Effects of sulphur fertilization in organically cultivated faba bean

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Optimal seed yield and quality requires that the sulphur (S) demand of faba bean (*Vicia faba* L.) is ensured. The effect of S fertilization on organic cultivated faba bean was investigated under field conditions during two growing seasons (2017–2018), in Sweden. Kieserite ($MgSO_4$) and gypsum ($CaSO_4$) were applied at a rate of 20 and 40 kg ha^{-1} to study the effect on faba bean growth, yield, crude protein (CP) content and amino acid (AA) composition. Gypsum and kieserite significantly increased S concentration of faba bean dry matter (DM) in 2017. The S concentration ranged from 0.20% to 0.23% of DM compared to 0.18% in the untreated control. In 2018, kieserite application at 40 kg ha^{-1} significantly increased S concentration to 0.15% compared to 0.12% in the untreated control. The faba bean plants did, however, not respond neither with increased growth nor increased seed yield. The seed quality in terms of CP and S-containing AA, was not affected by S fertilization, however, significant differences were observed between the experimental sites.

Key words: *Vicia faba* L, cysteine, methionine, crude protein

Introduction

Faba bean (*Vicia faba* L.) is an important protein crop in organic production and the cultivated area increased from 6000 ha in 2008 to 30000 ha in 2018. As the crop is sensitive to droughts the acreages have since the drought in 2018 decreased to 20000 ha in Sweden. The crude protein (CP) content of faba bean is higher than the CP content of pea (ca 30% vs. 23% of DM). Sulphur is one of the essential macronutrients required for normal growth of plants, and often considered as the fourth major nutrient ranking below nitrogen, phosphorous and potassium (Marschner 1995). Insufficient sulphur (S) availability in the soil is reported to affect yield, protein content and amount of S-containing amino acids (Scherer 2009). The reduction of sulphur dioxide (SO_2) emissions from different industrial sources and the reduction in usage of S-containing pesticides have led to a depletion of S in the soil (Scherer 2001). A substantial decrease of 80% in the measured S deposition in Sweden has occurred from 1997 to 2012 (Pihl-Karlsson et al. 2013), which is in line with the reduction of S deposition reported in other European countries (Klein et al. 2011). A sharp decline is shown from 2000–2011, and as a consequence suboptimal levels of S have been recorded in several forage leys in south Sweden by Jonsson (2012). Deposition of S from the atmosphere in Sweden is mainly originating from foreign sources, and there is a clear gradient from <1 kg ha^{-1} in the soil in the central part of Sweden to the highest concentrations in the south-west of Sweden, corresponding to > 4 kg ha^{-1} in soil water (Phil-Karlsson et al. 2013). Sulphur deficiencies in sulphur-demanding crops, especially oil seed rape, wheat and grass leys, became apparent in the early 1990s in Sweden (Andersson 1996) and, consequently, S was added in selected mineral fertilizers.

The consequences of S deficiency can become economically important for growers by reduced yield and impaired seed quality (Głowacka et al. 2019). The demand for fertilizers containing sulphur and proper recommendations to growers have, therefore, increased. In addition to its direct role in seed production and seed quality, lack of S influences other macronutrients, for example nitrogen, through inhibition of nitrogen fixation in legumes, which eventually reduces plant growth (Cazzato et al. 2012). However, the reduced nitrogen fixation may be expressed as small and few nodules on the roots. Atmospheric nitrogen fixation plays a major role in the protein synthesis of the crop, especially as faba bean synthesizes 62–74% of its nitrogen through biological nitrogen-fixation (Amanuel et al. 2000).

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The effect of S fertilization in yield and seed quality of faba beans has been investigated in other studies (Pötzsch et al. 2018). As a result, the legume proportion in mixed grass-legume leys decreases when the legumes suffer from S deficiency (Tallec et al. 2008a, Tallec et al. 2008b).

On farms with animal production, a large part of the nutrient supply consists of farmyard manure (FYM) containing small amounts of organically bound sulphur, which is largely inaccessible for the plant (Pedersen et al. 1998). It is very difficult to assess the importance of sulphur supplied by liquid FYM and it is considered to be of very little importance according to Eriksen and Mortensen (1999). Studies in Sweden have shown that liquid FYM contains lower levels of S ranging from 0.3 to 0.6 kg ha^{-1} (Jonsson 2012), compared to the guidelines specified by the Swedish Board of Agriculture (Jordbruksverket 2019). On the other hand, FYM was found to improve S mineralization in the soil (Boye et al. 2009). Other organic fertilizers, such as bone meal and meat bone flour, contain relatively small amounts of organically bound S, which is difficult for plant to absorb. Improved protein yield and digestibility of faba beans due to sulphur addition was reported by Cazzato et al. (2012). The presence of S-containing amino acids (cysteine and methionine) can potentially increase with increased S-uptake in faba bean and thus cause an altered amino acid composition (Josefsson 1970, Eppendorfer and Eggum 1996).

The aim of this study was to investigate the effect of S fertilizer applications in organically cultivated faba bean with focus on growth, yield, and contents of protein and S-containing amino acids. The overall objective is to produce recommendations for sulphur supply in organic faba bean production. We hypothesized that S application in faba bean results in; 1) increased growth and yield, 2) higher protein content and a modified amino acid composition.

Materials and methods

Experimental design of field trials

Faba bean field experiments were established in field crops of faba beans in four counties in Sweden in 2017 (-17) and 2018 (-18): Östergötland (ÖS), Skåne (SK), Västra Götaland (VG) and Örebro (ÖR), using different locations within each county between the years (Table 1). The pre-crops were as follows: ÖS-17 (spring barley); SK-17 (winter wheat); VG-17 (winter wheat); ÖR-17 (oats); ÖS-18 (winter wheat); SK-18 (spring barley); VG-18 (oats); ÖR-18 (spring barley). Weed control was performed by harrowing twice in SK-17 and SK-18 but not in the other fields.

Table 1. Location of the field experiments, date for sowing and harvest, precipitation and temperature at sowing and harvest

Location and county	Coordinates	Date		Precipitation (mm)		Temperature (°C)	
		Sowing	Harvest	Day 1–60[a]	Totally	Day 1–60	Average[b]
2017							
Hogstad, Östergötland (ÖG-17)	N 58.3 E 15.0	21 April	15 Sept	59	188	11.3	15.1
Österlöv, Skåne (SK-17)	N 56.09 E 14.26	10 April	30 Sept	101	346	9.7	15.3
Slätte Gård, Västra Götaland (VG-17)	N 58.8 E 14.2	23 April	28 Sept	85	303	10.9	13.6
Åkerby, Örebro (ÖR-17)	N 59.3 E 15.1	4 May	1 Oct	96	303	13.5	14.3
2018							
Vreta Kloster, Östergötland (ÖG-18)	N 58.5 E 15.0	10 May	4 Sept	40	91	16	17
Kristianstad, Skåne (SK-18)	N 56.1 E 14.2	13 April	6 Aug	25	86	14	17
Logården, Västra Götaland (VG-18)	N 58.2 E 13.0	27 April	-	0	133*	15	17
Röcklinge, Örebro (ÖR-18)	N 59.1 E 15.1	30 April	4 Sept	55	183	16	18

[a] = precipitation from sowing date to the day when the plants were cut by hand for amino acids analysis; [b] = average temperature over the whole growing period

Each field experiment was designed as a randomized block with four replications, each with a plot size of 12 m × 3 m. Sulphur was broadcasted and incorporated into the soil by a harrow as kieserite ($MgSO_4$) or gypsum ($CaSO_4$), each at application rates of 20 kg S ha^{-1} and 40 kg S ha^{-1} before sowing, which were compared to an untreated control. Faba bean was sown at 12.5 cm row spacing at a density of 60 germinated seeds m^{-2} with a seeder (Wintersteiger combi seeder). The cultivar Julia (Ssd) was used in the field experiments in 2017 and the cultivar Tiffany (Ssd) was used in 2018. Soil samples were collected, before sowing, from a depth of 0–30 cm, 30–60 cm and 60–90 cm, respectively with a soil auger (20 mm diameter). Soil chemical properties were analysed at Eurofins Food & Agro Testing AB, Kristianstad, Sweden (Table 2), as follows: soil pH determined according to (SS (Swedish standard)—ISO 10390), P-AL, K-AL (SS 028310/SS 028310T1), sulphur (Blair et al. 1991), organic matter (KLK1965:1), soil texture (SS ISO 11277 mod.) and mineral N (ADAS method 53).

Table 2. Soil type and chemical parameters at the field experiment locations in 2017 and 2018

		2017										2018									
	Soil layer	pH	P-AL	K-AL	Mg-AL	K/Mg ratio	Ca-AL	Organic matter	Clay	Sand	Sulphate	pH	P-AL	K-AL	Mg-AL	K/Mg ratio	Ca-AL	Organic matter	Clay	Sand	Sulphate
County	(cm)		(mg 100 g^{-1a})				(mg 100 g^{-1a})	(%)	(mg kg^{-1} DW)				(mg 100 g^{-1a})				(mg 100 g^{-1a})	(%)	(mg 100 g^{-1} DW)		
ÖG																					
	0–30	6.8	6.6	17	17	1	450	7.00	38	26	6	6.2	3.7	8.5	7.7	1.1	170	3.5	18	43	1
	3–60	7.5	6.5	15	15	1	540	1.70	59	12	8	7.0	7.8	8.3	17.0	0.5	310	1.6	34	28	3
	60–90	8.5	5.5	13	13	1	1000	0.75	53	10	9	7.4	12.0	10.0	17.0	0.6	310	0.9	36	17	3
SK																					
	0–30	6.6	6.2	5.2	6.4	0.8	160	3.20	12	62	4	6.5	13.0	12.0	9.3	1.3	170	2.7	14	54	2
	30–60	7.7	3.7	3.6	6.5	0.6	330	1.10	11	63	3	7.0	7.6	7.2	7.8	0.9	170	1.2	19	48	6
	60–90	8.4	5.2	3.1	19	0.2	>2000	0.41	7	64	3	6.8	7.3	6.4	6.3	1.0	180	0.7	14	57	2
VG																					
	0–30	7.3	8.4	13	43	0.3	250	2.40	40	7	3	6.0	2.8	8.1	5.7	1.4	120	6.0	9	69	4
	30–60	7.2	7.9	14	64	0.2	240	1.70	46	5	34	5.5	2.2	2.5	1.9	1.3	36	1.9	6	81	1
	60–90	7.2	8.3	14	84	0.2	230	0.84	49	7	99	5.8	1.5	5.8	13.0	0.4	56	0.5	11	67	1
ÖR																					
	0–30	6.5	3.8	11	18	0.6	140	3.60	25	12	3	6.6	7.3	12.3	25.6	0.5	172	2.1	20	8	23
	30–60	6.7	2.4	8.7	33	0.3	140	1.50	29	7	2	6.7	4.1	13.6	52.3	0.3	190	0.8	35	11	25
	60–90	6.9	6.2	8.7	52	0.2	140	0.74	32	4	2	7.0	6.7	15.7	80.6	0.2	205	0.5	45	14	32

[a] = air dried soil

Parameters investigated in field experiments

Registrations of crop canopy and analysis of nitrogen uptake and plant nutrient content

A visual rating of the crop canopy was performed on a scale of 0–100% at BBCH 60, to assess plant density. In addition, at BBCH 60 (Weber and Bleiholder 1990), at early flowering, faba bean shoots were harvested by hand by cutting two areas of 0.5 m^2 in each plot and both fresh and dry weights of plant biomass were recorded. Samples were dried in a forced-draught oven at 70 °C for 24 h and corrected for residual water content, by drying a subsample for 2 h at 105 °C for DM determination. Dry weight of whole-plant faba bean was not determined in field experiment SK-17. Plant macro- and micronutrients were analysed at Yara Megalab™, Yara UK Limited, Pocklington, UK, according to Bergmann (1992).

The nitrogen uptake was estimated with an N-sensor (Yara) at BBCH 60 in six out of the eight field experiments. Corresponding measurements were performed with Green Seeker handheld Crop Sensor (Trimble Agriculture, Westminster, Colorado 80021, USA) at the two remaining field experiments, field SK-17 and SK-18. The Green Seeker measures the crop's reflectance of light and from that it is possible to calculate the N content using complex agronomic calculations called NDVI.

Registrations of seed yield and analysis of crude protein and amino acids

The faba bean was harvested at maturity (BBCH 88–89), weighed and water content was determined. At harvest, undried bean samples were collected to determine CP content as well as the contents of the amino acids lysine, methionine, cysteine, threonine, valine and histidine. Total N was analysed by the Kjeldahl method and the CP was calculated as total N×6.25. The analyses were performed at LKS mbH, Lichtenenwalde, Germany. At the field experiment VG-18, only bean samples were taken from each plot and analysed for CP and amino acid contents due to poor plant growth caused by drought and high occurrence of weeds.

Statistics

The results were analysed by year using a mixed linear model with treatment, location and the interaction between treatment and location as fixed factors and block (location) as a random factor in JMP statistical software (ver.9.0) (USA) and SAS (ver. 9.3) (SAS Inst. Inc. Cary, NC). Four blocks were used per location and year. If the F-value for the main effect of treatment and location and the interaction effect between treatment and location was significant ($p < 0.05$), pairwise comparisons were performed using Tukey's HSD test to identify differences between individual means. The biomass weights in the beginning of flowering were transformed by logarithm to reduce the variance. Contrast analyses were performed, i.e. the untreated control was compared with the mean of all sulphur treatments.

Results

Crop canopy density and nitrogen uptake

The visual assessment of the crop density (% coverage of ground) at BBCH 60 showed in field experiment VG-17 that the crop density of the untreated control was significantly lower (80%) compared to the treatment with 20 kg S ha^{-1} gypsum and 40 kg S ha^{-1} kieserite (91%). The 2018 growing season was affected by drought, which resulted in low faba bean growth in comparison to the 2017 field experiments. The results of N uptake by measurements with Yara's N-sensor and Green Seeker, showed no significant differences between the treatments (not shown).

Biomass

The weight of plant dry mass (DM) at early flowering in 2017, was highest in ÖG- followed by ÖR and was lowest in VG, whereas the plant DM in 2018 was highest in SK-18, intermediate in ÖG and ÖR and lowest in VG ($p<0.001$; Table 3). There were no differences in biomass DM yield between the S fertilization treatments.

Plant sulphur concentration and N:S ratio

The application of gypsum or kieserite significantly increased the average S concentration of the dry biomass compared to the control at early flowering stage in field experiments conducted in 2017 ($p < 0.001$; Table 3). No significant additional increase was obtained by the application of 40 kg S ha^{-1}. In 2018, application of

40 kg S ha^{-1} kieserite to the soil significantly increased the S concentration in faba bean DM compared to the untreated control ($p < 0.001$). The S concentration was higher in ÖR than in all the other locations in both years ($p < 0.001$; Table 3). There were no interactions between treatments and locations (results not shown).

The S uptake by the crop at early flowering ranged from 2.4 to 18 kg S ha^{-1} in 2017 and from 2.3 to 4.3 kg S ha^{-1} in 2018 among locations ($p < 0.001$; Table 3). The S treatments did not affect the S uptake in 2017, whereas addition of 40 kg S ha^{-1} of kieserite in 2018 increased S uptake in the faba bean compared to the untreated control ($p < 0.05$; Table 3).

The ratio of nitrogen to sulphur content (N:S) in faba bean at early flowering differed between locations in both years (Table 3). The ÖG and VG locations had higher N:S ratios than the SK and ÖR locations in 2017, which did not differ. In 2018, the ÖG location had the highest N:S ratio, followed by SK, which had a higher ratio than VG and ÖR ($p < 0.001$). The S treatments did not significantly affect the N:S ratios (Table 3).

Table 3. Biomass dry matter, sulphur concentration, sulphur uptake, N:S ratio of faba bean at early flowering (BBCH 60) at the eight field locations in 2017 and 2018 with sulphur treatments

	Biomass (DW kg m^{-2})		S conc. (% DM)		Total S uptake (kg ha^{-1})		N:S ratio	
	2017	2018	2017	2018	2017	2018	2017	2018
Location (county)								
ÖG	0.97 a	0.22 b	0.19 b	0.12 bc	18.0 a	2.6 b	30 a	46 a
SK	–	0.37 a	0.18 b	0.11 c	–^{a}	4.0 a	17 b	30 b
VG	0.14 c	0.17 c	0.18 b	0.13 b	2.6 c	2.3 b	26 a	21 c
ÖR	0.52 b	0.25 b	0.29 a	0.17 a	14.9 b	4.3 a	18 b	25 c
p-value	<0.001	<0.001	<0.001	<0.001	< 0.001	< 0.001	<0.001	<0.001
Treatment								
Untreated	0.57	0.26	0.18 c	0.12 b	11.6	3.0 b	22	33
Gypsum 20 kg S ha^{-1}	0.53	0.25	0.20 b	0.13 ab	11.4	3.4 b	21	31
Gypsum 40 kg S ha^{-1}	0.58	0.23	0.22 ab	0.13 ab	13.1	3.1 b	22	29
Kieserite 20 kg S ha^{-1}	0.54	0.24	0.21 ab	0.13 ab	11.9	3.1 b	24	32
Kieserite 40 kg S ha^{-1}	0.50	0.26	0.23 a	0.15 a	11.3	3.7 a	23	28
p-value	ns	ns	<0.001	<0.001	ns	< 0.05	ns	ns

Different letters indicate significant differences between means within a column for the effects of location and treatment according to Tukey's HSD test ($p < 0.05$). ^{a} = not analysed; ns = not statistically significant

Concentrations of macro- and micronutrients

There were significant differences of all analysed macro- and micronutrients in faba bean at early flowering in 2017 and 2018 between locations (Tables 4 and 5). The experimental sites that showed the lowest or highest concentrations varied with the nutrient element. The average concentrations of N ranged from 3.3% to 5.5% in faba bean DM in 2017 and from 2.5% to 5.3% in 2018 (Tables 4 and 5). There were no effects of S fertilizer treatments on macronutrient- or micronutrient concentrations in any of the eight field experiments.

Faba bean seed yield

The effect of S fertilization on seed yield varied between locations in 2017 (Table 6) and 2018 (Table 7), but not between treatments. There was an interaction between location and sulphur treatment in 2017, but it was only at location SK-17 where there was a significant difference between treatments within the field. In general, the seed yield of 2017 was highest in ÖG, followed by SK, VG and ÖR (Table 6). In 2018, the yield was highest at SK followed by ÖG and ÖR, when averaged over S treatments (Table 7). No significant differences were found between the S fertilization treatments and the untreated control in 2018.

Table 4. Concentrations of macro- and micronutrients in faba bean at the beginning of flowering (BBCH 60) at the four field trial locations in 2017 with various sulphur treatments

	N	P	K	Ca	Mg	Mn	B	Cu	Mo	Fe	Zn
	(% DM[1])					(mg kg^{-1} DM)					
Location (county)											
ÖG-17	5.52a	0.61a	2.24b	0.96c	0.26d	23.4d	30.8a	16.3b	1.30b	142b	42.6c
SK-17	3.32d	0.28c	1.40c	1.58a	0.34c	57.0a	29.9a	10.2c	0.48c	471a	55.9b
VG-17	4.56c	0.33c	2.40b	1.10c	0.44b	30.8c	22.6b	10.4c	1.41b	262a	24.1d
ÖR-17	5.00b	0.41b	3.08a	1.36b	0.53a	49.6b	31.2a	21.3a	1.76a	368a	64.1a
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.002	<0.001
Treatment											
Untreated	4.59	0.41	2.27	1.23	0.39	41.4	28.7	14.1	1.34	308	46.0
Gypsum 20 kg S ha^{-1}	4.58	0.41	2.22	1.28	0.41	40.5	29.0	15.2	1.30	342	47.3
Gypsum 40 kg S ha^{-1}	4.61	0.40	2.15	1.28	0.40	41.1	28.7	14.4	1.15	291	47.3
Kieserite 20 kg S ha^{-1}	4.61	0.41	2.31	1.25	0.39	38.7	28.3	14.1	1.19	296	46.9
Kieserite 40 kg S ha^{-1}	4.62	0.41	2.44	1.21	0.39	39.4	28.5	15.0	1.20	316	46.1
p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Different letters indicate significant differences between means within a column for the effect of location according to Tukey's HSD test ($p < 0.05$).[1] = dry matter content

Table 5. Concentrations of macronutrients and micronutrients in faba bean at the beginning of flowering (BBCH 60) at the four field experiment locations in 2018 with various sulphur treatments

	N	P	K	Ca	Mg	Mn	B	Cu	Mo	Fe	Zn
	(% DM[1])					(mg kg^{-1} DM)					
Location (county)											
ÖG-18	5.33a	0.36a	2.04b	0.82c	0.26b	65.6ab	21.9bc	14.8a	0.26c	182c	53.6b
SK-18	3.21c	0.36a	2.43a	1.49a	0.20d	65.9a	26.1a	8.3b	0.98a	255b	45.7c
VG-18	2.50d	0.25b	2.27a	1.09b	0.23c	55.1b	20.3c	9.1b	0.81b	167c	42.5c
ÖR-18	4.13b	0.36a	1.95b	1.09b	0.46a	61.8ab	22.6b	15.7a	0.29c	366a	69.7a
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	<0.001
Treatment											
Untreated	3.80	0.34	2.2	1.17	0.29ab	63	23	12.1	0.62	238	54
Gypsum 20 kg S ha^{-1}	3.80	0.32	2.1	1.09	0.28b	60	22	11.6	0.61	241	50
Gypsum 40 kg S ha^{-1}	3.68	0.32	2.1	1.12	0.28b	61	23	11.7	0.51	239	52
Kieserite 20 kg S ha^{-1}	3.87	0.33	2.2	1.11	0.28ab	63	23	11.9	0.53	243	52
Kieserite 40 kg S ha^{-1}	3.83	0.35	2.2	1.12	0.30a	64	23	12.6	0.64	252	56
p-value	ns	ns	ns	ns	<0.001		ns	ns	ns	ns	ns

Different letters indicate significant differences between means within a column for the effects of location and sulphur treatment respectively according to Tukey's HSD test ($p < 0.05$).[1] = dry matter content

Crude protein and amino acids

The CP content of the faba bean seed was similar among the S treatments in 2017 but tended to differ between the S treatments in 2018 (Tables 6 and 7). However, there were differences in the seed CP content between locations, where SK had the highest CP content, followed VG and ÖR, and ÖG had the lowest CP content in 2017 ($p < 0.001$; Table 6). In 2018, the seed CP content was highest in ÖG and ÖR, followed by VG and SK ($p < 0.001$; Table 7). The protein yield of the seeds also was affected by location in both years. In 2017, the CP yield of the seeds ranked as ÖG > SK > ÖR > VG and as SK > ÖG > ÖR in 2018 (Tables 6 and 7).

Table 6. Content of crude protein; protein yield and dry matter content (DM) of faba bean supplied with various sulphur fertilizer applications in 2017

Main effect and interaction	Yield 15% WC[1] (kg ha^{-1})	Crude Protein (% of DM)	Protein yield (kg ha^{-1})	DM bean (%)
Location (county)				
ÖG-17	5531a	30.7c	1697a	70.9d
SK-17	4451b	33.0a	1467b	85.9b
VG-17	3943c	31.1bc	957d	75.4c
ÖR-17	3080d	31.9b	1256c	87.5a
p-value	0.001	<0.001	<0.001	<0.001
Treatment				
Untreated	4333	31.5	1365	80.0
Gypsum 20 kg S ha^{-1}	4308	31.6	1358	79.9
Gypsum 40 kg S ha^{-1}	4227	31.7	1339	79.9
Kieserite 20 kg S ha^{-1}	4265	31.7	1350	79.9
Kieserite 40 kg S ha^{-1}	4122	31.8	1308	80.0
p-value	ns	ns	ns	ns
Location × Treatment				
ÖG-17				
Untreated	5676a	31.0	1760a	71.1
Gypsum 20 kg S ha^{-1}	5651a	30.5	1725ab	70.9
Gypsum 40 kg S ha^{-1}	5265ab	30.5	1605abc	71.0
Kieserite 20 kg S ha^{-1}	5795a	30.7	1777a	70.7
Kieserite 40 kg S ha^{-1}	5266ab	30.7	1615abc	70.7
SK-17				
Untreated	4814bc	32.6	1567abcd	85.8
Gypsum 20 kg S ha^{-1}	4490bcd	33.1	1484bcde	85.8
Gypsum 40 kg S ha^{-1}	4480bcd	33.1	1482bcde	85.7
Kieserite 20 kg S ha^{-1}	4404cd	33.1	1457cdef	85.8
Kieserite 40 kg S ha^{-1}	4069d	33.0	1344defg	86.3
ÖR-17				
Untreated	3130ef	30.5	955h	75.5
Gypsum 20 kg S ha^{-1}	3090ef	30.9	954h	75.4
Gypsum 40 kg S ha^{-1}	3088ef	31.3	967h	75.3
Kieserite 20 kg S ha^{-1}	3015f	31.2	941h	75.3
Kieserite 40 kg S ha^{-1}	3074ef	31.6	971h	75.6
VG-17				
Untreated	3712def	31.8	1177gh	87.6
Gypsum 20 kg S ha^{-1}	4001d	31.8	1271efg	87.5
Gypsum 40 kg S ha^{-1}	4076cd	32.0	1303efg	87.5
Kieserite 20 kg S ha^{-1}	3845cd	31.9	1225fg	87.6
Kieserite 40 kg S ha^{-1}	4080cd	32.0	1304efg	87.4
p-value	0.001	ns	<0.001	ns
CV	4.9	2.0	5.0	0.4

Different letters indicate significant differences between means within a column for the effects of location and interaction between location and sulphur treatment, according to Tukey's HSD test ($p < 0.05$).[1] = water content

Table 7. Content of crude protein, protein yield and dry matter content (DM) of faba bean supplied with various sulphur applications in 2018

Main effect and interaction	Yield 15% WC (kg ha^{-1})	Crude Protein (% of DM)	Protein yield (kg ha^{-1})	DM bean (%)
Location (county)				
ÖG-18	1478b	31.5a	397b	81.3b
SK-18	2267a	27.5c	529a	85.7a
ÖR-18	850c	31.5a	228c	69.7c
VG-18	–[a]	30.0b	–	81.0b
p-value	<0,001	< 0.001	< 0.001	<0.001
Treatment				
Untreated	1535	30.5	390	79.5
Gypsum 20 kg S ha^{-1}	1581	30.3	400	78.0
Gypsum 40 kg S ha^{-1}	1592	29.8	395	81.0
Kieserite 20 kg S ha^{-1}	1519	29.8	380	78.8
Kieserite 40 kg S ha^{-1}	1430	30.2	360	79.8
p-value	ns	(0.094)	ns	(0.082)
Location × Treatment				
ÖG-18				
Untreated	1531	31.8	422	81.2
Gypsum 20 kg S ha^{-1}	1606	31.8	435	81.2
Gypsum 40 kg S ha^{-1}	1531	30.8	402	81.3
Kieserite 20 kg S ha^{-1}	1501	31.5	403	81.3
Kieserite 40 kg S ha^{-1}	1189	31.9	323	81.5
SK-18				
Untreated	2218	27.7	520	86.6
Gypsum 20 kg S ha^{-1}	2338	27.7	551	85.8
Gypsum 40 kg S ha^{-1}	2304	27.2	532	86.0
Kieserite 20 kg S ha^{-1}	2244	27.4	522	85.0
Kieserite 40 kg S ha^{-1}	2230	27.5	522	85.0
ÖR-18				
Untreated	826	32.1	227	68.7
Gypsum 20 kg S ha^{-1}	800	31.3	214	67.4
Gypsum 40 kg S ha^{-1}	1592	31.3	250	74.9
Kieserite 20 kg S ha^{-1}	1519	31.3	215	68.3
Kieserite 40 kg S ha^{-1}	1430	31.5	234	69.2
VG-18				
Untreated	–	30.5	–	81.6
Gypsum 20 kg S ha^{-1}	–	30.3	–	77.6
Gypsum 40 kg S ha^{-1}	–	30.1	–	81.7
Kieserite 20 kg S ha^{-1}	–	29.0	–	80.7
Kieserite 40 kg S ha^{-1}	–	30.0	–	83.5
p-value	ns	ns	ns	ns
CV	30.7	4.3	15.1	4.3

Different letters indicate significant differences between means within a column for the effect of location according to Tukey's HSD test ($p < 0.05$). [a] = not analysed

The analysed amino acids showed no differences between the S treatments and no interactions between the S treatment and location were found (Table 8). However, there were differences between the locations in both years, where ÖG and ÖR had the highest concentration of methionine compared to SK and VG ($p < 0.01$). Cysteine concentration was higher in ÖG than in SK and ÖR in 2017 ($p < 0.01$), whereas no differences in cysteine concentration between the locations could be found in 2018.

Table 8. Concentration of amino acids (g kg^{-1} DM) in faba bean treated with different products and various levels of sulphur applications at three field trial sites in 2017 and four field trial sites in 2018

	Lysin	Methionine	Cysteine	Threonine	Valin	Histidine
2017 Location (county)						
ÖG-17	17.3c	2.7a	3.6a	12.1a	12.9a	9.4a
SK-17	20.9a	2.3b	2.8b	11.9a	13.3a	8.6b
ÖR-17	19.3b	2.7a	2.7b	12.4a	13.3a	8.6b
p-value	<0.001	0.003	0.003	ns	ns	0.001
Treatment						
Untreated	18.6	2.7	2.6	11.9	13.2	8.6
Gypsum 20 kg S ha^{-1}	19.9	2.5	3.2	12.2	13.1	8.7
Gypsum 40 kg S ha^{-1}	19.2	2.5	2.9	12.1	13.1	8.9
Kieserite 20 kg S ha^{-1}	18.7	2.5	3.1	12.1	13.3	9.1
Kieserite 40 kg S ha^{-1}	19.4	2.7	3.5	12.5	13.2	9.0
p-value	ns	ns	ns	ns	ns	ns
2018 Location (county)						
ÖG-18	21.4a	2.4a	3.0a	10.5a	12.8b	8.6a
SK-18	18.7c	2.1b	3.0a	9.4b	12.0c	7.9b
ÖR-18	20.1b	2.4a	3.4a	10.9a	14.3a	8.9a
VG-18	21.4a	2.2b	3.2a	10.7a	13.2b	8.0b
p-value	<0.001	<0.001	(0.09)	<0.001	<0.001	<0.001
Treatment						
Untreated	20.3	2.3	3.0	10.4	13.1	8.4
Gypsum 20 kg S ha^{-1}	20.5	2.2	3.1	10.5	13.3	8.4
Gypsum 40 kg S ha^{-1}	20.7	2.3	3.2	10.2	12.8	8.3
Kieserite 20 kg S ha^{-1}	20.7	2.3	3.0	10.3	13.3	8.4
Kieserite 40 kg S ha^{-1}	19.9	2.2	3.4	10.5	13.1	8.5
p-value	ns	ns	ns	ns	ns	ns

Different letters indicate significant differences between means within a column for the effect of location according to Tukey's HSD test ($p < 0.05$).

Discussion

In this study, the influence of S fertilization on growth, yield and quality in terms of CP and amino acid content was investigated in organically produced faba bean for the first time under field conditions in Sweden. Sulphur fertilizer treatments increased both S concentration and S uptake in faba bean dry matter. However, our results were not in accordance with hypothesis 1, i.e., that the S fertilizers application in faba bean results in increased growth and yield. In general, S fertilization had no significant positive impact on the crop canopy density, except at the site VG-17. The S content in the topsoil (0–30 cm) at VG-17 (3 mg kg^{-1}) was at the same level, as experimental sites SK-17 and ÖR-17 (4 and 3 mg kg^{-1} respectively). In addition, the S-content in the biomass at VG-17 (0.18% DM) was comparable with the respective level at ÖG-17 and SK-17 (0.19 and 0.18% DM) respectively. Therefore, the positive effect in terms of canopy density observed in sulphur treatments as compared to control, is barely due to the reduced growth in the untreated control at this specific field experiment (VG-17), which in turn could probably be due to other factors such as weeds. In addition, there was no link between S concentration and plant dry mass weights. For example, in 2017, the dry mass weight was highest at ÖG, while S concentration was similar to that in faba bean grown at sites SK-17 and VG-17. The obtained average yield over the four field trials in 2017 was 4251 kg ha^{-1} and for such average seed yield the average S uptake was only 11.8 kg ha^{-1} (Tables 3 and 6).

These results correspond with the levels reported by Pötzsch et al. (2018) where the average seed yield was 3000 kg ha^{-1} and the average S accumulation in the shoots was 10 kg ha^{-1}.

The growing season of 2018 was affected by drought and heat stress, which resulted in low yields in several spring-sown crops. Furthermore, during 2018, the dry weight was highest in SK-18, while the S concentration in DM was significantly lower compared to the other sites. Statistical analysis did not show positive correlations between S in DM or seed yield. On the other hand, significant amounts of S might be mobilized to faba bean seeds, something that was not investigated in this study.

The CP content (%) and protein yield (kg ha^{-1}) were not affected by S fertilizer treatments. Thus, the results were not in accordance with hypothesis 2, i.e., that protein content would increase, and the amino acid composition would be altered by S fertilizer application. Differences between the experimental sites concerning the effect of the S fertilizers on protein yield indicate effects of other factors, such as soil properties and the prevailing environmental conditions (Pötzsch et al. 2018). The clear differences during both years between the experimental sites in macro- and micronutrient content in DM, protein content and protein yield, indicate the complexity of the effect of S fertilization on faba bean and the difficulty to generate guidelines for S fertilization to farmers, which was also discussed by Boye (2011).

Sufficient plant-available sulphur is also important for the sulphur-containing amino acids cysteine and methionine, which are normally found at lower concentrations than most other amino acids in faba bean (Barlóg et al. 2019). The low contents of cysteine and methionine compared to other amino acids (lysine, threonine, valine and histidine) were confirmed in the present study. Gypsum and kieserite treatments did not affect the contents of cysteine and methionine, nor the other amino acids. On the other hand, there were significant differences between the experimental sites in the content of methionine, cysteine and other amino acids, which may be due to differences in weather and soil properties and mineral contents. Similar to our results, Barlóg et al. (2019) showed no effect of applying 50 kg S ha^{-1} before sowing on the content of cysteine and methionine in faba bean. The authors explain that the soil contained sufficient amounts of S for the amino acid synthesis (8 mg kg^{-1}). However, Glowacka et al. (2019) reported an increase of cysteine and methionine in common beans fertilized with 50 kg S per ha^{-1} kieserite in sulphur-poor soils in southeast Poland.

Faba bean is generally sensitive to drought and the yield can be reduced by 52% under water stress conditions (Raderschall et al. 2021). Therefore, both growth and yield were lower in the 2018 field experiments compared to 2017. The field experiment in site SK-18 had the highest yield in 2018, possibly thanks to the late rain showers (10.6 mm) in July, which also prolonged the growing season. Significant differences in yield levels between years and experimental sites was reported by Pötzsch et al. (2018). It is also interesting to mention that the experimental site at ÖR had the highest S concentration in faba bean DM both experimental years, but the seed yields were significantly lower compared to the other field experimental sites. The DM content of the seeds and protein yield were also lowest at ÖR, while nitrogen uptake in the biomass showed normal levels. This site had more rainfall in total during 2017 compared to the site at ÖG (303 mm vs. 188 mm) and in 2018, the location at ÖR had the highest amount of rainfall in May and August, but the weather was very dry in July, hence the drought stress leading to decreased seed yield. In addition to total rainfall at each site, the amount of rainfall during the period of May–June is suggested to play a significant role since S content in dry matter and seed yield may negatively correlate with precipitation during the first 60 days (Pötzsch et al. 2018). One explanation for this might be that the vegetative growth increases in response to available water, which results in higher green biomass but eventually reduced seed yield (De Costa et al. 1997). In line with this, it can be speculated that one of several possible explanations for the highest seed yield in ÖG-17, was the lowest precipitation level during May to June (59 mm) compared to other experimental sites, which had an average rainfall of 94 mm. These differences between sites emphasize the importance of site characteristics over S fertilization treatments.

Conclusions

The results indicate that faba bean grown under the present conditions had no demand for sulphur fertilization. The experimental sites differed significantly in all tested parameters and therefore it can be concluded that the characteristics of a specific site plays a more significant role in faba bean growth and yield than sulphur fertilization.

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