# Tobacco regeneration

### Materials, for 2 students

Facilities

* Access to laminar flow hood. We will utilise:

|  |  |
| --- | --- |
| Location | Simultaneous users |
| Level 3 (South corridor) | 4 |
| 406 | 2 |
| 413 | 2 |
| 410 | 1 |

Laminar flow hoods need working Bunsen burners or gas or spirits burners, and one beaker or container (difficult to tip over) with alcohol to place the forceps and scalpels

* Plant growth incubator, at around 100 mol white light, for 4 weeks.

Materials

* 1x MS salts and micronutrients (Duchefa, Melford Laboratory Supplies)
* 1x Gamborg’s vitamins
* 3% sucrose
* 0.5 g/l MES
* pH to 5.7 with NaOH
* 0.8 % plant agar or microagar (Duchefa), add after adjusting pH
* Autoclave
* Pour about 35 ml/plate

Plant material

* 1 jar of sterile grown tobacco plants or explants. One plant or explant each enough, if containing several leaves. The plants should be grown under intense white light if possible (at least 100 micromoles light) for around 2 months. To raise these plants:
* Preparation of individual jars
* 1x MS salts, micronutrients and Gamborg’s vitamins (Duchefa, Melford Laboratory Supplies)
* 3% sucrose
* 0.5 g/l MES
* pH to 5.7 with KOH
* 0.8 % plant agar or microagar (Duchefa), add after adjusting pH
* Autoclave after stirring thoroughly. If unsure, melt fully in hot stirring plate and poor into jars before autoclaving in individual jars.
* Sterilise tobacco seeds (1’ ethanol, followed by 10’ 25% fresh bleach, followed by 5 washes with sterile water). To handle seeds individually, resuspend finally in 0.1% agar (they will stay in suspension) and pipette the solution with cut blue tip. Forceps are an alternative. Saw seeds on individual jars, at least five per jar.

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| --- | --- | --- | --- | --- | --- |
| Ingredient | Supplier | C- callus medium-old | B –shooting medium | D- rooting medium | A-callus medium new |
| MS salts & micronutrients\* | Melford Labs-Duchefa | 1x | 1x | 1x | 1x |
| Sucrose | Any | 3% | 3% | 3% | 3% |
| Agar (plant or micro agar) |  | 0.7% | 0.7% | 0.7% | 0.7% |
| Gamborg vitamins 1000x | Melford Labs-Duchefa | 1x | 1x | 1x | 1x |
| NAA\*\* |  |  | 0.05mg/l |  | 1mg/l |
| IAA\*\* |  |  |  | 1mg/l |  |
| IBA\*\* |  |  |  | 0.2mg/l |  |
| 2,4-D |  | 1mg/l |  |  |  |
| BAP\*\*\* |  | 0.5mg/l | 2mg/l |  | 0.5mg/l |
| Kinetin |  |  |  | 0.04mg/l |  |

Tissue culture material:

* 1 petri dish of each type of media/pair, at least 20mm tall plate with about 30ml media labelled as below

\*Set pH to 5.8 before autoclaving

\*\*NAA: 1-naphtylacetic acid. Readymade solution from Sigma or make as 2g/l in 0.1M NaOH. Or make as 10g/l in 1 ml acetone, add 10ml water, leave in fume hood to evaporate acetone off. Same for IAA (indol acetic acid) 0r IBA (indol butyric acid). Filter sterilise with a syringe filter

\*\*\*BAP: 6-benzylaminopurine, or Kinetin: 6-furfurylaminopurine. Readymade solution from Sigma or make at 2g/l in 0.1M HCl. Filter sterilise with a syringe

* Medium should be autoclaved with first 3 ingredients only, the last 3 prepared as sterile stocks (filter sterilised unless in organic solvent) and added once medium has cooled to around 50°C
* Make a few spare plates for the whole class, accidents, and plates not keeping aseptic, etc. Also spare forceps and scalpels

Concentrations of hormones in media:

Note: 1 mg/l is about 5 µM for all hormones, since their MW is about 200 g/mol for all.