Assessment of the environmental variability of a borage strain (*Borago officinalis* L.) by cultivation in different European regions

B. Galambosi[1], T. Moilanen[2], L. Angelini[3], F. Pank[4], J. Domokos[5], P.K. Svoboda[6], S. Dragland[7]

[1]Agrifood Research Finland - Mikkeli, Mikkeli, Finland

[2]Agrifood Research Finland, Kainuu Research Station, Sotkamo, Finland

[3]University of Pisa, Dipartimento di Agronomia e Gestione dell' Agroecosistema, Pisa, Italy

[4]Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Quedlinburg, Germany (retired)

[5]KHV Kozmetikai es Haztartasvegyipari Fejlesztö Kft, Budapest, Hungary

[6]The Scottish Agricultural College, Auchincruive, Ayr, Scotland UK

[7]The Norvegian Crop Research Institute, Apelswoll Research Centre, Division Kise, Norway.

Corresponding author: B. Galambosi, bertalan.galambosi@surffi.fi

Abstract

To study the environmental variability, a blue flowered borage genotype (*Borago officinalis* L.) was cultivated uniformly at various sites in Europe. Four plots of 1 m^2 were established at each region of which four were situated more in the North (Scotland, Norway and two in Finland) and two more in the South (Germany and Italy). The environment had a diverging impact on borage, depending on the characteristic under study. The environment had a particularly strong influence on the seed yield with a range of 159-837 kg/ha and on the germination capacity. The variability of the plant height and the duration of the growing period were smaller. The lowest variability revealed the thousand seed weight, ratio of black seeds, seed oil content with min. 27.6 % and max. 34 %, and the oil components, including γ-linolenic acid, ranging between 18.3 and 22.5 %. Basing on the results of this experiment it can be concluded that the selection of an appropriate cultivation site has high importance in particular for the seed yield, which seems to be favoured by cultivation in the northern regions of Europe. A high content of fatty seed oil and of γ-linolenic acid as its most important component are achievable under various environmental conditions.

Keywords

Borago officinalis, growth, seed yield, seed oil, fatty acids, environmental variability

Introduction

Seeds of many plants of several families contain gamma linolenic acid (GLA), a non-saturated fatty acid with wide importance for human health. Evening primrose (*Oenothera biennis* L.) and borage (*Borago officinalis* L.) are the primary sources of GLA (Barre, 2001; Janick et al, 1989). The main producers of borage are the United Kingdom (UK) and New Zealand.

To keep pace with the changes in agriculture, interest among the growers have arisen for the possible cultivation of evening primrose and borage as alternative minor crops in South Finland (Galambosi et al., 2003). Since the biennial evening primrose cultivation has more challenges in South Finland, the interest focused on the cultivation of borage as an annual crop. The advantages of borage are its annual life cycle, higher oil content in the seeds (24-34% as compared to 14-25 % in evening primrose*,* as well as higher GLA proportion in the seed oil with 23 % in borage and 10.5 % in evening primrose (de Haro et al., 2002).

Although borage is endemic in the Mediterranean (Hegi, 1927), it is being cultivated successfully in different regions. UK is considered to have the best environment for reliable and profitable production of borage seed with high GLA content, due to its favourable climatic conditions during the period of seed yield and seed oil formation (Lapinskas, P., 2000, personal communication). Several companies are involved in the cultivation in the North of England around York and in East England. The climatic conditions are similarly favourable for borage in New Zealand as well (Laurence, 2004). According to Polachic (1996), high yields can be achieved under humid conditions and borage thrives well on a wide range of different soils.

The dependence of the seed oil quantity and quality on environmental conditions have been studied in several important oil crops like sunflower (Harris et al., 1978), flax (Dybing and Zimmerman, 1967), in rape (Velasco and Goffman, 1999). It is observed, that cool temperatures enhance the accumulation of unsaturated fatty acids in seed oils. Canvin (1965) has grown oil crops in a phytotron at temperatures of 10, 16, 21, and 26.5 °C in the period of seed development.

It was observed that the highest oil content in rape and flax was found at the lowest temperature and a continual decrease was observed with rising temperature. Similarly the proportions of the more highly unsaturated fatty acids in the oil of rape, flax and sunflower decreased at higher temperatures. The levels of saturated fatty acids in all of the species were not affected by changes in temperature.

The environmental impact on pharmaceutically used seed oils was studied only on a few species, mainly on evening primrose. These investigations started when the production of high quality evening primrose oil failed in Israel, due to the low GLA content of the seed oil. It was assumed that the high temperature prevailing during seed ripening was the main cause. Levy et al. (1993) observed in a phytotron experiment under high temperature a substantial reduction of GLA content and an increase of oleic acid content of the seed of evening primrose. The importance of temperatures for GLA formation was demonstrated in a comparative experiment. It was shown that the GLA content of evening primrose grown in Turkey was much lower (5.8-6.9 %) than in Germany (10 %) (Reiner et al., 1989).

All these findings show that environmental factors can influence yield, oil content and oil composition of oil crops. The aim of the present experiment was not to investigate the influence of individual ecological factors on borage but to assess the extent of the ecological variability of some important characteristics by growing one genotype in different European regions. The results provide an indication of the extent of the dependence of the individual traits on environmental conditions.

Material and methods

The phenotypic expression of the characteristics of plants results from genetic, ontogenetic, morphogenetic and environmental factors. The aim of the design of this experiment was to keep constant all these factors except the environment.

Cultivation sites

The experiment was planned for eight experimental sites representing the factor "environment" during 2002. Four of them were situated in northern parts of Europe and four in Central and Southern Europe. The experiments in Hungary and in Switzerland as locations representative for Southern Europe failed. The coordinates of the experimental sites are presented in Table 1.

Table 1 The coordinates of the experimental sites

Country	Town	Direction	Abbre-viation	Coordinates		Institute
				North	East	
Italy	Pisa	South	IT	43° 40'	10° 10'	University of Pisa, Departimento di Agronomia
Germany	Quedlinburg	South	GE	51°47'	11°10'	Federal Research Centre for Cultivated Plants
Scotland UK	Ayr	North	SC	55°28'	4° 33' W	The Scottish Agricultural College
S-Finland	Mikkeli	North	MI	60°53'	10°55'	Agrifood Research Finland, Ecological Production
N-Finland	Sotkamo	North	KA	61° 44'	27° 18'	Agrifood Research Finland, Kainuu Research Station
Norway	Kise	North	NO	64° 06	28° 20'	The Norvegian Crop Research Institute, Division Kise

Genotype and cultivation measures

A Dutch origin blue flowered borage form was used. The seeds were purchased from Hyötykasviyhdistysry in Finland and it was imported from The Netherlands (Hem Zaden B.V.). Its seed germination capacity was 72 %.

Fertilisers (NPK=50-25-25 kg/ha) were incorporated into the soil before sowing. Plot size was 1 m² with four repetitions, seed rate 15 kg/ha, row distance 50 cm, sowing depth 2-3 cm. The plots were cleaned mechanically and irrigated whenever necessary. The flowering herb was cut on the individual plots, when the first brown seeds were falling down from the lowest second and third flowers. The herb was dried on paper under shade for 8-40 days. The dried and post ripened seeds were collected, cleaned and weighted. The sowing and harvesting times are presented in Table 2.

There were big differences in the climatic conditions between the growing sites. The mean temperatures of the southern growing sites (IT, GE) were significantly higher than those of the northern sites. The mean of the cumulative values of the effective heat sums of growing seasons in IT and GE was 2341 °C, while that of the four northern locations was 1483 °C. The time used for postharvest ripening during drying under shade was different: 17 d were used in GE, 20 d in SC and 40 d in IT. In the other sites the postharvest ripening time was 8-11 d.

Table 2 Dates of sowing and harvesting and meteorological data of the experimental sites

Country	Italy	Germany	Scotland	Finland		Norway
Town	Pisa	Quedlinburg	Ayr	Mikkeli	Kainuu	Kise
Abbreviation	IT	GE	SC	MI	KA	NO
Direction	South	South	North	North	North	North
Sowing (date)	21.03	17.05	17.04	8.06	28.05	23.05
Cutting (date)	28.06	26.08	15.08	16.08	23.08	15.08
Seed collection (date)	7.08	12.09	4.09	24.08	2.09	26.08
Mean temperature of the growing season (°C)	16.7	17.1	12.1	13.9	11.2	14.9
Precipitation of the growing season (mm)	237	232	409	260	239	258
Heat sum of the growing season (°C)	2572	2109	1856	1074	1138	1864

Measurement of trait expressions

The plant height (cm) was determined before the harvest. The length of the growing period (d) represents the number of days between sowing and cutting. The yield of cleaned seeds is indicated as kg/ha. The thousand seed weights (g) were determined by weighing of 5 x 100 seeds. The germination capacity (%) was determined in 9 cm Petri dishes, on light, at 20-23 °C day and 17-19 °C night temperature, with 4 x 50 seeds. GER1 represents the results of the germination capacity test during 25 November-5 December 2002 and GER2 of the second germination capacity test during 17-31 January 2003. To evaluate the seed colour, 2 g of seeds, in two repetitions, were visually separated into fully developed deep black seeds and gray coloured seeds. The portions were weighed and the ratios were calculated. In MI only one sample of nearly 100 g mixed seeds was used for this procedure.

The quantity of seed oil and the fatty acid composition were determined in Hungary, in the laboratory of the KHV by Dr. Domokos. The seed samples were extracted in a Soxhlet extractor by petroleum ether and the fatty oil content was determined. The analysis was carried out by the standard method (ISO 5508). The oils obtained, after preparation of fatty methyl esters, were analyzed for fatty acid composition by GC. The GC parameters were: Instrument: Varian Star 3400 CX, Column: Omegawax 320 30 m x 0.32 mm, Film 2000C, Injector: Tinj= 2200C, Detector: FID, Tdet=2700C.

The investigations covered the saturated fatty acids 16:0 palmitic and 18:0 stearic; the monounsatured fatty acids 18:1 oleic, 20:1 eicosenic and 22:1(ω-9) erucic; the polyunsaturated fatty acids 18:2 linoleic and 18:3 gamma linolenic.

Statistical analyses

The statistical analyses were carried out by SAS-software (SAS-package 9.1). After testing, the normal distribution (proc univariate), the distribution of all characteristics exhibited normality, except the eicosenic and erucic acids. Normality could also not be achieved by transformation of the original values. Therefore, no statistical evaluation of the eicosenic and erucic acids has been accomplished. The variance analysis was performed by the procedure glm. The means of the cultivation regions were compared by the Tukey-Test and the means of “southern” and “northern” growing sites by the t- test. The southern locations included Italy and Germany, the northern sites Norway, Scotland and the two locations in Finland. In tables and figures, the means marked with the same letter are not significantly different at $p \leq 0.05$.

The elementary statistics of the means of the 6 different cultivation sites included mean (MEAN), minimum (MIN), maximum (MAX), standard deviation (STD), variance (VAR) and coefficient of variation (CV) (Figure 1) and has been computed by proc means. The erucic acid content of 4.10 % seemed to be an outlier and therefore erucic acid was excluded from the elementary statistics.

Results and discussion

Comparison of the variability of the traits

The coefficients of variation of the investigated characteristics are plotted in Figure 1. They indicate the extent of variability due to the influence of environmental factors. The investigations reveal that the environment has a diverging impact on borage, depending on the characteristic under study.

The environment had a particularly strong impact on the seed yield and on the germination capacity. The influence of the environment was less strong, referring to the plant height and the duration of the growing period. The environmental impact on the thousand seed weight, the ratio of black seeds, the seed oil content and its composition was comparatively low.

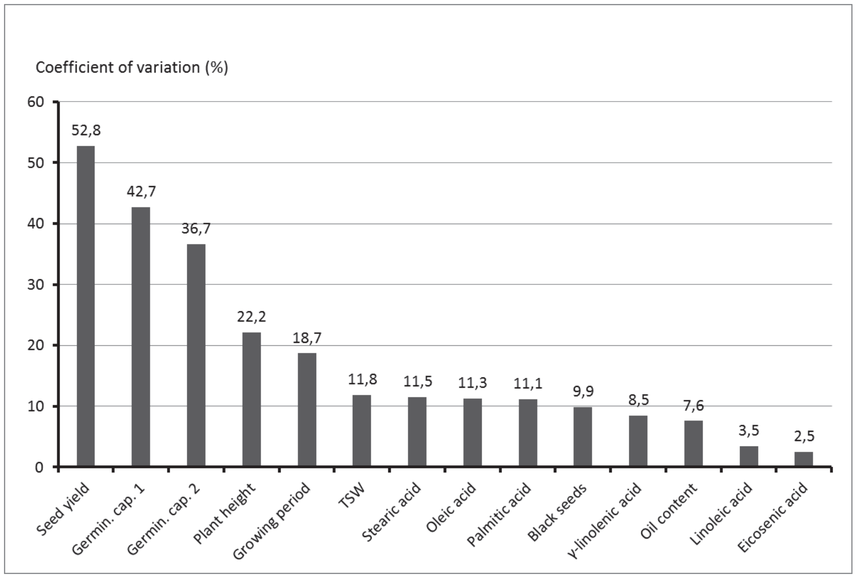

Figure 1: Coefficients of variation of the investigated characteristics

Seed yield, growth and seed quality

Seed yield

Table 3 shows data on traits characterising the growth of borage. The environment had a particularly strong influence on the seed yield. The seed yield was lowest in GE with 159 kg/ha and the highest in KA with 837 kg/ha and in SC with 735 kg/ha.

The following seed yields are reported in the literature: < 200 – 455 kg/ha by Francis and Campbell (2003) and 111-650 kg/ha by Laurence (2004) in Tasmania, 254-367 kg/ha by Simpson (1993) in Essex, England and 544 kg/ha by Gálvez and de Haro (2002) in Southern Spain. This range of the seed yield is in accordance with the observations in the present investigations.

In the present investigation, the average seed yield in the northern regions was three times higher than it was in the southern sites. These results comply with the conclusion of two borage development projects in Australia (Francis and Campbell, 2003) and in Argentina (Tremolieres et al., 1982). The authors proposed to transfer the borage cultivation areas to climatic cooler regions for increasing the seed yield and seed oil quality. Nevertheless, the cultivation of borage in Southern Spain resulted in a good yield of 544 kg/ha according to Gálvez and de Haro (2002).

Growth

The environment had also a considerable influence on the growth height and the duration of the growing period. But the coefficients of variation of these characteristics were lower in comparison to the CV of the seed yield. The plant height ranged between 90 cm in MI and 146 cm in SC. The average plant height exhibited no significant differences in the southern (116.8 cm) and the northern regions (109.5 cm). Schuster (1992) indicates a strongly varying growth height, ranging between 30 and 150 cm.

The duration of the period between seeding and harvesting was maximum 120 d in SC and minimum 69 d in MI and as an average only 10 d shorter in the northern regions in comparison to the southern ones. The reason for the short growing period in MI is the failure of the first sowing on 10th of May and the necessity to sow the borage anew on 8th June. The plants from this late sowing had an accelerated growth.

Table 3 Seed yield, plant height and the duration of the growing period

Region	Seed yield (kg/ha)	Plant height (cm)	Growing period (d)
IT	288 C	139.0 A	99
GE	159 C	94.5 C	101
SC	735 AB	145.8 A	120
MI	431 C	90.3 C	69
KA	837 A	91.2 C	87
NO	490 ABC	110.8 B	84
LSD_{Tukey}	368	12.9	
South	223 B	116.8 A	100
North	623 A	109.5 A	90
$p_{t\text{-}test}$	<0.0001	0.4920	
Elementary statistics overall			
MEAN	490	111.9	93.3
MIN	159	90.3	69
MAX	837	145.8	120
STD	258.5	24.83	17.44
VAR	6682	616.5	304.3

Seed quality

Table 4 presents data on seed quality characteristics.

The thousand seed weight (TSW) ranged between 19.2 g in KA and 14.2 g in IT. The average TSW was in the southern sites lower with 14.5 g than it was in the northern regions with 18.2. g. According to the literature the TSW of different accessions was 9-29 g (de Lisi et al., 2014; del Rio-Celestino et al., 2008; Gálvez and de Haro, 2002).

The germination capacity of the second trial (GER2) with a maximum of 82 % in MI revealed no significant differences except IT, where the germination capacity was only 20 %. The average germination capacity in the North was double in comparison to that of the South. Novák et al. (2010) determined the germination capacity of borage initially with 82.6 % and with 37.0 % after a ten years storage period.

The ratio of black seeds was highest in SC with 100 % and lowest in GE with 76 %. The seeds of the southern cultivation sites consisted of a lot of half-developed, smaller seeds, while the seeds of the Nordic cultivation sites were larger and deep black. Similar observations were recorded in Southern Spain by Gálvez and de Haro (2002). Under warm weather conditions 35 % of the harvested seeds were empty.

Table 4 Thousand seed weight (TSW), germination capacity (Ger1) and (Ger2) and ratio of black coloured seeds (Black seeds)

Region	TSW (g)	Ger1 (%)	Ger2 (%)	Black seeds (%)
IT	14.2 C	17.3 C	20.0 B	87.3 ABC
GE	14.8 C	43.3 B	56.0 A	76.0 C
SC	17.3 B	79.0 A	79.0 A	100.0 A
MI	17.9 B	70.0 A	82.0 A	91.0 AB
KA	19.2 A	43.3 B	77.0 A	98.8 AB
NO	18.4 AB	62.0 AB	73.0 A	86.1 BC
GD	1.22	20.6	26.4	12.9
South	14.5 B	30.3 B	38.0 B	81.6 B
North	18.2 A	63.6 A	77.8 A	94.4 A
$P_{t\text{-test}}$	<0.0001	<0.0001	0.0009	0.0150

Elementary statistics over all

MEAN	17.0	52.5	64.5	89.9
MIN	14.2	17.3	20.0	76.0
MAX	19.2	79.0	82.0	100.0
STD	2.004	22.39	23.7	8.91
VAR	4.016	501.41	559.5	79.37

Seed oil and its composition

The environmental impact on the oil content of the seeds and on the components of the seed oil was comparatively low (Figure 1). The differences in the studied parameters between the South-North sites were quite small and sometimes opposing. Table 5 shows the data on the oil content of the borage seeds and the composition of the fatty acids.

Oil content

The maximum of the fatty oil content of the seeds was reached in SC with 34 %. The minimum was 27.6 % in GE. The oil content in the southern cultivation sites was in average 28.9 %, while in the Nordic cultivation sites the average was significantly higher with 33.1 %. The observed range of the values of the oil content complies generally with data provided by literature with 22-38 % (Gálvez and de Haro,2002; Velasco and Goffman, 1999; Simpson, 1993).

Ratio of γ-linolenic acid

The variability of the GLA content as the most important component of the oil was comparatively low (Figure 1). It ranged between 18.3 in IT and 22.5 % in MI, but no significant differences were found between the averages in the southern (20.3 %) and the northern sites (21.2 %). The range of the GLA content of different accessions is wider with 12,6-28,6 % according to reports from literature (de Lisi et al., 2014; Mhamdi et al., 2009; Gálvez and de Haro,2002; Velasco and Goffman, 1999; Simpson, 1993).

Ratio of other oil components

There were significant differences in the other oil components between some regions. But the means of the proportion of the oil components ranged in narrow limits only. Stearic, linoleic and eicosenic acids showed no significant differences between the southern and the northern sites. Contrary to this, the portions of the palmitic and oleic acides were statistically significantly higher in the South. The content of the investigated oil components was in the range of data available from literature literature (de Lisi et al., 2014; Mhamdi et al., 2009; Gálvez and de Haro,2002; Velasco and Goffman, 1999).

Table 5 Content and composition of the fatty seed oil

Region	Oil content (%)	Fatty acids (%)						
		palmitic	stearic	oleic	linoleic	γ-linolenic	eicosenic	erucic
		C16:0	C18:0	C18:1c9	C18:2	C18:3	C20:1	C22:1
IT	30.2 C	13.8 A	4.15 B	21.1 A	33.7 B	18.3 C	3.98	1.38
GE	27.6 D	12.5 B	4.60 AB	19.3 A	36.7 A	22.2 A	3.80	2.30
SC	34.0 A	10.6 C	3.53 C	16.4 B	37.1 A	22.3 A	3.73	1.98
MI	33.1 AB	10.5 C	4.43 B	16.5 B	36.5 A	22.5 A	3.73	1.98
KA	33.3 A	10.9 C	4.30 B	17.0 B	35.5 AB	20.9 AB	3.80	4.10
NO	32.0 B	12.1 B	5.03 A	20.4 A	35.3 AB	19.2 BC	3.88	2.07
GD	1.3	0.66	0.54	1.98	2.66	2.44		
South	28.9 B	13.2 A	4.38 A	20.2 A	35.1 A	20.3 A	3.89	
North	33.1 A	11.0 B	4.32 A	17.6 B	36.1 A	21.2 A	3.78	
p t-test	<0.0001	<0.0001	0.8062	0.0016	0.1432	0.2483		

Elementary statistics over all

MIN	27.6	10.5	3.53	16.4	33.7	18.3	3.73
MAX	34.0	13.8	5.03	21.1	37.1	22.5	3.98
MEAN	31.7	11.733	4.34	18.45	35.8	20.9	3.82
STD	2.407	1.303	0.499	2.081	1.244	1.781	0.096
VAR	5.792	1.699	0.249	4.331	1.548	3.172	0.009

Conclusions

Referring to the results of the present investigation, the environment had a particularly strong impact on the seed yield and the germination capacity. The environmental influence was substantially lower on TSW, fatty oil content and its composition. This applies also to GLA, which is the most important component of the fatty seed oil.

It can be concluded that the choice of the appropriate environment has the first priority to achieve a high seed yield. Experiences reported in the literature and the findings presented in this paper suggest that the cultivation in cooler regions provides a higher seed yield. But generalizable evidence basing on scientifically sound experiments is still lacking. To check this hypothesis and to draw more reliable conclusions, a multi-year experiment series should be performed, including a selection of high performance cultivars and an adequate number of growing sites representing universe sets of the "North" and the "South" regions of Europe.

According to the presented investigations, good seed oil content and an adequate proportion of GLA are achievable under different environmental conditions. At all growing sites of this experiment, the composition of the fatty seed oil was in accordance with the requirements of Ph. Eur. (2010) (9-12 palmitic acid, 2-6 stearic acid, 12-22 oleic acid, 30-41 linolic acid, 17-27 GAL, 2.8-4.4 eicosenic acid, max. 3.0).

Besides the selection of the appropriate cultivation region and the provision of high performance cultivars, the weakest link in the production chain of borage seeds is an adequate harvest technology to collect the strongly scattering and gradually ripening seeds with low losses and low seed damages by mechanical procedures (Galambosi et al., 2014).

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