days 2 and 4 was ~30%, significantly ($P < 0.05$) higher than on day 0 (25.4%). The decreasing oil content from day 6 indicates that no major oil synthesis took place during germination. It is more likely that the stored oil in the seeds was used for growth energy [12].

The lowest dry matter content in the analyzed borage parts was observed in the cotyledons, leaves and petals (7.4, 8.5 and 8.8%, respectively), followed by those in the roots and stem (all ~11%), PB (13.7%), carpels (15.8%) and the central flower (18.0%) (Fig. 2). Developing seeds consisted of 37.4% dry matter, and mature seeds (without soaking in 6 h water, as for germination) had 69.2% dry matter. The oil content was lowest in the roots and stem, where it reached ~1% of the dry matter. The three flower parts, carpels, petals and the central flower including PB, contained less than 5% oil, while the leaves contained just above 5% oil. The oil content of cotyledons was relatively high (11.4%). During seed development, lipid bodies in cotyledons increase rapidly in both size and number, offering a large oil storage compartment in the seed [27]. In this study, the cotyledons contained high amounts of lipids after 7 days of growth. The developing and mature seeds had similar oil contents (22.9 and 22.1%, respectively). Baud et al. [28] have reported that during seed development, the water content decreases in an almost linear fashion and in parallel to the increase in oil content of the seeds. However, the results obtained in the current study indicated that lipid synthesis was completed well before the developing seed reached its final dry matter. Oil synthesis

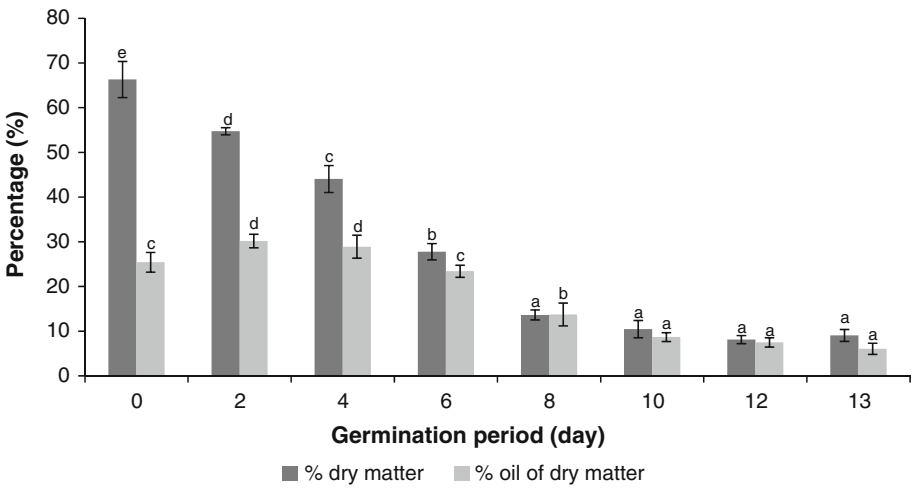

Fig. 1 Dry matter and oil content of dry matter (%) from day 0 to day 13 during 13 days of germination of borage seeds. Results are presented as mean values (±SD; $n = 6$); *different letters* on each column and series represent significant differences ($P < 0.05$)

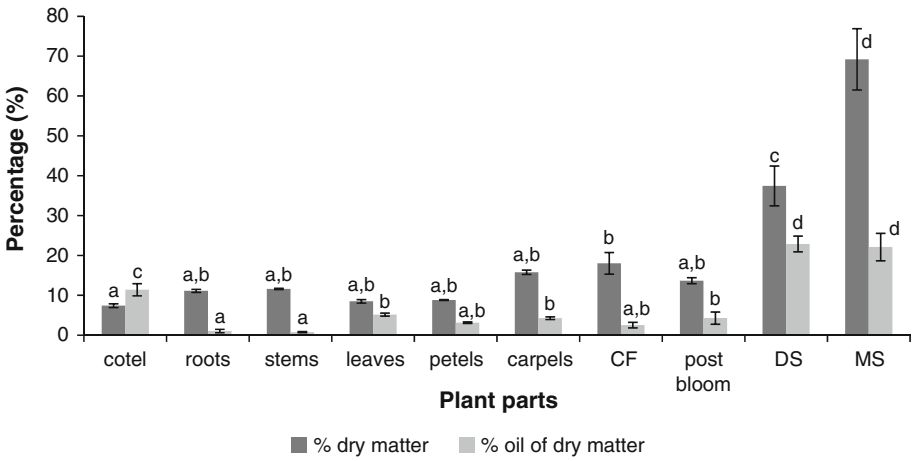

Fig. 2 Dry matter and dry matter oil content (%) of borage: cotyledons (cotel), roots, stems, leaves, petals, carpels, central flower (CF), post bloom, developing seeds (DS) and mature seeds (MS). Results presented as mean values (±SD; $n = 6$); *different letters* on each column and series represent significant differences ($P < 0.05$)

and storage took place at the time between the stage of PB and the sampling of the developing seeds. This period, in which four times more oil accumulated in the developing seeds than in PB, occurred over approximately 15–25 days after pollination. The oil production suggests that during this period, FA-synthesizing enzymes are most active in borage. The suggested activity of these enzymes was also confirmed by the results obtained for FA composition (Table 1) as the percentages of individual FA changed from PB to developing seeds.

Changes in Lipid Classes During Germination

During seedling growth, the percentage of TAG decreased significantly ($P < 0.05$) from 99.6 to 49.8%, with a corresponding increase in PL (from 0.2 to 39.1%), FFA (0.2 to 6.3%) and DAG (0.0 to 4.8%) (Table 2). The increase in DAG and FFA is probably due to the activity of lipases that generate FFA from DAG and TAG to supply energy via β-oxidation [29]. This reduction in TAG follows a similar pattern to that of the decreasing oil content. DAG on day 12 was lower (3.7%) than those on days 10 and 13 (4.3 and 4.8%, respectively), while FFA decreased from day 12 (6.7 to 6.3%).

The percentage of PL increased significantly ($P < 0.05$) (from 0.2 to 39.1%) as the amount of tissue increased in the seedling. PL are important for membrane development and this increase is due to their production (in particular, phosphatidylcholine) from DAG via cytidine diphosphate-choline: 1,2-diacylglycerol cholinephosphotransferase [30]. These changes in PL indicate that oil modification occurred over the complete germination period.

The changes in neutral lipids and PL in this study were greater than previously reported by Senanayake and Shahidi [12]. In the Senanayake and Shahidi study, over a germination period of 10 days, PL increased from 2.3 to 8.6% (compared with 39.1% in the current study), while neutral lipids consisting of TAG, DAG, monoacylglycerols, FFA and sterols decreased from 95.7 to 84.1% [12]. The dissimilarities in the results obtained in this study and the Senanayake and Shahidi study could be due to the slightly different germination conditions used, different seeds and the methods (column separation with a subsequent analysis by Iatroscan in the Senanayake and Shahidi study [12] as opposed to an Iatroscan analysis in this study).

Changes in Fatty Acid Composition During Germination

The n-6 FA were more than half of the total FA (%) in mature borage seeds, whereas n-3 FA were only observed as minor components, being less than 1% in total. The major FA in borage oil was LA (37.6%), followed by GLA (23.7%). The total amount of PUFA was 61.9% in the mature seeds. The FA composition determined for borage seeds in this study is similar to reported values [12, 31, 32].

During the 13-day germination period, there was a progressive change in the FA composition. There was a significant ($P < 0.05$) increase in palmitic acid (16:0) from 10.9 to 15.9% and stearic acid (18:0) from 3.6 to 5.1%. Of the monounsaturated FA, eicosenoic acid (20:1), erucic acid (22:1n-9) and nervonic acid (24:1) also increased significantly ($P < 0.05$) (from 4.0 to 5.6%, 2.7 to 3.8% and 1.5 to 2.5%, respectively). As the results are expressed as percentages, an increment in most saturated and monounsaturated FA does not necessarily reflect FA biosynthesis. It may indicate selective use or β-oxidation of a particular FA, which may be dependent on saturation (n-6 FA), chain length and/or position on the glycerol backbone.

Two C20 PUFA were detected from day 4 and day 8, respectively. The detection of these C20 PUFA at this later time of germination signifies possible FA biosynthesis, with enzyme activity involved in their production.

Table 1 Fatty acid composition (in % of total fatty acids) of borage plant parts

	Cotyledons	Roots	Stems	Leaves	Petals	Carpels	Central flowers	Post bloom	Developing seeds	Mature seeds
Σ Saturates	15.7 ± 0.5^{a}	25.2 ± 1.7^{d,e}	27.5 ± 5.5^{e,f}	21.5 (±2.5)^{b,c}	28.2 ± 0.7^{f}	23.8 ± 1.4^{c,d}	31.3 ± 0.9 ^{g}	21.5 ± 0.5^{b,c}	19.2 ± 0.7^{b}	16.2 ± 0.2^{a}
C6:0	n.d.	n.d.	n.d.	0.4 (±0.3)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C10:0	n.d.	n.d.	n.d.	0.8 (±0.7)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C14:0	0.3 ± 0.0	n.d.	n.d.	3.7 (±0.5)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C16:0	10.7 ± 0.3^{a}	25.2 ± 1.7^{e}	23.8 ± 1.4^{e}	12.5 (±0.3)^{a}	21.3 ± 2.4^{d}	17.5 ± 0.8^{c}	24.3 ± 0.9^{e}	15.0 ± 0.4^{b}	11.5 ± 0.1^{a}	11.3 ± 0.0^{a}
C18:0	3.2 ± 0.1^{a,b}	n.d.	4.8 ± 0.9^{c}	2.5 (±0.1)^{a}	3.6 ± 0.3^{a,b,c}	4.3 ± 0.2^{b,c}	7.0 ± 0.1^{d}	6.4 ± 0.9^{d}	4.5 ± 0.7^{c}	4.5 ± 0.7^{b,c}
C20:0	0.4 ± 0.0^{a}	n.d.	n.d.	0.7 (±0.2)^{b}	2.0 ± 0.0^{d}	1.0 ± 0.2^{c}	n.d.	n.d.	0.3 ± 0.1^{a}	0.3 ± 0.1^{a}
C21:0	0.4 ± 0.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C22:0	0.5 ± 0.1^{b}	n.d.	n.d.	0.6 (±0.5)^{c}	3.0 ± 0.1^{e}	1.1 ± 0.2^{d}	n.d.	n.d.	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}
C24:0	0.3 ± 0.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1 ± 0.0	n.d.
Σ Monounsaturates	13.3 ± 0.7^{c}	7.6 ± 0.6^{a,b,c}	8.4 ± 5.1^{b,c}	2.4 (±0.3)^{a}	4.5 ± 0.1^{a,b}	4.1 ± 0.4^{a,b}	4.0 ± 0.1^{a,b}	28.0 ± 1.2^{d}	24.9 ± 3.1^{d}	23.6 ± 0.9^{d}
C16:1	n.d.	n.d.	n.d.	0.3 (±0.3)^{b}	n.d.	1.0 ± 0.3^{c}	n.d.	n.d.	0.1 ± 0.0^{a}	0.1 ± 0.3^{a}
C18:1	6.4 ± 0.3^{a,b}	7.6 ± 0.6^{a,b}	8.4 ± 5.1^{b}	2.1 (±0.1)^{a}	4.5 ± 0.1^{a,b}	3.6 ± 0.2^{a,b}	4.0 ± 0.1^{a,b}	21.8 ± 2.3^{d}	16.5 ± 3.0^{c,d}	15.4 ± 0.5^{c}
C20:1	3.2 ± 0.2^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.1 ± 0.1^{a}	3.9 ± 0.2^{b}	3.7 ± 0.0^{b}
C22:1	2.2 ± 0.2^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.3 ± 0.3^{a}	2.8 ± 0.1^{b}	2.7 ± 0.1^{b}
C24:1	1.5 ± 0.1^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.5 ± 0.1^{a}	1.7 ± 0.1^{b}	1.7 ± 0.0^{b}
Σ Polyunsaturates	70.9 ± 0.8^{d,e}	66.3 ± 1.6^{c,d}	66.4 ± 5.5^{c,d}	76.5 (±2.8)^{e}	67.9 ± 2.7^{d}	72.1 ± 1.0^{d,e}	66.7 ± 1.5^{c,d}	50.5 ± 1.7^{a}	58.6 ± 3.9^{b}	60.1 ± 0.1^{b,c}
C18:2n-6	23.2 ± 0.6^{b,c}	42.5 ± 2.1^{f}	25.0 ± 1.8^{c}	9.3 (±0.5)^{a}	45.4 ± 3.0^{f}	20.4 ± 0.8^{b}	33.0 ± 0.7^{d}	26.4 ± 1.4^{c}	35.2 ± 1.4^{d,e}	36.8 ± 1.5^{e}
C18:3n-6	22.6 ± 0.4^{d}	13.7 ± 0.4^{b,c}	10.1 ± 0.6^{b}	5.9 (±0.2)^{a}	15.4 ± 0.7^{c}	15.0 ± 0.3^{c}	14.0 ± 0.2^{c}	15.5 ± 1.2^{c}	22.4 ± 2.6^{d}	22.7 ± 0.0^{d}
C22:2n-6	n.d.	n.d.	n.d.	n.d.	n.d.	0.8 ± 0.4	n.d.	n.d.	n.d.	n.d.
C20:2n-6	0.3 ± 0.0^{b}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}
C18:3n-3	15.6 ± 0.7^{d}	7.7 ± 0.2^{b}	21.5 ± 2.0^{e}	36.2 (±1.7)^{f}	5.9 ± 0.7^{b}	22.6 ± 1.3^{e}	11.3 ± 0.4^{c}	5.7 ± 0.7^{b}	0.6 ± 0.1^{a}	0.2 ± 0.0^{a}
C18:4n-3	9.3 ± 0.6^{d}	4.9 ± 0.3^{c}	9.8 ± 1.5^{d}	25.2 (±1.4)^{f}	2.3 ± 0.0^{b}	13.4 ± 1.2^{e}	8.4 ± 0.3^{d}	2.9 ± 0.4^{b}	0.3 ± 0.0^{a}	0.2 ± 0.0^{a}

Results presented as mean values (±SD; $n = 6$; for DS $n = 9$); different superscript letters in each row represent significant differences ($P < 0.05$); *n.d.* not detected

Table 2 Changes in the percentages of fatty acid composition and of triacylglycerols (TAG), free fatty acids (FFA), diacylglycerols (DAG) and phospholipids (PL) from day 0 to day 13 during germination of borage seeds; absolute amounts (g/100 g oil) are shown in parenthesis for the two unidentified fatty acids C20 polyunsaturated fatty acid (PUFA)(1) and C20 PUFA(2)

	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 13
Σ Saturates	15.3 ± 0.1^{a}	15.3 ± 0.3^{a,b}	16.1 ± 0.1^{a,b}	17.6 ± 0.2^{b}	21.6 ± 2.8^{c}	22.3 ± 1.9^{c,d}	22.6 ± 1.7^{c,d}	24.0 ± 0.9^{c}
C6:0	n.d.	n.d.	0.1 ± 0.0^{a}	0.3 ± 0.0^{a,b}	0.9 ± 0.5^{c}	0.9 ± 0.4^{c}	0.7 ± 0.2^{b,c}	0.9 ± 0.2^{c}
C14:0	0.1 ± 0.0^{a,b}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a,b}	0.1 ± 0.0^{b}	0.1 ± 0.0^{c}	0.1 ± 0.0^{d}	0.2 ± 0.0^{d}	0.2 ± 0.0^{e}
C15:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1 ± 0.0
C16:0	10.9 ± 0.1^{a}	11.0 ± 0.1^{a}	11.4 ± 0.0^{a,b}	12.2 ± 0.2^{b}	14.2 ± 1.2^{c}	14.8 ± 1.0^{c,d}	15.0 ± 0.8^{c,d}	15.9 ± 0.5^{d}
C17:0	0.1 ± 0.0^{a}	0.1 ± 0.0^{a,b}	0.1 ± 0.0^{a,b}	0.1 ± 0.0^{b}	0.1 ± 0.0^{c}	0.1 ± 0.0^{d}	0.2 ± 0.0^{d}	0.1 ± 0.0^{d}
C18:0	3.6 ± 0.1^{a}	3.6 ± 0.2^{a}	3.9 ± 0.1^{a}	4.2 ± 0.2^{a}	5.2 ± 0.9^{b}	5.1 ± 0.6^{b}	5.3 ± 0.8^{b}	5.1 ± 0.1^{b}
C20:0	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.5 ± 0.1^{b}	0.5 ± 0.0^{b}	0.5 ± 0.1^{b}	0.6 ± 0.1^{c}
C21:0	n.d.	n.d.	0.0 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2 ± 0.1^{b}	0.2 ± 0.1^{b}	0.2 ± 0.0^{b}	0.3 ± 0.1^{c}
C22:0	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.3 ± 0.0^{b}	0.3 ± 0.0^{c}	0.3 ± 0.0^{c}	0.5 ± 0.0^{d}
C24:0	0.1 ± 0.0^{a,b}	0.1 ± 0.0^{a,b}	0.1 ± 0.0^{a}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b,c}	0.1 ± 0.0^{c}	0.2 ± 0.0^{d}	0.3 ± 0.0^{e}
Σ Monounsaturates	22.7 ± 0.2^{a}	22.4 ± 0.1^{a}	22.6 ± 0.01^{a}	24.0 ± 0.3^{a,b}	26.4 ± 2.4^{c}	26.3 ± 2.1^{c}	25.6 ± 1.1^{b,c}	25.5 ± 0.6^{b,c}
C16:1	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.3 ± 0.0^{c}
C18:1	14.3 ± 0.2^{a,b,c}	13.9 ± 0.1^{a,b,c}	13.5 ± 0.2^{a,b}	14.4 ± 0.3^{b,c}	14.9 ± 1.3^{c}	14.5 ± 1.1^{b,c}	14.1 ± 0.8^{a,b,c}	13.2 ± 0.4^{a}
C20:1	4.0 ± 0.0^{a}	4.0 ± 0.0^{a}	4.3 ± 0.1^{a}	4.4 ± 0.1^{a}	5.1 ± 0.5^{b}	5.4 ± 0.5^{b,c}	5.3 ± 0.2^{b,c}	5.6 ± 0.1^{c}
C22:1	2.7 ± 0.0^{a}	2.7 ± 0.0^{a}	2.9 ± 0.1^{a,b}	3.1 ± 0.0^{b}	3.8 ± 0.4^{c}	3.8 ± 0.3^{c}	3.6 ± 0.1^{c}	3.8 ± 0.1^{c}
C24:1	1.5 ± 0.0^{a}	1.5 ± 0.0^{a}	1.7 ± 0.0^{a}	1.9 ± 0.1^{b}	2.3 ± 0.2^{c}	2.4 ± 0.2^{c,d}	2.3 ± 0.0^{c}	2.5 ± 0.1^{d}
Σ Polyunsaturates	62.0 ± 0.2^{b}	62.2 ± 0.4^{b}	61.2 ± 0.2^{b}	58.3 ± 0.5^{b}	51.9 ± 5.1^{a}	51.3 ± 3.9^{a}	51.7 ± 2.7^{a}	50.4 ± 1.5^{a}
C18:2n-6	37.6 ± 0.2^{d}	37.3 ± 0.1^{d}	35.6 ± 0.1^{c,d}	34.0 ± 0.3^{c}	29.2 ± 2.6^{b}	28.7 ± 1.9^{a,b}	28.6 ± 1.1^{a,b}	26.7 ± 0.7^{a}
C18:3n-6	23.7 ± 0.1^{b}	24.2 ± 0.4^{b}	24.4 ± 0.2^{b}	22.9 ± 0.5^{b}	20.2 ± 2.8^{a}	19.8 ± 2.0^{a}	19.9 ± 1.5^{a}	19.0 ± 0.9^{a}
C20:2n-6	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}
C18:3n-3	0.2 ± 0.0^{a}	0.3 ± 0.0^{a}	0.5 ± 0.0^{b}	0.6 ± 0.0^{b}	1.0 ± 0.0^{c}	1.3 ± 0.3^{c}	1.6 ± 0.2^{d}	2.6 ± 0.3^{e}
C18:4n-3	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.4 ± 0.0^{b}	0.5 ± 0.1^{c}	0.7 ± 0.1^{d}	1.0 ± 0.1^{e}
C20 PUFA(1)$^{+}$	n.d.	n.d.	n.d.	n.d.	0.1 ± 0.1^{a} [0.1 ± 0.0]^{a}	0.1 ± 0.1^{a} [0.0 ± 0.0]^{a}	0.1 ± 0.0^{a} [0.1 ± 0.0]^{a}	0.1 ± 0.0^{a} [0.0 ± 0.0]^{a}
C20 PUFA(2)$^{+}$	n.d.	n.d.	0.2 ± 0.0^{a} [0.1]^{a}	0.3 ± 0.0^{a,b} [0.2]^{a,b}	0.7 ± 0.3^{c} [0.4]^{c}	0.7 ± 0.3^{c} [0.2]^{b,c}	0.6 ± 0.1^{b,c} [0.4]^{c}	0.8 ± 0.1^{c} [0.4]^{c}
TAG	99.6 ± 0.1^{e}	99.5 ± 0.2^{e}	96.1 ± 0.6^{d}	91.5 ± 1.4^{d}	75.2 ± 9.4^{c}	68.2 ± 6.6^{b,c}	62.7 ± 7.2^{b}	49.8 ± 3.6^{a}
DAG	n.d.	n.d.	0.4 ± 0.2^{a}	0.6 ± 0.3^{a}	3.6 ± 3.2^{a,b}	4.3 ± 2.5^{b}	3.7 ± 1.3^{a,b}	4.8 ± 0.6^{b}
FFA	0.2 ± 0.1^{a}	0.1 ± 0.1^{a}	1.4 ± 0.1^{a}	3.0 ± 0.6^{a,b}	6.3 ± 3.5^{b,c}	7.0 ± 2.6^{c}	6.7 ± 1.8^{c}	6.3 ± 0.6^{b,c}
PL	0.2 ± 0.1^{a}	0.4 ± 0.1^{a}	2.1 ± 0.4^{a}	4.9 ± 1.0^{a}	14.8 ± 3.4^{b}	20.6 ± 2.4^{c}	26.9 ± 4.7^{d}	39.1 ± 3.4^{e}

Results are presented as mean values (±SD; $n = 6$–9); different superscript letters in each row represent significant differences ($P < 0.05$); *n.d*: not detected

$^{+}$ The amount of unsaturation and bond location was not assessable from mass spectra data

Unfortunately, the bond position of the C20 PUFA was unobtainable due to incomplete information from the MS data. The production of C20 PUFA takes place either via the conventional pathway involving Δ6-elongation and Δ5-desaturation, or via the Δ8-pathway, found commonly in microalgae, involving Δ9-elongation, Δ6- and Δ5-desaturation [33]. In terrestrial plants, gymnosperms also produce C20 PUFA, such as eicosatrienoic acid (20:3n-3) via the Δ8-pathway [34]. However, the pathway for the production of the two unidentified C20 PUFA in this study remains unknown.

In the polyunsaturated n-6 and n-3 FA pathway, the percentages of oleic acid (OA, 18:1n-9), the crucial precursor of GLA and SDA, remained approximately constant throughout germination, at ~14%. The n-6 FA, LA decreased significantly ($P < 0.05$) (from 37.6 to 26.7%) over the 13-day period. GLA started to decrease significantly ($P < 0.05$) from day 6 (from 22.9 to 19.0%). The reduction of these most abundant n-6 FA in borage can be connected with the parallel increase in other FA such as palmitic, erucic or nervonic acid. This point is further highlighted by the decrease in the percentage of total

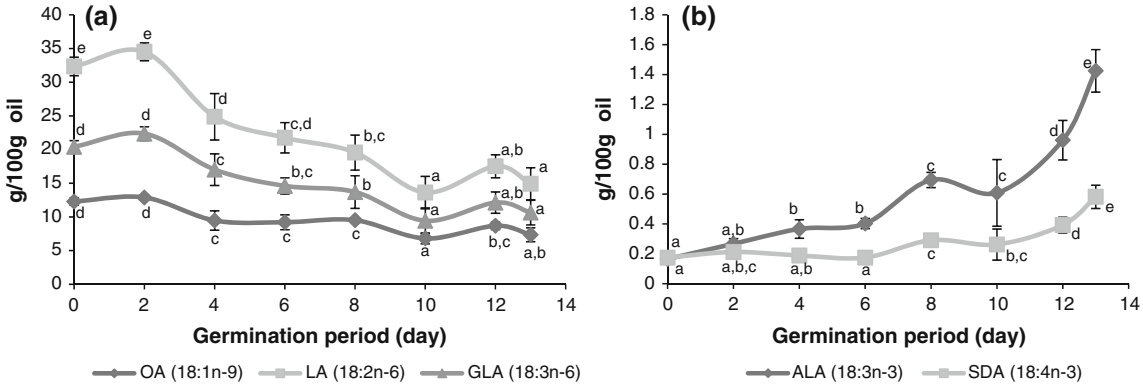

Fig. 3 Changes in fatty acid (FA) composition (g/100 g oil) of the n-6 (**a**) and n-3 pathway (**b**) during germination of borage seeds with light exposure on day 13. Results presented as mean values (±SD; $n = 9$); *different letters* on each graph and series represent significant differences ($P < 0.05$)

PUFA in borage oil compared with an increase in both, saturated and monounsaturated FA. The n-3 FA, ALA and SDA increased significantly ($P < 0.05$) (from 0.2 to 2.6% and 0.2 to 1.0%, respectively); however, their amounts remained relatively small compared with the n-6 FA. This increase may indicate that that the n-3 FA were selectively conserved in the growth phase. When these FA are expressed in absolute amounts (Fig. 3), OA, LA and GLA decreased over the 13-day germination period, while the n-3 FA ALA and SDA increased slightly, but significantly ($P < 0.05$). The increase in n-3 PUFA is likely due to FA biosynthesis via Δ6-desaturase.

The light exposure on day 13 did not have a large effect on the overall FA composition. However, a significant ($P < 0.05$) increase was detectable in ALA and SDA (from 1.0 to 1.4 g/100 g oil and 0.4 to 0.6 g/100 g oil, respectively) from day 12 to day 13. Chloroplasts in green photosynthetic tissue are proposed to have high levels of Δ15-desaturase, the enzyme responsible for producing ALA [35]. It is known that acyl-lipid desaturases such as Δ15- and Δ6-desaturase are localized in the membranes of plant plastids and/or endoplasmic reticulum with varying electron donors and substrate specificities [7]. Both, Δ15- and Δ6-desaturase enzymes are present at this stage of germination. The high abundance of n-3 PUFA was also detected in the total oil of borage leaves with high amounts of ALA and SDA (36.2 and 25.2%, respectively) (Table 1) and in the chloroplast lipid fraction MGDG (49.1 and

Table 3 Fatty acid composition (in % of total fatty acids) of total lipids, neutral lipids (NL), glyco- (GL), polar lipids (PL) and monogalactosyl diacylglycerol (MGDG) of borage leaves

	Total lipids	NL	PL	GL	MGDG
Σ Saturates	21.5 (±2.5)^{b}	47.0 (±4.8)^{d}	31.0 (±0.2)^{c}	11.8 (±0.3)^{a}	4.0 (±0.3)^{a}
C6:0	0.4 (±0.3)	3.0 (±0.1)	n.d.	n.d.	n.d.
C10:0	0.8 (±0.7)	4.9 (±0.5)	n.d.	n.d.	n.d.
C12:0	n.d.	0.6 (±0.5)	n.d.	n.d.	n.d.
C14:0	3.7 (±0.5)^{b}	17.1 (±1.1)^{c}	n.d.	0.2 (±0.2)^{a}	n.d.
C16:0	12.5 (±0.3)^{b,c}	13.8 (±3.2)^{c}	27.1 (±0.3)^{d}	9.3 (±0.4)^{b}	3.3 (±0.2)^{a}
C18:0	2.5 (±0.1)^{a,b}	5.0 (±1.4)^{c}	4.0 (±0.2)^{b,c}	2.3 (±0.4)^{a,b}	0.7 (±0.0)^{a}
C20:0	0.7 (±0.2)	2.6 (±0.4)	n.d.	n.d.	n.d.
C22:0	0.6 (±0.5)	n.d.	n.d.	n.d.	n.d.
Σ Monounsaturates	2.4 (±0.3)^{b}	6.5 (±0.3)^{d}	3.6 (±0.2)^{c}	1.1 (±0.2)^{a}	n.d.
C16:1	0.3 (±0.3)	1.9 (±0.3)	n.d.	n.d.	n.d.
C18:1	2.1 (±0.1)^{b}	4.6 (±0.4)^{d}	3.6 (±0.2)^{c}	1.1 (±0.2)^{a}	n.d.
Σ Polyunsaturates	76.5 (±2.8)^{c}	46.6 (±4.9)^{a}	65.4 (±0.3)^{b}	87.1 (±0.5)^{d}	96.0 (±0.3)^{e}
C18:2n-6	9.3 (±0.5)^{c}	10.4 (±0.2)^{c}	23.2 (±0.2)^{d}	5.2 (±0.8)^{b}	1.7 (±0.1)^{a}
C18:3n-6	5.9 (±0.2)^{b}	4.2 (±0.3)^{a}	8.4 (±0.2)^{c}	5.5 (±0.2)^{b}	5.5 (±0.1)^{b}
C18:3n-3	36.2 (±1.7)^{c}	16.4 (±3.2)^{a}	22.9 (±0.4)^{b}	44.6 (±0.7)^{d}	49.1 (±0.4)^{d}
C18:4n-3	25.2 (±1.4)^{c}	15.5 (±1.7)^{b}	10.8 (±0.3)^{a}	31.8 (±0.7)^{d}	39.8 (±0.6)^{e}

Results presented as mean values (±SD; $n = 3$); different superscript letters within each row represent significant differences ($P < 0.05$)

39.8%, respectively) (Table 3) (this aspect is further discussed in the following section). Our results demonstrate that the high n-3 FA production, resulting from activity of Δ15- and Δ6-desaturase, is connected with photosynthetic tissue and chloroplasts in borage.

The changes in individual FA during germination described here are more extensive than described in previous research into borage germination, where Senanayake and Shahidi [12] reported that palmitic acid remained constant with ~10.5%, LA decreased from 37.2 to 35.2%, whereas GLA increased slightly from 20.5 to 21.9% over a germination period of 10 days in complete darkness.

Fatty Acid Composition of Borage Plant Parts

The further the tissues were differentiated, the more variations in the FA compositions were observed, particularly with respect to the PUFA content (Table 1). The saturated FA myristic acid (14:0) was detected only in cotyledons and leaves. Similar studies conducted in southern Europe (Spain and Italy) [16, 17] support the presence of myristic acid, which is contrary to other studies in northern parts of Europe (Finland) [12, 13] and Canada [12]. The amounts of myristic acid observed in this and other studies could be due to factors including genetic, seasonal, climatic (colder regions might increase the need of the plant for more unsaturated FA) and experimental variations.

The major FA found in the roots was LA (42.5%), followed by palmitic acid (25.2%) and GLA (13.7%). The overall content of n-3 FA in roots was 12.6%, with approximately one third SDA. The stems consisted mainly of LA (25.0%), palmitic acid (23.8%) and ALA (21.5%), followed by GLA and SDA (10.1 and 9.8%, respectively). The SDA content in the stems is relatively high compared to the other tissues analyzed which could be connected to the presence of chloroplasts and photosynthetic activity. However, the low oil content of roots and stem (Fig. 2) demonstrate that these two tissues are not active sites of lipid storage.

Of the three flower parts, carpels had the highest amount of n-3 FA (ALA 22.6% and SDA 13.4%), compared with the central flower (11.3 and 8.4%) and petals (5.9 and 2.3%). The GLA content of these three parts was similar (15.0, 14.0 and 15.4%, respectively), but the content of LA (precursor of GLA) varied from 20.4, 33.0 to 45.4%, respectively. Therefore, activity of Δ6-desaturase is relatively higher during carpel or central flower development than during development of the petals.

The FA compositions of developing and mature seeds were similar, indicating that the majority of oil synthesis and modification took place in the initial phase of seed development. The major period when oil synthesis occurred was between the sample points of PB and developing seeds. The Δ6-desaturase was most active during the development of the green seeds on n-6 FA. The LA and GLA contents of developing and mature seeds were very similar (35.2 and 22.4% in developing seeds, 36.8 and 22.7% in mature seeds, respectively). The ALA content decreased by threefold from developing to mature seeds (0.6 to 0.2%), whereas the SDA content remained approximately the same (0.2%). The decreasing percentage of n-3 FA could be associated with the loss of chloroplasts (assessed by loss of green color) in which Δ15-desaturase is active [35] and usage of ALA for energy during the final maturation of the seed. In developing seeds, the main location of Δ6-desaturase is on the endoplasmic reticulum membrane, where this enzyme generally desaturates FA bound onto phosphatidylcholine (PC) in the sn-2 position [18, 36]. The FA composition of PB comprises a lower GLA content than the one in developing seeds (15.5% compared with 22.4%), but a higher n-3 FA content (8.6% with approximately one third SDA compared to 0.9%). This subsequent loss of n-3 FA could also be associated with the loss of photosynthetic tissue. A finer sampling scale between pollination and the sampling point of developing seeds would further define FA biosynthesis during seed development.

Cotyledons, the initial photosynthetic tissue of a plant enabling autotrophic function, contained mainly LA (23.2%) and GLA (22.6%). This indicates a high Δ6-desaturase activity during their development. In the leaves, n-3 FA were the major components of the oil (61.4%). Their n-6 FA content was approximately threefold lower versus cotyledons (15.2% compared with 45.8%). A similar FA composition in borage leaves has previously been reported, with up to 68.2% n-3 FA and 17.7% n-6 FA, depending on seasonal variations [13]. The high ALA content (36.2%) in the leaves indicates high activity of Δ15-desaturase, most likely in the chloroplasts. Although chloroplasts are able to import FA synthesized in the endoplasmic reticulum [37], it is more likely that SDA and its precursors ALA are produced within the chloroplast, which was found in developing cotyledons exposed to light [15]. The high content of n-6 FA in the cotyledons might be explained by their large oil storage compartments [27] and the lack of photosynthetic tissues during seed development.

The NL fraction (46.8% of total borage leaves oil) contained mainly myristic acid (17.1%), followed by ALA (16.4%) and SDA (15.5%) (Table 3). The main FA of the borage leaves PL fraction (6.3% of total oil) were palmitic acid (27.1%), LA (23.2%) and ALA (22.9%). The amount of GLA was approximately one third of its precursor LA (resulting in a GLA/LA ratio of 0.36) and that of SDA one half of its precursor ALA (resulting in a SDA/ALA ratio of 0.47). These relatively low ratios in the PL fraction may

indicate that Δ6-desaturase in borage leaves is less active in the endoplasmic reticulum. When bound to the endoplasmic reticulum membrane, Δ6-desaturase uses LA or ALA esterified on PC as substrates [18, 36] and enriches therefore, GLA and/or SDA in the PL fraction. Conversely, the GL fraction (46.9% of total oil) as lipids characteristic of the chloroplast membranes [14] showed the highest percentages of ALA and SDA (44.6 and 31.8%, respectively) within these three lipid fractions of borage leaves. The GLA content was with 5.5% relatively low. This might be explained by a high Δ15-desaturase activity in leaves, converting its substrate LA into ALA which is then further modified by the Δ6-desaturase enzyme. The GL fraction contained approximately 12.6% MGDG lipids. In leaves, Δ6-desaturase produces SDA esterified mainly on MGDG [15] and is bound to the chloroplast envelope membrane [7, 9]. As a proportion of total SDA in borage leaves oil, GL contains approximately 58.9% SDA and MGDG as part of the GL fraction, 8.6% SDA of the total oil. The percentage of SDA of leaf MGDG was found to be significantly ($P < 0.05$) higher compared to SDA of the total GL fraction (31.8% compared with 39.8%). The relatively high presence of SDA in the GL and MGDG fractions indicates that Δ6-desaturase is active in the chloroplast membrane, with specificity to ALA esterified on GL and probably MGDG. Therefore, leaves of the borage plant are a significant source of Δ6-desaturase due to their increased amounts of SDA, and the ability to produce SDA in the chloroplasts.

Conclusion

This study demonstrated that the large amounts of oil stored in borage seeds are most likely to be used to supply energy for growth of the new shoots during germination. There was no dramatic increase in the production of individual FA in the growing seeds. However, an increase of SDA indicates the existence of minor FA biosynthesis and the presence of Δ6-desaturase activity, in particular, when associated with the addition of light and subsequent greening of cotyledons on day 13. We demonstrated that the initial stage of seed development is the time when the majority of oil synthesis takes place. The deficiency of chloroplasts, which are connected with higher Δ15-desaturase activity, in the seeds may be the reasons for an increased production of n-6 FA rather than n-3 FA. FA profiles with high GLA contents in developing and mature seeds indicate high Δ6-desaturase activity during the initial stage of seed development. Of the other plant parts, the leaves demonstrated the presence of high Δ6-desaturase activity due to the high presence of SDA, especially in the GL and MDGD fraction, which are considered as typical chloroplast lipids. More n-3 FA than n-6 FA were determined in the total leaf lipids, which may be related to the Δ15-desaturase active chloroplasts. The major sources of Δ6-desaturase in borage are the seeds during their initial stage of development, and the leaves.

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References

1. Guil-Guerrero JL (2007) Stearidonic acid (18:4n-3): metabolism, nutritional importance, medical uses and natural sources. Eur J Lipid Sci Technol 109:1226–1236
2. Cahoon EB, Schmid KM (2008) Metabolic engineering of the content and fatty acid composition of vegetable oils. In: Bohnert HJ, Nguyen H, Lewis NG (eds) Bioengineering and molecular biology of plant pathways. Elsevier, Amsterdam
3. Ghasemnezhad A, Honermeier B (2008) Yield, oil constituents and protein content of evening primrose (*Oenothera biennis* L.) seeds depending on harvest time, harvest method and nitrogen application. Ind Crops Prod 28:17–23
4. Huang Y-S, Smith RS, Redden PR, Cantrill RC, Horrobin DF (1991) Modification of liver fatty acid metabolism in mice by n-3 and n-6 Δ6-desaturase substrates and products. Biochim Biophys Acta 1082:319–327
5. Tapiero H, Ba GN, Couvreur P, Tew KD (2002) Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies. Biomed Pharmacol J 56:215–222
6. Sperling P, Ternes P, Zank TK, Heinz E (2003) The evolution of desaturases. Prostaglandins Leukotrienes Essent Fatty Acids 68:73–95
7. Tocher DR, Leaver MJ, Hodgson PA (1998) Recent advances in the biochemistry and molecular biology of fatty acyl desaturases. Prog Lipid Res 37:73–117
8. Galle AM, Oursel A, Joseph M, Kader JC (1996) Solubilization of membrane bound Δ12- and Δ6-fatty acid desaturases from borage seeds. Phytochemistry 45:1587–1590
9. Schmidt H, Dresselhaus T, Buck F, Heinz E (1994) Purification and PCR-based cDNA cloning of a plastidial n-6 desaturase. Plant Mol Biol 26:631–642
10. Quettier A-L, Eastmond PJ (2009) Storage oil hydrolysis during early seedling growth. Plant Physiol Biochem 47:485–490
11. Jachmanián I, Mukherjee KD (1995) Germinating rapeseed as biocatalyst: hydrolysis of oils containing common and unusual fatty acids. J Agric Food Chem 43:2997–3000
12. Senanayake SPJN, Shahidi F (2000) Lipid components of borage (*Borago officinalis* L.) seeds and their changes during germination. J Am Oil Chem Soc 77:55–61
13. Sewón P, Tyystjärvi E (1993) Stearidonic and γ-linolenic acid contents of common borage leaves. Phytochemistry 33:1029–1032
14. Andersson MX, Dörmann P (2009) Chloroplast membrane lipid biosynthesis and transport. In: Sandelius AS, Aronsson H (eds) The chloroplast: interactions with the environment. Springer, Heidelberg

15. Griffith G, Brechany EY, Jackson FM, Christie WW, Stymne S, Stobert AK (1996) Distribution and biosynthesis of stearidonic acid in leaves of *Borago officinalis*. Phytochemistry 43:381–386
16. Del-Río-Celestino M, Font R, de-Haro-Bailón A (2008) Distribution of fatty acids in edible organs and seed fractions of borage (*Borago officinalis* L.). J Sci Food Agric 88:248–255
17. Peiretti PG, Palmegiano GB, Salamano G (2004) Quality and fatty acid content of borage (*Borago officinalis* L.) during the growth cycle. Italian J Food Sci 16:177–184
18. Galle AM, Joseph M, Demandre C, Guerche P, Dubacq JP, Oursel A, Mazliak P, Pelletier G, Kader JC (1993) Biosynthesis of γ-linolenic acid in developing seeds of borage (*Borago officinalis* L.). Biochim Biophys Acta 1158:52–58
19. Stolyhwo A, Mol J (2007) Changes in the content of γ-linolenic C18:3 (n-6) and stearidonic C18:4 (n-3) acids in developing seeds of viper's bugloss *Echium vulgare* L. Acta Biochim Pol 54:741–746
20. Simpson MJA (1993) A description and code of development of borage (*Borago officinalis*). Ann Appl Biol 123:187–192
21. Smedes F (1999) Determination of total lipid using non-chlorinated solvents. R Soc Chem 124:1711–1718
22. Barthet VJ, Chornick T, Daun JK (2002) Comparison of methods to measure the oil contents in oilseeds. J Oleo Sci 51:589–597
23. Volkman JK, Nichols PD (1991) Application of thin layer chromatography flame ionization detection to the analysis of lipids and pollutants in marine environmental samples. J Planar Chromatogr 4:19–26
24. Stern N, Tietz A (1993) Octadecatetraenoate synthesis in the unicellular alga *Isochrysis galbana*: studies with intact and broken chloroplasts. Biochim Biophys Acta 1167:248–256
25. Christie WW (2003) Lipid analysis: isolation separation identification and structural analysis of lipids. The Oily Press, UK
26. Hartman L, Lago RCA (1997) Rapid preparation of fatty acid methyl esters from lipids. Lab Pract 22:475–476
27. Mansfield SG, Briarty LG (1992) Cotyledon cell development in *Arabidopsis thaliana* during reserve deposition. J Can Bot 70:151–164
28. Baud S, Boutin J-P, Miquel M, Lepiniec L, Rochat C (2002) An integrated overview of seed development in *Arabidopsis thaliana* ecotype WS. Plant Physiol Biochem 40:151–160
29. Eastmond PJ (2006) SUGAR-DEPENDENT1 encodes a patatin domain triacylglycerol lipase that initiates storage oil breakdown in germinating *Arabidopsis* seeds. Plant Cell 18:665–675
30. Lung S-C, Weselake RJ (2006) Diacylglycerol acyltransferase: a key mediator of plant triacylglycerol synthesis. Lipids 41:1073–1088
31. Molero-Gómez A, Martínez-de-la-Ossa E (2002) Quality of borage seed oil extracted by liquid and supercritical carbon dioxide. Chem Eng J 88:103–109
32. Soto C, Chamy R, Zúñiga ME (2007) Enzymatic hydrolysis and pressing conditions effect on borage oil extraction by cold pressing. Food Chem 102:834–840
33. Qi B, Fraser T, Mugford S, Dobson G, Sayanova O, Butler J, Napier JA, Stobart AK, Lazarus CM (2004) Production of very long chain polyunsaturated omega-3 and omega-6 fatty acids in plants. Nat Biotechnol 22:739–745
34. Wolff RL, Christie WW, Pédrono F, Marpeau AM (1999) Arachidonic, eicosapentaenoic, and biosynthetically related fatty acids in the seed lipids from a primitive gymnosperm, *Agathis robusta*. Lipids 34:1083–1097
35. Harwood JL (1988) Fatty acid metabolism. Annu Rev Plant Physiol Plant Mol Biol 39:101–138
36. Galle-LeBastard AM, Demandre C, Oursel A, Joseph M, Mazliak P, Kader JC (2000) Phosphatidylcholine molecular species involved in γ-linolenic acid biosynthesis in microsomes from borage seeds. Physiol Plant 108:118–124
37. Browse J (1991) Glycerolipid synthesis: biochemistry and regulation. Annu Rev Plant Physiol Plant Mol Biol 42:467–506