Borago officinalis L. as an important source of natural aromatic compounds

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Abstract

Borage is planted for medicinal and culinary purposes and is also grown commercially to produce borage seed oil, high in GLA (γ-linolenic acid), which is used as a natural flavouring, food, and nutritional supplement. Borage antioxidants have potential in skin health products as UV absorbing components. The borage essential oil extracted by hydrodistillation contains important amounts of the valuable aroma compound 2.6 nonadienal (NDAL). Borage is grown mainly in Europe (England, the Netherlands) and in the United States (mostly in North Dakota) and Canada for commercial seed production. Seven varieties of borage were evaluated in the experiment. The average plant height ranged from 701 to 765 mm (locality Piešťany). The leaves of borage are characterised by a ground rosette at the base, the lower leaves with petioles 20-80 mm long are mostly elliptic, entire edged. The leaf characteristics were described: leaf length 115,6-166,0 mm, leaf width 69,9-99,4 mm, leaf circumference 311,4-450,5 mm and leaf area 5403-11426 mm^2. Borage flowers - predominantly large, long-stalked, 15-30 mm in diameter, mostly blue in colour, except for genotype 7/17 which had white flowers. 1000-seed weight varied 13.6-15.2 g. The evaluation of yield-forming elements, the average weight of fresh biomass per plant was quantified at 520 g (locality Piešťany) and 243-295 g (locality Plavnica), the average weight of biomass of dry plant was 115 g with a drying ratio of 4.52:1. The concentration of NDAL obtained from borage biomass ranged from 8.24-10.97 mg NDAL/kg for flower and 3.83-8.55 mg NDAL/kg for leaves, depending on the phenophase.

Keywords borage; variety; evaluation; characteristic; nonadienal

INRODUCTION

Borage is an annual, entomophilous plant with a large inflorescence that can grow to a height of 600-900 mm (Eskandari et al., 2011; Thom et al., 2016; Borowy et al., 2017). Seed germination is epigeic, hypocotyl. The germinating leaves are broadly elliptic (20 mm long, 18-20 mm wide) with short petioles and covered with stiff trichomes only on the adaxial side. The covering trichomes cover the entire plant. Their walls are often thick due to encrustation with silicic acid or calcium carbonate. The young cells of the trichomes are alive, the contents of the adult trichomes die, these fill with air and acquire a silvery appearance. The encrustation of the trichomes also serve as protection against animal pests (Volf et al., 1990; Chwil & Borowy, 2018). Glandular trichomes have a single spherical or ellipsoidal secretory head (glandule) in which the essential oil accumulates and a one to three-celled cell stalk. Authors Gupta & Singh (2010) and Chwil & Borowy (2018) report that the length of glandular trichomes ranges from 62-77 μm. Borage is cultivated for the high fatty acid content of the seeds. Borage, together with *Oenothera biennis*, represents the main natural source of γ-linolenic acid (Tewari et al., 2019). Borage seed oil also contains 26-38% of gamma-linolenic acid and 35-38% of linoleic acid, oleic acid (16-20%), α-linolenic acid (10-28%), palmitic acid (10-11%), stearic acid (3.5-4.5%), eicosanoic acid (3.5-5.5%) and erucic acid (1.5-3.5%) (Pieszak et al., 2012; Asadi-Samani et al., 2014; Borowy et al., 2017). Alphalinolenic acid plays a key role in human growth, development, and disease prevention. It serves as a precursor of longer chain omega-3 fatty acids such as eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) (Griffiths et al., 1996; Del Río-Colestino et al., 2008). The important flavour compound of borage is NDAL, also referred to as violet leaf aldehyde or cucumber or melon aldehyde. It has a low odour threshold (treshold, 40 ng/l) and can therefore be smelled even at low concentrations. NDAL acts as a repellent to insects (Germinara et al, 2015), and its antimicrobial activity was also found (Cho et al., 2004).

These properties and the unique aroma of NDAL are already commercially used in food products, furthermore in cosmetics, perfumes, and detergents. Due to the lack of natural, economically viable source, the synthetic form of NDAL s often used (Kula & Sadowska, 1993).

Cucumbers appear to be a promising source of natural NDAL. For example, a fresh cucumber as the main source, with a diameter of 36 mm, usually produces 8-12 mg/kg of NDAL (Buescher & Buescher, 2001). *Borago officinalis* L. (borage) has also been described as a potential source of natural NDAL (Sitkey et. al., 2022; Jaffel et al., 2022).

The aim of the present research work was to evaluate the production potential of borage from experimental results on the basis of morphological characters and to experimentally verify the potential possibilities for the preparation of 2.6-nonadienal and 2.4-decadienal from fresh biomass.

MATERIAL AND METHODS

Plant material

Five borage genotypes were used in 2019, with the following designation: 4/17, 5/17, 6/17, 7/17, 8/17. During the following years 2020 and 2021 we worked with only one genotype from the company Bohemiaseed s.r.o., which was selected based on the results of chemical analyses as well as the availability of seed for large-scale experiments.

Methodology and instruments for measuring morphological characters

The morphological characters of borage - plant, flower and trichomes were measured in the laboratory of medicinal plants of NPPC - VÚRV in Piešt'any. Some of the morphological traits measured: plant height, leaf length and width, flower diameter or trichome length, each trait was measured from ten plants of all genotypes. CI-203 laser analyser for leaf morphological analyses was used, which is a portable laser scanner designed for rapid and non-destructive measurement of leaf area. The measurement is performed by simply moving the scanner over the leaves, thus different parameters can be obtained: area, width, length, circumference, leaf shape factor. The leaf circumference is calculated using the function: $\Delta p = 4\Delta l2 + (W0 - W1)2$ where Δp is the leaf circumference, Δl is the length (usually 1 mm), W0 is the last measurement, and W1 is the previous measurement.

CarlZeiss Discovery V20 microscope with an AxioVision 4 modular system was used. The basic functions of the microscope software allow for: imaging, microscope control, image processing, image analysis and documentation. Morphological evaluation of flowers and trichomes was performed directly in the basic microscope program. The research was able to measure the length of covering and glandular trichomes, document the distribution of trichomes on the stem, leaves and flower.

All the borage varieties were grown on the experimental base of the National Agricultural and Food Centre (NPPC) - VÚRV in Piešt'any, where all the genotypes obtained were evaluated in field conditions. During 2020 and 2021 only one genotype from the company Bohemiaseed s.r. o was evaluated on all experimental locations. Field experiments on cultivation of borage were performed in 2019 in the region of Stará Ľubovňa in cooperation with agricultural cooperative in Plavnica (locality Plavnica) and with Agrokarpaty company (locality Plaveč) and continued in 2020-2021.

Method for the determination of natural aromatic aldehydes and alcohols (hexenal, trans-2-hexenal, trans-2-cis-6-nonadienal)

Natural aromatic substances were determined by analysis of the extracted homogenate (plant and water) in ethyl acetate, the ratio of organic extraction reagent to homogenate being 1:1. The extraction of the plant-water homogenate was carried out in a two-step procedure: the organic extractant (in the same volume as the homogenate) was divided in half and the homogenate was extracted twice. Into centrifuge tubes (15 ml plastic), 1g of NaCl was added and 2ml of ethyl acetate was added, followed by pipetting 4 ml of the plant homogenate. This mixture was vortexed for 2 min and then centrifuged (5000 rpm, 5 min, 15°C). The separated organic phase was pipetted into another marked centrifuge tube. The extraction process was repeated with a further addition of 2ml of ethyl acetate. An organic mixture with aromatic products after biotransformation was obtained and analysed by gas chromatography. The analysis was carried out on an Agilent 7890N gas chromatograph with a flame ionization detector (FID). A DBWAX capillary column (methyl dodecanoate PEG film: 30 m length x 0.250 mm column diameter x 0.15 mm film thickness, Agilent technologies, CA, USA) was used. The carrier gas used was hydrogen at a flow rate of 1.5 ml/min and the injections were 1ml in split mode 20:1. The following temperature program was used: an oven temperature of 90°C for 6 min (first isotherm), followed by a gradient of 10°C/min up to 120°C in the oven, and immediately followed by a faster gradient of 20°C/min up to 230°C in the oven, which was maintained for 17 min (second isotherm). During the first isotherm, C5-C8 volatile odorants were detected, and the starting temperature was adjusted to 70°C if the peaks overlapped. In subsequent gradients, less volatile C9-C10 odorants were detected. In the second isotherm, esters, and acids, even substrate acids (C16 to C18) were detected. The injector temperature was 270°C and the detector temperature was 300°C. For the determination of substrate components (unsaturated fatty acids) we also used an FFAP Optima capillary column (nitro terephthalic acid PEG film: 30 m length x 0.250 mm column diameter x 0.25 mm column thickness).

Instruments used

Homogenization of the plants with water and with the substrate (biotransformation) was performed in a Concept SM3410 mixer (P.R.C.) with a 2-litre glass container. Subsequent extraction and vortexing of ethyl acetate with the homogenate were carried out on a Vortex IKA MS3B (USA). Centrifugation to separate the organic phase with the aromatic products was carried out in a Hettich Universal 320R centrifuge (Germany). Analysis of the extracted mixtures was performed on an Agilent 7890N gas chromatograph (GC, Agilent technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (FID). Validation of the structures was performed on an Agilent 5977B GC/MSD gas chromatograph equipped with a mass spectrophotometer and an Agilent 7693A autosampler (GC, Agilent technologies, Palo Alto, CA, USA).

RESULTS AND DISCUSSION

Morphological, anatomical, and physiological plasticity may play different roles in plant adaptation to environmental changes. The influence of the environment on plant morphological expression is significant and can induce changes at the morphological and physiological levels. These changes may be critical for plant survival under variable environmental conditions.

Morphological evaluation of borage

Plant height

It is a functional trait that applies to the whole plant, is easily measurable and is an important trait for understanding the ecological strategies of plants. Plant height represents the competitive ability of plants for light, as taller plants have better access to light and higher photosynthetic activity. In the case of biomass production, plant height determines biomass yield and profit per hectare of production. Plant height is one of the most plastic traits and depends largely on specific abiotic and biotic factors. Plant height is one of the yield-forming traits and was assessed by mechanical analyses (Figure 11). The average plant height of the genotypes studied over the time evaluated was 796.8 mm. The tallest genotype was found to be 7/2017 originating from Germany. In the other years under study, the average plant height ranged from 701-765 mm. Our measurement results correspond with the work of Sachivko & Bosak (2018), who studied local populations of borage in Belarus and found a plant height range of 500-1000 mm. They described the borage variety Blakit, which has been registered in the State Register of Varieties of the Republic of Belarus since 2016. This variety is densely hairy, 70-80 cm tall, the stem is straight, thick, ribbed, hollow, branched, green at the top. The flowers are dark blue, clustered in whorls on long peduncles; the calyx is densely stiffly hairy, divided almost to the base into linear lanceolate; the corolla is longer than the calyx with a short tube; the pigmentation of the calyx is weak. Fruits are oblong-ovate; the weight of 1000 seeds are 13-18 g. In Poland borage is a rare plant, usually found as a garden weed; the plant reaches a height of 30-60 cm (Pieszak et al., 2012). Our measured values agree with other authors Eskandari et al. (2011), Thom et al. (2016) and Borowy et al. (2017), who report a height range of 600-900 mm for borage plants.

The flower diameter parameter is of importance in terms of the yield of the aldehydes DDAL and NDAL, which are most localized in the leaves and flowers. The borage has a regular saucer-shaped crown, its diameter is 15-25 mm, some botanists report 10-50 mm. Bussmann et al. (2019) have observed a crown diameter of 15-20 mm, which is consistent with our measurements.

Trichomes are special unicellular or multicellular structures that the epidermis forms on its surface. Glandular trichomes are generally described as bio factories with the unique capacity to biosynthesize specialized metabolites, which are critical for the capacity of plants to adapt to their environment and to overcome biotic and abiotic stresses. Secondary metabolites produced by medicinal plant cumulated in glandular trichomes are exploited by pharmaceutical and food industries in order to gain specific constituents and substances. Trichomes protect the plant from reduction of heating effect of sunlight, excessive transpiration, provided general protection of plant body from harmful insects, herbivores, and other agencies., they serve for fruit and seed dissemination Using a CarlZeiss DISCOVERY V 20 microscope, the lengths of the cover and glandular trichomes were measured (Table 2). The cover trichomes measured were from flower pedicels and the glandular trichomes were from leaf veins.

We found that both sides of the leaves are densely covered with trichomes, and glandular trichomes are best visible on the main vein of the leaf. We detected the most prominent covering trichomes on the flower pedicel and flower stem. At high magnification (more than 50x) it was possible to observe glandular trichomes with a typical round head filled with essential oil.

Results of the chemical evaluation

Biotransformation reactions of NDAL and DDAL production using the proprietary enzymes in borage were carried out both under laboratory conditions and under production conditions in lo-

Table 2. Average lengths of cover and glandular trichomes on leaves and flowers of borage at the flowering growth phase of the plant in 2019

Genotype	Cover trichomes (mm)	Glandular trichomes (mm)
4/2017	2,39	0,072
5/2017	2,27	0,052
6/2017	2,12	0,061
7/2017	1,94	0,052
8/2017	2,06	0,056

Figure 1. Measurement of trichomes on the flower petiole (CarlZeiss Discovery V20, magnification 12.5x)

Table 1. Average values of leaf characteristics of borage in the growth phase of the beginning of flowering in 2019-2021 at the locality NPPC-VÚRV Piešťany

Year	Area (mm^2)	Lenght (mm)	Width (mm)	Circumference (mm)	Shape factor	Ratio width/ length
2019	5403	115,6	69,9	311,4	1,66	0,7
2020	6341	126	81,8	332,8	1,47	0,72
2021	11426	166	99,4	450,5	1,61	0,71

cality Plavnica. In addition to the biotransformation of the substrates (Oleon HE30, the main component is linoleic acid; and Oleon LE80, the main component is linolenic acid), experiments without the addition of substrate were also performed in the laboratory. After initial homogenization in the blender (the maximum performance), the homogenate of the plant with water, was left in the blender for further reaction (minimum performance of blender). The blender was aerated, and the desired experimental temperature was maintained (18°C to 26°C). Samples were taken after 6 min of reaction. Finally, the homogenate was hydro distilled in the apparatus Clevenger type. Figure 2 illustrates the GS MS record measured from the borage organic extract after hydro distillation and Table 3 identifies the individual peaks of the chromatogram. The arrows show the C9 volatiles on the chromatogram.

To enhance production, elicitors - substances that can interfere with the cascade of enzymatic reactions and thus favour one pathway for product formation - are used in complicated enzyme systems in plants (Sharma et al., 2011). In our case of oxylipin metabolism, since a common substrate (linolenic acid hydroperoxide, HPOT) for multiple enzyme pathways is involved, methyl jasmonate was used as an elicitor, which is the product of the allene oxide synthase (AOS) pathway that competes with hydroperoxide lyase (HPL) in secondary metabolism. A similar situation was described in their paper by the authors Halitschke et al. (2004). The following Figure 17 documents the competition and interactions of the oxilipin enzyme cascade. Martinez-Espla et al. (2017) used methyl jasmonate directly on artichoke to increase phenolic production with a positive effect.

Figure 3 illustrates the positive effect of using an 8.83 mM concentration of elicitor (Plants with flowers, marked as Flowers 5) on the maximum production of 11.536 mg NDAL/kg borage, compared to a plant without elicitor (Plants with flowers, marked as Flowers 0) with only 8.512 mg NDAL/kg borage production.

Moreover, we tested also various plants parts for their NDAL production. The stems and frozen leaves did not produce any NDAL as illustrated in the figure 4. On the opposite, plants with flowers exhibited maximum NDAL production used oleon LE 80 or methyl jasmonate as stressing factors / elicitors.

Table 3. Identified C9 volatiles by GC MS

ret. Time	% GC	name
7,529	1,821	cis-2-nonenal
8,642	17,135	trans-2-nonenal
8,976	14,172	4-nonanan
9,216	55,898	trans-2-cis-6-nonadienal
12,769	1,185	acid trans-2-nonanoic

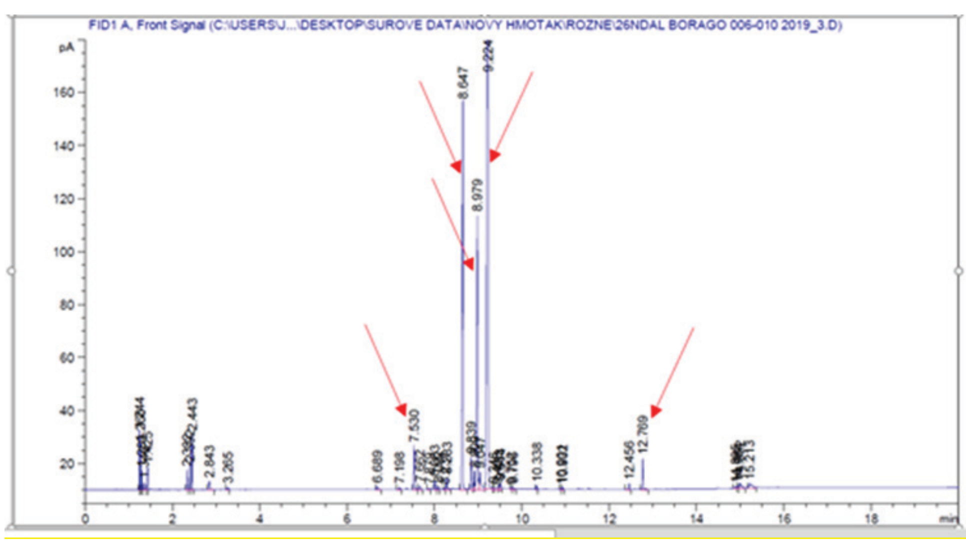

Figure 2. GS MS record of borage organic extract after hydro distillation arrows marked C9 volatiles (see table 3).

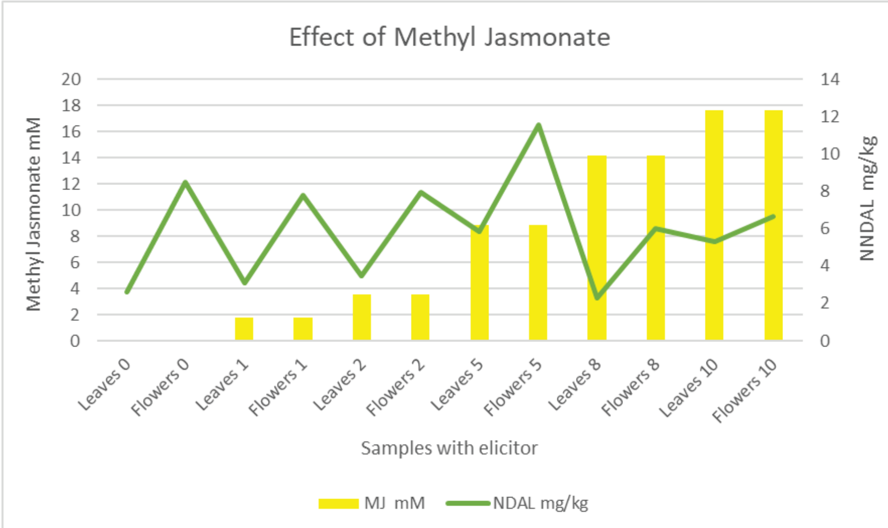

Figure 3. Positive effect of elicitor (methyl jasmonate) on NDAL production by Borago plants with flowers (sample Flowers 5)

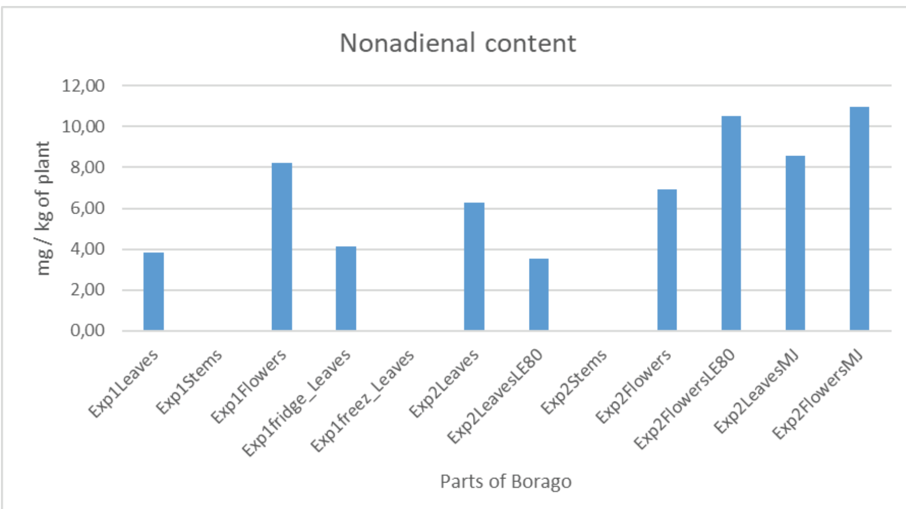

Figure 4. NNDAL content in various plant parts, comparison of various stress factors/elicitors: low temperature (fridge or freezer); methyl jasmonate; mixture of unsaturated fatty acids (oleon LE80). Plants parts compared: stems; leaves; flowers (leaves with flowers).

CONCLUSIONS

The results of this experimental work serve to increase the knowledge on the cultivation and introduction of a new plant species in specialty crop production in selected production areas of Slovakia. Borage is suitable for large-scale cultivation in almost all agro-climatic regions of Slovakia and at the same time provides a suitable raw material for processing in the food and chemical industry to produce natural flavouring.

All borage genotypes evaluated in the experiment were characterized by an erect plant growth habit, green colour of the stem and leaves as well as densely hairy vegetative organs. The average plant height varied from 701 to 765 mm (locality Piešťany). The leaves of borage are characterised by a ground rosette at the base, the lower bull leaves with petioles 20-80 mm long are mostly elliptic, entire edged, with a distinct reticulate venation. In the evaluation of rosette leaf parameters, the following average leaf characteristics were found leaf length

115.6-166.0 mm, leaf width 69.9-99.4 mm, leaf circumference 311.4-450.5 mm and leaf area 5403-11426 mm2. Borage flowers - predominantly large, long-stalked, 15-30 mm in diameter, mostly blue in colour, except for genotype 7/17 which had white flowers. In terms of seed morphological traits, its average length was found to be 5.698 mm and width 2.714 mm with HTS 13.6-15.2 g. In terms of evaluation of yield-forming elements, the average weight of fresh biomass per plant was quantified at 520 g (locality Piešt'any) and 243-295 g (locality Plavnica), the average weight of biomass of dry plant was 115 g with a drying ratio of 4.52:1.

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