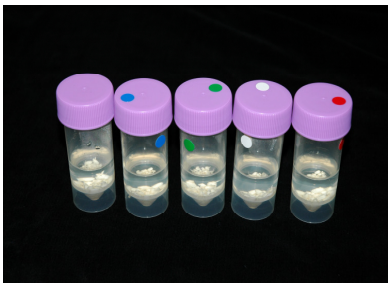
The Lab of Bioactive Molecule, Tohoku University

Hydroponic Culture Growing System for Rice and Arabidopsis

(Shoot Branching Inhibition Test for Strigolactones)

Rice hydroponic culture system

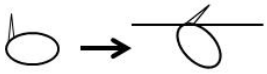


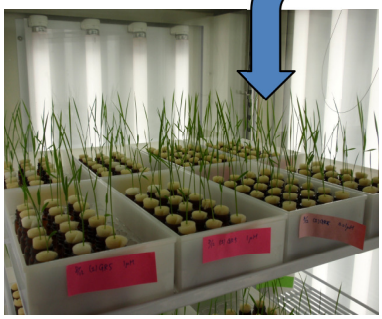
1) Remove the huskss of rice seeds using a special instrument shown in the following picture. After washing with 70%EtOH for 30 sec, sterilize the seeds in 2.5 % sodium hypochlorite solution for 15 min. Then rinse with sterile water 5 times, and incubate in sterile water at 28°C in the dark for 2 days.

(Fujiwara Scientific CO LTD)

2) Select the seeds whose bud length is about 1-2mm after germination. Transfer into hydroponic culture media solidified with 0.6% agar (pH 5.7) and culture at 25°C under fluorescence white light (150–200 mmolm22 s21) with a 16 h light/8 h dark photoperiod for 5 days. One glass tube holds 20 mL of medium, and about 5 seeds are grown in a tube.

Strigolactone





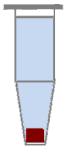
3) Transfer to a glass vial containing a sterilized hydroponic culture solution (13 ml), fix with a piece of sponge at the root-shoot junction to the top of the vial, and grow under the same condition for an additional 7 days. When the shoot branch inhibiting activity of strigolactones is tested, we can add the chemical in hydroponic culture. Fill the vial with the hydroponic solution (total 13 mL) every 3 days.

4) For large scale cultures, we can change the glassware from small bottles (Screw bottle No.4, Maruemu Corporation), to middle bottles (Screw bottle No. 7, Maruemu Corporation), 500mL chemical bottles, and wagner pots as shown in left picture. Using this method, we can finally harvest seeds.

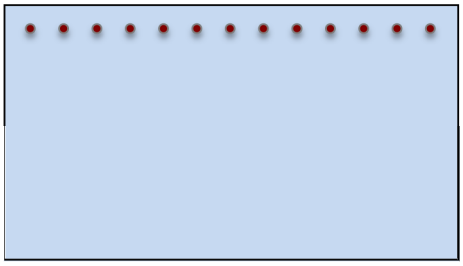
*For strigolactone activity test, the cheimcal is dissolved in acetone at 10,000 times concentration, and dilute with hydroponic culture media (final acetone concentration is 0.01%).

All glassware and hydroponic culture media were used after autoclave.

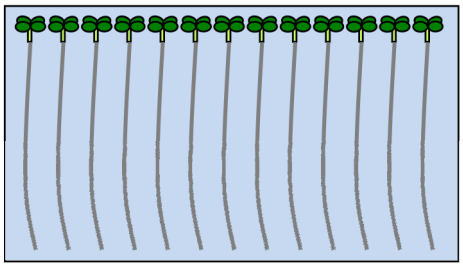
Arabidopsis hydroponic culture system



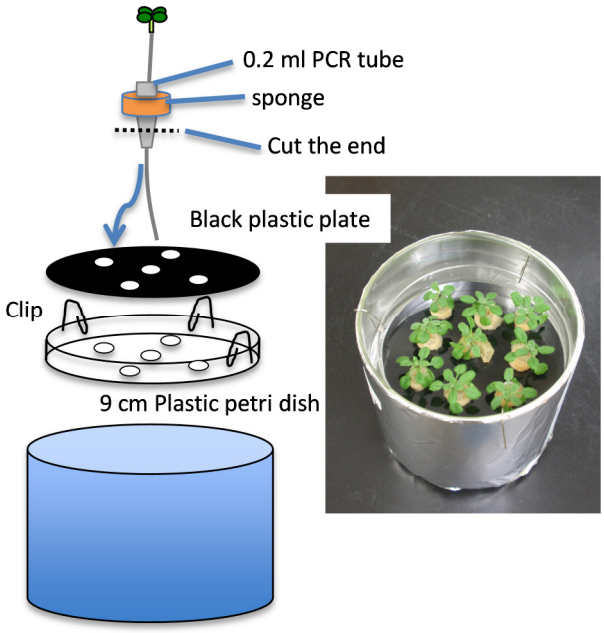
1) Sterilize seeds in 1% sodium hypochlorite solution for 5 min, rinse with sterile water 5 times, and stratify for 1-2 days at 4°C.



2) Place on the 1/2 Murashige and Skoog (MS) medium containing 1% sucrose and 1% agar (pH5.7). Each plate contains 40 mL of medium, and about 12 seeds are placed on one agar plate. The plate is sealed with surgical tape.



3) Grow at 22°C under fluorescence white light (60–70 μmol/m^{-2} s^{-1}) with a 16 h light/8 h dark photoperiod for 15 days.



4) Wrap the outside of glass pot with aluminum foil to block out light. Prepare the instruments as shown in left figure. Transfer plants one by one. Grow under the same condition for an additional 15 days. The pots should be covered with plastic wrap for about first 3 days after transfer. When the shoot branch inhibiting activity of strigolactones is tested, we can add the chemical in the hydroponic culture.

(Petri dish and black plastic plate = the holes are made using 1cm Cork borer)

*For strigolactone activity test, the cheimcal is dissolved in acetone at 10,000 times concentration, and dilute with hydroponic culture media (final acetone concentration is 0.01%).

Composition of hydroponic culture for rice (pH5.7) kamachi et al (1991) Plant Physiol. 96: 411-417

Stock No.	Atom	Chemical	Final conc	MW	1,000× stock
1	N	NH_4NO_3	1 mM	80.4	24 g/300ml
2	P	NaH_2PO_4 $2H_2O$	0.6 mM	156.0	28.08 g/300ml
3	K, S	K_2SO_4	0.3 mM	174.3	15.72 g/300ml
4	Ca	$CaCl_2$ $2H_2O$	0.2 mM	147.0	8.85 g/300ml
5	Mg	$MgCl_2$ $6H_2O$	0.4 mM	203.3	24.39 g/300ml
6	Fe	Fe-EDTA	45 μM	421.1	5.68 g/300ml

Stock No.	Atom	Chemical	Final conc	MW	10,000× stock
7	micro	H_3BO_3	50 μM	61.8	9.06 g/300ml
		$MnSO_4$ $5H_2O$	9 μM	241.0	6.54 g/300ml
		$CuSO_4$ $5H_2O$	0.3 μM	249.7	0.228 g/300ml
		$ZnSO_4$ $7H_2O$	0.7 μM	287.6	0.6 g/300ml
		Na_2MoO_4 $2H_2O$	0.1 μM	242.0	72 mg/300ml

Composition of hydroponic culture for Arabidopsis (pH5.7) Noren et al (2004) Physiol. Plant. 121:343-348

Stock No.	Atom	Chemical	Final conc	MW	1,000× stock
*1	N,K	KNO_3	5 mM	101.1	30.33g/300ml(200×stock)
2	P,K	KH_2PO_4	1 mM	136.1	40.83 g/300ml
*3	Mg,S	$MgSO_4$ $7H_2O$	1 mM	246.5	73.94 g/300ml
4	N,Ca	$Ca(NO_3)_2$ $4H_2O$	1.5 mM	236.2	70.85 g/300ml(667×stock)
5	N	NH_4Cl	1 mM	53.49	16.05 g/300ml
6	Fe	Fe-EDTA	50 μM	421.1	6.32 g/300ml

*Please notice that stock No.1 and 3 are not 1,000×stock

Stock No.	Atom	Chemical	Final conc	MW	1,000× stock
7	micro	H_3BO_3	46 μM	61.8	853 mg/300ml
		$MnSO_4$ $5H_2O$	10 μM	241.0	723 mg/300ml
		$CuSO_4$ $5H_2O$	0.32 μM	249.7	24 mg/300ml
		$ZnSO_4$ $7H_2O$	0.77 μM	287.6	66.4 mg/300ml
		Na_2MoO_4 $2H_2O$	0.58 μM	242.0	42.1 mg/300ml
		NH4VO3	0.25 μM	117.0	8.8 mg/300ml