

Rice seed germination

All ddH₂O used for this process should be sterilized.

1. Homogenization

1. Dehusked the seeds. Select only seeds that are well-developed, fully-filled, clean, and transparent.
2. Dry-incubate the seeds in dark under 45°C for 24 hours.

2. Sterilization

1. Soak the seeds in 3% bleach solution (add one drop of detergent) and gently inverting / shaking for 15 minutes.
2. Wash at least 6 times with ddH₂O, until the water is clear.

3. Priming and imbibition

1. Remove the water thoroughly with pipette.
 2. (optional) Seed priming. Soak the seeds in 3% KNO₃ solution under room temperature for 24 hours;
 3. Soak the seeds in 1 ~ 2% Plant Preservative Mixture™ (PPM™) solution for 16 ~ 24 hours.
- Add 300 μL PPM into 30 mL sterilized ddH₂O.
 - The PPM™ stock solution (100%) is stored at 4°C (a).

4. Sowing

1. Remove the PPM solution.
2. Sow the seeds on suitable medium.

Optional: Add 0.05 ~ 0.2% (v / v) PPM™ to the medium, if it is difficult to control contamination. The PPM™ can be autoclaved together with the media.

Half-MS medium preparation

Chemicals	Stock Concentration	Addition	Final concentration	Location
MS basal salt	—	8.66 g	2.165 g / L	II
Sucrose	—	10 g	2.5 g / L	I
MES hydrate	—	2 g	0.05% (w/v)	III
PPM™ (optional)	100%	2 mL	0.05%	4°C (a)
KOH ※	5 M	~ 710 µL	—	VII
ddH ₂ O	—	~ 4 L	—	—
Total volume		4 L		

(optional) The gelling agent: [Gelzan \(G1910\)](#) or [Phytigel \(P8169 \)](#) → 1.5 g / L (0.15%). Located at I or IX. Generally, we make 400 mL per bottle, so weigh 0.6 g / 400 mL for each bottle.

The final pH should be around 5.7 ~ 5.8. Autoclave under 121°C for 20 mins.

※ KOH is used for pH adjustment.

To prepare 5 M KOH solution, dilute 330 g of 85% KOH in 1 L ddH₂O. Since KOH releases heat during dissolution, it is important to add the KOH granules into the water gradually and slowly.