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Aspects on cultivation of vegetable soybean in Sweden – cultivars, soil requirements, inoculation and nitrogen contribution

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ABSTRACT

Background. Global soybean production is expected to double in the coming decades, driven by a request for animal feed to meet increasing meat consumption. Demand for locally produced food and feed is also increasing, making it interesting to explore the potential for soybean production in high-latitude regions. *Scope*. To present information on the potential for cultivating vegetable soybean, edamame, under cold-temperate conditions. *Conclusions*. For the successful establishment, sowing at low soil temperatures should be avoided. Commercial inoculants are effective, irrespective of soybean cultivar. There is no need to re-inoculate fields if the crop has been included in the crop rotation. Little nitrogen remains in the soil after harvest, resulting in a low risk of leaching but a need for nitrogen fertilisation of the following crop. Suitable vegetable soybean cultivars are available on the market and are preferred compared to cultivars intended for dry harvest. The cropping system needs to be improved, e.g. by moderating the microclimate by plastic tunnels, in order to secure harvest.

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Bradyrhizobium japonicum; cold temperate conditions; pre-crop effects; re-inoculation; sustainable agriculture; edamame

Introduction

Over the past 50 years, global soybean (*Glycine max* (L.) Merr.) production has increased 10-fold and now occupies 1 million km^2 worldwide. This is because it produces more protein per hectare than any other major crop. Increasing the use of soybean as animal feed to meet the increasing global demand for meat is the most important reason for the rapid expansion in cultivation.

Annual imports of soybean into Sweden comprise approximately 250,000 Mg, so there is scope for domestic production to replace some of these imports. Attempts have been made in the past to grow soybean in Sweden (Holmberg 1973). In 1949, the first Swedish soybean cultivar was released, followed by the Fiskeby III cultivar in 1950. The last cultivar to be released, Fiskeby V, was registered in 1968. In field trials at the Swedish University of Agricultural Sciences, conducted during 1974–1977, recorded yield varied from 80 kg ha^{-1} at the northernmost experimental site (59°48′N, 17°39′E) to 1360 kg ha^{-1} at the southernmost field sites (55°94′N, 13°08′E) (Bengtsson and Larsson 1979). The mean yield for all sites and years was 971 kg ha^{-1} when compensating for 30 % loss at harvest (Bengtsson and Larsson 1979).

It is noteworthy that the disappointing experiences during the 1970s are still resulting in a negative attitude to the production of soybean in Sweden. However, in trials using updated modern cultivars, it has been shown that soybean can be grown from Skåne (approximately 55°N) up to Mälardalen county (approximately 59°N), with yield on commercial scale of 2000–2400 kg ha^{-1} and with a protein content of up to 44.3 % (Fogelberg and Lagerberg-Fogelberg 2013). A northward movement of the agroecological zones due to climate change will most likely result in even higher yields (Iglesias et al. 2012; Stagge et al. 2017).

To be successful, the choice of cultivars from maturity group 000 is important. Soybean cultivars are divided into 13 maturity groups there the 000-group consists of cultivars suitable for northern Europe. As the maturity groups are based on a visual assessment of maturity in combination with degree days using the Crop Heat Unit (CHU) system adapted for North American cropping, a direct comparison with the European systems for degree days is impossible. In general in southern Sweden, the temperature sum above 5°C from May 25th to October 1st varies between 1500 and 1700 (Fältforsk 2021).

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The main obstacle to increasing soybean cultivation under sub-optimal conditions prevailing at high latitudes is the low temperature, which restricts or prevents soybean plant growth. Fact is that soybean growth and completion of critical stages during plant development, such as nodulation and nitrogen fixation, is reduced at soil temperatures below 25°C (Schmidt et al. 2015). The cool climate conditions therefore pose the idea if the production of vegetable soybeans (edamame), that is pods harvested immature, similar to sugar snaps, could be an option for Sweden as this will remove the problem of late maturation of seeds for dry harvest. As for many other crops, there are soybean cultivars especially suitable for the harvest of fresh green pods – edamame – as these cultivar seeds and pods have attractive green colour, crispiness and sweetness compared to standard food and feed cultivar although these latter cultivars *per se* also can be used for fresh harvest. Edamame is currently sold to consumers either as fresh chilled pods or as precooked frozen pods/kernels for use in salads or various Asian-inspired food dishes.

In addition, when cultivating soybean on juvenile soils, which would be the main case in Sweden, the seed needs to be inoculated with its symbiotic partner, the nitrogen-fixing bacterial species *Bradyrhizobium japonicum*. Therefore, an important question for Swedish growers is whether inoculation is necessary. The current advice is to always inoculate, supported by a study conducted in Canada under similar climate conditions to those in Sweden, which reported that the bacteria were unable to survive between years in Canadian prairie soils (Bailey 1989). Another question is whether the commercial inoculants currently available are effective when used with the soybean cultivars developed for high-latitude production. Pre-inoculated cultivars are available on the European market, but storage and transport may affect the viability of pre-inoculum. At present, it is not clear if inoculum used is suitable for Swedish conditions. Hence, an on-farm inoculation could be preferred until seed companies have tested the viability of pre-inoculated seeds in Swedish soil conditions. There are also concerns that applying less effective inoculants can result in low yields. In Argentina, for example, the use of less effective soybean bacterial inoculants has resulted in problems with exploiting the benefits of the symbiotic process in soybean (Brutti et al. 2001).

As soybean is a nitrogen-fixing crop, leftover soybean crop residues in the soil following harvest may contribute positively to the nitrogen (N) pool in the soil, reducing the need for N fertilisation of the next year's crop. Under the specific climate conditions in Australia, Bergersen et al. (1992) reported that one-third of the N in an oat crop grown immediately after incorporation of soybean leaves was derived from those nitrogen-rich leaves.

Given this the hypotheses tested in the present study were thus that:

- Inoculation is necessary each time soybean is cultivated, irrespective of the previous inoculation
- Nitrogen-rich soybean root residues contribute N to the following crop.
- There are interesting cultivars suitable for fresh harvest in Sweden

Specific objectives were to investigate suitable cultivars for vegetable soybeans, the fate of introduced inoculants and the need for re-inoculation of soybeans in the cropping sequence and to determine the contribution of the soybean crop to the soil N pool in a field experiment.

Materials and methods

Indoor experiments

Early emergence of edamame soybean – growth chamber

Three seeds each of three cultivars of soybean (Bohemians – standard food cv, Chiba Green, Midori Giant – edamame cvs, respectively), were sown in trays (diameter 25 cm; seven replicates per cultivar) containing either garden soil consisting of highly humified peat or mineral soil with a clay content of 15 % (both pH 7.1). The trays were randomly distributed and kept for 15 days in growth chambers at 10, 15, 18 or 22°C and light duration 17 h day^{-1}, light intensity 250–350 µmol m^{-2} s, and humidity 60 %, and watered when needed. Emergence and later on growth, was visually recorded every 24 h.

Inoculation and strain-cultivar interactions – Greenhouse

Three seeds each of four standard food cultivars of soybean (cvs. Bohemians, Silesia, SL960663, Tundra) were sown in plastic pots (diameter 12 cm, volume 700 mL; four replicates per cultivar) containing potting compost consisting of highly humified peat (pH 7.1). Before sowing, 75 seeds of each cultivar were shaken together with either pure culture of *B. japonicum* obtained from Instituto Nacional de Tecnologia Agropecuaria, Castelar, Argentina (strains E11, E109 and E367) or the commercial inoculant HiStick (BASF). All pots were thinned after emergence to give one plant per pot. The pots were randomly distributed and kept in a greenhouse for four weeks at 18 °C, light duration 17 h day^{-1}, light intensity 400 µmol m^{-2} s, and humidity 70 %, and watered

Table 1. Properties of the four field soils used to study the effect of previous soybean seed inoculation.

Soil	pH	Total-C (%)	Total-N (%)	Readily soluble K (mg·100 g dry matter of soil^{-1})	Less soluble K (mg·100 g dry matter of soil^{-1})	Readily soluble P (mg·100 g dry matter of soil^{-1})	Less soluble P (mg·100 g dry matter of soil^{-1})	Clay content (%)
Berga	5.32	3.81	0.29	5.0	92.2	6.4	62.2	11
Edsberg	5.78	1.66	0.05	3.5	76.3	7.1	94.8	7
Munktorp*	5.55	2.50	0.20	15.4	389.5	5.2	59.6	16
Sjöö**	6.32	2.15	0.18	23.0	550.7	11.2	72.1	19

*Munktorp, the values are mean values from the two investigated fields, differing by less than 10 % from each other.
**Sjöö, the values are mean values from the three investigated fields, differing by less than 10 % from each other.

when necessary. At harvest, dry weights of plants and presence of nodulation were recorded.

Inoculation and need for re-inoculation – Greenhouse

To determine the need for re-inoculation, soil samples were collected from four Swedish farms which had been inoculated with HiStick (BASF) before previous field production of soybean. The farms were: Berga (59°11′N, 14°53′E), where sampling was conducted in fields in which a soybean crop had been grown in the previous year; Edsberg (59°22′N,13°15′E), where sampling was conducted in fields in which a soybean crop had been grown in the previous year; Munktorp (59°32′N,16°9′E), where sampling was conducted in fields in which a soybean crop had been grown one and three years earlier; and Sjöö (59°43′N, 17°30′E), where sampling was conducted in three fields in which soybean had been grown one, two and three years earlier. The soil samples were collected in the topsoil (0–20 cm) diagonally across the fields (field size varied between 100 and 2000 m^2). A total of 20–30 samples each consisting of 1–2 L soil were collected at each field, pooled and immediately transported to the laboratory and mixed with pumice stone (1:0.3 v/v) to avoid compaction. For each soil seven pots containing soil/pumice mixture (1.3–1.4 L soil and 0.6–0.7 L pumice) were prepared and five seeds of soybean (Moravians – standard food cv) were sown in each pot. The pots were randomly distributed in the greenhouse, conditions as above. No additional nutrients were applied to the plants during growth. After seedling emergence, the plants were thinned to give two plants per pot. After six weeks of growth, the plants were harvested and aboveground plant material was dried and weighed. The presence of nodules was noted. The soils from each field were analysed for pH and content of carbon (C), nitrogen (N), phosphorus (P) and potassium (K), using standard procedures (Table 1). Soil pH was determined by potentiometry in 0.01 mol L^{-1} calcium chloride (CaCl 2) solution, carbon was estimated by loss on ignition, N was quantified colorimetrically in potassium chloride extracts (2 M) by an auto-analyser (TrAAcs800, Bran & Lubbe, Hamburg, Germany). P and K were measured in ammonium acetate lactate by ICP (Perkin Elmer Optima 300 DV, Perkin Elmer, Norwalk, CT, U.S.A.). All laboratory soil sample determinations included three replicates.

Field trials

Suitable vegetable soybean cultivars

As there is no earlier experience on vegetable soybeans field cropping in Sweden, pilot trials were established in the province of Skåne, (southern Sweden 55°4′N,14°18′E), in 2015, on the island of Öland (south-east Baltic Sea, close to the Swedish mainland, 56°33′N,16°925E), in 2016–2017 and in the province of Västergötland (Götala in southwest Sweden 58°38′N,13°49′E), in 2017–2018. As edamame should be harvested green and transported to a processing facility for cooking and freezing the test sites in Skåne and in Götala were selected as they represent possible production areas. The Öland site was selected due to the early spring conditions and long tradition on cropping of beans and peas (Table 2).

True edamame cultivar seeds were obtained from Japan (Snow Brand Seeds Co. Ltd) and U.S.A. (Wannamaker Seeds Inc. and Gourmet Seeds International). Soybean cultivars originating from Czech Republic (Prograin-ZIA) and Canada (SG Ceresco Inc) intended for mature harvest and further use in food/feed industry were used as a comparison. The field trials were designed as randomised complete block trials with four replicates using plot sizes of 20 m^2 and three rows (Table 3). Seeding, maintenance and weed control were carried out by the Rural Economy and Agricultural Societies, a NGO organisation for advisory services in Sweden. Fields were cultivated in spring and 300 kg ha^{-1} mineral fertiliser (NPK 11-5-18) was applied prior to seeding in accordance with recommendations from seed supplier Prograin-ZIA, Czech Republic. Seeding took place in the period May 10th to May 24th depending on weather conditions. A seeding depth of about 4 cm was used with a row spacing of 50 cm and 15 seeds per row meter resulting in about 12 plants per row meter. Chemical (pre-emergence spraying with Sencor 0.4 l ha^{-1}) as well as

Table 2. Selected soya bean cultivars, experimental field site and year site and test year.

Variety	Origin	TKW* (g)	Year 2015	2016	2017	2017	2017	2018
Anser	Canada	223	Skåne		Öland			
Bohemians	Czechia	240			Öland	Skåne	Götala	Götala
Silesia	Czechia	224			Öland	Skåne	Götala	
Aoshizuku	Japan	315	Skåne	Öland				
Kaohiung no 9	Japan	453	Skåne					
Sapporomidori	Japan	362	Skåne					
Sayakomachi	Japan	384	Skåne					
Sayamusume	Japan	420	Skåne	Öland	Öland			
Be Sweet	U.S.A.	418		Öland				
Chiba Green	U.S.A.	394	Skåne	Öland	Öland	Skåne	Götala	Götala
Envy	U.S.A.	330		Öland				
Ginza	U.S.A.	315	Skåne	Öland				
Midori Giant	U.S.A.	360	Skåne	Öland	Öland	Skåne	Götala	Götala

*TKW represents thousand kernel weight.

mechanical weed control were used. Fresh green pods were harvested at stage R6 (Licht 2014) from 4 row meters randomly selected in each plot where seeds had developed (last week in September to third week in October) and yield was calculated as fresh pod weight in kg ha^{-1}.

Nitrogen contribution of the soybean crop to the following crop

To study the contribution of symbiotic nitrogen fixation by soybean plants to the following crop, the field experiment at Götala was used. The soil at the site contains 15 % clay, with an organic matter content of 2.2 % and (per 100 g dry weight of soil): 2.2 mg poorly soluble P, 16 mg readily soluble P, 155 mg poorly soluble K and 9.8 mg readily soluble K.

Three soybean cultivars (Bohemians, Chiba Green, Midori Giant), inoculated with HiStick, were sown in mid-May in four replicate plots measuring 9 × 16 m laid out in a randomised complete block design of four blocks. At sowing, the authors took soil samples from each plot by extracting nine cores each from the 0–15 cm and 15–30 cm soil layers, respectively, using a drill with a diameter of 3 cm. The samples were then pooled per layer, frozen immediately and stored for analyses of nitrate-N and ammonium-N content. Similar sampling of the 0–15 cm and 15–30 cm soil layers was performed at harvest of the soybean crop in late September. Before sowing of the next crop (barley) in early May of the following year, samples were taken from the 0–15 cm, 15–30 cm and 60–90 cm soil layers, using a similar technique as at previous samplings, but with the number of cores from the deepest sampled layer reduced to six. At harvest (stage R6; Licht 2014) of the soybean crop in September, fresh dry weight was recorded for all plants and presence of nodules in inoculated plots was determined visually by noting presence of nodules on roots on three randomly selected plants in each plot.

Sampling and statistical analyses

Sampling was generally carried out by the authors. In the indoor experiments, all pots were used for sampling and visual assessments. In the field trials investigating suitable cultivars, the centre row was used for sampling of yield and measure of plant height. Visual assessments were made of the entire plot and soil sampling was distributed to cover the entire plot. We used certified seeds in all experiments with a viability of 88–93 %.

In the analyses of results, Multifactor ANOVA with Tukey's HSD (Statgraphics Centurion XVI) was used as a standard method for statistical difference between treatments. Results are presented as mean values using the 95 % level for statistical difference, i.e. if $p < 0.05$ a statistical difference between mean values is present.

Results

Indoor experiments

Early emergence of soybean – growth chamber

The fastest emergence of the soybean plants was observed at a temperature of 22°C. However, after two

Table 3. Seeding and harvest dates of the edamame trials 2015–2018.

	Year							
	2015	2016	2017	2018				
Site	Seeding	Harvest	Seeding	Harvest	Seeding	Harvest	Seeding	Harvest
Skåne	June 1	Oct 16			May 24	Oct 12		
Öland			25 May	29 Sept	May 31	Oct 28		
Götala					May 23	Oct 2	May 29	Oct 12

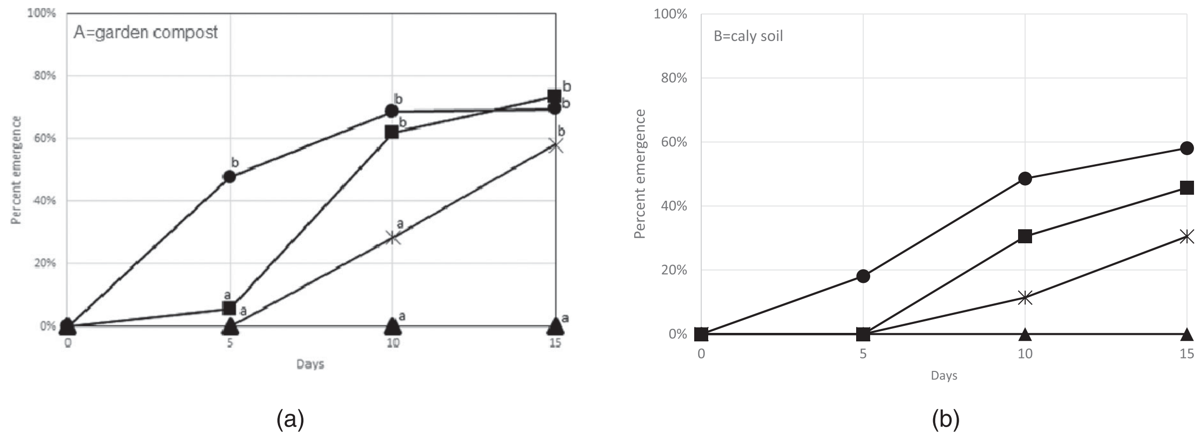


Figure 1. Emergence of soybean in (A) garden compost and (B) clay soil at four different temperatures. Values followed by different letters are significantly different ($p \leq 0.05$) ▲ = 10 °C, ✱ = 15 °C, ■ = 18 °C, ● = 22 °C.

Table 4. Effect of inoculation with *Bradyrhizobium japonicum* strains and commercial inoculant on aboveground dry matter production (g/pot) in four soybean cultivars. Values shown are mean ± standard error ($n = 4$).

Cultivar	*B. japonicum* E11	*B. japonicum* E109	*B. japonicum* E367	HiStick	Mean
Bohemians	3.50 ± 0.37Ba*	3.31 ± 0.59Bb	1.91 ± 0.16Aa	2.45 ± 0.32Aa	2.79 ± 0.68AB
Silesia	1.77 ± 0.16Aa	2.45 ± 0.15Aa	2.11 ± 0.24Aa	2.20 ± 0.31Aa	2.08 ± 0.32A
SL960663	1.95 ± 0.16Aa	2.88 ± 0.34Ba	3.04 ± 0.33Bb	3.62 ± 0.55Bb	2.79 ± 0.61AB
Tundra	3.27 ± 0.28Bb	2.52 ± 0.27Aa	2.65 ± 0.29Aab	2.68 ± 0.11Aa	2.68 ± 0.33AB
Mean	2.58 ± 0.76ab	2.79 ± 0.66ab	2.23 ± 0.74ab	2.67 ± 0.82ab	

*Values within each row or column followed by different letters (lowercase in columns, uppercase in rows) are significantly different ($p \leq 0.05$).

weeks, the difference in emergence between 15, 18 and 22 °C was negligible (Figure 1). All cultivars tested, (Bohemians, Chiba Green and Midori Giant) performed equally well at all temperatures. No cultivars were able to emerge at 10 °C. Soybean emergence was least favoured under cool temperature on the clay soil, resulting in only 30 % emergence of the seeds at 15 °C compared to 60 % emergence in the garden soil.

Inoculation and strain-cultivar interactions – Greenhouse

The four soybean inoculants tested did not differ with respect to the ability to induce nodules and all soybean cultivars tested developed well with respect to plant growth. However, there were some differences for individual strain-cultivar combinations. The cultivar Silesia grew well with all soybean inoculants, whereas Bohemians developed best with *B. japonicum* strains E11 and E109, cv. SL960663 with *B. japonicum* E367 and HiStick, and cv. Tundra with *B. japonicum* E11 (Table 4). There were no differences between the pure cultures of *B. japonicum* and HiStick (BASF) or among the pure cultures with respect to the ability to induce nodule formation (data not shown).

Inoculation and need for re-inoculation – Greenhouse

Soybeans cultivated in farm soils which had hosted a soybean crop one year previously or with a longer gap of two and/or three years in soybean cultivation were able to induce soybean nodules, data not shown. Soybean plants developed well in all studied soil, dry matter production ranged between 6.5statistical difference between mean 15.1 g per pot depending on soil. No statistical analyses were performed on the mean differences between the plants grown on different soils, since the soil conditions differed widely between sites.

On comparing the impact of soil factors on the ability to induce soybean nodules at the sites where soybean had been grown in the previous year, soil pH and soil K content negatively affected these abilities. A weak negative relationship was found between soil pH at the sites and plant dry weight (Figure 2(a)) and a stronger negative relationship between K content at the sites and plant dry matter production (Figure 2(bc)). In contrast, soil P status in terms of readily soluble P had a positive influence on the soil to induce nodulation (Figure 2(d)). However, the soil content of less soluble soil P did not affect the soil's abilities to induce nodules (data not shown). No correlations between plant dry weight and soil N and soil C concentrations have existed (data not shown).

Field trials

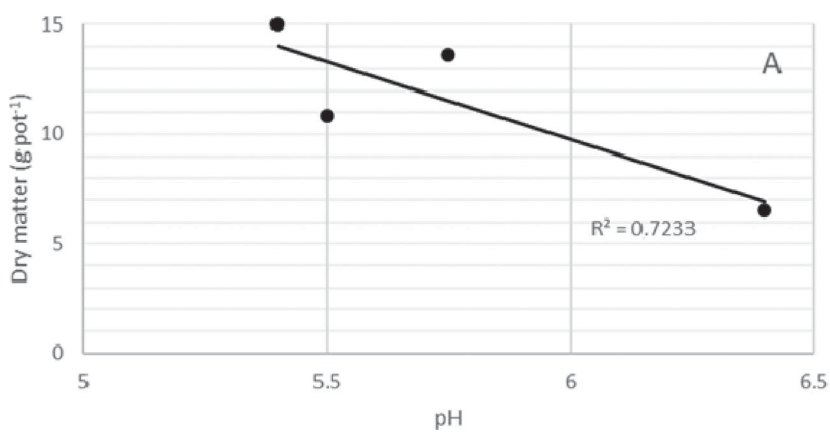
Suitable vegetable soybean cultivars

In 2015, the Japanese cultivar Ginza did not develop at all. Sayakomachi, Aoshizuku and Kaohiung no 9 did not set pods, and were excluded from further studies. There were no statistical differences in yield, ($p = 0.88$). Sapporimidori and Sayamusume yielded 4350, respectively 3980 kg ha^{-1}, whilst Anser gave 3670 kg ha^{-1}, Chiba Green 3410 kg ha^{-1} and Midori Giant 3330 kg ha^{-1}. No pest damages were reported observed, but two of the cultivars, Midori Giant and Sayakomachi had a visually lighter green leaf colour and some pale or even yellow leaves. The annual average temperature in the region in 2015 was 9.5°C compared to the 30-year mean of 7.9°C with 18 summer days above 25°C.

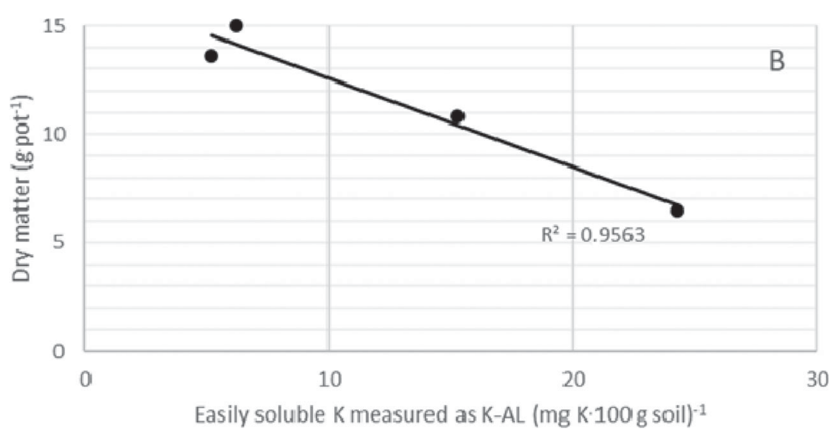
In the Öland experiment 2016 three cultivars resulted in yield: Chiba Green, Midori Giant and Sayamusume. Again Ginza together with Be Sweet and Envy failed to establish. There was a significant difference in yield between cultivars ($p = 0.0015$) with Sayamusume as the best with 5150 kg fresh pods ha^{-1}. Chiba Green (4170 kg ha^{-1}) and Midori Giant (4330 kg ha^{-1}) did not differ statistically. The annual average temperature in the region was 8.8°C compared to the 30-year mean of 7.0°C with 16 summer days above 25°C.

The trials in 2017 partly confirmed the earlier results, however, the Skåne trial was partly damaged by birds which makes the results less credible. Chiba Green and Midori Giant yielded (fresh pods) 2300 and 2460 kg ha^{-1}, respectively. Bohemians and Silesia yielded 2720, respectively, 2300 kg ha^{-1}. There were no statistical differences ($p = 0.23$) in yield between cultivars. The annual average temperature in the region was 9.3°C compared to the 30-year mean of 7.8°C with 6 summer days above 25°C.

The Öland trial included the Japanese cultivar Sayamusume. As in 2016, Chiba Green and Midori Giant gave the highest yields (5380 and 5800 kg ha^{-1}respectively), Bohemians (2960 kg ha^{-1}) and Silesia' (2470 kg ha^{-1}) were comparable to the yields in Skåne. The Canadian cultivar Anser, intended for mature harvest, yielded 2020 kg ha^{-1}. Chiba Green and Midori Giant were statistically different from Anser, Bohemians and Silesia ($p = 0.043$) while there were no differences between the three latter

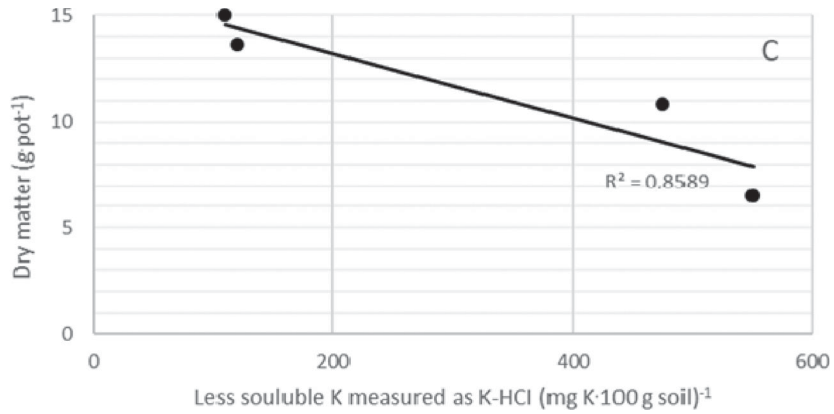


(a)

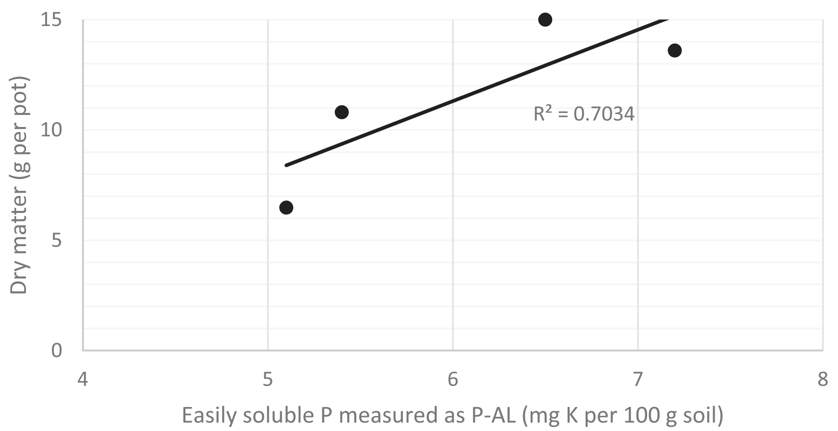


(b)

Figure 2. Effect of (A) soil pH and of soil content of (B) easily soluble potassium (K), (C) less soluble K and (D) easily soluble phosphorus (P) on plant biomass (g pot^{-1}) of soybean grown in soils where soybean had been grown one year previously.



(c)



(d)

Figure 2 *Continued*

cultivars ($p = 0.67$). The annual average temperature in the region was 8.5°C compared to the 30-year mean of 7.0°C without any summer days above 25°C.

The 2017 trial in Västergötland (Götala in southwest Sweden) suffered from drought during July and early August but had a fresh green stand at harvest. Chiba Green and Midori Giant did not perform as well as on the other test sites. Yields were 1160 and 2170 kg ha^{-1} each and pods without seeds in both cultivars were observed. In contrast Bohemians and Silesia yielded 5360, respectively, 6410 kg ha^{-1}. There was a statistical difference between the true edamame cultivars and the standard cultivarss ($p = 0.002$) but not within the two groups. The annual average temperature in the region was 7.4°C compared to the 30-year mean of 5.9°C with 5 summer days above 25°C.

The 2018 trial in Västergötland (Götala in southwest Sweden) was partly weed-infested due to insufficient control measures. The soybeans suffered from drought during the growing season which is reflected in low yields. Bohemians yielded 1635 kg ha^{-1}, Chiba Green 3000 kg ha^{-1} and Midori yielded 3345 kg ha^{-1}, there were no statistical differences ($p = 0.214$) in yields. The annual average temperature in the region was 8.0°C compared to the 30-year mean of 5.9°C with 56 summer days above 25°C.

Nitrogen contribution of the soybean crop to the following crop

All the plants visually inspected for presence of nodulation had nodules except for those in the uninoculated control, demonstrating the necessity of inoculating soybean seeds. The N levels in the soil profile, monitored as ammonium-N and nitrate-N, when cultivating soybean decreased during the cropping season (Figure 3). The uninoculated soybean plants extracted more soil ammonium-N and nitrate-N from the 15–30 cm soil layer by the time of harvest. Some cultivar differences with respect to soil N were observed at sowing, indicating an uneven distribution of soil N at the field site. In May of the next year (2019), a month before sowing the following crop, the soil showed

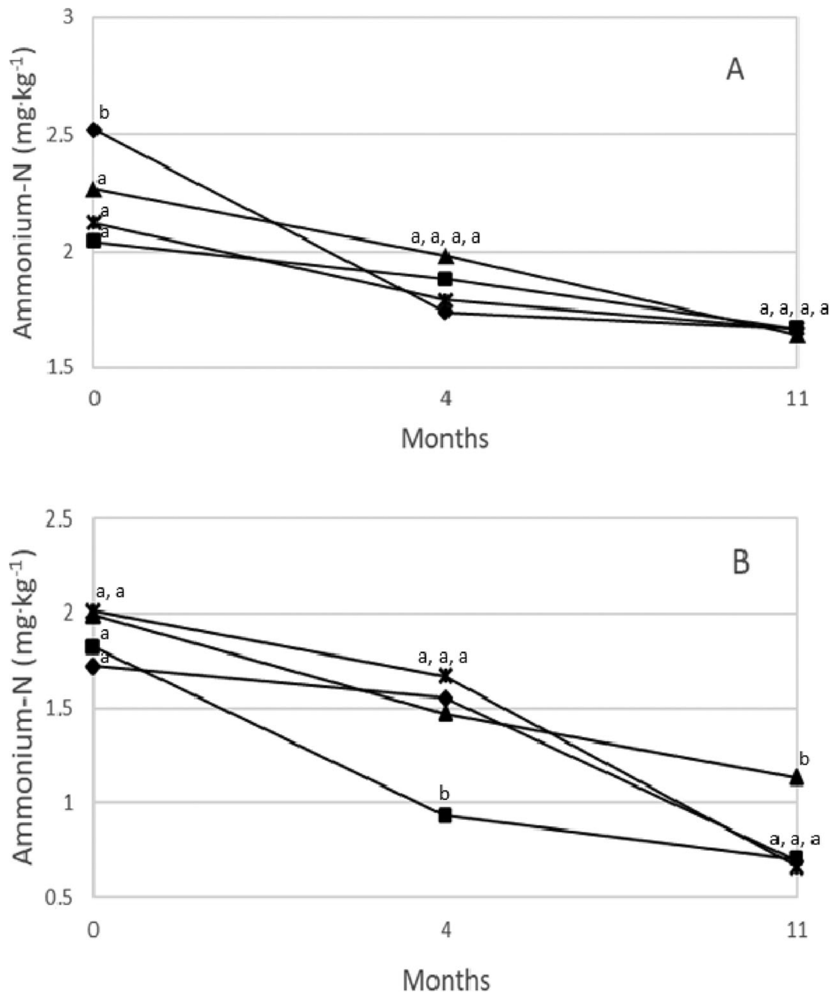


Figure 3. Changes over time in soil nitrogen (N) content, measured as (A, B) ammonium-N and (C, D) nitrate-N at (A, C) 0–15 cm depth and (B, D) 15–30 cm depth, when cultivating three edamame cultivars. —✱—= Bohemians, —■—= Chiba Green, —◆—= Midori Giant, —▲—= uninoculated,

elevated nitrate-N levels in the inoculated treatment at 60–90 cm soil depth compared with the uninoculated control (Table 5).

Discussion

Early emergence of soybeans

The fastest emergence of soybean plants was observed at 22 °C, but after two weeks the difference in emergence between 15, 18 and 22 °C had disappeared (Figure 1). Low root (soil) temperature has previously been reported to inhibit germination, rhizobial infection and plant growth in soybean (Wang et al., 2011; Leibovitch et al. 2001). In farming in the Nordic countries and Northern Europe, the soybean crop should therefore not be sown too early as this may result in poor emergence and later problem with weed competition, bird damage, or soil crusting. Light soil which warms up early during the spring can be recommended as well as covering the soil with non-woven plastic.

Inoculation and strain-cultivar interactions

It is well known that there are strain-cultivar interactions between *B. japonicum* and soybean (Vlassak et al. 1997). However, in this study, there was no marked difference in efficiency between the four strains studied when comparing the results of the entire experiment, although some individual strain-cultivar combinations were better than the average (Table 4). In theory, one should match the right strain to the specific soybean cultivar, but in a practical farming situation that is seldom possible. Based on the results, it can be concluded that HiStick is a suitable option. However, HiStick may not be the best commercial inoculant for use under cold climate conditions, as a study by Pannecoucque et al. (2018) comparing the commercial inoculants HiStick,

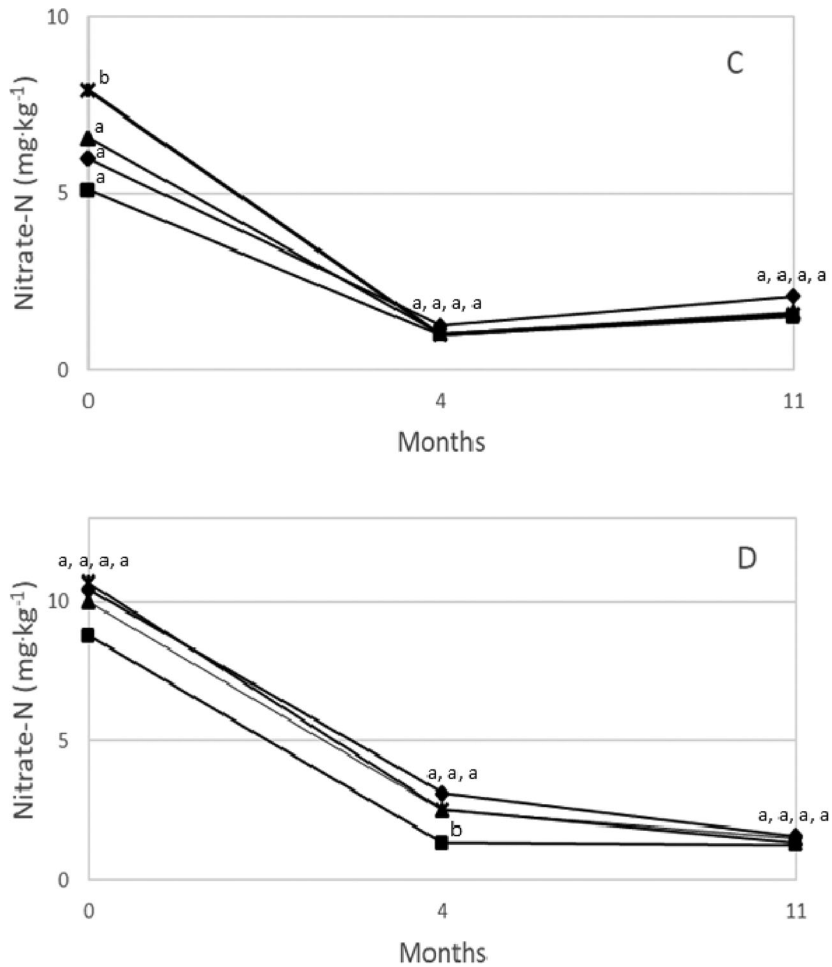


Figure 3 *Continued*

Force 48, Biodoz and Optimize found that the bacteria in Biodoz had better capacity to grow at low temperatures. Artificial selection could be an option, as rhizobial inoculants have been reported to adapt to local environmental conditions (Alves et al. 2003). For instance, Lynch and Smith (1993) found that strains of *B. japonicum* isolated from low soil temperature conditions in Hokkaido, Japan, were more effective at cold temperatures than commercially available inoculant strains. In order to develop soybean cropping in Northern Europe and especially the Nordic-Baltic countries, further research attention should be devoted to finding inoculant strains adapted for cool soil-cool climate conditions.

Table 5. Impact of soybean cultivation on soil ammonium-nitrogen (N) and nitrate-N content (mg· kg^{-1}) at 60–90 cm depth, measured seven months after harvesting the soybean crop (one month before sowing the next year's crop). Values shown are mean ± standard error (n = 4).

Soybean cultivar	Ammonium-N	Nitrate-N
Bohemians	0.69 ± 0.17a*	3.49 ± 0.76b
Chiba Green	0.75 ± 0.03a	4.44 ± 1.05b
Midori Giant	0.83 ± 0.19a	3.84 ± 0.57b
Uninoculated cultivar mixture	0.75 ± 0.09a	2.08 ± 0.31a

*Values within each column followed by different letters are significantly different ($p \leq 0.05$).

Inoculation and need for re-inoculation

As presented, in field trials on commercial farms, the soils kept their ability to induce soybean nodulation. This contradicts findings by Bailey (1989), who reported that no soybean bacteria were present in Canadian prairie soils a year after the latest inoculated soybean crop and suggested that low winter temperature killed the inoculated bacteria. However, in a study by Perrineau et al. (2014) on soils in Malaysia and Senegal, the inoculants were found to be still present after 15 years, with varying ability for symbiotic nitrogen fixation. The ability of soybean-associated N-fixing bacteria to survive longer than a year was also reported by Galli-Terasawa et al. (2003), who recovered *B. japonicum* from soil 15 years after introduction in the Cerrados in Brazil.

Soil C and N content had varying and inconsistent effects on the ability of the soils to induce soybean nodulation, making it difficult to estimate their impact. Higher pH correlated negatively with plant dry matter production (Figure 2(a)), whereas many previous studies have found that low pH negatively affects the survival of soybean bacteria (Taylor et al. 1991; Albareda et al. 2009; Atieno and Lesueur 2019). However, slow-growing *Bradyrhizobium* strains are reported to be generally more acid-tolerant than fast-growing species (Bordeleau and Prévost 1994). The results may be explained by the higher solubility of many essential plant nutrients at lower pH, which promotes plant fitness and thereby improves the symbiotic process. This explanation is supported by the observation that increasing levels of soluble P improved the symbiotic performance, measured as plant growth (Figure 2(d)). The amount of K in soil appeared to impair the ability of the soils to induce soybean nodulation (Figure 2(b,c)). This is contrary to results by Premaratne and Oertli (1994) and Faé et al. (2020), who found that increased K concentration increased plant dry matter and nodule weight. Their findings were confirmed by a meta-analysis by Santachiara et al. (2019) showing positive plant and *Rhizobium* fitness following application of K fertiliser. The negative impact of K observed in the present study could be due to a link between K content and clay content, as Swedish clays are heavy and rich in K, as e.g. Albareda et al. (2009) observed a better survival rate of *B. japonicum* in lighter soils than in loamy-textured soils.

Nitrogen contribution of the soybean crop to the following crop

When considering the data obtained in this study, it must be borne in mind that the field experiment was carried out in the summer of 2018, which was extremely dry in Sweden. The average yield of faba bean (*Vicia faba minor*) in the study region (Västra Götaland) in that year was only 35 % of the average yield for the previous five years (Jordbruksstatistisk årsbok 2014, 2015, 2016, 2017, 2018, 2019). The results of the field experiment are thus not fully representative of a typical year for soybean cropping in western Sweden. As shown in Figure 3, all the inoculated cultivars tested took up ammonium-N equally in the upper soil profile, while the uninoculated cultivar exploited it least. The soil nitrate-N content also decreased during the growing season, most in the uninoculated treatment.

After the winter period, the levels of surplus N were very low and the N level decreased during the growing season (Table 5). The contribution of symbiotically fixed N to the soil pool is therefore probably negligible. In a study in Australia, it was found that 33 % of the N in an oat crop following a soybean crop derived from soybean residues incorporated into the soil, but that less than 1 % derived from incorporated soybean roots (Bergersen et al. 1992). The study also showed that most of the N from the soybean residues remained in the upper (0–10 cm) layer of the soil profile, from which leaching N losses can be expected to occur during wet conditions. Kearney et al. (2019) confirmed that substantial losses of N from soybean residues can occur during winter fallow under wet conditions. The contribution of N-rich residues from the soybean crop to the following crop should therefore not be overestimated.

Suitable vegetable soybean cultivars

The field trials in 2015–2018 have shown that the production of true edamame cultivars only to some extent can be cropped in the open field under Swedish climatic conditions. The main obstacle for production is likely the low soil temperature at seeding. To safeguard plant development and subsequent nodulation 'starter N' was therefore applied as suggested by (van Kessel and Hartley 2000 Namvar et al. 2011;). In mid of May in southern Sweden, soil temperatures seldom exceed 10–14°C. The low field emergence of Be Sweet, Envy and Ginza is probably due to a high soil heat requirement as the seeds were delivered with a certified germination ability. The growth chamber study showed that seeds in general did not germinate at temperatures of 10°C. Seeding on raised beds or covering the rows with plastic tunnels (often used for the production of raspberries or strawberries) can be a technically simple method to enhance germination and plant development. This production system will however also influence production costs.

In mid of May in southern Sweden soil temperatures seldom exceed 10–14°C. The low field emergence of Be Sweet, Envy and Ginza is probably due to a high soil heat requirement as the seeds were delivered with a certified growth ability. The growth chamber study showed that seeds in general did not germinate at temperatures of 10°C, which highlights the importance for growers to control the soil temperature before sowing Seeding on raised beds or covering the rows with plastic tunnels (often used for the production of raspberries or strawberries) can be a technically simple method to enhance germination and plant development. This production system will however also influence production costs.

Chiba Green and Midori Giant originating from U.S.A. and Sayamusume from Japan, showed yields up to 5800 kg ha^{-1} fresh pods, but with large variations in yields (1200–5800 kg ha^{-1}) making it difficult to safeguard an economic production. Regarding multiplication of edamame seeds, there seems to be no such possibility under Swedish conditions. We observed that non-harvested rows did not mature even as late as in early December. In future production, seeds need to be imported to Sweden in case true edamame cultivars are to be harvested green. The possibility to use cultivars intended for mature harvest is an option, but these pods are in general less attractive to consumers due to the small-sized pods and seeds. A promising tool for the selection of soybean cultivars suitable for Swedish conditions may be the approach suggested by Schoving et al. 2020 combining semi-controlled conditions for phenotyping with a simple algorithm.

The results from this pilot study make it possible to formulate cultivation advices for edamame cultivation in Sweden. Prospective growers are recommended to inoculate their seeds when introducing soybean, but will not have to repeat inoculation if soybean is grown around once every four years. There is little to be gained by a thorough investigation of interactions between soybean cultivars and type of *B. japonicum* strain/commercial inoculant used. A standard commercial *B. japonicum* product intended for Europe is most likely the best choice for the majority of farmers in the Nordic countries. Edamame needs high soil temperature to emerge, therefore careful monitoring of soil temperatures before sowing can be recommended. An alternative could be the use of raised beds to quickly raise soil temperatures and safeguard plant development.

Conclusions

- Only a handful of true edamame cultivars can be cropped in Sweden as they require higher soil and air temperatures for flowering and seed setting.
- Development of the cropping system, such as raised beds or plastic tunnels, could enhance soybean development and secure yields.
- Using standard soybean cultivars instead of true edamame cultivars may be an applied solution for promoting Swedish production of vegetable soybeans.
- Annual inoculation is not necessary if soybean has been cultivated up to 3 years previously.
- Under Swedish conditions, soybean root residues are not a major source of nitrogen to the following crop.

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