

## Screening for corky root phenotype in lettuce seedlings in the growth room

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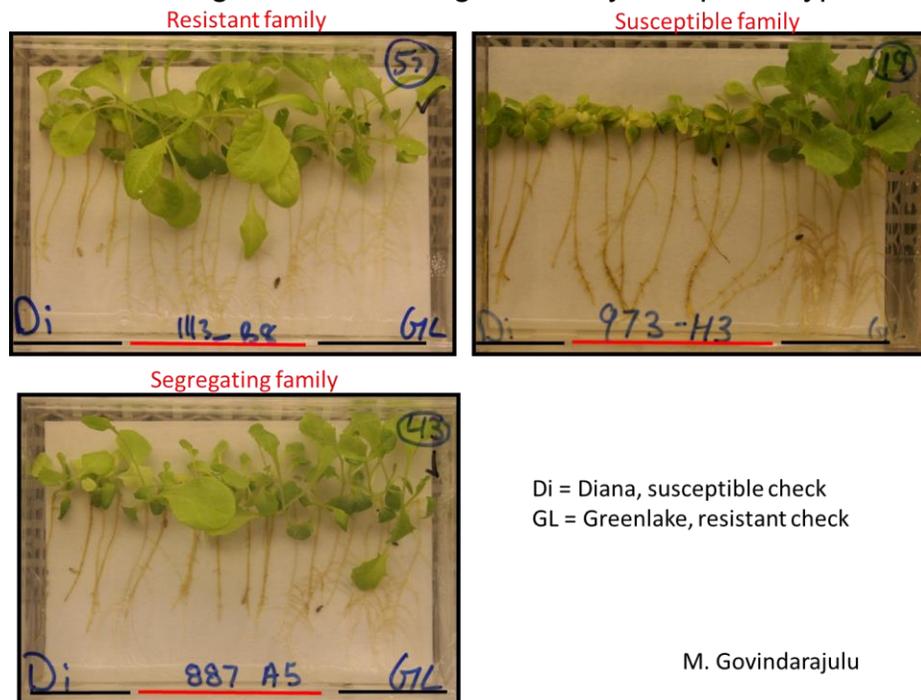
### Preparation of lettuce seedlings:

1. Place chromatographic paper into plastic boxes. Add 15 mL of Hewitt's solution (see below).
2. Place lettuce seeds on to the moist paper.
3. Incubate boxes on a slant at 14°C (16 h/8 h day/night cycle). On the 3rd day, transfer boxes to 25°C (16 h/8 h day/night cycle) chamber.
4. On the 6th day, inoculate the seedlings with suspension of *Rhizorhapis suberifaciens*.

### Inoculation of lettuce seedlings:

1. Add ~10 mL of the suspension of *R. suberifaciens* to each box. Lay the boxes in a horizontal position after addition of bacteria for 5 minutes for uniform inoculation.
2. After 5 minutes, pipette off liquid containing the bacteria. Add 15 mL of fresh Hewitt's solution gently to the boxes without disturbing the roots.
3. Maintain in 25°C chamber (16 h/8 h day/night cycle).
4. Add additional Hewitt's solution to the boxes as and when needed to keep the paper moist for continual growth of lettuce seedlings.
5. Score the boxes for corky root phenotype on the 14th day.

### Screening lettuce seedlings for corky root phenotype



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## Media preparation:

### S-Broth for *Rhizorhapis suberifaciens* (1 L)

- 5g casein hydrolysate (enzymatic)
- 2.5g glucose
- 1.3g of  $K_2HPO_4 \cdot 3H_2O$
- 500mg  $KNO_3$
- 500mg  $MgSO_4 \cdot 7H_2O$
- 60 mg of  $Ca(NO_3)_2 \cdot 4H_2O$

Adjust pH 7.2. Autoclave media and cool. Inoculate cultures with *R. suberifaciens*. Incubate cultures at 29-30°C for 5-7 days in 1 L flask on rotary shaker at 100 rpm. Use these cultures directly for inoculation of lettuce roots.

## Hewitt's Solution:

Stock solutions:

### SOLUTION A (1000x)

FERRIC CITRATE 4.9g/100ml

\*add 500mL diH<sub>2</sub>O, dissolve over heat while stirring for a long while.

### SOLUTION B (5x)

Prepare this solution in the order the chemicals are shown:

Dissolve the chemicals in 3L and then make up to 4L

- $NaH_2PO_4 \cdot H_2O$  3.68g
- $KNO_3$  4.04g
- $MgSO_4 \cdot 7H_2O$  15.10g  
(or  $MgSO_4$  7.38g)
- $Ca(NO_3)_2 \cdot 4H_2O$  18.9g

### SOLUTION C (1000x)

- $MnSO_4 \cdot 4H_2O$  558mg  
(or  $MnSO_4 \cdot H_2O$  475mg)
- $NiSO_4 \cdot 7H_2O$  7mg  
(or  $NiSO_4 \cdot 6H_2O$  6.5mg)
- $CuSO_4 \cdot 5H_2O$  60mg
- $CoSO_4 \cdot 7H_2O$  7.0 mg
- $ZnSO_4 \cdot 7H_2O$  72.5mg
- $H_3BO_3$  65mg
- $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$  8.75mg
- H<sub>2</sub>O to 250mL

## Hewitt's Working solution (1 L)

### SOLUTION A (1000x)

1 mL

### SOLUTION B (5x)

200 mL

### SOLUTION C (1000x)

1 mL

Adjust pH from 3.8-4.0 to 4.5-4.7

Add 10 mg ROVRAL/liter fungicide to prevent fungal growth.