

Thermal hysteresis measurement

Material

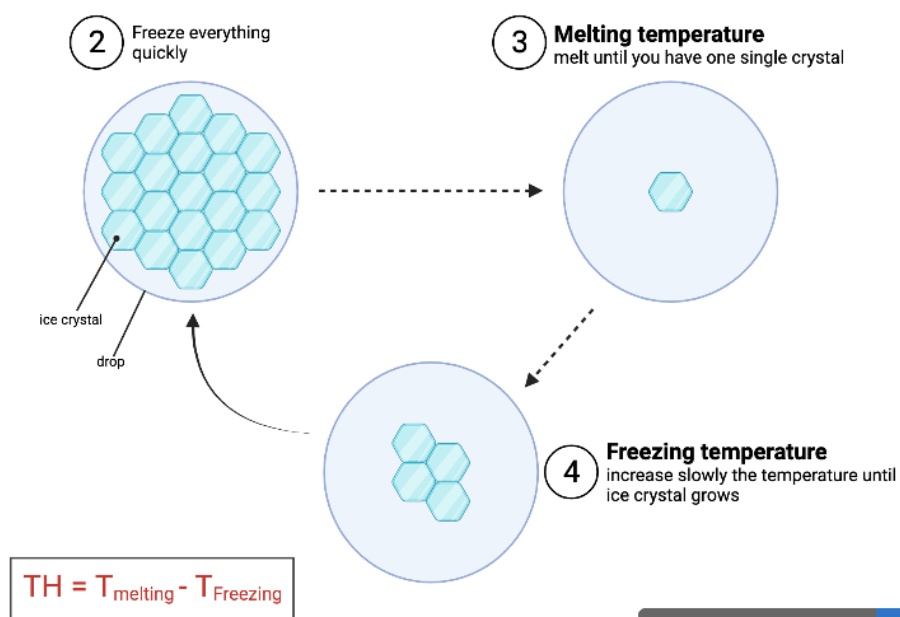
In order to measure the Thermal hysteresis you will need:

- a Nanoliter osmometer (in our case FROZONE)
- softwares:
 - to control the Nanoliter Osmometer
 - to record the screen
- microscope (or stereoscope)

Methods

As mentioned in the measurements section, we used the ISF ("I Said Freeze") assay in order to monitor the thermal hysteresis.

- 1) Put a drop of 10 μ L of your solution on your Osmometer Nanoliter under microscope
- 2) Reduce temperature at -20°C in order to freeze everything
- 3) Then slowly increase the temperature until you see only one ice crystal. Write down this temperature, this is the melting temperature.
- 4) Once you have one crystal, slowly decrease the temperature until you see that your crystal is growing rapidly. write down this temperature, this is the freezing temperature.
- 5) Subtract the melting temperature with the freezing temperature in order to find the Thermal Hysteresis of your solution.



Time To Freeze

The idea of our TTF assay is to monitor the freezing time of our different solutions. Thanks to FROZONE, we can precisely set a temperature, in our case -10°C , and time when our drop freezes.

Material

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- softwares:
 - to control the Nanoliter Osmometer
 - to record the screen
- microscope (or stereoscope)

Methods

As mentioned in the measurements section, we used the TTF assay in order to monitor the time before freezing.

- 1) Put a drop of $10\mu\text{L}$ of your solution on your Osmometer Nanoliter under microscope
- 2) Stabilise the temperature at 0°C .
- 3) Start the screen recording.
- 4) Set the temperature to -15°C
- 5) Once your drop freezes, increase the temperature to 0°C and wait for everything to thaw.
repeat at least three times point 7 to 10,
- 6) Thanks to a video editor, measure the time before freezing.

versus

In this assay, we placed two drops of liquid solutions that we wish to compare using our FROZONE device. Thanks to EDNA (the FROZONE's controller), we set the temperature at -15°C and observed which drop is freezing first. We compared the freezing time of two $10\text{ }\mu\text{L}$ drops of buffer both supplemented with *P. syringae syringae* ($\text{OD} = 1$), where one sample was first incubated for one hour with tailocins. If tailocin does reduce the effect of *P. syringae syringae*, we should observe a difference in the freezing time between the two drops.

Material

- a Nanoliter osmometer (in our case FROZONE)
- softwares:
 - to control the Nanoliter Osmometer
 - to record the screen
- microscope (or stereoscope)

Methods

As mentioned in the measurements section, we used the TTF assay in order to monitor the time before freezing.

- 1) Put two drops of $10\mu\text{L}$ of solutions you want to compare on your Osmometer Nanoliter (under microscope).
- 2) Stabilise the temperature at 0°C .
- 3) Start the screen recording.
- 4) Set the temperature to -15°C
- 5) Once your two drop freezes, increase the temperature to 0°C and wait for everything to thaw. repeat at least three times point 7 to 10,
- 6) Thanks to a video editor, measure the time before freezing.

FDT Results

iGEM UniLausanne

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1 Introduction

This year, our team tried to reduce the damage done to apricot trees due to frost. In order to do this we came up with two solutions. The first solution is using Antifreeze Proteins (AFP), a type of protein that can bind to ice crystals and reduces their growth. the second solution is targeting a bacteria, *Pseudomonas Syringae* (PSS), that live on apricot trees. This bacteria can produce a protein, an Ice Nucleation Protein (INP), that will act like a nucleation site for the ice and therefore boost the formation of ice crystals. In order to avoid the production of INP we have two possibility. First one is to kill the bacteria with tailocin, a protein that will kill specifically the bacteria. The second possibility, is using some phages (a virus specific to bacteria). these phages are genetically modified in order stop the production of INP. all of these solutions should reduce ice crystal formation and therefore reduce frost damage on apricot trees.

In order to simulate the cold spring temperatures that apricot trees were exposed to we used *A.thaliana* Col-0 plants and a thermal chamber. Next we quantified the damage inflicted by the frost and PSS to our plants as well as test the efficacy of our protective solutions, we used a trypan Blue based staining method. This method specifically stains dead cells. We next use a microscope to capture images of our samples and run them through a custom image processing software to quantify the damage done to the plant samples.

2 Treatment

2.1 Materials

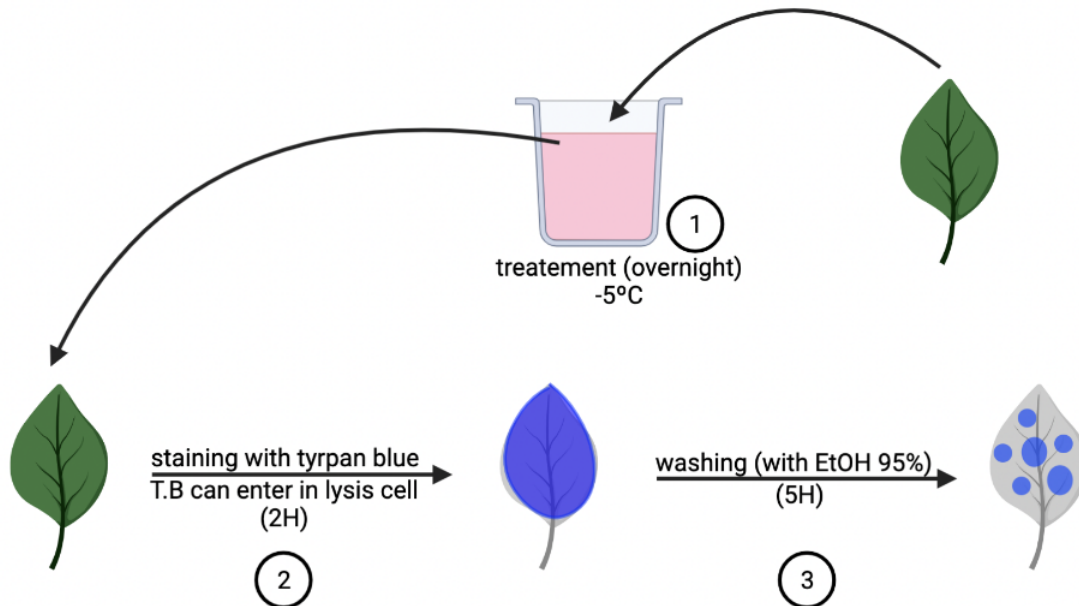
- MS growth medium (for 1/2 L):
 1. Add water (1/2 L)
 2. Add MS-half strength 1/2 ([0.5x] 1.2 g)
 3. Add sucrose (0.3% 1.5g)
 4. Adjust pH with KOH to pH 5.7-5.8
 5. Adjust Volume
 6. Add plant propagation agar in separate bottle (1% 5g)
 7. Add liquid medium to the agar bottle
 8. Microwave until homogeneous
- Sterilized Col-0 seeds:
 1. Put seeds in eppendorf
 2. Add 1 ml EtOH 70%. Wait 5 minutes
 3. Replace EtOH 70% with EtOH 100/95% and mix.
 4. Leave to dry under hood

2.2 Methods

A.thaliana Col-0 seeds were plated onto MS. After spending 3 days at 4° in total darkness, they were allowed to grow 2-3 weeks at 25° with 8h light cycles.

2.3 Treatment

Next the plants were fully immersed in eppendorf tubes containing the desired solutions to be tested and left overnight (12h) in the thermal chamber at -5° . The plant samples were not previously acclimated to the cold temperature.



3 Staining Protocol

3.1 Material

- Trypan blue solution:
 - 10 ml lactic acid 85 %
 - 10 ml phenol (TE buffer equilibrated at pH 8)
 - 10 ml glycerol 99%
 - 10 ml distilled H₂O
 - 40 mg trypan blue (final concentration: 10 mg/ml)
- EtOH 95 %solution
- EtOH 75 % solution
- Incubator
- Eppendorf tubes (or any adapted container)

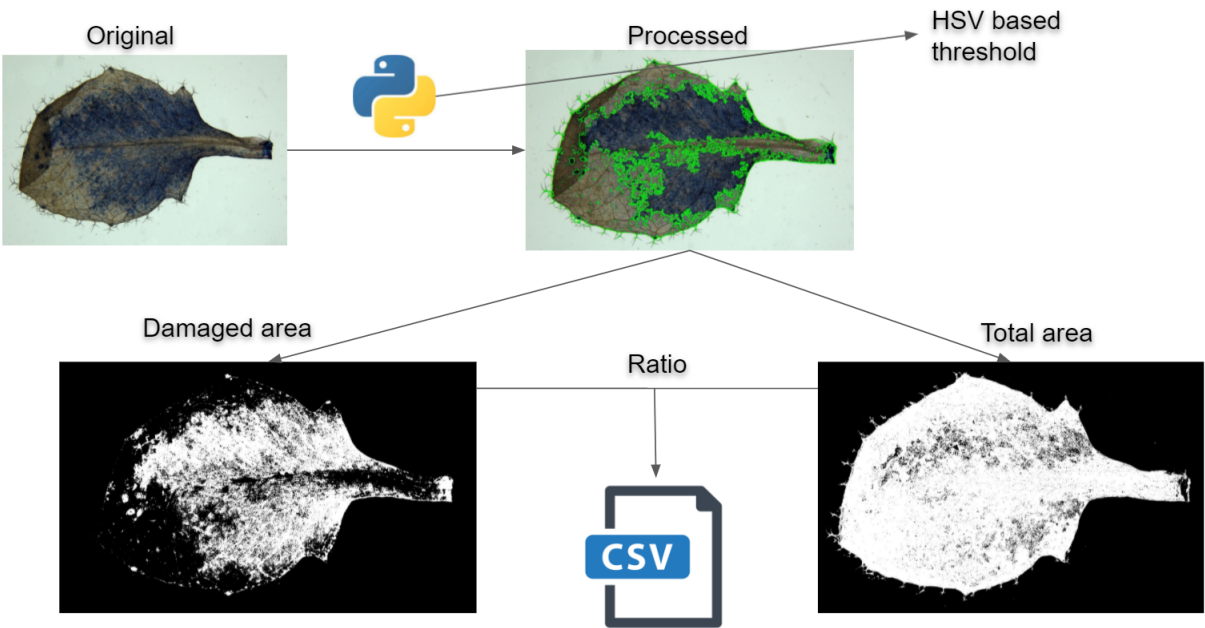
3.2 Methods

- After treatment of the samples, place them in the trypan blue solution in an eppendorf (make sure they are totally submerged), work under hood. Incubate at 30° for 2h.
- Remove trypan blue solution (it can be reused) and replace it with EtOH 95 %. Wait 5-6h.
- Transfer the samples to a 75 % EtOH solution for storage.

4 Image Analysis- Vision software

In order to quantify the damage done to the leaves, we have written a python script and used the opencv library. The script takes an image and measures the area of pixels in a certain range of HSV values. The

thresholds are determined manually. A ratio is then calculated between the damaged area and the leaf area.



5 First tests

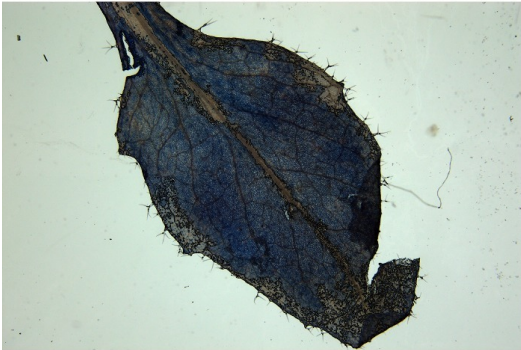
6 Data 1

7 Data 2

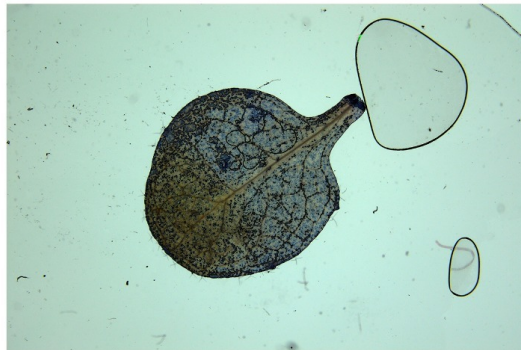
8 Data 3

9 Conclusion

Buffer

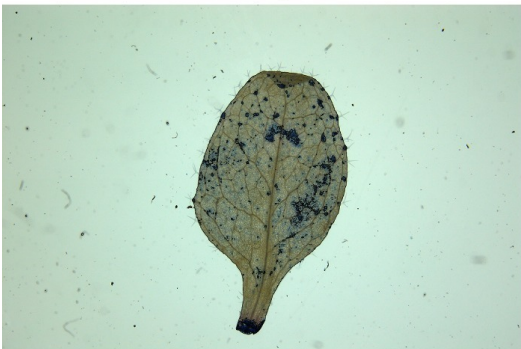


Test at -4°C during overnight

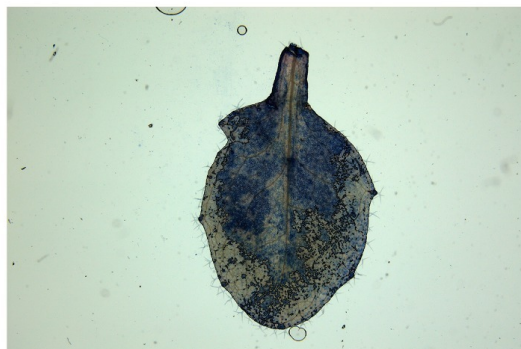


Control

FfIBP - 71 μM

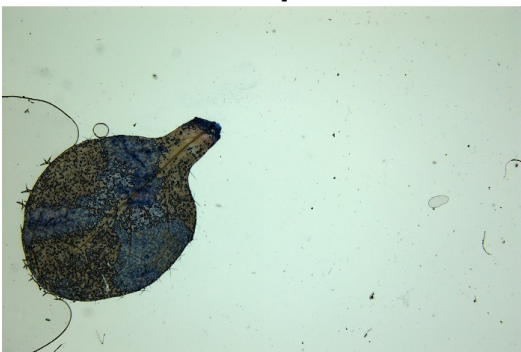


Test at -4°C during overnight

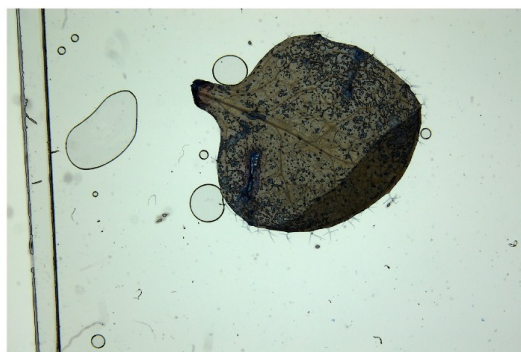


Control

FfIBP - 44 μM



Test at -4°C during overnight



Control