

Cotyledon Explant Shoot Regeneration Protocol (M.Raizada)

Media B

(from Carol Auer, Univ. Conn. from Arabidopsis Genetics Meeting 2000 Abstracts)

SIM recipe from Arabidopsis, A practical Approach (pp.129)

SIM + 4.4uM iP + 0.5 uM NAA =

Gamborg's B5 medium with vitamins (Sigma)

0.5g/L MES

20g/L glucose

pH 5.8

2g/L phytigel

Autoclave, then add:

4.4uM 2-iP (isopentenyl-adenine)

0.5uM NAA

1. Use 100 x 25mm petri dishes.
2. Cut off cotyledons at petiole and place 25 shoots on each plate. Place transplants such that cotyledons are touching the media (*keep this placement very consistent*). Regeneration will be from the cotyledons themselves.
3. *We will have to determine whether we excise cotyledons based on age after germination or cotyledon size and then be consistent about this selection criterion.*
4. Use surgical tape to seal plates to allow good gas exchange.
5. Growth conditions:
 - 20 uE/sec/m² cool white fluorescent light, 24 hour light, 25C.
 - Make sure that all ecotypes receive same light intensity exposure.*
6. After 2-3 weeks, score number of shoots regenerated on standard "scoring sheet" as a percentage of plantlets transplanted.