

# Symbiosis between Pisum Sativum and Nodule Bacteria in varying temps

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# Chapters

- Introduction
- Materials & Methods
- Results
- Discussion

# Introduction

Climate change → impact on symbiosis → mutual benefits

Presence or absence of Rhizobial bacteria

Genotypes: WT vs SYMRK

Higher temperature is good for symbiosis?

What differences can we expect in varied temperatures (RT vs 26 vs 30 )

# Materials and Methods

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# Pretest

- aluminium foil
- cardboard
- bubble wrap
- thermometer

## Plant supply

- tray
- units
- farmbot
- pods
- sterilized sand
- sterilized soil
- heating pads
- WinRhizo

## Plants

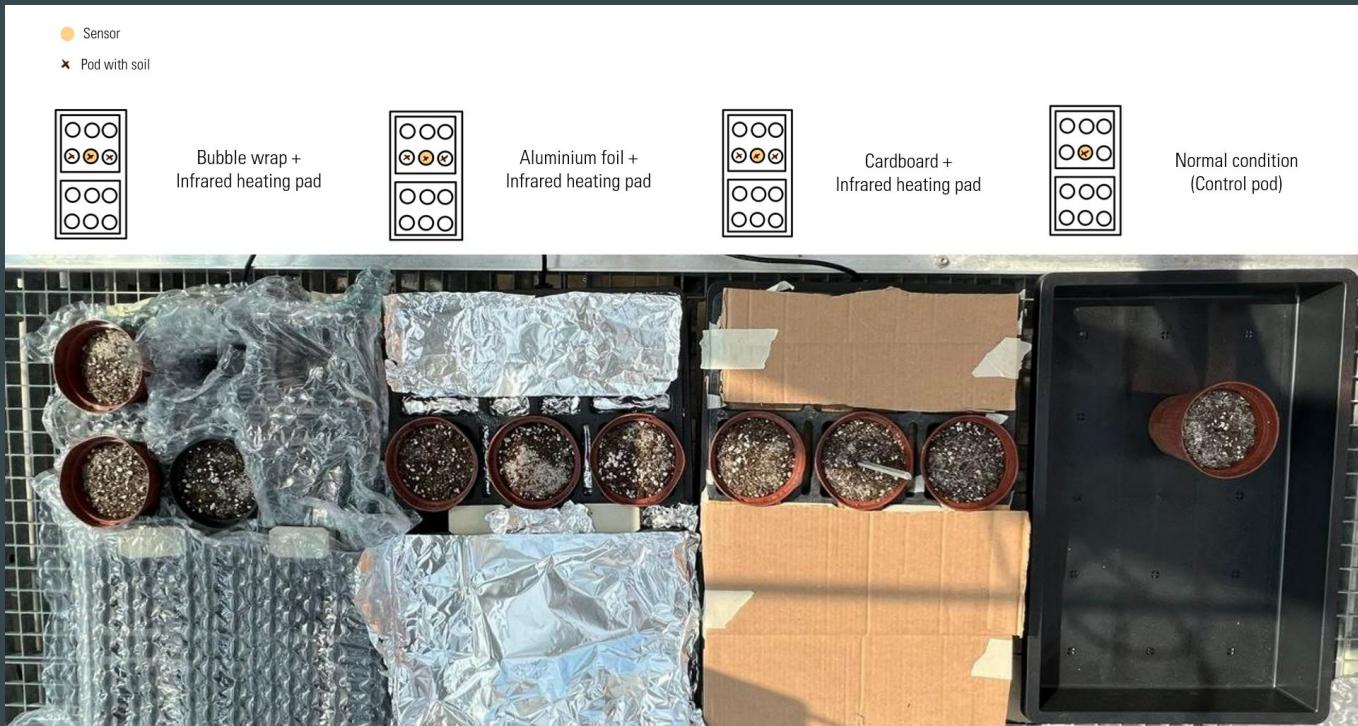
- pea plant seeds
- pea plant seeds with SYMRK mutant
- nodule bacteria

## Extra material

- papertowel
- deionized water
- scale
- ruler
- pipette

# Task 1 - Pretest

- setup of the pretest
- testing three different insulating foil



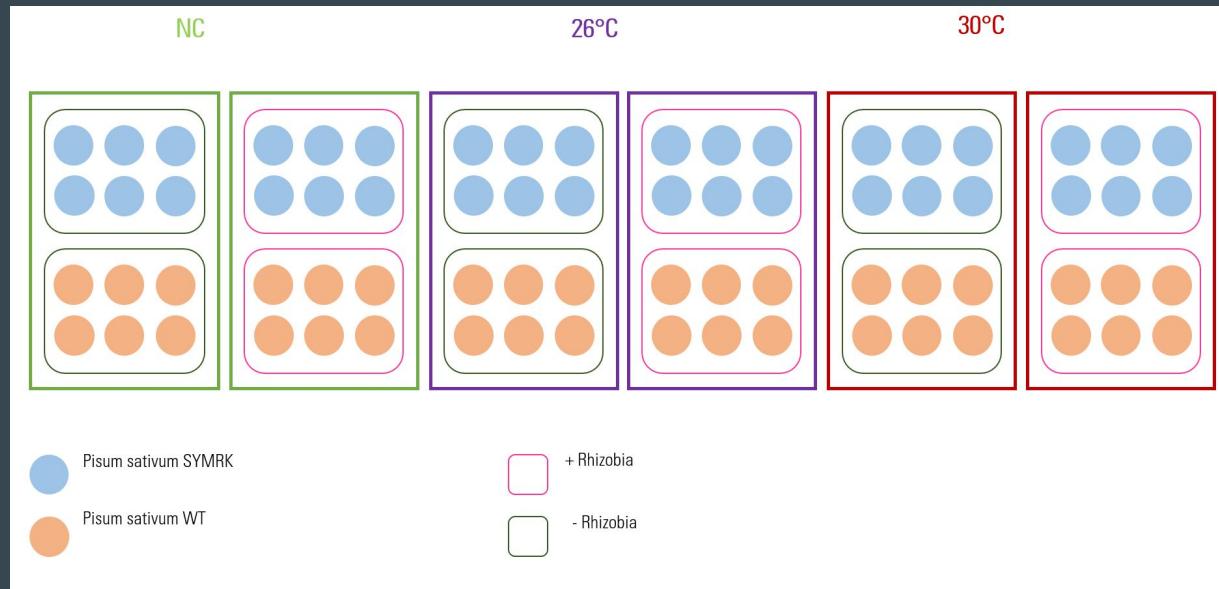
# Task 2 - Filling the pods

- Filling the pods  
with soil and sand



# Task 3 - planting the seeds

- seeds are planted in the middle of the pods
- hook facing upwards
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# Task 4- adding the bacteria

- bateria is diluted in a solution
- added with a pipette close the seed



# Task 5 - plants growth

-plants get watered by the farmbot



# Task 6 - Harvesting

## Taking out the plants

- softening the pod by squishing it
- softly loosen the soil by hand

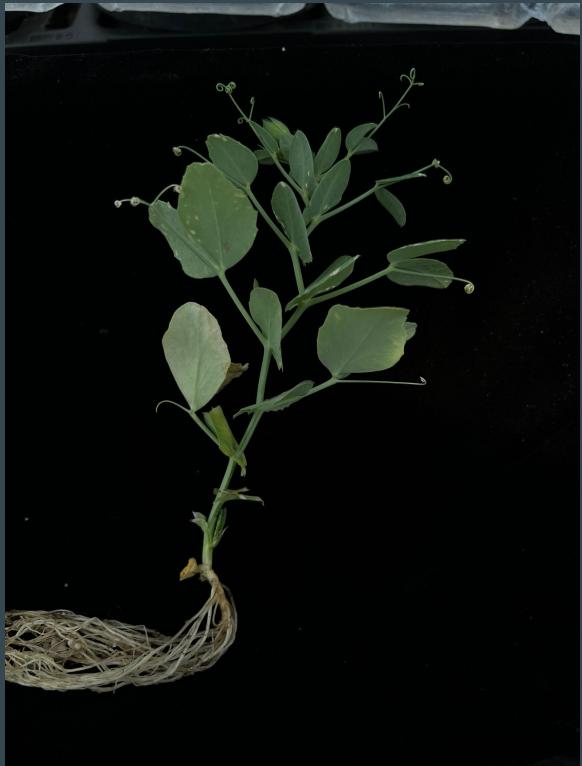
## Cleaning the roots

- washing the roots in the deionized water
- patting the roots dry with a papertowel

## Measuring the plant

- root and shoot axis get weighted
- shoot axis get measured with a ruler
- leaf counted
- three roots of each testing group gets send to the winRhizo
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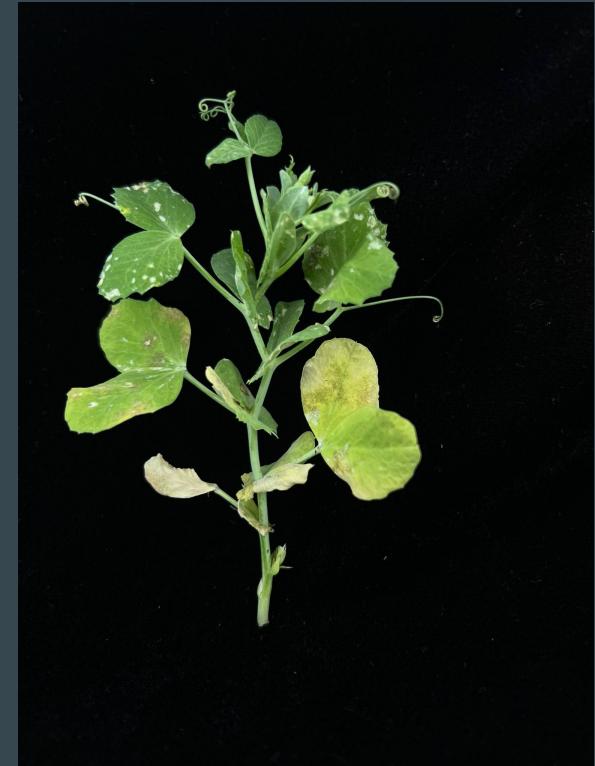
Whole plant



Root with nodules

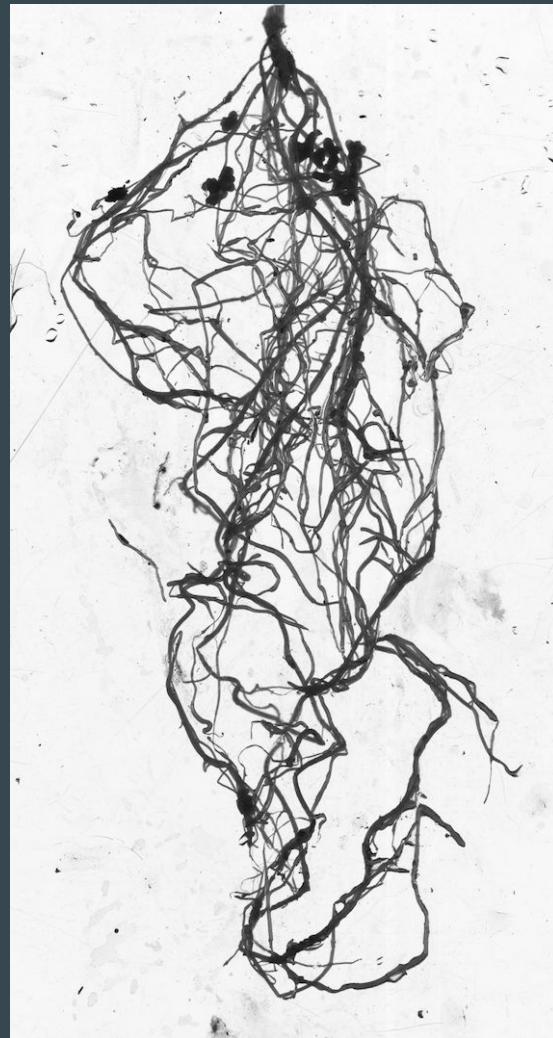


Shoot axis



# Task 7 - winRhizo

- 3 roots per root were analysed by the winRhizo



# Results

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Collected at 26.07.2023

# Division of theme

general  
information

The  
informations  
we gathered  
for “Normal  
Temperature”

The  
informations  
we gathered  
for “26°C”

The  
information  
we gathered  
for “30°C”

# Generell information

- one plant died during the experiment
- most of the plants were sick
  - leaf lice (3 of the plant)
  - white spots ( more than half)
  - yellow and white leaves (almost all of them)

# General information temperature

- on the day when the highest temperatures were recorded, the sensors did not record any temperature difference
- during the night when the lowest temperatures were recorded, the sensors were able to pick up a difference that was five degrees below our desired temperature but had the correct difference

# Normal Temperatures

		Average of Shoot axis height	Average of leaf count	Average of weight shoot axis	Average of root weight	Average of weight plant	Average of nodule count
Normal temperature	Pea Plant	10,78	16,17	1,51	2,27	3,78	0
	SYMRK	7,85	13,83	1,07	2,22	3,29	0
	Pea Plant + Bacteria	10,40	15,67	1,34	2,13	3,46	19,67
	SYMRK + Bacteria	5,35	5,00	0,50	0,88	1,37	0

# Normal Temperatures

- **Nodules:** only to find in pea plant and bacteria -> 19,67
  - **leaf count:** SYMRK and bacteria got far less leaves -> about 30 percent of the other conditions
  - **shoot axis height:** SYMRK and bacteria again got the smallest number -> 5,35 cm (50 %), but SYMRK also just got 7,85 cm
  - **weight of plant:** again pea plant with bacteria got far less centimeters than the other conditions -> about 30 percent of the other conditions
- > the pea plant with bacteria had in every category less outcome. (-> excluded nodule count)

26 °C

		Average of Shoot axis height	Average of leaf count	Average of weight shoot axis	Average of root weight	Average of weight plant	Average of nodule count
26°C	Pea Plant	8,07	9,50	0,85	1,40	2,25	0,83
	SYMRK	4,55	6,50	0,45	0,69	1,14	0
	Pea Plant + Bacteria	8,83	10,17	0,92	1,10	2,02	0
	SYMRK + Bacteria	5,13	6,00	0,47	0,82	1,29	0

26°C

- **nodules:** only to find pea plant (without bacteria) -> should not be there
- **leaf count:** both SYMRK variants had less leaves (6,0 and 6,5)
- **shoot axis height:** SYMRK (without bacteria) has 4,55 cm, SYMRK with bacteria has alittle bit more (5,13 cm) and the normal plants have both over 8 cm
- **plant weight:** Again Both SMYRK conditions where half as heavy then the wildtype plants

-> Both SYMRK variants did not have as much outcome as the normal plants (-> excluded nodules)

30°C

		Average of Shoot axis height	Average of leaf count	Average of weight shoot axis	Average of root weight	Average of weight plant	Average of nodule count
30°C	Pea Plant	8,23	9,50	0,92	1,11	2,03	6,33
	SYMRK	5,78	6,83	0,53	0,83	1,35	0
	Pea Plant + Bacteria	9,80	12,67	1,22	1,11	2,34	12,00
	SYMRK + Bacteria	5,62	6,33	0,51	0,70	1,21	6,17

# 30°C

- nodule: all conditions had nodules except the SYMRK mutant without bacteria
- leaf count: both SYMRK mutants had around 50 percent less leaves.
- shoot axis height: both SYMRK mutants had around 55 percent of what the non mutant plants had in centimeter
- plant height: the SYMRK mutants both had around 55 percent of the height of the wildtype plants.

-> both SYMRK mutants did not have as much outcome as the wildtype plants (-> excluded nodule)

# Conclusion

- both SMYRK conditions did not grow as good as the wildtype plants-> In normal conditions the difference is not as high
- the nodules just grew correctly in normal temperature condition-> in the other two temperature conditions the nodules formed symbiosis with the wrong plants

# Discussion

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# Main ideas:

- Mutant grows much worse than wild type peas under the same conditions
- Our assumptions were not confirmed
- At normal temperature, WT not inoculated with *Rhizobia* have higher average plants parameters than inoculated WT. Same with *symrk*.
- At 26°C WT not inoculated with *Rhizobia* have lower plants parameters (except higher roots and the whole plant weight) inoculated WT. Also the mutants without *Rhizobia* have all parameters lower than the mutant with it. None of the plants in which we added *Rhizobia* did not form nodules.
- *Rhizobia*, even without forming symbiosis, has a positive effect on soil properties and at plant growing

- At 30°C not inoculated WT have lower average plant parameters (except same average root weight), than inoculated WT. The mutants without Rhizobia have all parameters lower than the mutant with it, except for the average weight of the plants, which is bigger in mutants without Rhizobia.
- Leaf yellowing and white dots on plant leaves can be caused by several reasons:
  - 1.Improper temperature conditions
  - 2.Nutrient deficiencies
  - 3.Pest attack and disease
- Inaccuracies in our results could be for:
  1. Technical faults and human error
  2. High outside temperature

## Interesting:

- Two of the wild type pea plants, in which we don't add bacteria, form nodules: 1 at 26°C and 1 at 30°C. Also 1 *symrc*, plant, in which we add bacteria, at 30°C form nodules and all general values of this certain plant are closer to the wild type pea than to the mutants.
- 30°C is more suitable to form symbiosis than 26°C, but less than normal conditions. Also at 30°C more plants form nodules, even than at normal temperature, but they have worse plants parameters.

# Sources

-[https://o.aolcdn.com/images/dims3/GLOB/legacy\\_thumbnail/630x315/format/jpg/quality/85/http%3A%2F%2Fi.huffpost.com%2Fgen%2F5066758%2Fimages%2Fn-ANDREW-BROOKES-OGM-628x314.jpg](https://o.aolcdn.com/images/dims3/GLOB/legacy_thumbnail/630x315/format/jpg/quality/85/http%3A%2F%2Fi.huffpost.com%2Fgen%2F5066758%2Fimages%2Fn-ANDREW-BROOKES-OGM-628x314.jpg) (Slide nr. 6)