LSOP Title	In vitro single node split plate assay
LSOP No.	LSOP20
Version	1.1
Location	UQ Node/Centre-wide
Policy/Procedure Link	*UQ- Equipment* *UQ -waste* *OGTR*
Risk Assessments	
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Date Approved	14/09/2021
Date Effective	14/06/2021
Next Review Date	14/06/2026
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1.0 Scope

This procedure covers in vitro single node split plate assays. It includes growth conditions, split plate preparation, nodal segment collection, phenotyping and image analysis.

2.0 Definitions

PPM - preservative plant mixture

MS – Murashige and Skoog

3.0 Materials and Equipment

1. Pots (68mm)
2. Square 11cmx11cm Plates
3. MS
4. Agar
5. PPM
6. Autoclave
7. Scalpel
8. Water (for node segments)

9. Ethanol
10. Camera
11. Image J (on computer)

4.0 Prescribed Actions

Growth conditions:

1. 16 hrs light (150-200 $\mu m^{-2}s^{-1}$).
2. 22/19°C (day/night).
3. One plant per pot.

Split plates preparation:

1. For one plate prepare 50 ml medium
    a. 1/2 MS
    b. 0.9% agar
    c. 0.1% PPM
    d. pH 5.8.
2. Add the required amount of sugar, hormone or other chemicals (check if autoclavable before).
3. Autoclave and pour in square plates (11cm x 11 cm).

NB: You can generally keep plates in the fridge for weeks.

NB: Before harvest, dig a 1 cm large gap in the middle of the plate with a spatula. Use the attached model to help you. For Arabidopsis it is better to have only one row. For pea and rose you can do two.

Nodal segment collection:

1. Harvest nodes when rosette branches are 10-20 cm long (Fig 1, appendix)
2. Collect preferentially node segments on secondary rosette branches (cauline branches are fine too). Avoid harvesting very basal nodes where the stem is very thick and purplish. Buds must be very small and the bud stem should be practically invisible (See Figure 2, appendix). Harvest stem

segment with 2 cm of stem above and below the bud. Collect segments in water. You need 10-12 nodes per petri dish.

NB: some nodes have 2 or 3 buds. Is OK to use them if you thin them to leave one bud.

3. Under the laminar flow bench, briefly transfer the stem segments in ethanol 70% (if you use PPM in the media, this step is optional). Then place them on the lid of a petri dish on which you have drawn a 1.5cm long mark to help cutting the segments at that size (Figure 3, appendix). When you cut, the bud should be in the middle with 0.75cm of stem at each side.

   NB: Sterility is not really important as nodes will be grown for a short period (up to 10 days) and PPM is enough to prevent contamination during this period.

4. Once you have cut the stem segments, place them in the gap that you have dug in the growth medium with each extremity in the medium (Figure 4, appendix). All buds should be pointing in the same direction. If you have several plates, repeat the steps from the beginning. It is better to decrease the time between excision and *in vitro* set up.

5. Put the plates in a growth chamber (same conditions as above). Plates should be kept vertically with an angle to let buds growing out of the medium (Figure 5, appendix).

<u>Phenotyping:</u>

1. Take a picture at T0 and every 24 hrs. The picture should be taken for the top, with an angle so that the camera is perpendicular to the buds and not to the stem segments (Figure 6A & B, appendix). This angle will be changing as the branch will be growing.

2. Use the attached model to have the scale in the same ground than the buds as on the picture below (on the picture the angle of the camera and the zoom are not good, Figure 6A&B).

3. Take pictures with very high resolution and zoom as much as possible to have all the buds and the scale on the same picture (example at T0, Figure 7).

   NB: If you notice water in the plate when you take pictures, remove it with a tissue.

NB: Some buds may die or have abnormal growth (curling or growth in the medium). Keep record of this for further analysis. These buds will need to be removed.

Image analysis:

1. Open pictures with ImageJ.
2. Zoom as much as possible on the scale.
3. Use the "straight line" tool to draw a line along the scale bar.
4. In "Analyze" and "set scale" write 20 in the field near "known distance" and click ok.
5. Then measure with the same tool the buds in order from the left to the right.
6. To do so draw a line on the bud and press "T" (a new window will pop up).
7. Repeat this step for all the buds.
8. Click "measure" in the window with values.
9. The length of each bud is given in mm in a new window.
10. Copy and paste these values in an excel file and calculate the average of each date for each treatment to plot the kinetics.

5.0 Appendix A

Figure 1: Arabidopsis size for harvesting nodes

Figure 2: Arabidopsis nodes with small buds

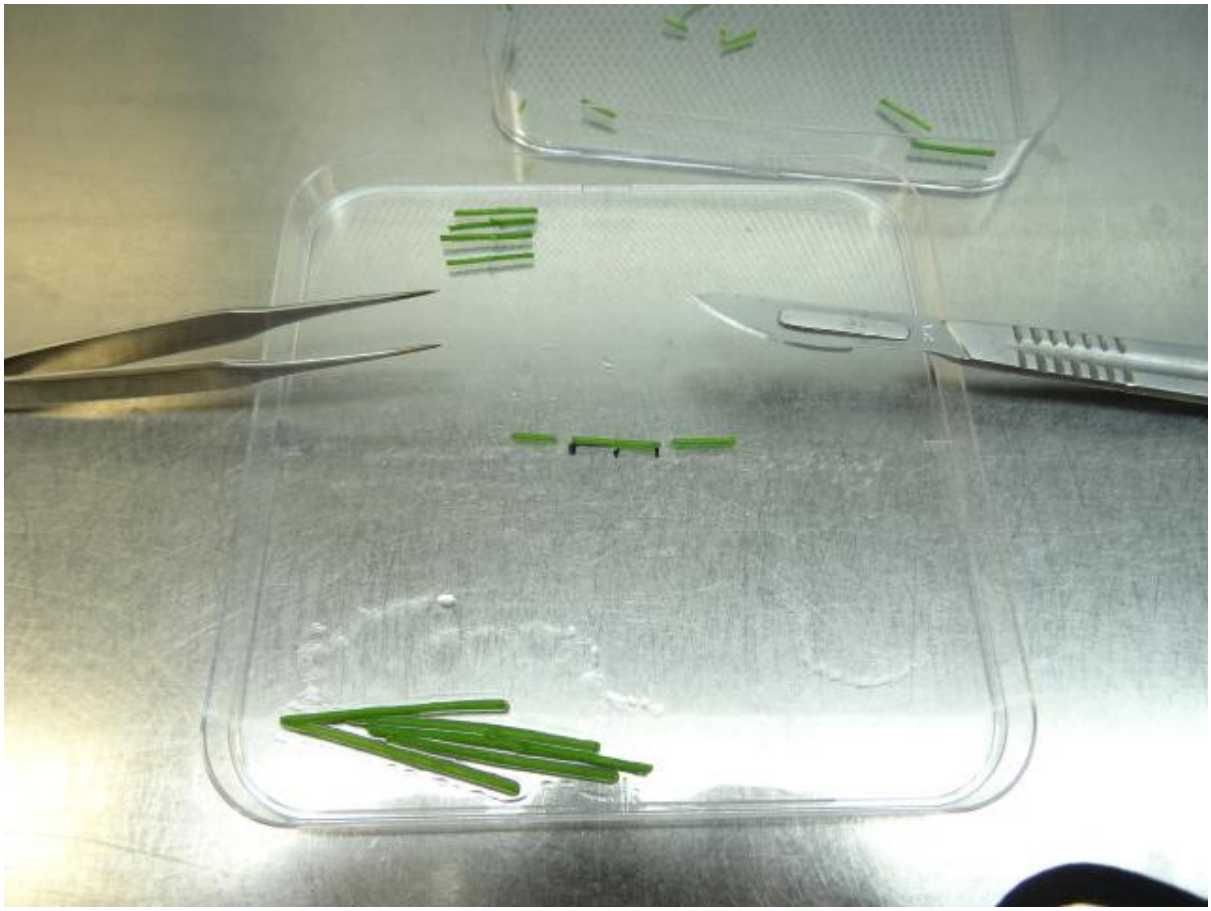


Figure 3: Stem segments cut to 1.5cm as indicated by the black lines on the lid of the petri dish

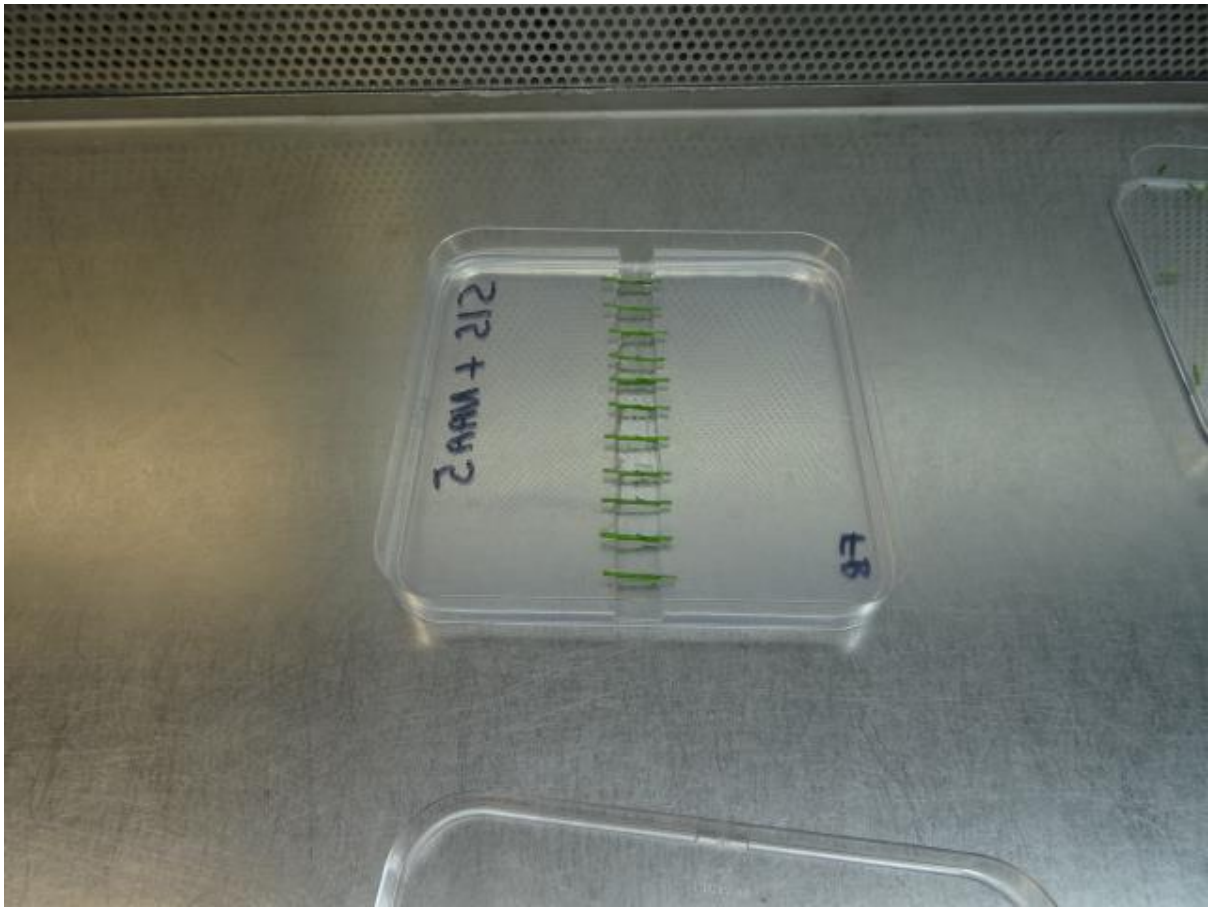


Figure 4: Place cut stem segments in the gap of the growth medium

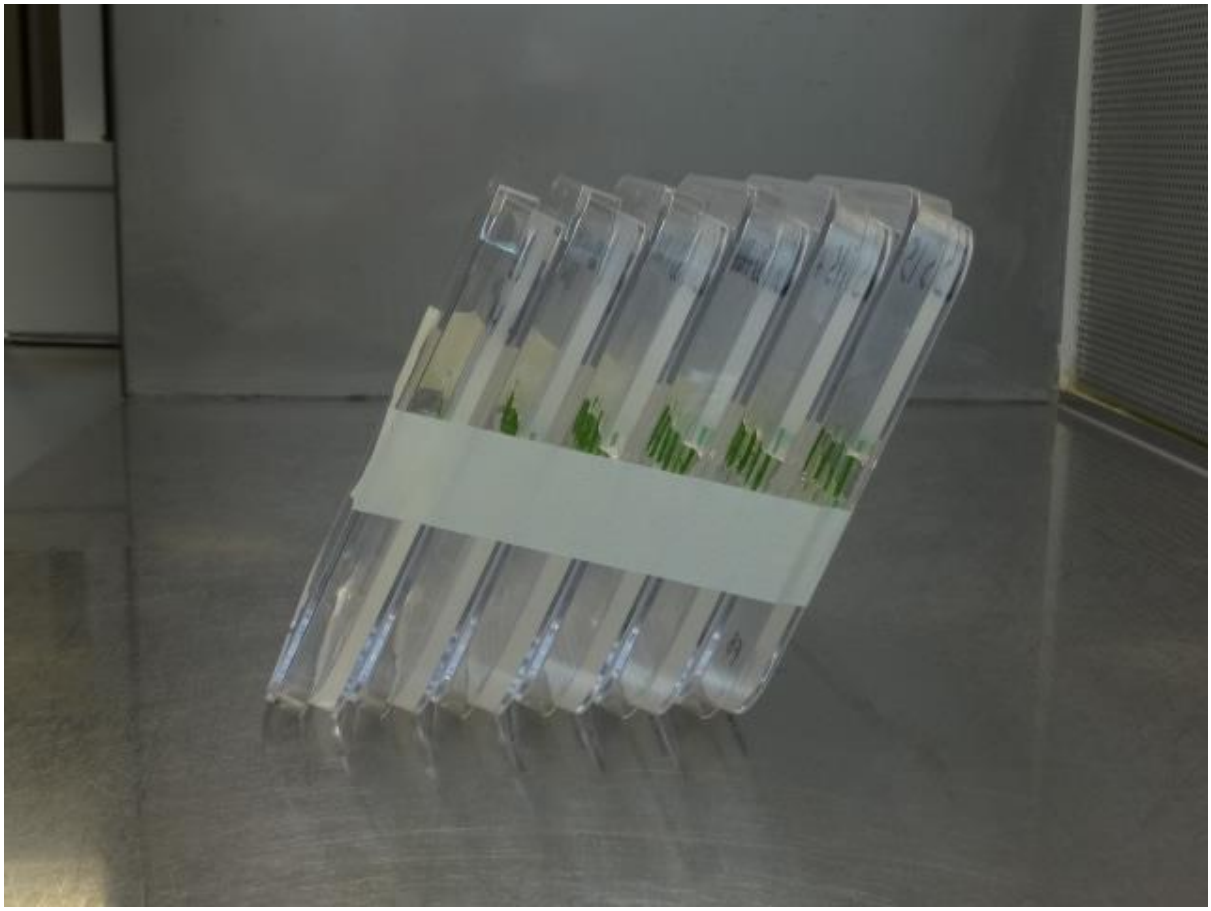
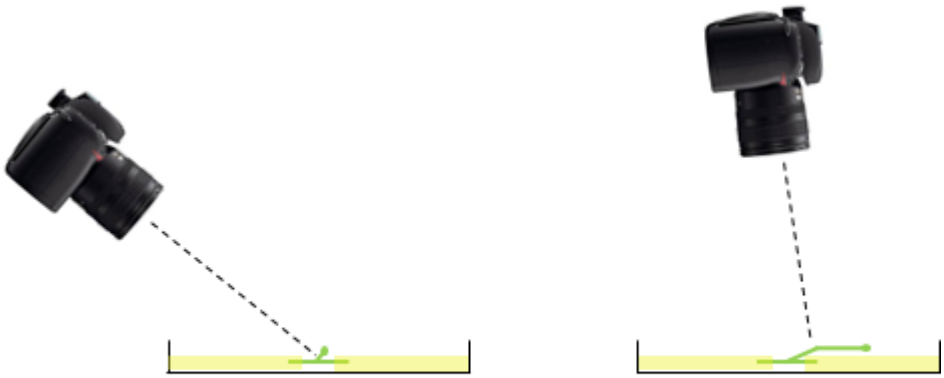


Figure 5: Plates should be kept vertically



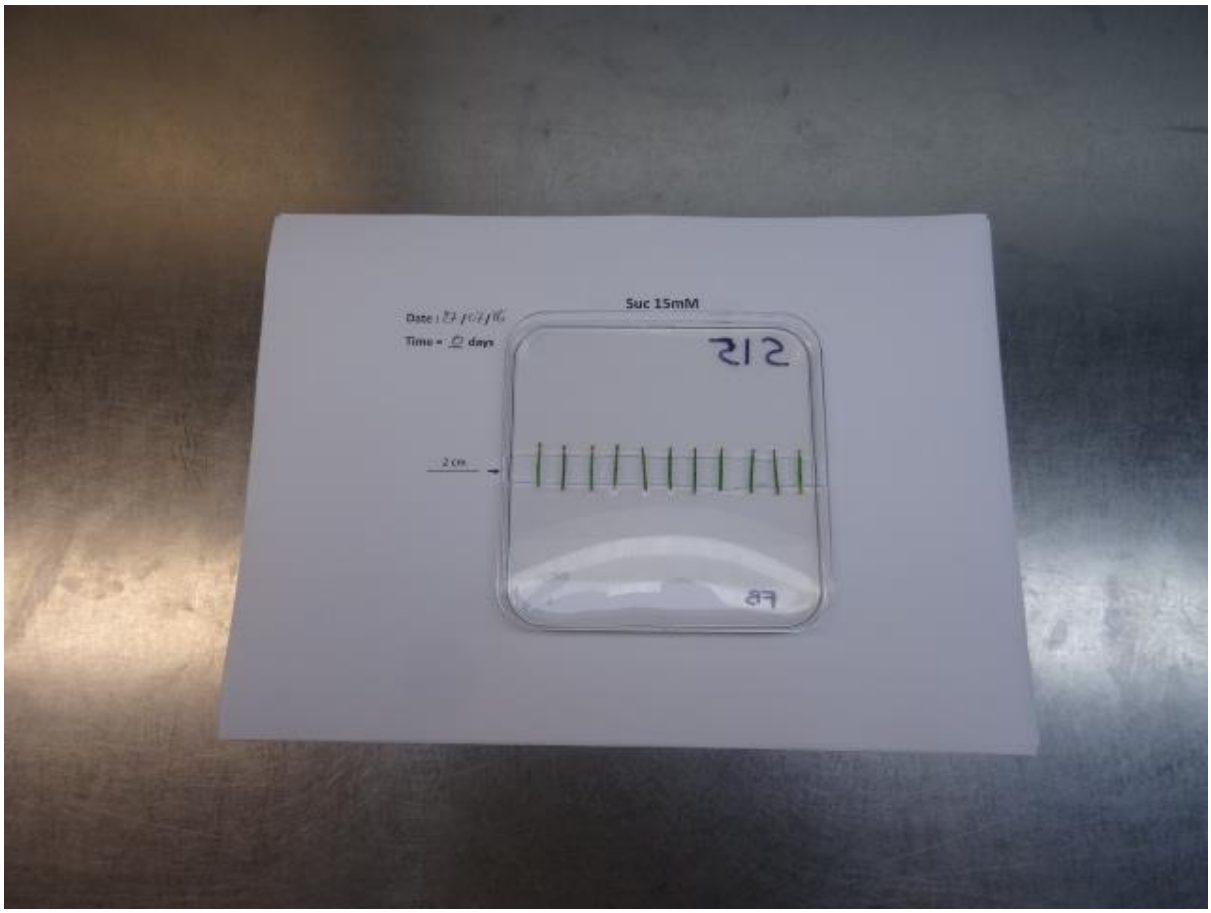


Figure 6 A & B: Zoom in as much as possible and have all the buds and scale in the same picture.

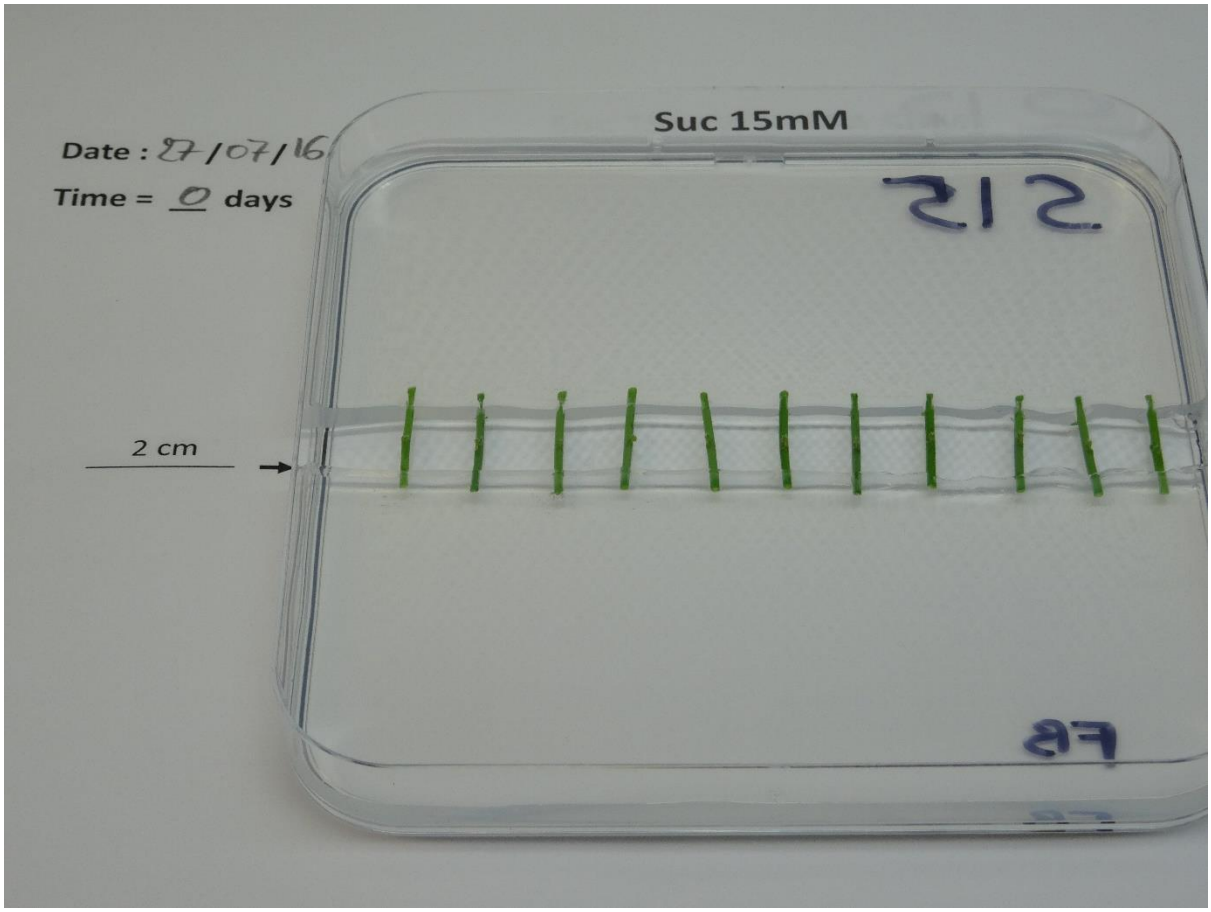


Figure 7: Example of picture taken at T0