

## Ronald lab rice growing protocol (February, 2020)

### 1. Seed germination:

Before germination, remove the husk from each rice seed by grinding gently with a mortar and pestle. Treat dehusked seeds with 30% household bleach for 30 minutes. Rinse thoroughly at least 3 times with sterile water.

Place 20 disinfected seeds into a Solo cup (Dart Solo SD5, and SDL58) containing ½ MS media [for 1 liter, add 2.2g MS powder (Caisson, MSP09-50LT) and 20g sucrose, adjust pH to 5.8, add agar to 0.8%, autoclave 121°C for 15mins] and incubate at 28°C with 14 hours fluorescent light and 26°C at dark for 7 days.

### 2. Growing rice in tubs in the greenhouse

After 1 week in MS media, transplant rice seedlings either to soil (for greenhouse growth) (described below) or to a hydroponic system (for growth chamber) (see point 5).

Fill 5" square pots with sandy soil mix (80% sand, 20% Peat; Redi-Gro, Sacramento, CA; <http://www.redi-gro.com/>) to 1 inch from the top rim. Plant three seedlings per pot, which will be appropriate for most purposes. Place the pots in 24"x 36" black tubs and then fill tubs with fertilized water (below soil surface level; don't overfill). To make the fertilizer water, prepare concentrated stocks A and B (see below), mix 1:1 and dilute 200 times with reverse osmosis (RO) water before use. Use an electrical conductivity (EC) meter (HM Digital, AP-2) to measure the EC. The final EC reading should be 800 -1000 uS/cm.

200X Concentrated fertilizer water			
Tank A, 10 gallons		Tank B, 10 gallons	
Components	Mass (g)	Components	Mass (g)
Calcium Nitrate	1076	Monopotassium Phosphate	666
Potassium Nitrate	800	Ammonium Sulfate	1330
Iron EDDHA	189.2	Magnesium Sulfate	1313
Zinc EDTA	2.7	Potassium Sulfate	166
Copper EDTA	1.08		
Manganese EDTA	29.2		
Sodium Molybdate	0.19		

### 3. Water, fertilizer, temperature, humidity, and light:

Top off tubs in greenhouse with fertilizer water daily. After flowering, plants can be topped off with RO water.

The temperature in greenhouse ranges from 27-29°C during the day and 19.5-21.5°C at night. Relative humidity (RH) varies from 50-95%.

The Ronald lab main greenhouse uses 20 fixtures of 1000w High Pressure Sodium bulbs. From April to October 1st, natural light is sufficient for rice grown in Davis, CA.

The greenhouse staff turns off lights May 1st of each year and turns them on again October 1st each year. Beginning October 1st, the greenhouse staff supplements with artificial lights from 6 am until 10 PM when outside light falls below 400W/square meter.

#### 4. Pesticide spraying:

Monitor greenhouse for aphids, spider mites, thrips and other pests. A pesticide spray may be needed. Follow regulations for safe spraying.

#### 5. Hydroponic Growth in growth chambers

An alternative approach for growing rice plants is to use a hydroponic system in a growth chamber.

After 1 week in MS media (see above), transplant rice seedlings into 2" A-Ok Starter Plugs™ inside 10"x20" flats soaked with 1 X Hoagland Nutrient Solution. Move rice to a growth chamber (14 hours florescent light and 26°C; 10 hours dark and 24°C, 85% relative humidity). Change the nutrient solution every 2-3 days.

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<b>1000X-Hoagland Nutrient Solution</b>	
NH <sub>4</sub> NO <sub>3</sub>	80g
NaH <sub>2</sub> PO <sub>4</sub> ·2H <sub>2</sub> O	93g
K <sub>2</sub> SO <sub>4</sub>	52.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	44.2g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	122g
FeEDTA	19g
H <sub>2</sub> BO <sub>3</sub>	3.01g
MnSO <sub>4</sub> ·5H <sub>2</sub> O	2.17g
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.075g
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.2008g
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.024g
pH	5.6-5.8

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#### 6. Seed harvesting and storage:

When seeds turn brown (usually 1 month after flowering) collect seeds in envelopes.

After harvest, immediately dry seeds in a 50°C oven for 5-7 days and store seeds at 20°C for short-term storage or 14°C with 14% relative humidity for long-term storage.

#### 7. For seed increase and crosses:

For a good seed set of Kitaake rice, optimum planting time in Davis California is between January and August. Rice planted between late August and early December set fewer seeds because our greenhouses cannot maintain sufficiently high temperatures during the winter months for optimum seed set.

To obtain seeds that are true-to-type, you must bag the panicles right before flowering to avoid cross-pollination, or isolate the same rice genotype by time or space. The glassine bag can be removed after flowering in order to obtain healthy seeds (without discoloration). Seeds must be harvested when mature for maximum germination. If harvested too early or late, seeds will not germinate well.

For crosses, cut the top each spikelet in a panicle off at a slight angle using small sharp scissors, remove anthers from each spikelet using fine pointed-forceps or vacuum in the late afternoon (after 5:00pm) or in the early morning (before 9:00am) when most of the panicle emerges from the sheath. Chop off the top spikelets (which could be self-pollinated) and the basal spikelets (which are too young to cross) from the panicle. Bag the panicle of fully emasculated spikelets and the pollen parent panicle with a glassine bag. Gently tap (flick) the glassine bag every half hour during the flowering period (11:00am to 2:00pm). Pollination should be done for two to three consecutive days to obtain maximum hybrid seeds.