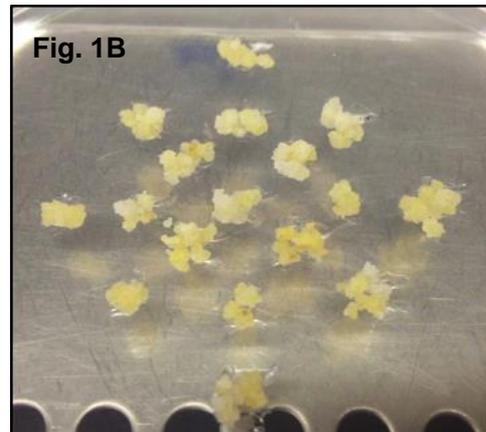
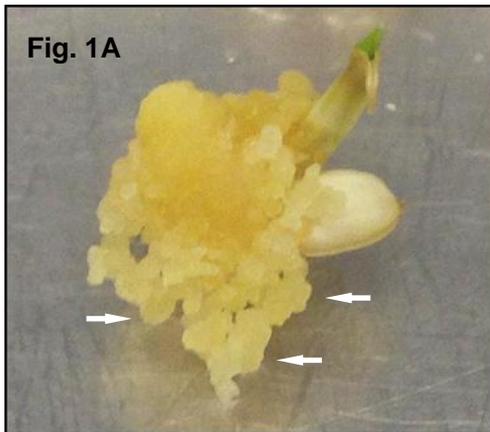


Kitaake rice transformation

Callus induction:

Sterilize dehulled rice seeds with 70% ethanol for 2 min, and further sterilize with bleach (~2.5% sodium hypochlorite solution) for 15 min, then wash five times in sterile water. Inoculate sterilized seeds on "R1" plate and culture in the light at 32°C for 2-3 weeks (Fig.1A). Transfer fresh secondary calli (as indicated by arrows) onto new "R1" plate and grow for 3-5 days (Fig.1B), then use for transformation.

Medium R1: 4.3 g/L MS salts & vitamins + 30 g/L Sucrose + 0.5 g/L MES + 300 mg/L Casamino acid + 2.8 g/L L-Proline + 2 mg/L 2, 4-D + 4 g/L Phytigel, pH 5.8 with KOH, autoclave.



Agro preparation:

Streak agrobacterium strain EHA105 harboring binary vector on LB plate containing appropriate antibiotics, and culture for 2 days at 28°C in the dark. Scrape agrobacterium culture from the plate and suspend with AAM medium (10 ml for example) in a sterile tube to yield an OD600 of approximately 0.3~0.5.

AAM medium: 4.3 g/L MS salts & vitamins + 68.5 g/L Sucrose + 0.5 g/L MES + 36 g/L Glucose + 500 mg/L Casamino acid + 100 ml **10 x AA amino acids**, pH 5.2 with KOH and aliquot by 100 ml. Autoclave and store at 4°C. Add 200 ul of 20 mg/ml acetosyringone per 100 ml before use.

10 x AA amino acids: Dissolve 8.76 g L-Glutamine, 2.66 g L-Aspartic acid, 1.74 g L-Arginine and 75 mg Glycine in 900 ml distilled water and make up the volume to 1,000 ml. Filter sterilize, aliquot by 100 ml, and store at 4°C.

Co-culture:

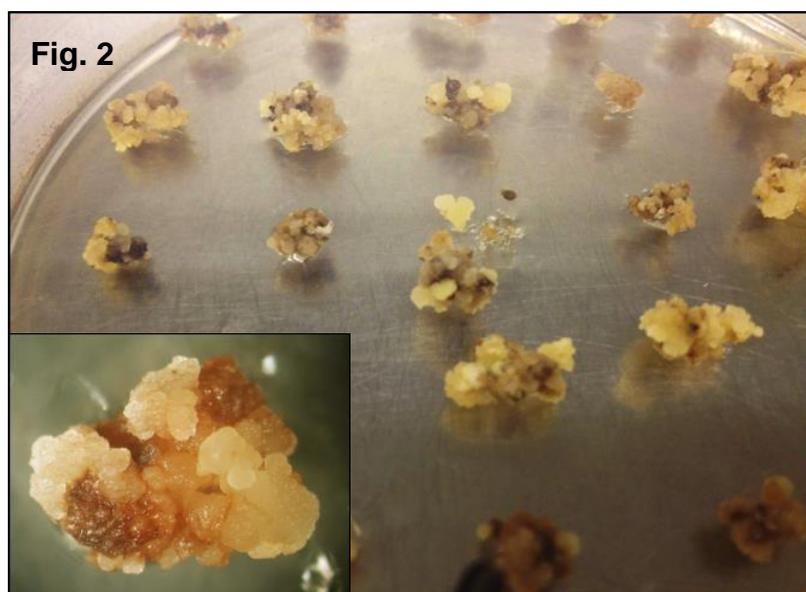
Immerse Calli in the agrobacterium suspension by gently shaking the tube for 5 min, then blot dry with sterilized filter paper to remove excess agrobacteria. Transfer the infected calli onto sterilized filter paper on top of "R2" medium, and culture in the dark at 25°C for 3 days.

Medium R2: 4.3 g/L MS salts & vitamins + 30 g/L Sucrose + 0.5 g/L MES + 10 g/L Glucose + 300 mg/L Casamino acid + 2 mg/L 2, 4-D + 4 g/L Phytigel, pH 5.2 with KOH. Add 1 ml of 20 mg/ml acetosyringone to 1 L medium after autoclaving.

Selection:

Transfer calli to "R3" plates and culture in the light at 32°C for 2 weeks. Transfer living calli to new "R3" plates for 2 more weeks (Fig.2).

Medium R3: 4.3 g/L MS salts & vitamins + 30 g/L Sucrose + 0.5 g/L MES + 300 mg/L Casamino acid + 2.8 g/L L-Proline + 2 mg/L 2, 4-D + 4 g/L Phytigel, pH 5.8 with KOH. Add 200 mg/L Timentin and 30 mg/L Hygromycin (final concentration) after autoclaving.



Re-generation & rooting:

Transfer proliferating calli to "R4" plates and grow in the light at 32°C for 2 weeks or more (Fig.3A). Transfer plantlets arising from the calli (better with roots initiated) to "R5" tubes to facilitate root growth (Fig.3B). Replace "R5" medium with water in the tube and open the lid for training before transplanting to soil.

Medium R4: 4.3 g/L MS salts & vitamins + 30 g/L Sucrose + 0.5 g/L MES + 2 g/L Casamino acid + 30 g/L Sorbitol + 2 mg/L Kinetin + 1 mg/L NAA + 4 g/L Phytigel, pH 5.8 with KOH. Add 200 mg/L Timentin and 20 mg/L Hygromycin (final concentration) after autoclaving.

Medium R5: 2.15 g/L MS salts & vitamins + 15 g/L Sucrose + 0.5 g/L MES + 2 g/L Phytigel, pH 5.8 with KOH. Add 200 mg/L Timentin and 20 mg/L Hygromycin (final concentration) after autoclaving.

Fig. 3A



Fig. 3B



20 mg/ml Acetosyringone stock:

Dissolve 200 mg Acetosyringone in 10 ml DMSO. Aliquot by 1 ml and store at -20°C.

200 mg/ml Timentin stock:

Dissolve 1000 mg Timentin in 5 ml H₂O, filter sterilize, aliquot by 1 ml and store at -20°C.

50 mg/ml Hygromycin stock:

Dissolve 500 mg Hygromycin in 10 ml H₂O, filter sterilize, aliquot by 1 ml and store at -20°C.

1.0 mg/ml 2,4-D stock:

Dissolve 50 mg 2,4-D in 500 µl Ethanol, add H₂O to 50 ml. Store at 4°C.

2.0 mg/ml Kinetin stock:

Dissolve 20 mg Kinetin in 200 µl of 1N NaOH, add H₂O to 10 ml. Store at 4°C.

1.0 mg/ml NAA stock:

Dissolve 50 mg NAA in 200 µl of 1N NaOH, add H₂O to 50 ml. Store at 4°C.