Germination medium (GM) **Protocol written by Nicole Lettner 30.10.2013**

This medium was originally developed to optimize Arabidopsis protoplast culture (Masson and Paszkowski (1992) Plant J. 2: 829-833). Under different growth conditions, it is superior to media based on the commercially available MS mixture with respect to plant growth speed and chlorophyll content, and also better for selection experiments. Therefore, the Mittelsten Scheid lab uses it for most experiments with in vitro *grown material. Companies and order numbers are given for the chemicals used in the Mittelsten Scheid lab, but other brands are likely fine (beside of agar, this is critical). Preparation of the stock solutions and buffers takes some time, but (except for the Sucrose Solution) they can be stored.*

Solutions needed:

MES Solution

Conc. = 0.65 M pH = 6.0
Dissolve 70 g MES-monohydrate (M = 213.25 g/mol, AppliChem A1074.0500, buffer grade) in about 400 ml H_2O.
Adjust to pH 6.0 by slowly adding 2 M NaOH solution.
Bring to a final volume of 500 ml with H_2O.
Filter sterilize.
Store at 4°C and always handle under hood to keep sterile.

Ferric Citrate Solution

Conc. = 10 g/l
Dissolve 5 g ammonium-iron(III)citrate (VWR BDH Prolabo, 83887.290, technical grade) in about 450 ml of H_2O and bring to a final volume of 500 ml with H_2O.
Filter sterilize.
Store at 4°C and always handle under hood to keep sterile.

MS Macro Solution

Dissolve completely, one component at a time, and in this order:

- 76.0 g KNO_3 (potassium nitrate, M = 101.11 g/mol, AppliChem A1041.1000),
- 66.0 g NH_4NO_3 (ammonium nitrate, M = 80.04 g/mol, AppliChem A1031.1000),
- 17.6 g $CaCl_2$ x 2 H_2O (calcium chloride dihydrate, M = 147.02 g/mol, AppliChem A3587.1000),
- 14.8 g $MgSO_4$ x 7 H_2O (magnesium sulfate heptahydrate, M = 246.48 g/mol, AppliChem A1037.1000),
- 6.8 g KH_2PO_4 (potassium dihydrogenphosphate, M = 136.09 g/mol, AppliChem A3620.0500),

in 3.8 l H_2O and bring to a final volume of 4 l with H_2O.
Aliquot and sterilize by autoclaving.
Store at room temperature and handle under hood to keep sterile.

B5 Micro Solution

Dissolve completely, one component at a time, and in this order:

- 2.0 g $MnSO_4$ x H_2O (manganese(II)sulfate monohydrate, p.a., M = 169.02 g/mol, AppliChem A1038.0250),
- 0.6 g H_3BO_3 (boric acid, buffer grade, M = 61.83 g/mol, AppliChem A0768.0500),
- 0.4 g $ZnSO_4$ x 7 H_2O (zincsulfate heptahydrate, p.a. ACS, M = 287.54 g/mol, ROTH K301.1),
- 0.15 g KJ (potassium iodide, M = 166.00 g/mol, Fluka 60405),
- 0.05 g Na_2MoO_4 x 2 H_2O (sodium molybdate dihydrate, M = 241.95, AppliChem A2193.0025),
- 0.005 g $CuSO_4$ x 5 H_2O (coppersulfate pentahydrate, p.a. ACS, M = 249.69 g/mol, ROTH P024.2),
- 0.005 g $CoCl_2$ x 6 H_2O (cobalt(II)chloride hexahydrate, M = 237.93, Fluka 60820)

in 190 ml H_2O and bring to a final volume of 200 ml with H_2O.
Filter sterilize.
Store at 4°C and always handle under hood to keep sterile.

Sucrose Solution

Conc. = 100 g/l
Dissolve 40 g D(+) sucrose (M = 342.30 g/mol, p.a., AppliChem A4734.1000) in about 120 ml H_2O.
Under a sterile hood (to avoid contamination of stock solutions) add

- 200 ml MS Macro Solution,
- 4ml B5 Micro Solution,
- 20 ml Ferric Citrate Solution.

Stir well.
Adjust to pH 5.4 +/- 0.02 by slowly adding 2 M NaOH solution.
Bring to a final volume of 400 ml with H_2O.
Stir at least 30 minutes at room temperature. A precipitate may form, this is normal.
Filter sterilize.

Make always fresh before use.

GM agar plates

To 4.0 g of agar (Merck 1.016.14.5000) add 450 ml H_2O and, under a sterile hood (to avoid contamination of stock solution), 2.5 ml MES solution.
Autoclave.
Cool to 56°C (water bath).
Add 50 ml Sucrose Solution.
Stir well but take care to avoid bubbles.
Pour plates in a sterile hood and leave them to cool overnight.
Wrap them and store at 4°C.

GM liquid medium

= same as plates but without agar.