

JA sensitivity root assay protocol (using agar plates with shelves)
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Make up plant growth media as follows:

- ½ strength MS
- 1% (10g/L) sucrose
- 1% (10g/L) agar
- pH 6.0
- Methyl jasmonate (MeJA; at appropriate concentration)*

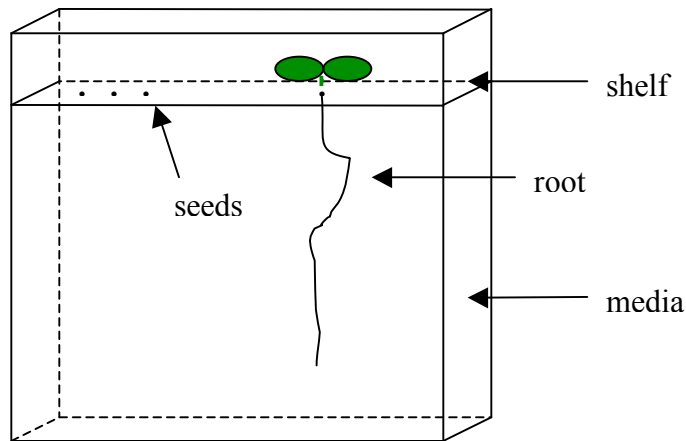
*MeJA must be added after autoclaving and letting agar cool to ~55°C.
In a typical dose-response assay we use: 0, 10 and 50 µM MeJA.

We use square Petri dishes, 100 x 100 x 15 mm, with grid. The grid allows easier estimation and comparison of root lengths.

Pour dishes about half full of media. It is best to let the plates sit overnight to dry a bit.

Using a sterilized scalpel, cut a strip of media out of each plate. If using gridded plates, it is easiest to cut out the top grid square. This forms a shelf of agar that you will place your seeds on. A diagram illustrating this is provided below.

Use a toothpick to individually place sterilized seeds along this shelf, centered with respect to the depth of the media. For plates of the indicated dimension, evenly space 15-20 seeds along each plate.



Wrap the edges of the plate with Parafilm. It is essential that you use Parafilm or a similar substance that is permeable to O₂ and CO₂, but not to water to prevent the plates from drying and shrinking as this will allow the roots to escape from the media at the thin spots, and begin to grow along the surface of the agar.

Plates should be placed vertically with the shelf as the top side. This will cause the majority of roots to grow down into the media and remain within it (and thus exposed to MeJA) for the entirety of the growth process.

After the desired growth period (e.g. 10 days.), the roots can be photographed, measured, or otherwise examined.

When transplanting seedlings from this condition, it is easiest to simply transplant a small agar plug around the root rather than trying to extract the root from the agar.