

Growth chamber care for transgenic rice plants of Japonica cv. 'Nipponbare' or 'Kitaake' – soilless mix

Materials

⌘ Containers

1. F1020 flat without drainage holes (Hummert Cat# 11-3050-1)
2. Plastic pots 3 ½" x 3 ½" (Hummert Cat. # 14-0404-1)
3. Clear plastic Humi-dome (Hummert Cat. # 14-3850-1) or Saran-type plastic wrap

⌘ Soil

1. Sunshine Universal Mix SB300 (Consumer Supply)

⌘ Fertilizers

1. Osmocote 17-7-12 controlled release fertilizer for greenhouse crops (B&T Grower Supply, Inc Cat # FZ17712)
2. Sprint 330 chelated iron (Hummert Cat. # 07-1510-1)

Transgenic Rice Plants

⌘ Transferring Plants from Tissue Culture to Soil

1. Plantlets are germinated in 100 x 25 mm petri dishes containing MSS media (MS major and minor salts and vitamins, no hormones, 3% sucrose and 0.3% gelrite, pH 5.8). They are incubated at 25°C in 80-100 $\mu\text{E m}^{-2} \text{s}^{-1}$ intensity light (16:8 photo-period) in a Percival incubator (Model # CU36L5).
2. When the leaf is 4-6 cm long and has developed a good-sized root, transfer the plantlets from the Petri dish to soilless mix using sterile conditions. Be sure to select strong green plants. This transfer should be done on an individual plant basis and may range from 7 to 14 days after germination begins. When one plantlet reaches the appropriate size, remove it from the petri dish regardless of the stage of the other plants. Replace the lid and reseal the dish with micro pore tape to allow the remaining plants to grow.

⌘ **Plant hardening in the growth chamber**

1. The PTF growth chamber (Conviron, CMP3244) is set at 12:12 photoperiod with a temperature setting of 28°C/25°C (day/night) and a light intensity of 350 μ E m⁻² s⁻¹ at plant height (12 inches). ‘Nipponbare’ is a short-day cultivar that requires \leq 12 hours of daylight to flower, whereas ‘Kitaake’ is day neutral.
2. Fill the necessary number of plastic pots with soilless mix. For each pot, 1 tsp of Osmocote and 1 tsp of iron is mixed into the bottom half of soilless mix in pot. Then place into a greenhouse flat without drainage holes. Fill flat with water and allow soilless mix to hydrate by wick action. Discard excess water from flat.
3. In a laminar flow bench, to maintain sterility, take the regenerating plantlet from the petri dish and remove as much media as possible (favor those plantlets with high root/leaf ratio). Plants should always be manipulated by the callus ball at the base of the stem to avoid leaf-breakage. Avoid manipulation of fragile stem parts.
4. Place the plantlet into a hole made into the middle of the soil and cover the roots completely. Do not bury the stem. Gently water pot from above to settle soilless mix around roots. Only a small amount of water should be in the bottom of flat after transplanting is completed.
5. Cover plants with a plastic Humi-dome with one ventilation hole opened; place the flat in a growth chamber. Optionally, cover plants with plastic wrap with some small holes to allow for ventilation. Be sure to support the wrap so it does not touch the top of the rice plants.
6. Check the amount of water in the flat daily. Make sure the pH of the water used in the flat had been adjusted to between 5.2 to 5.8. If possible, adjust the lamps height to be within 12 inches of the top leaves of the rice plants and adjust as the plants grow.
7. Every couple of days allow for more ventilation by opening more holes or pulling up corners of the plastic wrap. The transplants should be weaned of their plastic humidity cover within one to two weeks.
8. Once the rice plants have their cover removed, fill flat $\frac{3}{4}$ full with water and maintain constant water availability in the flat until dry down stage.

⌘ **Flowering and Seed Harvest**

1. Flowering begins at approximately 6 weeks post transplant. The size of the pot allows for more plants per growth chamber however they will require support at flowering through seed harvest for top heaviness. Four plants tied together with string keeps them upright. Rice plants self pollinate in general. Continue to water as described above throughout the flowering process. At about 10 weeks after transplant, the seeds enter milk stage and continue through ripening. After kernels start to fill out, reduce the amount of water in tray to force the plants to finish setting seed. The plants should then be allowed to dry down and watering should be stopped.
2. When the plant is completely dry, (an indication that nutrition has stopped being sent to the seed from the rest of the plant), harvest the seed. This is usually at 17 weeks post transplant.
3. If seeds are contaminated by fungus, use 70% ethanol to clean the surface before storage or further use of the seed. Please see references for articles and websites on rice seed storage and ways to break dormancy.

Non-Transgenic Rice Plants

⌘ Non-transgenic rice from seed

1. Non-transgenic plants are needed to produce mature seed as starting material for transformation experiments.

⌘ Seed germination in the growth chamber

1. Seeds are imbibed for 24H in distilled water before planting into pots. Pots are prepared the same as for transgenic rice. See references for information on breaking seed dormancy if necessary.
2. Place the rice seed in the middle of the pot (about 1/4" below the surface of the soilless mix). Gently water pot from above to settle soilless mix around the seed. Only a small amount of water is needed in the flat at this time.
3. The seeds should germinate in approximately 8 days.
4. Check the amount of water in the flat daily. Make sure the pH of the water used in the flat has been adjusted to 5.2 to 5.8. If possible, adjust the lamps height to be within 12 inches of the top leaves of the rice plants and adjust as the plants grow.
5. Once the rice plants have reached 6" tall, fill the flat ¾ full with water and maintain constant water availability in the flat until the dry down stage.
6. Seed is harvested from the plants as indicated for transgenic rice plants.

Troubleshooting

⌘ Monitoring pests

1. The most common pest found in the growth chamber is fungus gnats. Placing yellow sticky cards near the soil surface will help to control them.

Vendors

Consumer Supply: 1509 E. Richland, Storm Lake, IA 50588, 1-800-274-6810

Hummert International: 4500 Earth City Expwy., Earth City, MO 63045, 1-800-325-3055

B&T Grower Supply, Inc 10462 Hwy 165, Forest Hill, LA 71430, 1-800-748-6487

References:

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Roberts EH. 1961. Dormancy in rice seed. I. The distribution of dormancy period. *Journal of Experimental Botany* 12:319-329.

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<http://www.knowledgebank.irri.org/GRCManuals/Appendix%203-4.pdf>

http://www.knowledgebank.irri.org/seedMgmt/Seed_Dormancy.htm