

May 25, 2025

Salicornia Germination on Agar Plate

DOI

<https://dx.doi.org/10.17504/protocols.io.kqdg394xpg25/v1>

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Protocol Citation: Lina Maria Caceres Leal 2025. Salicornia Germination on Agar Plate. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.kqdg394xpg25/v1>

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Protocol status: Working

We use this protocol and it's working

Created: September 12, 2022

Last Modified: May 25, 2025

Protocol Integer ID: 69844

Keywords: Salicornia, in vitro, seed germination, halophytes, salicornia germination on agar plate, salicornia germination, seeds of various salicornia species, germination, various salicornia species, seed, collected seed, sterile condition, sterile conditions for different purpose, agar plate, free plant, tissue culture

Abstract

This method describes the steps to germinate seeds of various *Salicornia* species under sterile conditions for different purposes; germination of wild-collected seeds, tissue culture and where disease/pest-free plants are required for hydronic experiments.

Guidelines

It is advisable to perform all the steps under sterile conditions.

Materials

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 - ThermoFisher

Gibberellic acid - Sigma-Aldrich



Media preparation

4h

1 *Salicornia* medium germination.

One liter of medium is enough for approximately 20 Petri dishes (100mm x 100mm x 15mm).

	A	B	C	D
Component		500 mL	1000 mL	2000 mL
Murashige and Skoog Basal Medium (MS with vitamins) M0404-10L (Sigma Aldrich)		2.2 g	4.4 g	8.8 g
Sucrose 3% (w/v)		15 g	30 g	60 g
100 mM NaCl*		2.922 g	5.844 g	11.688 g
Preservative for Plant Tissue Culture Media (PPM)**		0.5 mL	1 mL	2 mL
Adjust pH 5.8				
Agar 0.5% (w/v)		2.5 g	5 g	10 g

*NaCl is optional and dependent on the aim of the project.

**PPM is optional; it is used to prevent microbial contamination and recommended for wild-collected seeds.

2 Store the Petri dishes at room temperature for short-term storage or at 4 °C for long-term storage.

Sterilization of seeds

2w 0d 0h 16m

3 Place the seeds in a 2 mL Eppendorf tube. Add 1 ml 70% (v/v) Ethanol and mix for 1 minute.

1m

Remove the ethanol by pipetting carefully to avoid discarding the seeds.

4 Prepare a solution containing 20% (v/v) bleach and 0.2% (v/v) TWEEN 20. Add 1 mL of this solution to the seeds and incubate for 10 minutes, mixing occasionally. After incubation, remove the solution and wash the seeds four times with Milli-Q water. Carefully remove the supernatant by pipetting to avoid discarding the seeds.

15m

- 5 Place the sterilized seeds on agar plates. Use forceps to place them one by one, or use a 1000 μL pipette to distribute them onto the agar. Cover the Petri dishes with aluminum foil during the first 24 hours.

If germination is low, add one drop (50 μL) of 500 μM Gibberellic Acid directly onto the seeds and keep them in the dark for 24 hours to enhance germination.

500 μM Gibberellic Acid = 17 mg/100 mL water

Keep the plates in a controlled-environment growth chamber for approximately two weeks. Grow the seeds under a 12-hour light / 12-hour dark photoperiod at 28 $^{\circ}\text{C}$.



Salicornia bigelovii seeds after two weeks on petri dish.



Transplanting of seedlings

- 6 Once the seedlings reach a height of 1 cm on the agar plates, they are ready to be transferred to soil or hydroponics.