

Hydroponic Culture of 'Micro-Tom' Tomato

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[Abstract] We use 'Micro-Tom' to study tomato fruit ripening and development mechanisms. 'Micro-Tom' is suitable for cultivation and experiments due to its small size of 10 to 20 cm in height and short life cycle of 3 months. There is also an abundance of publically available information on 'Micro-Tom' including EST, full-length cDNA clones and transcriptome data. 'Micro-Tom' plants are grown in hydroponic culture under fluorescents using Arabidopsis cultural shelves in greenhouses or plant rooms to get data with reproducibility for transcriptome and proteome analyses.

Materials and Reagents

1. 'Micro-Tom' (Prof. Ezura and Prof. Mizoguchi of Tsukuba University, *Solanum lycopersicum* cv. 'Micro-Tom', a model plant in the *Solanaceae* family). 'Micro-Tom' is a dwarf phenotype cultivar, originally first reported of in 1989, fixed by crossbreeding Florida Basket and Ohio 4013-3 (12th filial generation) (Martí *et al.*, 2006) (Figure 1)



Figure 1. *Solanum lycopersicum* cv. 'Micro-Tom'

2. Nutrient solution [Ootsuka house 5 (OAT house 5) OATagurio Co. Ltd. 2776 (<http://www.oat-agrio.co.jp/en/product/index.html>)
3. MS (Murashige and Skoog) mineral salts (Wako Pure Chemical Industries, Siyaku, catalog number: 392-00991)
4. Gamborg's B-5 vitamin (Sigma-Aldrich, catalog number: G1019)
5. Sucrose (Wako Pure Chemical Industries, Siyaku, catalog number: 193-00025)
6. Agar (Bacto agar) (BD, catalog number: 214010)
7. Nutrient solution (see Recipes)

8. MS (Murashige and Skoog) medium (Li, 2011) (see Recipes)

Equipment

1. Rock wool (a kind of mineral wool) (A0 25/40, 25 x 25 x 40 mm) (Nitoubou Co. Ltd. catalog number: 762-090)
2. Blower (Air pump, 100 V, working pressure 0.012 MPa) (YASUNAGA CORPORATION, model: LP-30A) (Figure 2 A)
3. Three-way tube (metal 6 mm caliber) (Nissei Sangyo Corporation, catalog number: 25424) (Figure 2B and 2C)
4. 6 mm silicon tube (bore diameter 4 mm) (Figure 2B)
5. Air stone (17 x 17 x 60 mm) (Figure 2D)

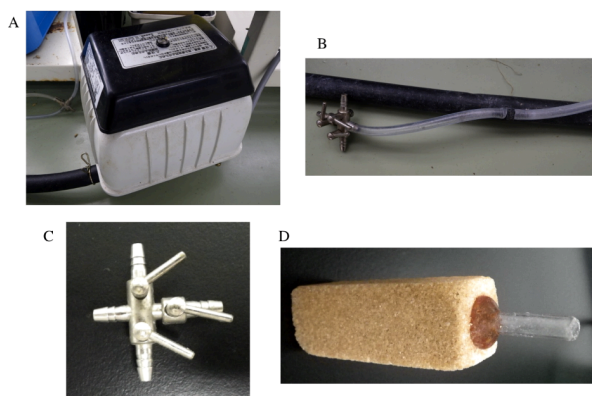


Figure 2. Parts of hydroponic culture system. A. Blower, B. 6 mm silicon tube, C. Three-way tube, D. Air stone (17 x 17 x 60 mm).

6. Shallow container (Size accordingly to match the scale of your project. In this case we are using a container 10 cm deep, a good example is Tupperware. We should use light-proof type of containers, because they protect to occur algae.)
7. Perforated polystyrene foam board (2.5 cm thickness, 3.5 cm hole diameter, distance between holes is approximately 12 cm) (Figure 3)

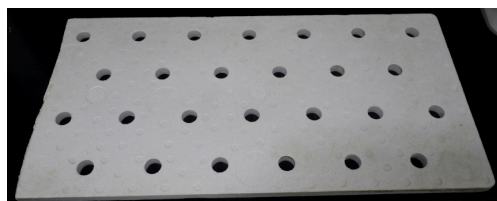


Figure 3. Perforated Polystyrene Foam Board (2.5 cm thickness, 3.5 cm hole diameter)

8. Plant boxes (DUCHEFA Biochemie, Steri Vent Low container, catalog number: S1682.0048 and Standard Closure lids, catalog number: S1681.0032)
9. Sponge (household daily-use type)

Procedure

A. In greenhouse (Figure 4A-B)

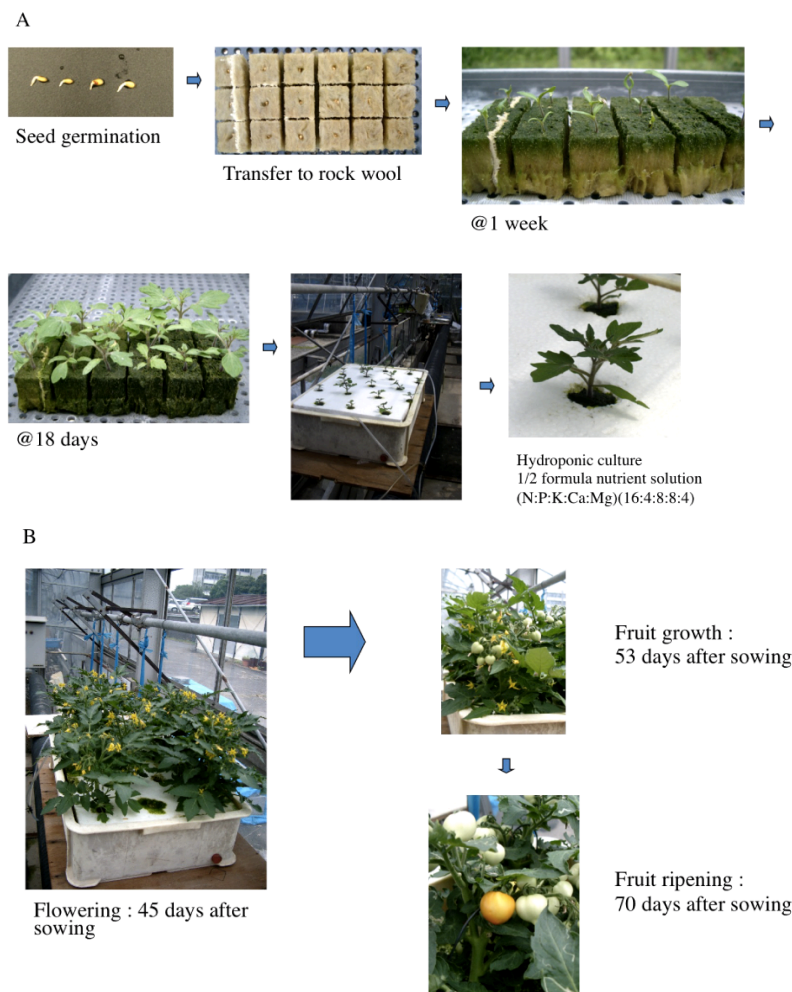


Figure 4. Growing 'Micro-Tom' plants using hydroponic culture. A. From seed germination to moving seedlings into hydroponic culture; B. From flowering to ripening.

1. Soak seeds in distilled water overnight in a shallow container to germinate (Figure 4A).
2. Transfer germinated seeds to rock wool (Figure 4A).
3. Grow 'Micro-Tom' seedlings for approximately 2 weeks (Figure 4A).
4. Transfer 'Micro-Tom' seedlings by removing them with rock wool still attached and placing them into the holes in the polystyrene foam board (Figure 4A).

5. Add 1/2 formula nutrient solution (diluted to half concentration with water) into the container (filling it to about 2/3), and use the blower to send air bubbles into the root zone of the tomato plants (Figure 5). Flowers bloom at approximately 45 days after sowing, and fruits begin to grow larger at approximately 53 days after sowing. Ripening begins at approximately 70 days after sowing (Figure 4B).

Note: By using the three-way tube, you can send air bubbles from 1 blower into multiple containers for increased efficiency. It is worth mentioning that improper air bubble supply to root zones will usually result in poor plant growth and ultimately rot symptoms. Hydroponic culture does not rot as it is constantly receiving air supply, but needs to be changed about once every 7 days (with a new nutrient solution). Polystyrene foam board that is smaller than the container may leave hydroponic culture exposed to light, which can lead to algae growth. As plant growth continues hydroponic culture will slowly be consumed, which can leave a thin air space between the polystyrene foam board and the culture, leading to problems with growth. The foam board needs to be the same size and fit well into the container, preferably with sponge/rock wool being soaked in culture.

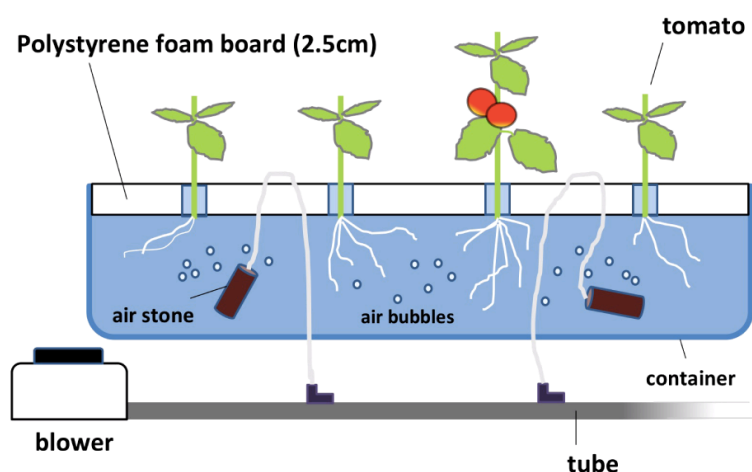


Figure 5. Hydroponic culture system



Figure 6. 'Micro-tom' plants are grown in hydroponic culture (16 h light/8 h dark at 26 °C)

B. In a plant room

In addition to growth in greenhouse conditions, we also developed a protocol for hydroponic growth in a plant room (6 h light/8 h dark at 26 °C).

1. Sow sterilized seeds and grow 'Micro-Tom' plants in plant boxes (MS medium containing 2% sucrose) for 3 weeks (Figure 7) (Li, 2011).
2. Extract 3-week-plants from the boxes so as not to hurt their roots (Figure 7).
3. Gently wind sponge around the stems of extracted seedlings (Figure 7).



3-week-plants

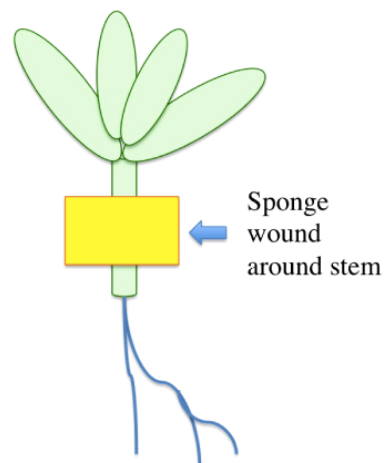


Figure 7. 3-week-plants grown in MS medium are ready to be transferred into hydroponic culture. Sponges are wound stems of seedlings and placed into holes of polystyrene foam board.

4. Transfer seedlings with sponge attached into the holes of polystyrene foam board (Figure 5).
5. Send air bubbles to hydroponic culture using a blower in the same protocol as above (in greenhouse). Roots grow within the hydroponic culture after 2 to 3 weeks (Figure 8).



Figure 8. Roots of 'Micro-Tom' in hydroponic culture after 2~3 weeks

Note: Plant density per container is an important consideration, as higher density can lead to increased mildew and other problems. For a container of about 30 x 50 cm size, consider growing 15 plants or less to avoid diseases and other issues (Figures 5-6).

Recipes

1. Nutrient solution
 - KNO₃ 808 mg/L
 - MgSO₄/7H₂O 492 mg/L
 - Ca(NO₃)/4H₂O 944 mg/L
 - NH₄H₂PO₄ 152 mg/L
 - Microelement 50 mg/L
 - Mn 0.77 ppm
 - B 0.32 ppm
 - Fe 2.85 ppm
 - Cu 0.020 ppm
 - Zn 0.040 ppm
 - Mo 0.020 ppm
2. Murashige and Skoog (MS) medium
 - a. Murashige and Skoog (MS) mineral salts
 - b. Gamborg's B-5 vitamin

- c. 2% sucrose
- d. 0.8% agar (Bacto agar)
- e. Adjust pH to 5.7 with KOH

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