Table 1
Mikkelson's Nutrient Solution[a]

Constituent	Stock solution, g/L	Nutrient solution, mL stock solution/L
KNO_3	101.1	6.0
$Ca(NO_3)_2 \cdot 4H_2O$	236.2	4.0
$NH_4H_2PO_4$	115.1	2.0
$MgSO_4 \cdot 7H_2O$	246.5	1.0
Micronutrient stock solution	[b]	1.0
Iron cheleate 330	[c]	0.2 g

[a]Make a separate stock solution of micronutrients and each macronutrient. Dilute stock solutions to prepare final nutrient solution.

[b]For micronutrient stock solution, dissolve the following in 1 L water while mixing: 3.728 g KCl, 1.546 g H_3BO_3, 0.338 g $MnSO_4 \cdot 4H_2O$, 0.575 g $ZnSO_4$, 0.125 g $CuSO_4 \cdot 5H_2O$, 0.081 g H_2MoO_4 (85% MoO_3).

[c]Add iron cheleate as a solid to the final nutrient solution.

6. Reverse osmosis purified or deionized water.
7. 1-gal Plastic bags (e.g., Ziplock freezer bags, Dow, ~11 × 11 in., 2.7 mil).
8. Mikkelson's ***(17)*** nutrient solution (*see* **Table 1**).

3. Methods

3.1. Preparation of Embryos

1. Collect panicles 12 d after flowering and keep the cut ends submerged in water until use. Choose seeds that are almost fully formed and are close to the end of the milky stage. The embryos should be approx 2.0 mm long (1.7–2.2 mm) (*see* **Note 1**).
2. On a clean area, but not necessarily in a laminar flow hood, remove the spikelet from the panicle by cutting through the rachilla at the base of the grain. Be especially careful to cut high enough to facilitate removal of the lemma and palea, but low enough to avoid damaging the embryo.
3. Using a jewelers forceps, peal away the lemma and then the palea, being careful not to damage the pericarp (which should be light green) on the surface of the grain. This can be difficult, since the lemma and palea are interlocked and do not separate easily.
4. The dehulled seeds should be immediately submerged in sterile water and left there while subsequent seeds are being processed.
5. Transfer the dehulled seeds to the bleach solution (100 mL for each 100–150 seeds) in a flask and vacuum infiltrate (~1 mmHg) for 5–10 min, gently swirling each 1–2 min. Adequate vacuum is being applied if all the seeds initially float and the surface of the liquid is covered with fine bubbles. By the end of the vacuum treatment, many of the seeds should sink and the bubbles on the surface will be much larger.