Jun 23, 2025

Hydroponic Screening Protocol for Salinity Tolerance in Galapagos Tomato Seedlings

DOI
https://dx.doi.org/10.17504/protocols.io.eq2lyqoxmvx9/v1

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Protocol Citation: Yveline Pailles 2025. Hydroponic Screening Protocol for Salinity Tolerance in Galapagos Tomato Seedlings. **protocols.io** https://dx.doi.org/10.17504/protocols.io.eq2lyqoxmvx9/v1

Protocol status: Working
We use this protocol and it's working

Created: June 15, 2025

Last Modified: June 23, 2025

Protocol Integer ID: 220271

Keywords: hydroponic screening protocol for salinity tolerance, salinity tolerance, galapagos tomato seedlings this protocol, galapagos tomato seedling, growth responses under salt stress, hydroponic screening protocol, galapagos tomato, salt stress, salt treatment, hydroponic cultivation, solanum cheesmaniae, sodium, hydroponic system, standardized procedures for seed germination, supported hydroponic system, seed germination, genotype

Abstract

This protocol describes a method to evaluate salinity tolerance in *Solanum cheesmaniae* (Galapagos tomato) using a supported hydroponic system. It enables screening of a large number of genotypes for variation in sodium (Na^+) exclusion capacity and growth responses under salt stress. The method includes standardized procedures for seed germination, hydroponic cultivation, salt treatment, and phenotypic evaluation.

Materials

A. Seed Germination

- ¼ MS medium (0.8% agar, no sucrose) in 100×100×15 mm square Petri dishes
- 1 mL sterile pipette tips
- 2.7% Sodium hypochlorite solution
- Tap water
- Seed strainer
- Small Petri dishes
- Seeds (40 per genotype)
- Sterile forceps and spatulas
- Parafilm
- Permanent marker
- Plastic beads
- 3" square pots
- Cheesecloth
- Bleach
- Rectangular trays (with and without divisions)
- Tray covers
- Waterproof labels

B. Hydroponic Growth

- General Hydroponics Flora Series nutrient mix
- Tap water
- Multi-ion electrode (e.g., CleanGrow Europe)
- pH meter
- 2 L beakers and volumetric flasks
- Spatulas
- Timers
- Econo-Tray Hydroponic System (American Hydroponics)
- Custom metal support structure
- Submersible aquarium pumps (ViaAqua Powerhead 360)
- Voltage transformer (120V/60Hz)

C. Salt Treatment Solutions

- 4 M NaCl stock solution (1,168.8 g NaCl in 5 L)
- 2 M $CaCl_2$ stock solution (588.08 g $CaCl_2$ in 2 L)
- Tap water
- Stirring rod

D. Phenotyping and Data Collection

- Paper towels
- Empty container (for bead disposal)
- Ruler
- Beakers with 10 mM $MgSO_4$
- Sterile scissors and forceps
- 70% ethanol spray
- Labeled envelopes and tubes
- Photosimile lightbox

- Digital camera and EOS Utility software
- Scanner
- WinFOLIA software with USB key
- High-precision scale
- Data recording laptop with Excel spreadsheet

Safety warnings

Important note: Avoid using green or yellow labels, pots, markers, or backgrounds to prevent interference with green pixel-based image analysis.

Seed Germination

1 Select 8 genotypes; sow 40 seeds per accession to allow for 6 replicates × 3 treatments × 2 seeds per pot, plus 2 extra pots per condition.

2 Sterilize trays, covers, pots, and plastic beads in bleach solution; rinse thoroughly.

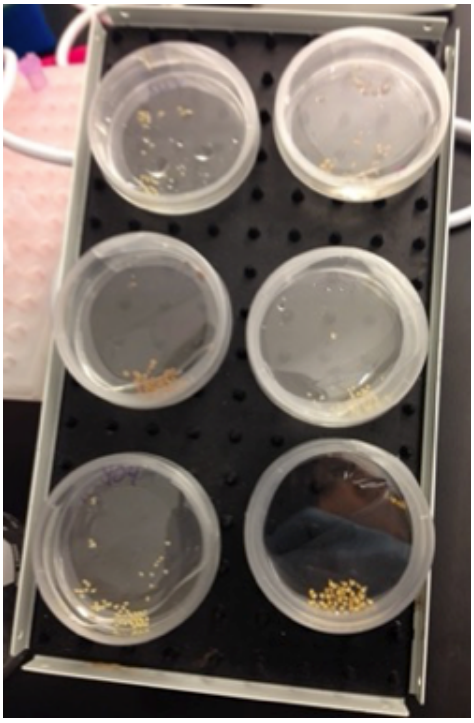
3 Cut cheesecloth squares to line the bottom of each pot to retain beads.

4 Fill each pot with plastic beads and position in trays.

5 Add tap water to trays until the water reaches just below the bead surface.

6 Using a sterile 1 mL pipette tip, cut agar cylinders from MS plates and embed 3 per pot into the beads.

7 Surface sterilize seeds (20 min in 2.7% NaOCl), rinse well, and place one seed per agar plug.



8 Label trays and seal with tape. Place on nursery shelves and monitor daily.

9 Transfer trays to well-lit conditions once germination begins. Record germination dates.

10 Once cotyledons are fully expanded, transfer seedlings to the hydroponic system to avoid stress.

Hydroponic System Setup

11 Mark desired water level in each hydroponic tank (e.g., 22 cm for ~100 L).

12 Determine number of EconoTray units required (each holds 118 pots).

13 Fill tanks with pre-filtered tap water and assess quality using a multi-ion electrode.

14 Add nutrients and salt solutions per treatment group. See Table 1 for $CaCl_2$ volumes to maintain Ca^{2+} activity at 0.4.

Table 1. $CaCl_2$ Supplementation for Target NaCl Concentrations

	Final [NaCl] (mM)	Final [$CaCl_2$] (mM)	Ca^{2+} Activity (α)	Volume NaCl (4M) per L	Volume $CaCl_2$ (2M) per L
	75	1.0	0.40	18.75 mL	0.50 mL
	150	1.2	0.40	37.50 mL	0.60 mL
	225	1.3	0.39	56.25 mL	0.65 mL
	300	1.4	0.40	75.00 mL	0.70 mL
	375	1.4	0.39	93.75 mL	0.70 mL
	450	1.5	0.41	112.5 mL	0.75 mL

Note

Nutrient Solution: Use 33 mL each of FloraGro, FloraBloom, and FloraMicro per 100 L tap water.

15 Program pumps to cycle every 15 minutes (15 min on/off).

16 Monitor pH (~5.8), nutrient levels, and salt concentration weekly; refresh solution every 1–2 weeks.

17 Track emergence of the 4th leaf for initiating salt treatments.

Salt Stress Application

18 Once the 4th leaf emerges, transfer seedlings from control tanks to salt treatment tanks.

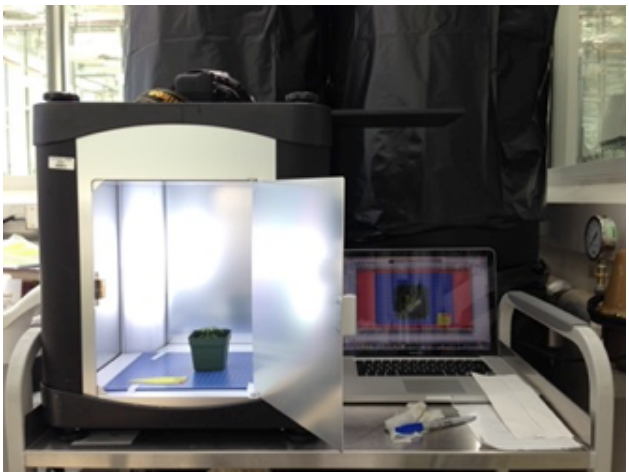
19 Gradually increase salinity: 100 mM NaCl for 12 hours → 200 mM NaCl full treatment.

Phenotyping and Biomass HarvestUntitled section

20 Power on camera, Photosimile lights, and EOS Utility software.

21 Remove pot label, place plant on Photosimile mark, and straighten it in the live view.

22 Capture image and replace label.

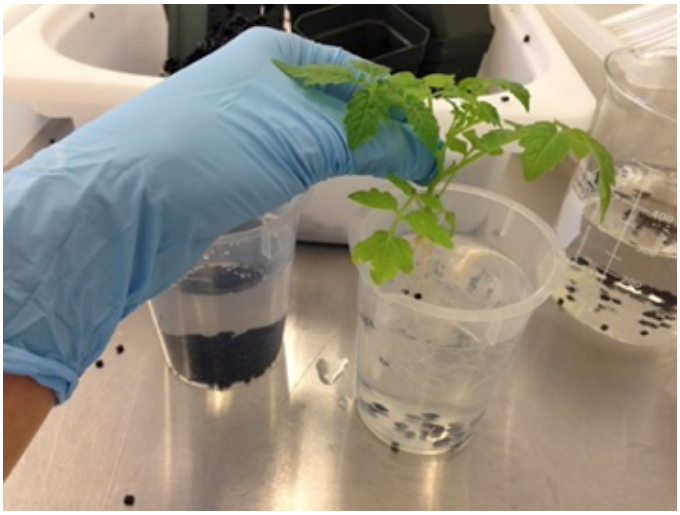


23 Prepare pre-labeled envelopes and tubes.

24 Record total number of true leaves (exclude cotyledons).

25 Remove plant by inverting pot and collecting beads. Avoid damaging roots.

26 Wash roots twice in 10 mM $MgSO_4$; pat dry with towel.



27 Measure root length; weigh fresh root biomass.

28 Cut root and store in envelope/tube.

29 Measure shoot length and fresh shoot weight.

30 Cut and scan the 4th leaf using WinFOLIA; save images with proper ID.

31 Separate leaf blades from petiole. Weigh blades; store tissues separately.

32 Place all samples in a 60°C drying oven for 48 hours.

33 Record dry weights of all tissue components.

Protocol references

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