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Hydroponic cultivation of *Salicornia*

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We use this protocol and it's working

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Abstract

This method describes how to cultivate *Salicornia species* in a hydroponic ebb and flow system (flood-drain) using an inert substrate and liquid nutrient fertilizers. This method enables application of very precise nutrient/ionic/osmotic stress and for ease of harvesting plant tissue (shoots and roots) for downstream physiological or molecular assays.

Attachments

PDF

WATER BOX FRAME_hydr...

515KB

Guidelines

Always ensure electrical equipment near hydroponic systems and wet areas is properly grounded, waterproof, and installed with ground-fault circuit interrupters (GFCIs) to prevent electrical shock hazards.

Materials

Seed germination:
- Murashige and Skoog Basal Medium - Sigma-Aldrich
-Sucrose - Sigma-Aldrich
-NaCl
-Preservative for Plant Tissue Culture Media (PPM) - Plant Cell Technology
-Agar - Sigma-Aldrich
-Ethanol Absolute (>99.5%) EMPARTA
-Bleach (household grade)
-TWEEN 20 - TermoFisher
-Gibberellic acid - Sigma-Aldrich
- 1ml pipette tips (sterile)
- 70% (v/v) sodium hypochlorite (bleach)
- 70% (v/v) ethanol
- Tap water
- Strainer
- Petri dishes (100mm x 100mm x 15mm)
- *Salicornia species* seeds
- Sterile forceps and spatulas
- Parafilm
- Plastic beads (high density polyethylene supplied by companies who produce plastic buckets or crates; ensure that they do not float)
- Squared black pots (3″)
- Cheesecloth
- Commercial Bleach
- Rectangular trays and trays with divisions
- Tray covers
- Pot tags with QR code sticker labels
- Growth chamber

Hydroponics dimensions:
-920mm x 920mm x 970mm (see manufacturing instructions for the frame attached)

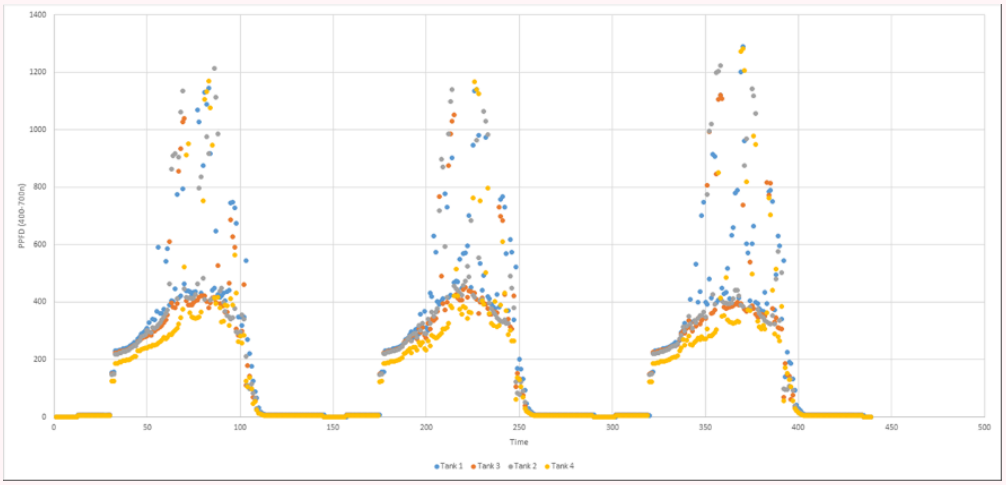
Growing phase:
- FloraGrow nutrient stock
- FloraMicro nutrient stock
- Tap water
- EC meter
- pH meter
- 2 L plastic beaker
- 2 L volumetric flask
- Spatulas

- Timers
- Econo-Tray Hydroponic systems: 87 × 87 cm (American Hydroponics)
- Metal support structure for Hydroponic systems: 92 × 92 × 97 cm (custom made)
- Submersible aquarium pumps (ViaAqua Powerhead Pump 360)
- Voltage transformer for aquarium pumps (120V/60Hz)

Salt treatments:
- NaCl
- 2M CaCl2 stock solution
- Tap water
- EC meter
- pH meter
- Long-handled stirring rod

Safety warnings

The light conditions should be homogeneous across all hydroponic systems. Consider analyzing the light intensity spatially across the greenhouse to ensure uniformity across tank treatments. This is an example.

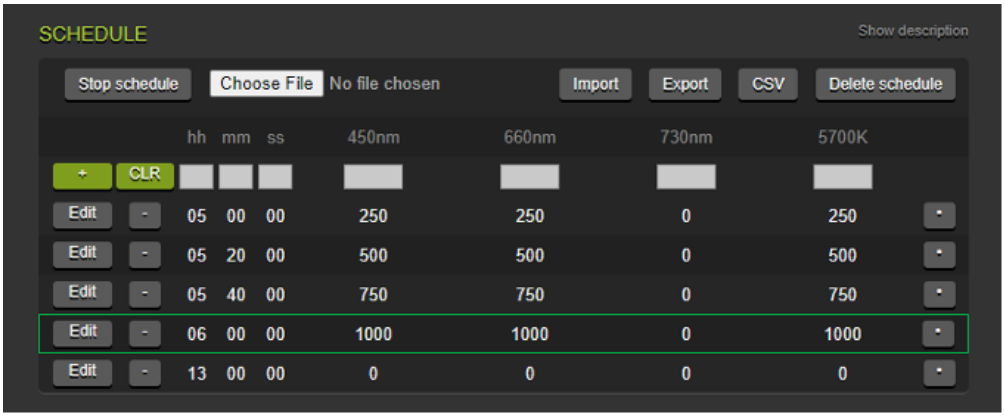


Comparable light intensity was achieved in three positions. Tank 4 received the least light and was therefore not used.

Before start

Set up the growing and light conditions of the room where the experiment will run.

Growth room conditions
Day/night 28/24°C
Using LED supplemental lights
The example below shows a ramp-up scenario.



Light wavelength and distribution settings in the greenhouse.

Seed Germination

2w

1 Select required number of genotypes.
Work out the number of repetitions (take more seeds than you need) (For example, 6 replicates * 2 treatments * 2 seeds per pot) (note. later thinned to one seedling per pot).

2 Sterilize the seeds and place them on the germination agar medium following the steps described in protocol '*Salicornia Germination on Agar Plate*'.

Citation

Lina Maria Caceres Leal. Salicornia Germination on Agar Plate. protocols.io.

https://protocols.io/view/salicornia-germination-on-agar-plate-cgfuttnw LINK

3 After two weeks the seedlings are ready to move to establishment stage in the hydroponics system. (step 8).

Hydroponic System Set up and Cleaning

1d

4 Before starting:
Measure ideal water level (with a metered hose or a 20 L container) and put a mark inside the tank to fill to that level. (100 liters final volume).

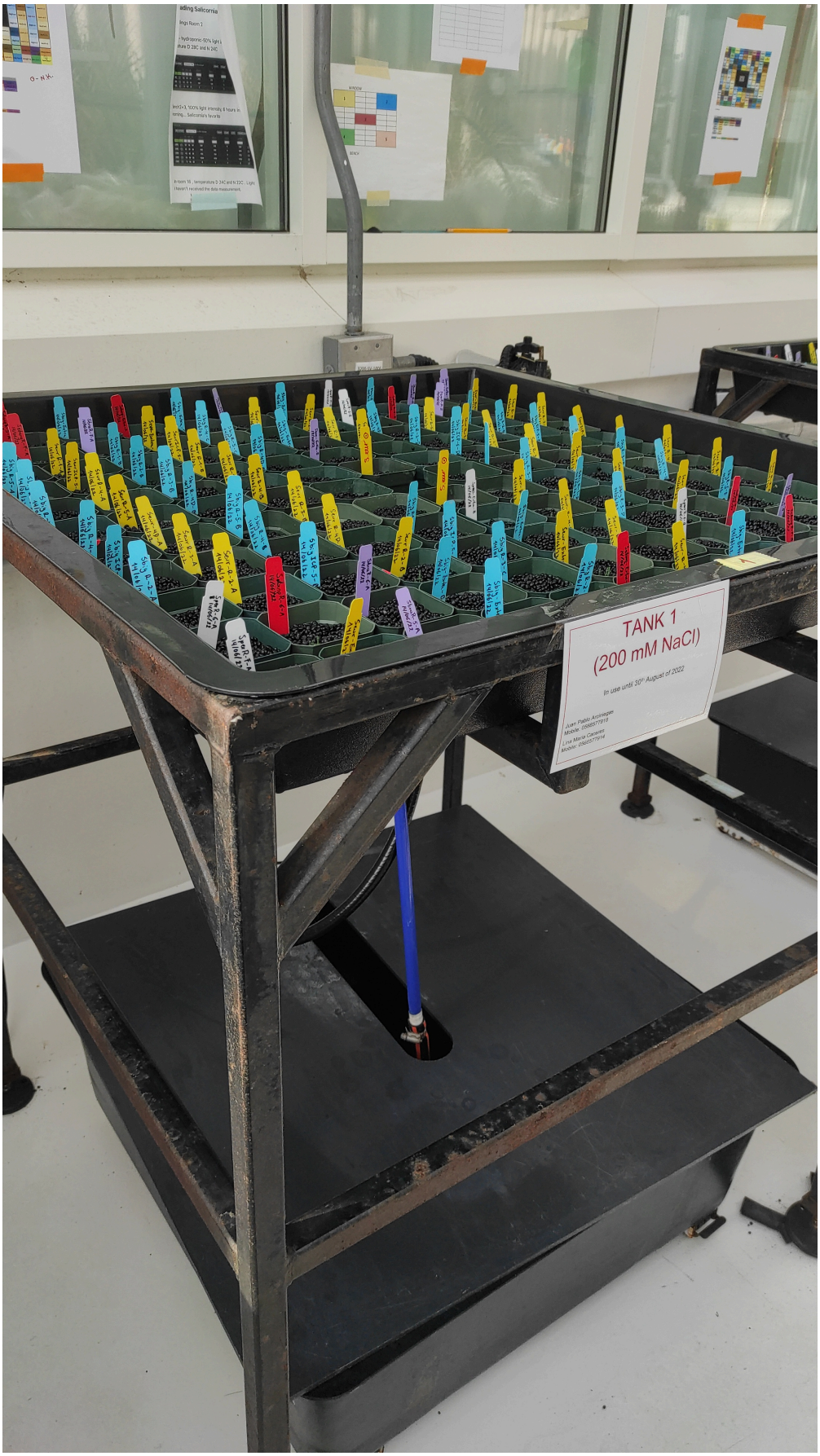
5 To prepare the pots:

- Sterilize the pots and plastic beads using 20%(v/v) commercial bleach solution, then rinse thoroughly with clean water.
- Cover the bottom of each pot with a square of cheesecloth to prevent the plastic beads from falling through the drainage holes.
- Fill the pots with plastic beads up to the filling line.
- Place the pots in the trays. Each tray can hold up to 118 small square pots (3″).

Location of the pots in the hydroponics.

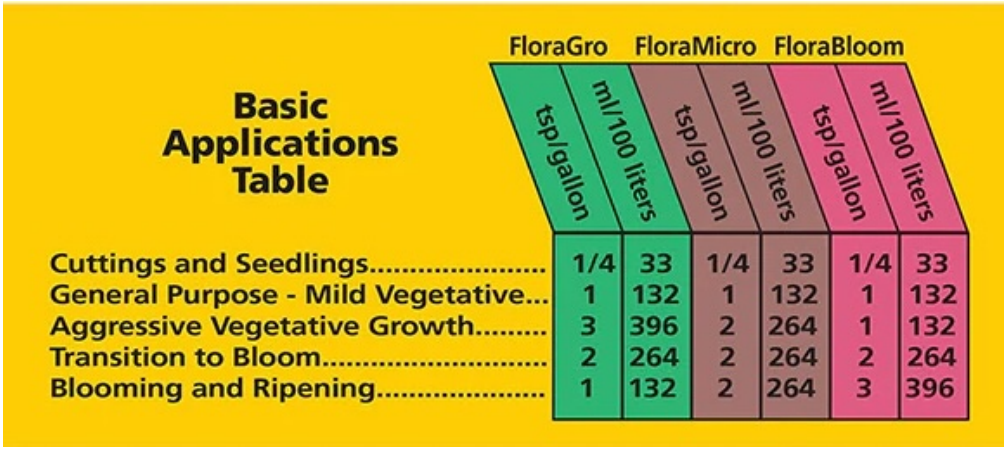
6 Prepare the ebb and flow hydroponic system and ensure the pumps are functioning correctly. Set the timer to run 15 minutes on and 15 minutes off. Run the cycle for one day before moving the seedlings to confirm that everything is working properly.

The nutrient solution reaches the pots with the seedlings during the 15 minute 'on' cycle. When the timer ends, the pump stops and the solution drains.


TANK 1
(200 mM NaCl)
In use until 30th August of 2022

Hydroponic tank assembled.

7 The nutrient solution use for the hydroponics is "General Purpose – Mild Vegetative" from Flora Series- General hydroponics.



FloraSeries® Feedcharts

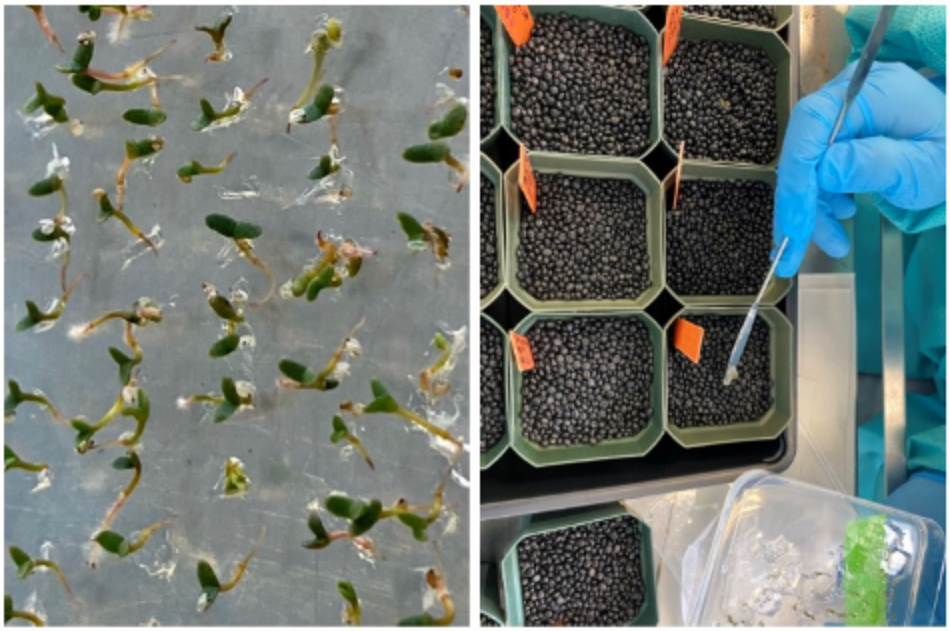
Seedling establishment

2w

8 Prepare the nutrient solution and fill the thank. Add the nutrient solution stock, follow the instructions for General Purpose – Mild Vegetative from Flora Series- General hydroponics, (see step 7).

9 Once all the pots are positioned on the trays and labeled, take one agar plate with the seedlings and use a spatula to scoop out a portion of agar with one seedling. Bury it in the plastic beads until it is completely covered, with the surface still visible. Place two or three seedlings per pot.

2w



Salicornia spp. seedlings after 10 days growth. Move from agar plates to hydroponics system.

10 Cover the trays with cling wrap for two days to maintain humidity and prevent contamination. The seedlings reach a length of 1-2 cm, they are strong enough to begin the treatment.

Salt Treatment

6w

11 Add the nutrient solution stock ('General Purpose – Mild Vegetative' from Flora Series- General hydroponics, see step 7.), plus NaCl stock to reach the desired final concentrations.

It is important to add NaCl incrementally during the initial salinity treatment. For example, cultivating *Salicornia spp.* with a final target salinity of 200 mM NaCl, apply 75 mM on Day 1 and 125 mM on Day 2.

Note: More salt sensitive plant species may require smaller daily increments of the salt treatment (e.g., increasing in 50 mM NaCl increments per day). Increasing salt incrementally allows for proper osmotic adjustment.

Maintain an activity coefficient of 0.4 by supplementing the NaCl treatment with $CaCl_2$ according to the table below.

	A	B	C	D	E
	Final [NaCl] (mM)	Final [CaCl2] (mM)	Ca2+	Volume of 4M NaCl per litre (ml)	Volume of 2M CaCl2 per litre (ml)
	75	1	0.4	18.75	0.5
	150	1.2	0.4	37.5	0.6
	225	1.3	0.39	56.25	0.65
	300	1.4	0.4	75	0.7
	375	1.4	0.39	93.75	0.7
	450	1.5	0.41	112.5	0.75

$CaCl_2$ supplement for a NaCl solution, to keep a Ca^{2+} activity coefficient of 0.4

12 Monitor the pH and Electrical Conductivity (EC) twice per week until plants are 5 weeks old and then every day thereafter. Optimize the pH between 5.8 and 6.2 and salinity at EC target measured after complete dissolution of NaCl (200 mM).

- Replace the nutrient solution when your pH is out of the range of 5.8-6.2
- Take 50 ml samples of your nutrient solution per week and store samples with date and tank number at 4°C. Samples are collected at critical stages of your experiment for elemental analysis to record nutrient depletion rates.

13 Change nutrient solution (+NaCl and +$CaCl_2$ to treatment tank) every week or when pH or EC is out of expected range. Mix the solution thoroughly using the long-handled stirring rod.

14 Assess plant growth responses to salt or collect tissue for further analysis following guidelines from Negrao et al. (2017).

Citation

S. Negrão, S. M. Schmöckel, M. Tester (2017)
. Evaluating physiological responses of plants to salinity stress.
Annals of Botany, Volume 119.

https://doi.org/10.1093/aob/mcw191 LINK

Protocol references

S. Negrão, S. M. Schmöckel, M. Tester, Evaluating physiological responses of plants to salinity stress, *Annals of Botany*, Volume 119, Issue 1, January 2017, Pages 1–11, https://doi.org/10.1093/aob/mcw191