Effects of temperature on germination and juvenile growth in Spinach (*Spinacia oleracea L.*)

Olika Temperaturers effekt på groning och tidig tillväxt hos spenat *(Spinacia oleracea L.)*

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Independent project • 30 credits
Swedish University of Agricultural Sciences, SLU
Department of crop production ecology
Agriculture programme - Soil and Plant sciences
Series title, Part number

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Credits:	30 credits
Level:	Second cycle, A2E
Course title:	Master thesis in Biology
Course code:	**EX0898**
Programme/education:	**Agriculture programme – Soil and Plant sciences**
Course coordinating dept:	Department of Aquatic Science and Assessment
Place of publication:	**Uppsala, Sweden**
Year of publication:	**2022**

Keywords:	Indoor farming, vertical farming, hydroponics, spinach, seed germination

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Abstract

As the worlds urban population grows more and more every year, it becomes increasingly important that cities become more self-sufficient on food. Indoor vertical hydroponic farms offer a solution to this by placing the farm in an urban environment and growing plants in a controlled environment. Nära Sverige AB is doing just that, by growing kale, pak choi and salad hydroponically in an ebb and flow system, two stories below the ground in Liljeholmen, Stockholm. At Nära Sverige AB, constant seed trials are performed to find new suitable crops for cultivation, with spinach being on top of the list. The yield of hydroponically grown spinach has been found to be higher than field grown spinach. It is also economically sound for a vertical hydroponic system because of its short production cycles. Short production cycles require a uniform emergence of the crop for it to be economically viable. Compared to other crops grown in hydroponic systems, spinach presents a challenge, because of its frequently non-uniform and low seed germination, and germination is highly affected by temperature.

By exploring the effects of different temperatures and water soaking of the seeds on seed germination and juvenile plant growth, this thesis aimed to find the most efficient way of germinating spinach for use in a hydroponic system. This was done in two phases, the germination phase where the aim was to see how the different treatments affected germination percentage, speed of germination and uniformity of germination. And the propagation phase, where the aim was to examine if the different treatments had any lingering effects on the growth and morphology of the plants after a week in the hydroponic system.

Four temperatures were tested, 12, 18, 21 and 27 °C and a soaking treatment was included at 18 °C, the seeds were germinated for seven days and a total of four replicates was performed. After the germination phase half of the seeds were dried and weighed for biomass. The other half were transferred to the hydroponic system for seven days and then measured for height, leaf length, chlorophyll content and biomass.

The results showed highest germination percentage at 12 °C, with decreasing germination rate with increasing temperature. Speed of germination was highest at 27 °C, while the most uniform germination occurred at 12 °C. The soaking treatment had no significant effects on the germination percentage, Temperature had significant effects on plant height, leaf length, chlorophyll content and biomass after propagation. Plant height increased linearly with temperature. Leaf length increased between 12 and 18 °C, before decreasing with temperature.

The most efficient germination method was discussed with the best compromise between high germination, high yield, and uniform growth in mind. Effects on juvenile growth and optimal days of germination were taken into account. Germination temperature of 27 °C showed significantly worse germination percentage, with germination inhibition indicated at 21 °C. Yield inhibition was indicated at 21 °C and above, with decreasing leaf length and chlorophyll content, albeit only significantly at 27 °C.

The high germination percentage, high uniformity, low plant height and favourable effects on juvenile growth all pointed to 12 °C as the most efficient temperature, with six days being the optimal germination time.

Sammanfattning

Delen av befolkningen som lever i städer växer mer och mer varje år, i och med detta blir det viktigare att stadsbefolkningen är självförsörjande när det kommer till mat. Inomhusodlingar med vertikala, hydroponiska system kan leda till en högre självförsörjningsgrad genom att odla mat i en kontrollerad miljö, mitt i staden. Två våningar under marken i Liljeholmen, Stockholm odlar Nära Sverige AB grönkål, pak choi och krispsallat vertikalt och hydroponiskt, i ett så kallat Ebb and Flow system. Där sker också fortlöpande odlingsförsök för att hitta nya grödor att odla, med spenat på toppen av listan. Hydroponiskt odlad spenat har tidigare visat högre avkastning än fältodlad spenat. Spenat lämpar sig också för att odlas hydroponiskt ur ett ekonomiskt perspektiv, på grund av den korta odlingscykeln. Ju kortare en odlingscykel är desto viktigare är det med en enhetlig uppkomst hos grödan. Detta utgör en större utmaning hos spenat jämfört med andra hydroponiskt odlade grödor, på grund av låg och ej enhetlig frögroning. Något som har visat sig vara till stor del påverkat av temperatur.

Syftet med detta arbete är hitta det mest effektiva sättet att gro spenatfrön, genom att utsätta fröna för olika temperaturbehandlingar och blötläggning. Effekterna av behandlingarna undersöktes i två etapper. Först i relation till procent grodda frön, groningshastighet och enhetlighet. Sedan undersöktes huruvida behandlingarna hade någon påverkan på växternas tillväxt och morfologi efter en vecka i det hydroponiska systemet.

Fyra temperaturer undersöktes, 12, 18, 21 och 27 °C samt en behandling med blötlagda frön vid 18 °C. fröna lämnades att gro i sju dagar och totalt fyra replikat utfördes. Efter groningsfasen torkades hälften av fröna och vägdes. Den andra hälften skickades till den hydroponiska systemet i sju dagar och mättes sedan för höjd, bladlängd, klorofyllhalt och biomassa.

Antalet grodda frön var högst vid 12 °C och minskade sedan med ökad temperatur. Groningshastigheten var högst vid 27 °C, medan 12 °C ledde till den mest enhetliga groningen. Blötläggning hade inga signifikanta effekter på antalet grodda frön. Temperatur visade signifikanta effekter på höjd, bladlängd, klorofyllhalt och biomassa efter en vecka i det hydroponiska systemet. Höjd hade ett linjärt samband med ökande temperatur. Bladlängd ökade mellan 12 och 18 °C och minskade sedan med temperaturen.

Den mest effektiva groningsmetoden diskuterades utifrån en avvägning mellan hög grobarhet, hög avkastning och enhetlig tillväxt i åtanke. Behandlingarnas effekt på tillväxt efter sju dagar och optimal groningstid togs med i beräkningen. 27 °C ledde till betydligt färre grodda frön, 21 °C visade en tendens att leda till betydligt färre grodda frön. Mätningarna av bladlängd och klorofyllinnehåll indikerade en hämning av avkastning vid 21 °C och högre, effekterna var dock endast signifikanta vid 27 °C

Det höga antalet grodda frön, den höga enhetligheten, plantornas låga höjd och de gynnsamma effekter som kunde ses efter sju dagar i det hydroponiska systemet visade alla att 12 °C var den mest effektiva temperatur att gro spenatfrön på. Sex dagar bestämdes vara den optimala groningstiden.

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T12	The 12 °C treatment
T18	The 18 °C treatment
T21	The 21 °C treatment
T27	The 27 °C treatment

1. Introduction

As the world's urban population grows more and more every year (Kozai 2018), it becomes increasingly important that cities become more self-sufficient on food. With the Swedish agricultural sector becoming more and more dependent on fossil fuel inputs, and with import of food on the rise (Martin & Molin 2019). An increased self-sufficiency has the added benefit of decreasing emissions twofold. Especially in Northern Europe, where the distances that our food must travel also increase during winter. Indoor vertical farms offer a solution to these challenges, by placing the farms in an urban environment. By using LED lighting, recirculating water, no pesticides and growing on stacked levels, they are less affected by outside conditions, require less input and able to more effectively use the space they occupy (Kozai 2018). For the citizens of a city such as Stockholm, this could enable a year-round supply of locally produced crops that would otherwise travel long distances before they reach the customer. However, as of 2022, the crops that are being grown in these system are still limited and there are few studies on their sustainability (Martin & Molin 2019). It is therefore of the utmost importance that more research is put into the indoor cultivation of new crops so that the percentage of locally produced food in our diet increases.

1.1 Indoor vertical farms

Indoor vertical farms usually use hydroponic systems to grow plants. A hydroponic system is a method of growing plants in nutrient rich water solution. There are several types of hydroponic systems, such as the nutrient film technique (NFT), the deep flow technique (DFT), and the ebb and flow system (Kozai 2018). In the ebb and flow system used at Nära Sverige AB, irrigation water is enriched with nutrients and pH is adjusted. It is then pumped to cultivation benches with raised edges at certain intervals per day, where it stays for a set amount of time before being drained, filtered, pumped back to the water tanks, and used for irrigation again. The nutrients used for hydroponic cultivation are usually commercial synthetic fertilizers that are soluble in water. Vertical farms have the added benefit of greatly reducing the prevalence of pests and diseases and therefore eliminating the use of pesticides (Tidke 2017).

1.2 The Nära Sverige farm

Two stories below ground in a parking garage in Liljeholmen, Stockholm, the company Nära Sverige AB grows lettuce, pak choi and kale in a vertical, hydroponic growth facility. The farm has a total growing area of 800 m^2 on four levels. The location was in part selected because of its vicinity to Årsta partihallar, an area with a high concentration of food related retailers.

Plants are seeded, harvested, packed, and delivered five days a week with one plant cycle consisting of 28 days divided into three phases: germination, propagation, and maturation. Germination takes place in special tents outside the growth room with no lights, close to 100% relative humidity and at 23 °C. After two days, the germinated seeds are moved to the propagation area of the growth room where they stay for five days before being transplanted to maturation for the final three weeks. The growth room is supplied with CO_2, and temperature and humidity are controlled, LED lights that are on for 18 h a day and are placed on each level with increased intensity over time and nutrient rich water is added. Constant seed trials are performed to find new suitable crops for cultivation and the best way of growing them in their system. One crop that has been on the wish list for a long time is Spinach.

1.3 Spinach

Spinach (*Spinacia oleracea* L.) is a leafy cool season vegetable that produces a rosette during its vegetative stage, which is harvested for consumption. It is originally native to central Asia, probably Iran, and is a member of the Chenopodiaceae family. Spinach has high amounts of beta carotene, folate, vitamin C, calcium, iron, phosphorus, sodium and potassium (Morelock & Correll 2008). It has a shallow root system and grows best under slightly acidic conditions when traditionally cultivated (Morelock & Correll 2008).

The yield of hydroponically grown spinach has been found to be much higher than field grown spinach (Tidke 2017). It is also economically suitable for a vertical hydroponic system because of its short production cycles (Brandenberger et al. 2007). Short production cycles require a uniform emergence of the crop for it to be economically viable (Magnée et al. 2020). Compared to other crops grown in hydroponic systems, spinach presents a challenge in this aspect because of non-uniform and low seed germination. The spinach "seed" is in fact a fruit consisting of an embryo, perisperm, endosperm and testa, surrounded by a pericarp (Deleuran et al. 2013). Due to varying flowering pattern of the spinach plant, harvested seeds will vary in size and maturity along with varying thickness of the pericarp (Deleuran et al. 2013). According to (Katzman et al. 2001), seed dormancy in spinach can be attributed to chemical inhibitors or physical properties of the pericarp, with the pericarp reducing germination by blocking emergence of the radicle or limiting the oxygen available for the embryo. Optimization of spinach germination has been studied by using chemical and physical means to break seed dormancy, such as decoating of the seed and soaking in water and chemicals such as NaOCl and H_2O_2 (Katzman et al. 2001). Germination has also been found to be greatly affected by temperature. Published reports vary on the optimal germination temperature of spinach. Chitwood et al. (2016) found maximum germination between 15 °C and 20 °C, with germination inhibition above 20 °C, while Røeggen (1984) found that germination decreased when the temperature was raised above 12 °C. Leskovar et al. (1999) and Katzman et al. (2001) both found the optimal germination temperature to be 18 °C. All these studies used different cultivars.

1.4 Temperature and germination

Temperature related seed dormancy in spinach has been attributed to the physical properties of the pericarp. The removal of the pericarp has been shown to facilitate germination at higher temperatures, due to limiting the amount of oxygen available (Katzman et al. 2001). Heydecker & Orphanos (1968) proposed that temperature affects the amount of oxygen available to the embryo, because oxygen becomes more soluble in water at colder temperatures as well as the fact that metabolism is slower and therefore requires less water.

1.5 Aims and hypothesis

By exploring the effects of different temperatures and water soaking of the seeds, this thesis aimed to find the most efficient way and optimal time of germinating spinach for use in a hydroponic system. To fully achieve this, the experiment was split into two phases: The germination phase, where the aim was to see how the different treatments affected germination percentage, speed of germination and uniformity of germination; and the propagation phase, where the aim was to examine if the different temperature treatments had any lingering effects on the growth and morphology of the plants after a week in the hydroponic system.

With a basis in the reports I had read when designing this experiment, I expected the highest seed germination rate at 18 °C, and that germination rate would be lower at 12 °C and 21 °C, with a significant drop in germination percentage for the seeds germinated at 27 °C. I also expected that the soaked seeds would have a better germination percentage than the unsoaked ones.

2. Materials and Methods

2.1 Plant material and growth facilities

Seeds of spinach (*Spinacia oleracea* L. cv. ‘Matador’) were obtained from Suba seeds (Longiano, Italy). The cultivar ’Matador’ was recommended by the seller as well suited for growth in hydroponic systems. The seeds were sown in substrate sourced from Quick-plug, a company based in the Netherlands. The substrate consisted of peat and coconut fibre, which were formed into plugs with a hole in the middle. The plugs were delivered in trays consisting of 192 plugs (Fig. 1). Seeds were sown by hand and the plugs were sprayed with water and covered with plastic sheets to prevent evaporation. The seeds were then placed in dark tents for the duration of the experiment, one for each temperature. The temperature of the tents was controlled with radiators and an air condition unit. The tent with 12 °C was cooled down using an air conditioner and the tents with 21 °C and 27 °C were heated with radiators. The tent with 18 °C was not heated nor cooled since the surrounding temperature where the tents were placed was around 18 °C.

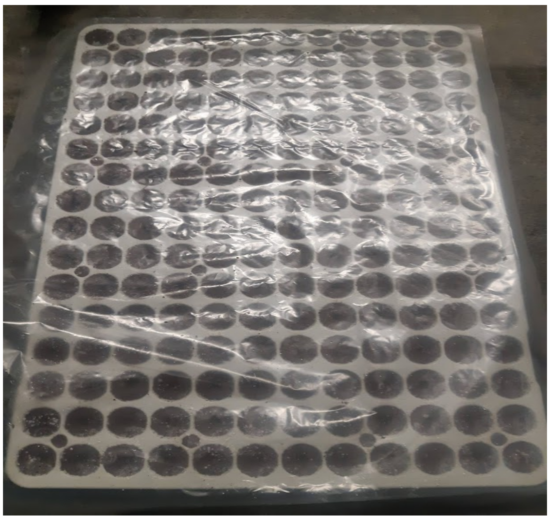


Figure 1. Picture of the trays of plugs used for the experiment. It is covered in plastic to prevent evaporation

2.2 Treatments

The experiment involved four different temperature treatments: T12, T18, T21 and T27 corresponding to 12 °C, 18 °C, 21 °C and 27 °C. A soaking treatment consisting of two parts was also added at 18 °C. One where seeds had been soaked in water 10 h prior to germination, and a control where the seeds had not been soaked. The temperature treatments consisted of 30 seeds each and the soaked treatment and control consisted of 15 seeds each. The soaked treatment and the control were placed in the tent with 18 °C since it was assumed to be the optimal temperature for germination. During the germination phase, the seeds were left to germinate for 7 days, with the day of seeding counted as day 0 and the day of harvesting counted as day 7. After 7 days, half of the germinated seedlings from each treatment were dried and weighed to measure biomass, while the other half went on to the propagation phase where they were put in the hydroponic system for one week (Fig. 2). The treatments were replicated for a total of four times (i.e., temporal replication was applied), and each time a new growth phase was initiated the temperatures in the germination tents were changed in a random manner, so that in the end (after one month) each tent had hosted all four different temperatures.

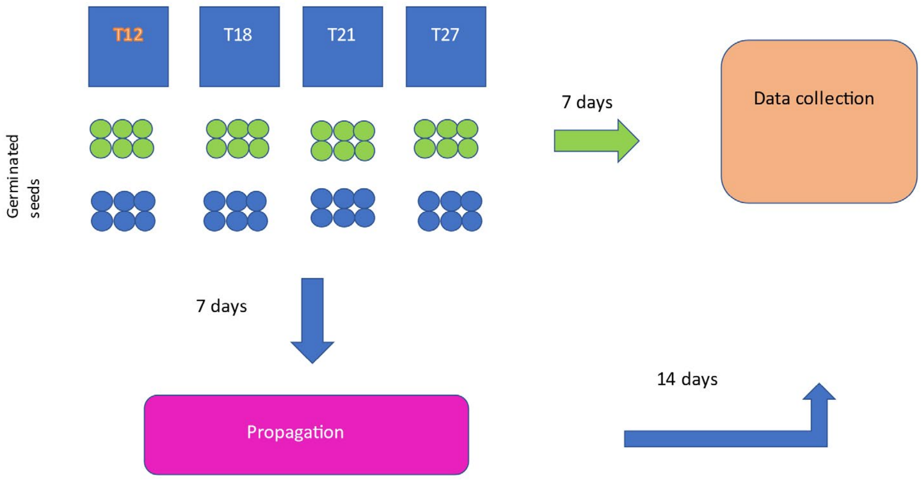


Figure 2. Schematic outline of the flow from the germination phase to the propagation phase. Seeds were kept in the germination tents for 7 days. After 7 days, half of the seedlings from every treatment were harvested and measured for biomass. The remaining seedlings where moved to the propagation area of the growth room for another 7 days, before being harvested for data collection. A total of 14 days had elapsed by this time.

2.3 Hydroponic system

At Nära Sverige, an ebb and flow hydroponic system is used. The benches used are irrigated with water for 20 minutes every two h, the water then stays in the bench for 30 minutes before being fully drained. LED lights are placed 30 cm above the bench. The system is divided into two parts: propagation and maturation. The two parts differ in the intensity of light, pH and electrical conductivity of the nutrient solution. The germinated spinach seeds were placed in the propagation area of the growth room for one week. In the propagation area, the light intensity was 100 µmol m^{-2} s^{-1}, the pH was regulated to 6.5 and no nutrients were added.

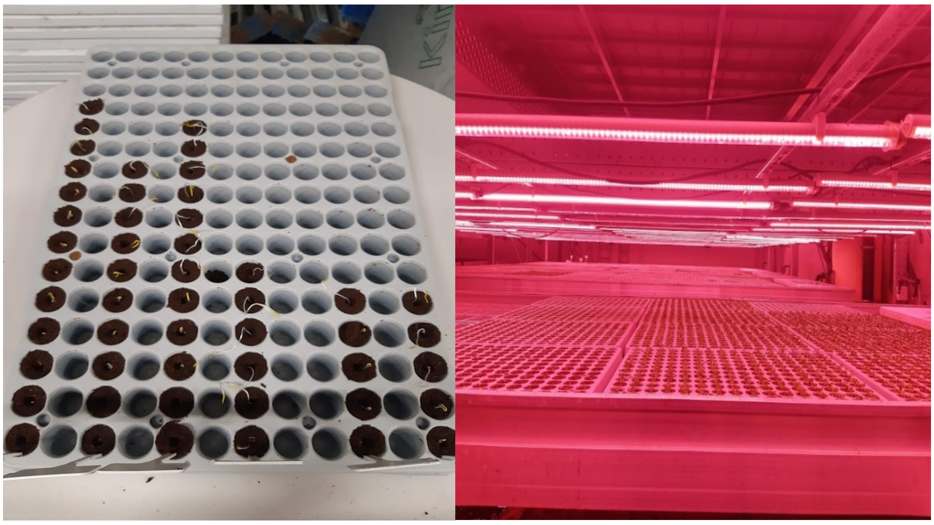


Figure 3. To the left a picture of plugs with germinated seeds after spending 7 days in the germination tents. To the right is a picture of the propagation area of the grow room where the seeds were placed for the final 7 days of the experiment.

2.4 Data collection

Germination counts were recorded at 24-h intervals. The seeds were counted as germinated when 1 mm of the radicle had protruded through the seed coating. When a seed was registered as germinated, it was moved to a different tray but kept in the same germination tent. After 7 days, the final germination count was examined. The seeds were then arranged in order of size and every other seed was dried in a microwave oven for one minute and weighed for biomass. The remaining seeds were taken into the propagation area of the grow room for another 7 days, before being measured for plant height, leaf length and chlorophyll content. Measurements of plant height and leaf length were carried out with a ruler. Plant height was measured from the plug to the top shoot, and the length of the biggest leaf was measured. Chlorophyll content was measured non-destructively using a SPAD meter (Model SPAD-502, Konica Minolta Sensing, Japan). Once the non-destructive measurement had taken place, the plants were carefully removed from the substrate, dried for one minute in a microwave oven and weighed to measure biomass. All plants from individual treatments were dried and weighed together, in order to come up to the minimum detection level of the scale.

2.5 Data analysis

The Program RStudio was used for the statistical analysis of the data (RStudio team 2020). Analysis of variance (ANOVA) and correlation test was used to assess the differences and impact of the treatments on the morphological traits, growth, and chlorophyll content of the plants. Graphs were created in Microsoft Excel. Speed of germination, T_{50}, representing the number of days required for half the seeds to germinate, was used as a measure of the speed of germination. T_{90-10}, representing the number of days passed from 10 % of germination to 90 % of germination, was used as a measure of uniformity of germination (Atherton & Farooque 1983). T_{50} and T_{90-10} were calculated in RStudio.

3. Results

3.1 Germination percentage

The T12 treatment showed the highest germination percentage (88 %), and the germination percentage then decreased with increased temperature as follows: T18 > T21 > T27 (Table 1)(Fig.4). The only statistically significant differences between treatments were found between T12 and T27, and T18 and T27 (Table 2). There was no significant difference between the germination percentage of the soaked and unsoaked seeds (Table 2).

Table 1. Germination percentage and standard deviation of spinach seeds germinated at four different temperatures for one week. Soaked indicates seeds that had been soaked in water for 10h prior to germination. Unsoaked indicates a control treatment. Soaked and unsoaked seeds were germinated at 18 °C.

	12 °C	18 °C	21 °C	27 °C	Soaked	Unsoaked
Germ (%)	88	81	68	51	73	78
SD (%)	8.3	5	8.8	12.8	19.2	6.4

*Table 2. Results from the Tukey HSD test in regards to germination percentage. The mean of all the termperature treatments was compared each other. The same was done between the soaking treatments. The p – values are documented in the top diagonal half. Significance levels are indicated in the bottom diagonal half. Significance levels are shown as follows. **$p \leq 0.01$, *$p \leq 0.05$and n.s.=not significant.*

	12 °C	18 °C	21 °C	27 °C	Soaked	Unsoaked
12 °C		0.738	0.052	0.001		
18 °C	n.s.		0.269	0.003		
21 °C	n.s.	n.s.		0.080		
27 °C	**	**	n.s			
Soaked						0.645
Unsoaked					n.s.	

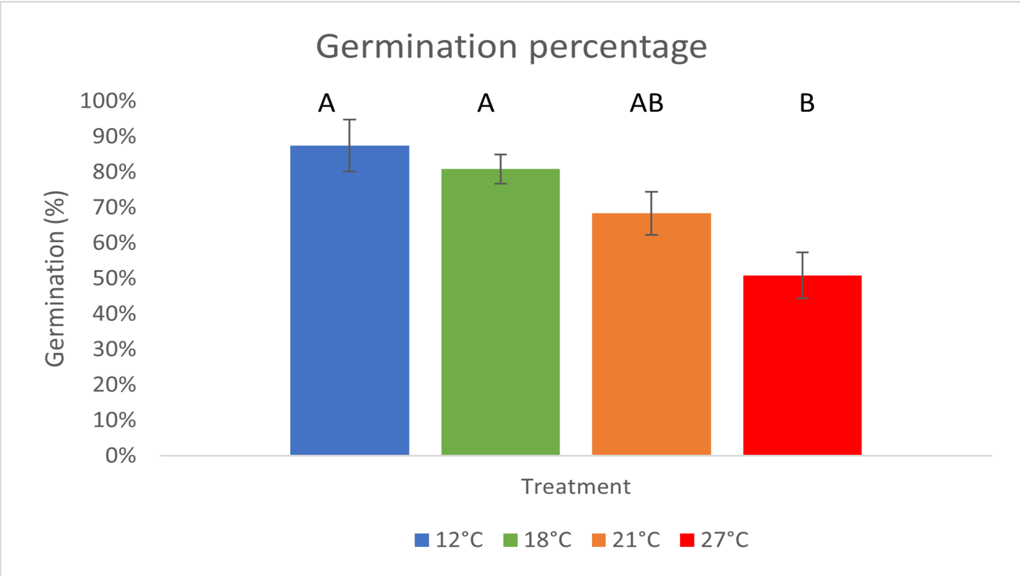


Figure 4. Mean percentage (±SD) of spinach seeds germinated at four different temperatures for one week. Germination percentage is shown on the y-axis and the treatments are shown on the x-axis. Bars that have the same capital letter above them are not significantly different.

3.1.1 Speed and uniformity of germination

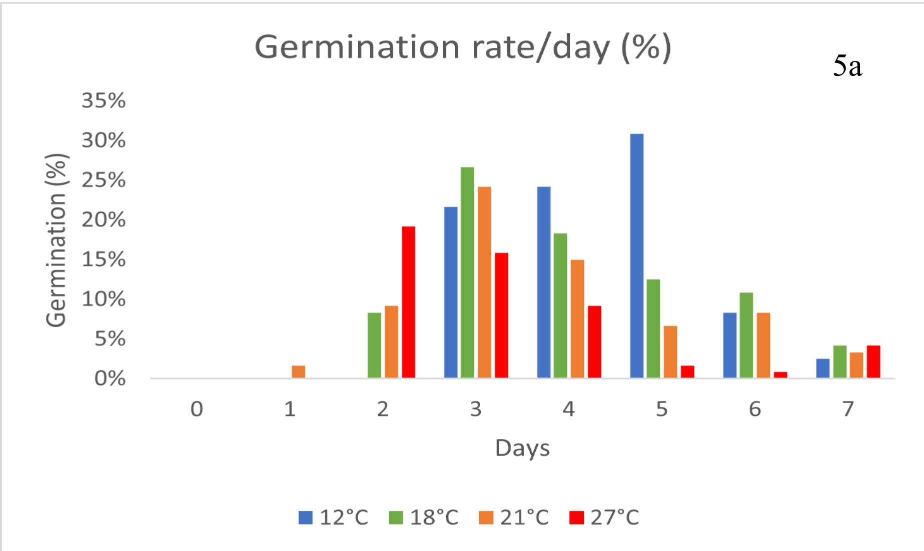
The T27 treatment showed the highest speed of germination, with results as follows: T12 < T18 < T21 < T27 (Table 3). The highest uniformity of germination was found in the T12 treatment, with results as follows: T12 < T18 = T27 <T 21.

Table 3. Speed of germination, T_{50}, the numbers of days required for half the seeds to germinate, was used as a measure of the speed of germination. $T_{90\text{-}10}$, the number of days passed from 10 % of germination to 90 % of germination, was used as a measure of uniformity of germination.

	12 °C	18 °C	21 °C	27 °C	Soaked	Unsoaked
T_{50}	3.92	3.30	2.97	2.40	2.60	2.90
$T_{90\text{-}10}$	2.85	3.70	4.02	3.70	3.30	3.20

3.1.2 Germination percentage per day

In the T21 treatment, one seed germinated on day one. In T18, T21 and T27, the first seeds germinated on day 2, and seeds in T12 started to germinate on the third day. T27 showed the highest initial seed germination of all treatments, after that its germination rate decreased for the rest of the seven days, except for a small resurgence on day 7. T18 and T21 had the highest germination per day on day three, and T12 showed a maximum in single day germination on day 5 (Fig. 5a). On day 4, the number of germinated seeds was similar across treatments, but by day 5, T12 had passed the other treatments and stayed on top (Fig. 5b).



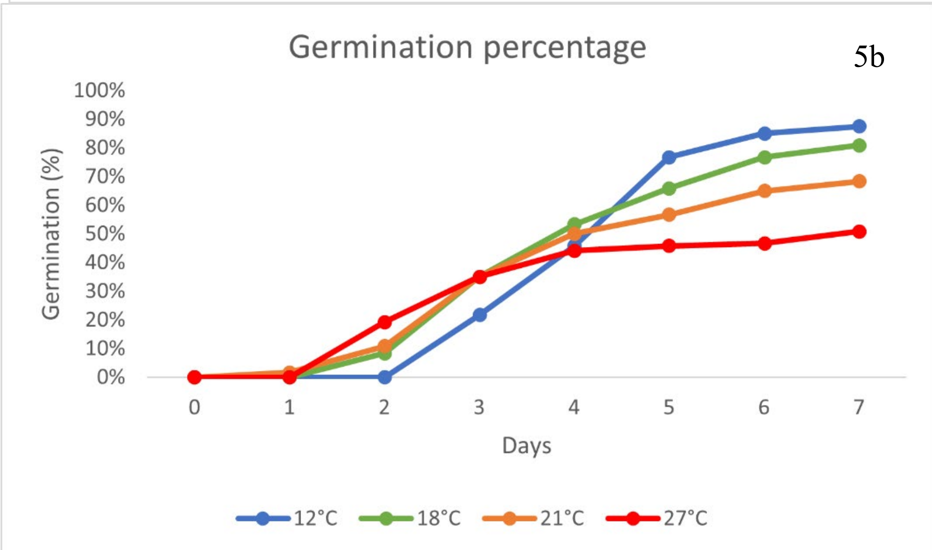


Figure 5. a) Mean percentage of spinach seeds germinated at four different temperatures for one week, per individual day of the germination phase. b) Mean germination percentage as it increased per day for seven days. Germination percentage is presented on the y-axis and days on the x-axis.

3.2 Growth and morphology

The ANOVA showed that the different temperature treatments had significant effects on all the growth and morphology traits except for biomass after germination (Table 5). Soaking showed no significant effect on any of the traits.

Table 4. Mean values of the measured height (mm), Leaf length (mm), Chlorophyll content (SPAD), Biomass1 (mg/plant) and Biomass 2 (mg/plant) of spinach seeds germinated at four different temperatures for one week (germination phase) and hydroponically grown for one week (propagation phase). Soaked indicates seeds that had been soaked in water for 10h prior to germination. Unsoaked indicates a control treatment. Soaked and unsoaked seeds were germinated at 18 °C. Biomass measured after the germination phase is labelled as biomass 1. Biomass measured after the propagation phase is labelled as biomass 2.

	12 °C	**18 °C**	**21 °C**	**27 °C**	**Soaked**	**Unsoaked**
Height (mm)	16.6	31.8	52.2	57.8	31.0	29.3
Leaf length (mm)	57.6	59.5	55.7	46.8	56.1	59.8
SPAD	40.5	39.2	38.0	31.4	37.5	38.8
Biomass 1 (mg/plant)	3.24	6.55	5.13	3.67	2.20	1.61
Biomass 2 (mg/plant)	14.37	17.09	12.24	9.68	14.68	15.92

*Table 5. Results from the Tukey HSD test showing the difference between spinach plants germinated at four different temperatures for one week (germination phase) and hydroponically grown for one week (propagation phase). plant height, leaf length, Chlorophyll content (SPAD) and biomass after the propagation phase (Biomass2). P-values are shown in top right diagonal and significance levels are shown in the bottom left diagonal. **p ≤ 0.01, *p ≤ 0.05and n.s.=not significant.*

	Treatment	**12 °C**	**18 °C**	**21 °C**	**27 °C**
Plant height	12°C		0.062	<0.001	<0.001
	18°C	n.s.		0.011	0.002
	21°C	**	*		0.729
	27°C	**	**	n.s.	
Leaf Length	12°C		0.785	0.775	0.001
	18°C	n.s.		0.280	<0.001
	21°C	n.s.	n.s.		0.003
	27°C	**	**	**	
SPAD	12°C		0.944	0.726	0.018
	18°C	n.s.		0.956	0.039
	21°C	n.s.	n.s.		0.080
	27°C	*	*	n.s.	
Biomass 2	12°C		0.532	0.708	0.134
	18°C	n.s.		0.117	0.012
	21°C	n.s.	n.s.		0.578
	27°C	n.s.	*	n.s.	

3.2.1 Biomass

The ANOVA showed no significant differences between biomass of the plants after the germination phase. After the propagation phase, the ANOVA only showed a significant difference between the biomass of T18 and T27 (Table 5). T18 produced the highest biomass after propagation, followed by T12 < T21 < T27 (Fig. 6).

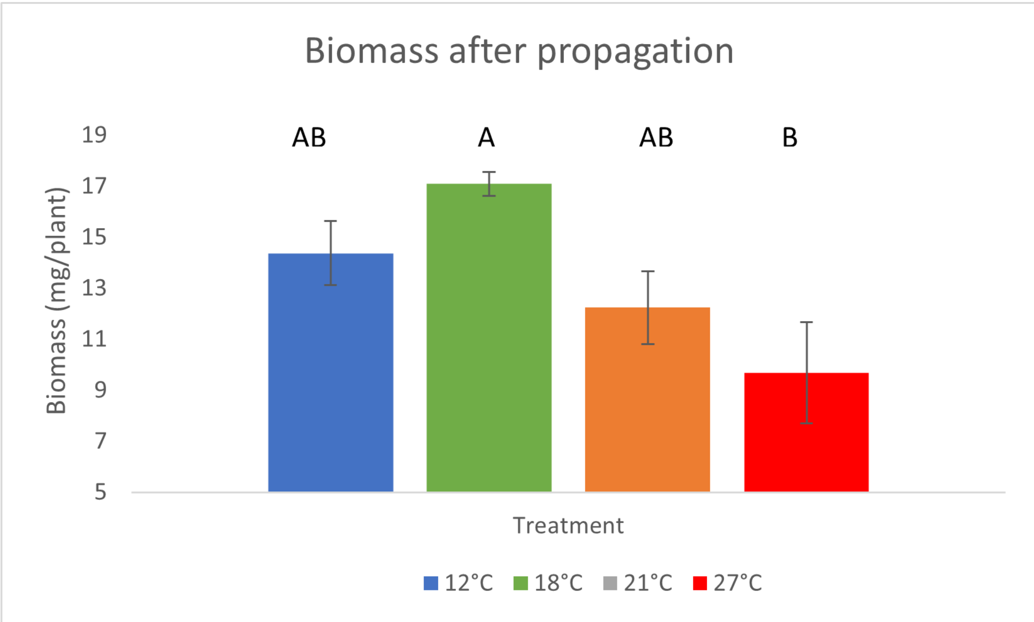


Figure 6. The mean (±SD) biomass after propagation of spinach seeds germinated at four different temperatures for one week and hydroponically grown for one week. measured by drying the plants in a microwave and weighing them. The plants from each treatment were weighed together and then divided by the number of plants. Biomass (mg/plant) after the propagation phase, is shown on the y-axis. The temperature treatments are shown on the x-axis. Bars that have the same capital letter above them are not significantly different.

3.2.2 Plant height

Plant height increased consistently with temperature (Fig. 7, 8b). The treatments T12 and T18 differed significantly from T21 and T27 (Table 5). The treatments T21 and T27 showed a larger variance between samples than the other treatments Fig 7).

Figure 7. The mean (±SD) height of spinach plants after being germinated at four different temperatures for one week and hydroponically grown for one week Plant height (mm) is shown on the y-axis and the temperature treatments are shown on the x-axis. Bars that have the same capital letter above them are not significantly different.

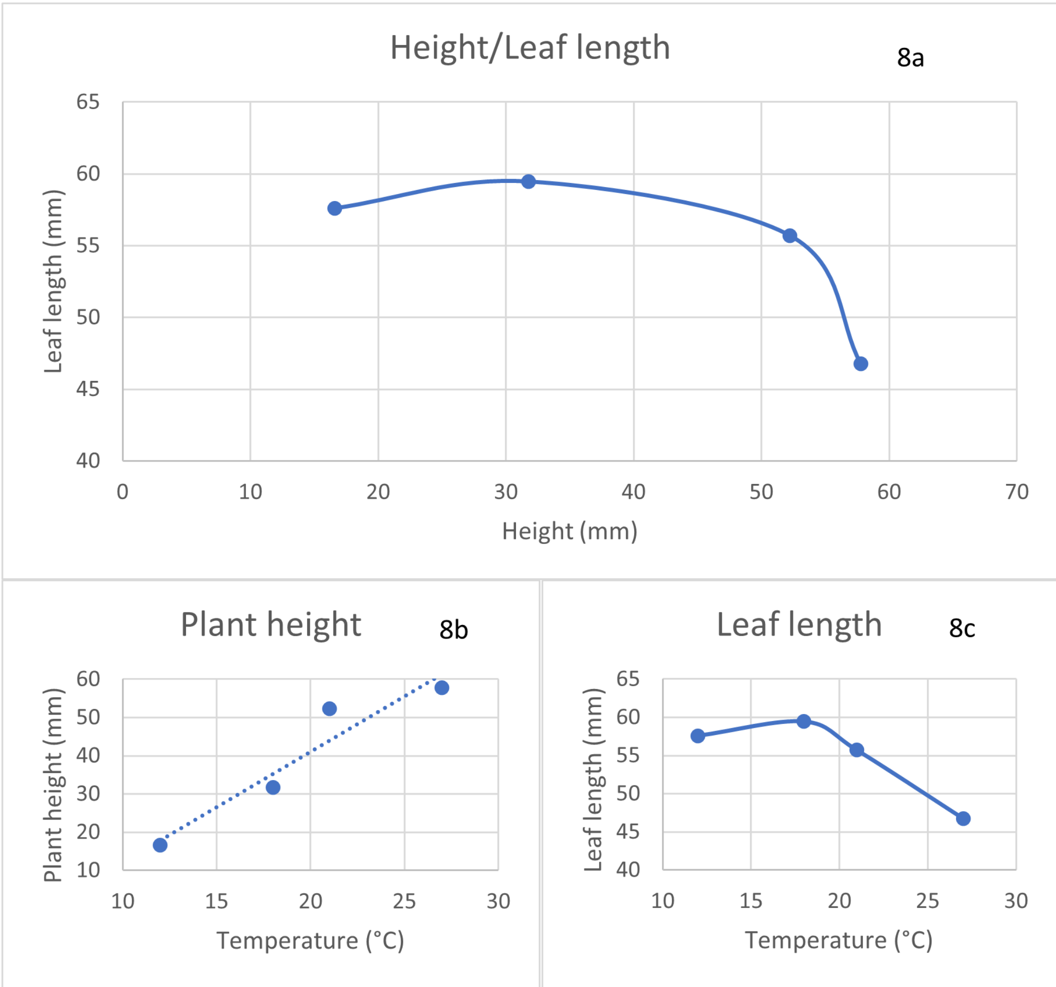


Figure 8. a) Correlation between plant height and leaf length of spinach plants after being germinated at four different temperatures for one week and hydroponically grown for one week. Leaf length is shown on the y-axis and height is shown on the x-axis. b) linear regression of plant height vs. temperature ($y = 2.902x - 16.99$, $r^2 = 0.91$). Plant height is shown on the y-axis and temperature on the x-axis. c) leaf length vs. temperature. Leaf length is shown on the y-axis and temperature is shown on the x-axis.

3.2.3 Leaf length

T18 showed the highest mean leaf length (Fig. 9). There was no significant difference between the leaf length produced in T12, T18 and T21, whilst T27 produced significantly shorter leaves than the rest of the treatments (Table 5). Leaf length increased from T12 to T18 but decreased with increasing temperature above 18 °C (Fig. 8c). As a result, leaf length increased with plant height up to 18 °C and decreased thereafter (Fig. 8a).

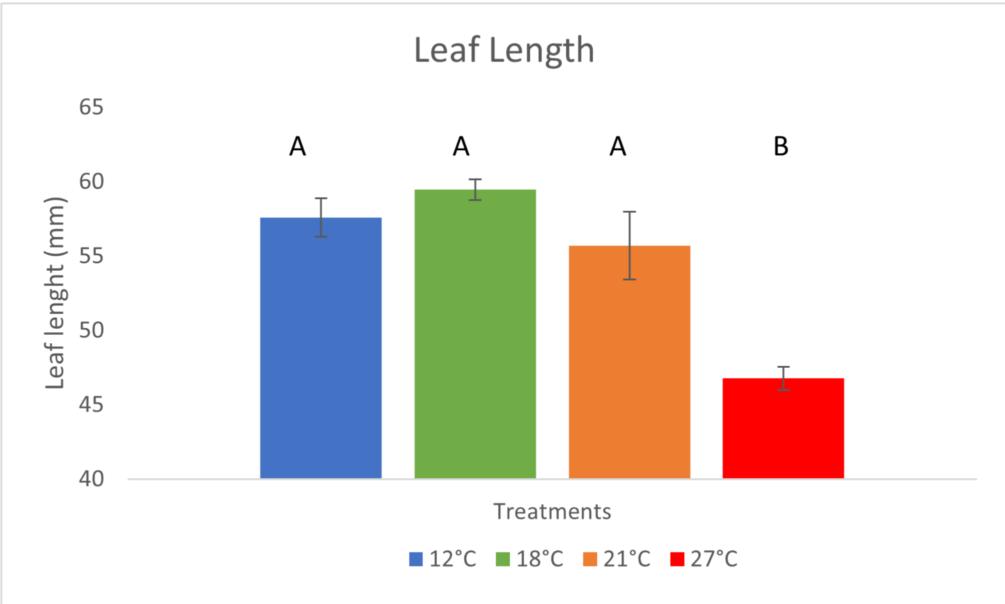


Figure 9. The mean (±SD) length of the biggest leaf was measured on Spinach plants that were germinated at four different temperatures for one week and hydroponically grown for one week. Leaf length (mm) is shown on the y-axis and the temperature treatments are shown on the x-axis. The bars with the same capital letter above them are not significantly different.

3.2.4 Leaf chlorophyll content

The highest chlorophyll content was found in the plants from T12 (Fig. 10). It then decreased with increasing temperature. Chlorophyll content in T27 was significantly lower than in T12 and T18 (Table 5).

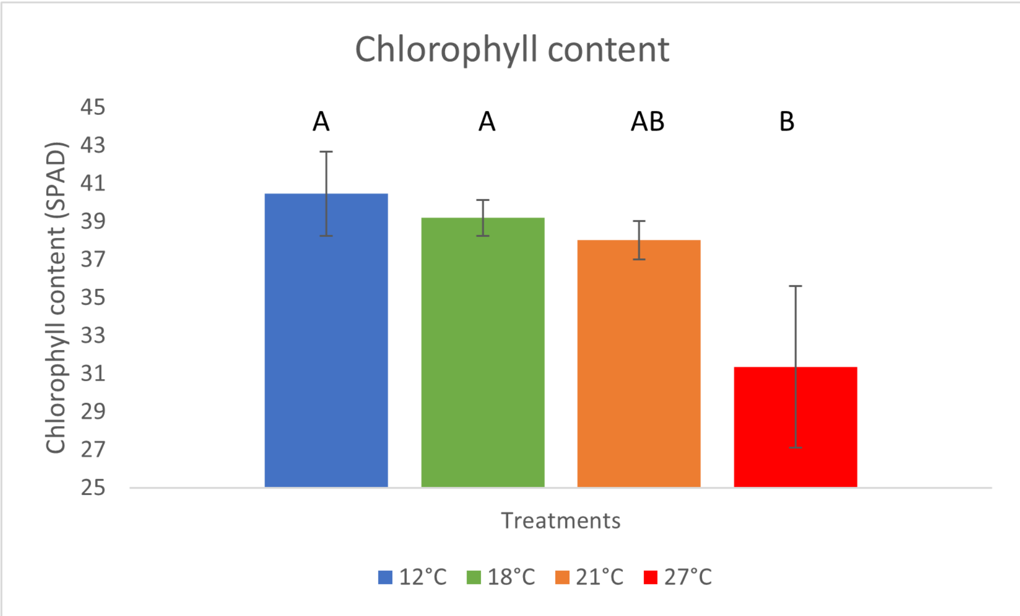


Figure 10. Mean (±SD) chlorophyll content as measured with a SPAD-meter on the biggest leaf of spinach plants germinated at four different temperatures for one week and hydroponically grown for one week. Chlorophyll content (SPAD) is shown on the y-axis and the temperature treatments are shown on the x-axis. The bars with the same capital letter above them are not significantly different.

3.3 Correlations between traits

Leaf length decreased significantly with increasing plant height (Table 6)(Fig. 8a), whilst chlorophyll content showed a tendency towards decreasing significantly with increasing plant height. Leaf length increased with increasing chlorophyll content and biomass after propagation (Table 6). Chlorophyll content increased significantly with increased biomass after propagation (Table 6). The was no significant correlation between biomass after germination and any of the traits (table 6).

*Table 6. Correlation matrix showing the results from the correlation analysis of the plant height, leaf length, chlorophyll content and biomass measured in spinach plants germinated at four different temperatures for one week and hydroponically grown for one week Biomass measured after the germination phase is labelled as biomass 1. Biomass measured after the propagation phase is labelled as biomass 2. The pearson r-value is documented in the upper diagonal half, whilst the p-values are documented together with the significance levels in the bottom diagonal half. ** $p \leq 0.01$, * $p \leq 0.05$ and n.s. =not significant.*

	Height	Leaf length	SPAD	Biomass 1	Biomass 2
Height		-0.55	-0.52	0.02	-0.450
Leaf length	0.026*		0.734	0.36	0.72
SPAD	0.063 (n.s)	0.007**		0.034	0.72
Biomass 1	0.942 (n.s.)	0.893 (n.s.)	0.918 (n.s.)		0.152
Biomass 2	0.08 (n.s.)	0.002**	0.008*	0.575 (n.s.)	

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4. Discussion

The primary aim of this study was to find the most efficient way of germinating spinach seeds for use in a hydroponic system. This was done by exposing seeds to different temperatures, as well as soaking in water for 10 h. I will discuss the soaking treatment before moving on to the rest of the results. The soaking treatment was added to see if there was an easy way to improve on the most effective temperature treatment, the soaked seeds were therefore inoculated at 18 °C since I had expected the highest germination to occur at this temperature. This was not the case. However, since there was no significant difference between the germination percentage of T18 and T12, where the highest germination percentage was found, I can conclude that soaking of spinach seeds does not significantly increase germination. I will therefore not discuss it in relation to the further results of the temperature treatments and rephrase the aim as which temperature leads to the most efficient germination.

By efficient germination I mean the outcome that generates the best compromise between high germination, and high, uniform growth. I will discuss which temperature is most effective by discussing germination percentage, speed and uniformity of germination, and lingering effects on the juvenile growth of the plants. I will also discuss optimal days of germination

4.1 Germination percentage

The total germination percentage was highest at 12 °C (Table 1). It then showed a tendency toward decreasing significantly at 21 °C, with a significant decrease at 27 °C (Table 4). These results are somewhat in line with two prior studies on the subject. Røeggen (1984) found germination to decrease above 12 °C, whilst (Chitwood et al. 2016) found germination inhibition above 20 °C. The temperatures tested, and the cultivar of spinach seeds used in those studies differed however, both from each other and from my study, which might account for the difference in results. Since one of the cultivars tested in the study by (Chitwood et al. 2016) showed its highest germination at 10 °C there is a possibility that optimal germination of the “Matador” cultivar can be performed at even lower temperatures. But speed and uniformity of germination also needs to be considered. If the goal would have been 50 % germination, 27 °C would have been the most effective temperature to germinate the seeds at (Table 3). $T_{50,}$ which reflects the number of days required for half the seeds to germinate, was lowest with T27 and got progressively higher with decreased temperature. $T_{90\text{-}10,}$ the number of days passed from 10 % of germination to 90 % of germination, was around one day less in T12 than the rest of the treatments (Table 3). This indicates that if a germination percentage higher than 50 % is desired, 12 °C is the temperature that results in the most uniform germination. When only focusing on germination, a preliminary conclusion is therefore that 12 °C is the most effective temperature for high spinach germination, but the final conclusion might change when looking also at the lingering effects of different temperature treatments on juvenile growth.

4.2 Effects on juvenile growth

Juvenile growth is interesting to look at because it might say something about the final yield of the plant. Since it is the vegetative leaf biomass of spinach that is harvested, the vegetative growth after one week is likely to mirror final yield. Yield is important, but also uniformity within the sample. Large variance might affect the final yield if some plants grow larger at the expense of others. To evaluate the lingering effects on the juvenile growth. Plant height, Leaf length, Chlorophyll content and biomass was measured. Variance between the replicates was also considered.

Plant height turned out be an interesting parameter to look at. Plant height increased linearly with temperature and from what I observed, final plant height was heavily linked to the height of the seedlings after the germination phase. The longer germinated seeds are left in the dark, the more stretched they will be, mirroring being sown at greater depths (Fogelfors 2015). Speed of germination increased with temperature, while the uniformity of germination was lower in the top three temperatures (Table 3). This implies that seeds that germinated early at higher temperatures had a longer time before and better conditions to stretch before being moved to propagation, leading to a larger variation of growth. Plant height increased with temperature (Table 4), while leaf length decreased above 18 °C and chlorophyll content decreased above 12 °C. While this admittedly could result in longer plants in the end, it will affect the final yield adversely since it is the leaves that are harvested. The measured biomass followed the same pattern as leaf length with a decrease above 18 °C (Fig. 6), indicating that most of the biomass was made up of the leaves. An increased height in juvenile plants, could be evidence that germination temperature has a detrimental effect on final leaf length and chlorophyll content. Since seedlings that are more stretched, will have spent more of their reserve nutrients on its stem (Fogelfors 2015), resulting in a slower start and less competitiveness in relation to surrounding plants once they reach the propagation phase. I did however not measure the length of the seedlings after the germination phase, so I have no clear data on this subject and can only speculate from what I observed. Fogelfors (2015) states that lower germination temperatures lead to an increase in root development, which would support this speculation.

Plant height also has a practical disadvantage when it comes to handling of the plants. The longer the stem the harder it is for the plants to stay upright. I noticed that the plants had difficulty staying upright with plant heights over 30 mm. T21 and T27 showed a larger variance in plant height between replicates (Fig. 7). There is a possibility that this could start showing later in the growth cycle, with weaker plants being outcompeted by stronger ones.

It was interesting to see that the T18 treatment produced the longest leaves, while the T12 treatment generated the highest chlorophyll contents (Table 4). A reason for this could be that the leaves are mainly built from cellulose containing mostly carbon, while chlorophyll consists of mostly nitrogen. Carbon is supplied from CO_2 from the stomata and is therefore more readily available than nitrogen that needs to be supplied by the roots (Fogelfors 2015).

Since there is no significant difference in leaf length, chlorophyll content and biomass between 12, 18 and 21 °C, temperatures around 12 °C still appear to be optimal for germination and growth when considering total germination percentage, uniform germination, and effects on juvenile growth. Could this change when looking at the germination per day?

4.3 Optimal days of germination

With the results from both the germination phase and the propagation phase in mind, I will try to find the optimal days of germination. The answer lies in a compromise between higher germination and final yield of the plant. Maximizing germination percentage is desirable, but not at the expense of a lower yearly yield. The longer it takes the plant to reach maturity, the less amount of grow cycles can be achieved per year. If there are 12 growth cycles of 30 days in a year, an extra day of germination could mean 12 days of lost growing time over a year. Longer time in germination might also have detrimental effects on the continued growth of the plant, as the plant becomes more stretched with a larger variance from the non-uniform emergence.

In discussion with the team at Nära Sverige AB, we decided that at least 80 % germination is desirable. This was only achieved by germinating the seeds at 12 and 18 °C. The seeds germinated at 12 °C took 6 days to reach 85% germination (Table 5b). Since T12 also showed the most uniform emergence and promising results in relation to juvenile growth, I will only consider it as the only relevant treatment from now on.

Given that none of the treatments apart from T27 showed a resurgence in germination for the final days, it can be assumed that it would not be effective to leave the seeds in the germination tent for more days. The 3 % increase that T12 showed for the last day does not merit the loss in yield from having one less day of growth in the hydroponic system. In relation to the conditions of this experiment, I agreed with the team at Nära Sverige AB that 6 days is the optimal number of days for germination.

4.3.1 Limitations of the experiment and possibilities of improvement

There are several things that could be improved for future experiments. To start with, the temperatures of the treatments might not have been the most optimal. I wanted to design the experiment with the tools available at Nära Sverige AB. Since they did not have any thermostats that could control the temperature of the tents, I needed to choose four temperatures that would be as steady as possible. The plan was to work around 18 - 20 °C as optimal temperatures and have one temperature above that and one temperature below. 18 °C and 21 °C were chosen beforehand and worked well, but I had initially chosen 15 °C and 25 °C as the outlying temperatures. In the end it worked better than I had thought at showing the effects of temperature on germination with the results of the 12 °C and 27 °C treatment.

According to Røeggen (1984), germination temperature for spinach seeds could be even lower than 12 °C, so for future experiments it would be interesting to add more temperature treatments at closer intervals. Soaking of the seeds would be interesting to examine further as earlier studies found it to lead to higher germination percentages for spinach, contradicting my results (Suganuma & Ohno 1984). For a future experiment it could be relevant to add a soaking treatment at each of the temperatures tested as well as different soaking times to see if that has an impact on the result.

Data collection could have been improved. When thinking back on the experiment, it would have been valuable to measure the length of the seedling after the germination phase to see the correlation between the radicle length and the final plant height since this corresponds to what I observed. Measuring of biomass after the germination phase could also been improved. I weighed the entire seedling along with the seed in this experiment and that could have been one of the reasons that the biomass after germination did not show any significant differences between the treatments, even though I could see that there was a difference in length of the radicle. Fresh weight could also have been useful to measure since the weight of the seedlings was so low. One factor that was not accounted for in my experiment is moisture. Increased moisture has been found to lead to germination inhibition both at lower and higher temperatures (Røeggen 1984; Magnée et al. 2020). While I took care to irrigate the seeds with equal amounts of water, this method might not have led to the most optimal results, germination wise.

The supply of spinach seeds was kept in the refrigerator at 4 °C for the duration of the experiment. Magnée et al. (2020) mentioned stratification of the seeds as a way of increasing germination percentages. This may have impacted the results of my study since there was a three-week time gap between the start of the first replicate and the start of the fourth, final replicate. T12 and T27 showed an increase in germination from replicate 1 to replicate 4. T18 and T21 did not however, so there is no clear indication that leaving the seeds in refrigerator for longer improved germination. One way of increasing germination percentage is to simply sow more seeds, for Nära Sverige AB this could be a simple way of both ensuring a higher germination as well as decreasing the days of germination. When examining this, the limited space in the plugs that were used needs to be considered though, but it would be highly relevant to include this factor in future studies.

Something I did not address in this thesis is that the germination tent with 12 ° had to be cooled with an air condition unit. This could have a direct effect on the sustainability of germinating spinach seeds at this temperature. However, since I did not measure the amount of electricity used this will have to be further studied. When designing further experiments on the subject of spinach germination, I would have temperature treatments at 2,5 °C intervals from 5 – 35 °C, with seeds soaked in water for different periods, added at each temperature. I would have different moisture treatments at each temperature to examine the impact moisture has on spinach germination at different temperatures. Finally, I would also like to measure the amount of electricity used to cool the tent at the lower temperatures to further examine the sustainability of germinating spinach seeds at lower temperatures.

5. Conclusion

The optimum temperature for germination of spinach seeds was found to be 12°C from this study. 12°C showed the highest germination percentage as well as a more uniform germination than the higher temperatures. It was also clear that an increase in temperature had a negative effect on the juvenile growth. The optimal number of days for germination was found to be 6 days.

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Popular science summary

The amount of people living is cities gets higher every year, it is therefore important that they have easy access to food. A way of doing this is to put the farm in the city. Some farms grow plants indoor on floors stacked on each other and without using soil. This is called vertical hydroponic farming, hydroponic is the name of the method of growing plants in water. These types of farms make it possible to control the environment of the plants by using grow lights, they also use less water by reusing the same water over and over. A company called Nära Sverige AB is doing this in Liljeholmen, Stockholm. There they grow three types of salad in their farm two stories below the ground. They are always looking for new plants to grow and spinach is the one they want to grow the most. Spinach has been found to grow better in water than in fields. You can also make more money from it since it grows very fast. One problem though is that not all spinach seeds are as good. Some take a longer time to start growing, and some don't start growing at all. How well a spinach seed grows depends on the temperature.

I wanted to find out the best way of making a spinach seed start growing. I did this by putting seeds in different tents with different temperatures and putting some seeds in water overnight before putting them in the tents. I used four different temperatures, 12, 18, 21 and 27 °C. After leaving the seeds in the tents for a week, I took half of them and dried them in a microwave before weighing them. I then put the rest of the seeds in the water growing system under grow lights for a week. After a week under grow lights, I measured the height of the plant, the length of the biggest leaf and I dried them in a microwave. I also used a special device that could see how much chlorophyll was in the leaves. Chlorophyll is the thing that makes it possible for plants to get energy from light, and by measuring it you can see how healthy the plant is.

I found out that the seeds that were put in the tent with 12 °C were best at starting to grow, there were fewer and fewer that started to grow the higher temperature was. The seeds in the tent with 12 °C started to grow faster than the other in the beginning though. The seeds in the tent with 12 °C all started to grow within a short time of each other, while there was a longer time between the first seed that started to grow and the last seed that started to grow in the tents with higher temperatures.

Putting the seeds in water overnight did not make more start to grow that not putting them in water. You could see that the plants looked different after a week under grow lights, depending on which temperature they were in before. The height of the plants, the length of the leaves, the weight of the plants and the amount of the thing that make them take up energy from light was different depending on the temperature of the tents where they started to grow. You could also see that the plant that were taller had smaller leaves.

As I said earlier, I wanted to find out the best way of making a spinach seed grow. To do that I looked at which temperature made the most seeds start growing, how big and healthy they were after a week under grow lights and how much they looked the same. I also looked at how many days they should be left in the tents before putting them under light. The difference between the seeds that started to grow at 27 °C and the other temperatures was too big, and the difference between the seeds that started to grow at 21 °C and the other temperatures was almost too big, so they were counted as bad temperatures. When looking at how big and healthy the plants were I found the same. 27 °C was very bad for the plants and 21 °C was bad. This leaves the seeds that were put in tents with 12 and 18 °C.

12 °C had the highest number of plants that started to grow, the plants that looked most alike, the shortest plants and the overall biggest and healthiest plant. Because of that I decided that the best way of making a spinach seed start to grow, was by putting them somewhere where it was 12 °C. I also found out that they should be there for six days before putting them under a grow light.

Acknowledgements

I want to start off by giving an immense thanks to my supervisor Martin Weih, without whom this thesis would not be. Thank you Martin, for the support and guidance, and for the fun dinners with your research group at OPA! Thank you to the team at Nära Sverige for all the support, and for all the patience with my loud air conditioners. A special thank you to Karin Nilsson, lead grower at Nära Sverige for believing in me and helping me with my project.

Appendix 1

Appendix 1. Raw mean data used to calculate the statistical analysis for all the treatments. Mean values of the measured height (mm), Leaf length (mm), Chlorophyll content (SPAD), Biomass1 (mg/plant) and Biomass 2 (mg/plant) of spinach seeds germinated at four different temperatures for one week (germination phase) and hydroponically grown for one week (propagation phase). Soaked indicates seeds that had been soaked in water for 10h prior to germination. Unsoaked indicates a control treatment. Soaked and unsoaked seeds were germinated at 18 °C. Biomass measured after the germination phase is labelled as biomass 1. Biomass measured after the propagation phase is labelled as biomass 2.

Treatment	Height	Leaf length	SPAD	Biomass1	Biomass2	Germ.percent.
	(mm)	(mm)		(mg/plant)	(mg/plant)	(%)
12°C	16.61282	57.59358974	40.45893	3.236	14.366	88
18°C	31.77273	59.45454545	39.18822	6.554	17.087	81
21°C	52.23611	55.69444444	38.0213	5.125	12.241	68
27°C	57.7875	46.76607143	31.36952	3.659	9.679	51
Soaked	31.0375	56.11785714	37.51905	2.196	14.679	73
Not soaked	29.27083	59.74583333	38.8	1.607	15.917	78

Appendix 2. Standard deviation of the means from each replicate of the temperature treatments. Standard deviation is calculated from the measured height (mm), Leaf length (mm), Chlorophyll content (SPAD), and Biomass 2 (mg/plant) of spinach seeds germinated at four different temperatures for one week (germination phase) and hydroponically grown for one week (propagation phase. Standard deviation of the germination percentage was calculated from the means of the total germinated seeds after the germination phase.

Treatment	Height	Leaf length	SPAD	Biomass2	Germ.percent.
12°C	2.39061	2.587444	2.219063	2.503822	8
18°C	2.561099	1.368678	0.930759	2.503822	5
21°C	9.653927	4.532802	2.894602	2.847859	8.8
27°C	11.02636	1.536798	4.245796	3.95618	12.9

Appendix 3. Mean percentage of germinated seeds per day, from each of the treatments. Number of germinated seeds were counted at 24-hour intervals for seven days.

Days	Seeds germinated					
	12°C	18°C	21°C	27°C	Soaked	Unsoaked
0	0%	0%	0%	0%	0%	0%
1	0%	0%	2%	0%	2%	2%
2	0%	8%	11%	19%	20%	13%
3	22%	35%	35%	35%	50%	42%
4	46%	53%	50%	44%	60%	63%
5	77%	66%	57%	46%	70%	73%
6	85%	77%	65%	47%	73%	78%
7	88%	81%	68%	51%	73%	78%

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