



Jul 04, 2025

Version 2

## Spinach Germination Protocol V.2

DOI

<https://dx.doi.org/10.17504/protocols.io.5qpvodxebg4o/v2>

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**Protocol Citation:** Alexander Hernandez, Michael Dzakovich, Suraj Chandramouli 2025. Spinach Germination Protocol. protocols.io <https://dx.doi.org/10.17504/protocols.io.5qpvodxebg4o/v2> Version created by **Michael Dzakovich**

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** July 04, 2025



**Last Modified:** July 04, 2025

**Protocol Integer ID:** 221753

**Keywords:** germination, plant biology, spinach, hydroponics, research, horticulture, botany, genetics, plant breeding, spinach germination protocol, spinach seed, germinated seedling, germination inhibitor, spinach, germination protocol, germination inhibitors present on the surface, seedlings into hydroponics system, seed, storage condition of seed, cultivated variety, pollinated landrace, crop

**Funders Acknowledgements:**

USDA-ARS CRIS Funds

Grant ID: 3092-10700-066-001S

## Abstract

This protocol was developed for spinach (*Spinacia oleracea* L.) to overcome difficulties associated with germinating this crop. The following protocol helps degrade germination inhibitors present on the surface of spinach seeds (e.g. tannins) and helps sterilize their outer surface. Additional steps for moving germinated seedlings into hydroponics systems is also included. Depending on the age and storage condition of seeds, we find >90% germination success with most cultivated varieties and similar success with open-pollinated landraces.

## Materials

### Bleach/Detergent Wash:

- Spinach seeds (~2 times as many as the final desired quantity of plants)
- 18.2 MΩ water
- Tween 20 detergent
- Household bleach
- Platform shaker
- Transfer pipette
- 50 mL centrifuge tubes
- 50 mL tube rack
- 200 μL pipette
- 250 mL beaker
- 100 mL graduated cylinder

### Osmopriming:

- Sterile petri dishes and petri dish lids (100 mm W x 20 mm H)
- Whatman No.1 Filter paper (100 mm W)
- 18.2 MΩ water
- Household hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)
- Washed spinach seeds
- 100 mL beaker
- 100 mL graduated cylinder
- Tape

### Transplanting to slant boards:

- Slant boards
- Slant board holders (<https://3d.nih.gov/doi/021396/1>)
- Tub (20 inch L x 15 inch W x 7 inch H)
- 70% Ethanol Spray bottle
- Tweezers
- Petri dishes of osmoprimed seeds
- Calcium nitrate-tetrahydrate
- DI water
- DI water squirt bottle
- Seedburo 10"x14" Germination Blotters (product number: SKU 41)
- KimWipes
- Paper towels
- Analytical balance
- Weigh boats
- 2000 mL volumetric flask

### Transplanting into neoprene collars:



- 2" neoprene cloning collars
- Polyester felt cut into 8 cm long x 4 cm wide wicks
- Plastic container (500 – 1000 mL)
- Tweezers
- DI water
- DI water squirt bottle



## Bleach/Detergent Wash Protocol (based on Lindsey et al. 2017)

- 1 Add 100 mL of bleach to a 250 mL beaker.
- 2 Add 100 mL of 18.2 MΩ water to the same beaker.
- 3 Add 50 μL of Tween 20 to bleach solution with a 200 μL pipette.
  - 3.1 Thoroughly mix the solution.
- 4 Add spinach seeds to separate 50 mL tubes.
  - 4.1 Make sure to label tubes to avoid mixing seed varieties.
  - 4.2 Use ~2x as many spinach seeds as the desired quantity of plants (ex: add 20-25 spinach seeds if you want 10 final plants of that variety).
- 5 Add 40 mL of the bleach/detergent solution to each 50 mL tube of seeds. Close the tubes.
- 6 Place tubes on rack and then place rack on platform shaker.
  - 6.1 Allow platform shaker to agitate the seeds and solution for 15 minutes.
  - 6.2 Use a speed setting to ensure seeds are rapidly moving within the liquid.
- 7 Discard majority of solution and use transfer pipette to aspirate the remaining liquid.



- 7.1 If working in a laminar flow hood (recommended for hydroponics applications) this step and the following rinse steps will be significantly sped up if using a vacuum aspiration system.
- 7.2 Do not aspirate or remove seeds.
- 8 Drill 4 small holes into tube cap prior to screwing cap onto tube.
- 8.1 This step can be done in advance if extra 50 mL caps are available.
- 9 Fill the tubes with 40 mL of 18.2 MΩ water.
- 10 Close tubes and invert repeatedly to mix while holding.
- 11 Allow seeds to settle to bottom of the tubes.
- 12 Allow water to drain through the holes in the tube or vacuum aspirate if in a laminar flow hood.
- 13 Repeat steps 9 through 12 six more times (for a total of 7 rinses with 18.2 MΩ water).

## Osmopriming Protocol

- 14 Measure out and add 90 mL of 18.2 MΩ water to the 100 mL beaker.
- 15 Measure out and add 10 mL of Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) to the same beaker. Mix solution.
- 15.1 Make additional solution or scale up volumes as needed.
- 16 Lay out a petri dish and its lid.

- 16.1 Optional: This step and the following steps can be done in a laminar flow hood if increased sterility is desired. For hydroponic applications where pathogens like *Pythium* can be a problem, we recommend using aseptic technique as much as possible.
- 17 Place one piece of filter paper into petri dish.
- 17.1 Filter paper and petri dishes can be pre-saturated in 70% ethanol if desired and allowed to dry in a laminar flow hood prior to use.
- 18 Cover filter paper with hydrogen peroxide solution until saturated, with no more than 1 mm of standing solution on top.
- 18.1 It should take about 7 mL of solution to saturate the filter paper.
- 19 Sprinkle the washed spinach seeds of one variety on top of the filter paper and spread evenly.
- 20 Cover the spinach seeds with a second layer of filter paper.
- 21 Apply more solution until the second piece of filter paper is also saturated without leaving more than 1 mm of standing solution in the petri dish.
- 21.1 Do not apply more than 7 mL of solution.
- 22 Place the lid on the petri dish.
- 23 Span a piece of tape across the petri dish to secure the lid and label/date/initial the petri dish with the cultivar name.
- 24 Repeat steps 16 through 23 for the other varieties of spinach if required.
- 25 Leave the petri dishes in a growth chamber (20/15 °C day/night; ~100-200  $\mu\text{mole/m}^2/\text{s}$  PPFD) until ready to transplant (ideally no more than 3 days).



## Transplanting seeds to slant boards protocol (based on Langenfeld and Bugbee 2022)

- 26 Sterilize the slant boards, slant board holders, and tub with 70% ethanol.
  - 26.1 Spray thoroughly before wiping dry with paper towels.
  - 26.2 Allow to air dry after.
- 27 Lay the slant board down on a flat, clean surface.
- 28 Cut a piece of germination paper that is 10 cm high and the same length as the slant board.
- 29 Place germination paper on top of the slant board.
- 30 Use tweezers to move the seeds (of one variety) from the petri dish to on top of germination paper in a line parallel to the top and bottom of the slant board.
  - 30.1 Seeds should be about 5 mm apart.
  - 30.2 The line of seeds should be 9 cm above the bottom of the board and 1 cm below the top of the germination paper.
- 31 Place a layer of single-ply KimWipes over the seeds.
- 32 Use the DI water squirt bottle to gently wet the KimWipes and the entire germination board.
  - 32.1 This action should lock both the germination paper and KimWipes in place.
- 33 Place the slant board into a slot on the slant board holder.

33.1 The slant board should be angled at 70 to 80 degrees and the seeds should be on the side facing "up".

34 Repeat steps 27 through 33 for the other varieties of spinach.

34.1 Make sure to label each board with the variety of spinach it has.

35 Place the slant board holder and the slant boards into the tub.

36 Make 6000 mL of 1 mmol calcium nitrate tetrahydrate.

37 Pour the calcium nitrate tetrahydrate solution into the slant board tub until the solution reaches the top of the slant board holders.

38 Place the tub and slant boards in the growth chamber. Monitor health and growth of seeds for a few days.

38.1 The growth chamber should be set to a light intensity of 100 micromoles per m<sup>2</sup> per second for 12 hours a day.

39 Once the first cotyledon appears, peel back the KimWipes so that the cotyledons are exposed but the roots are still covered.

## Transplanting seeds to slant boards protocol (based on Franz, Joly, and Mitchell 2000)

40 Soak 4 cm wide x 8 cm long polyester felt wicks in DI water.

40.1 Cutting wicks into an upside down "L" shape works well.

41 Carefully remove germinated seedlings from slant board using tweezers.

- 41.1 Work with seedlings individually to minimize stress and time away from moisture.
- 42 Place seedling on upside down “L” shaped polyester wick so that the cotyledons and most of the epicotyl extend beyond the wick.
- 42.1 All roots should be below the top of the wick.
- 43 Wrap the short excess of wick over the seedling and nestle inside a 2” neoprene cloning collar so that the felt is flush with the surface of the collar.
- 43.1 Excess exposed felt can wick moisture out of the hydroponics system and develop algae in some cases.
- 44 Install collar with plant into your hydroponics system.
- 45 Use a DI water squirt bottle to hydrate the top surface of each planted wick to ensure sufficient moisture prior to root system establishment.

## Protocol references

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## Acknowledgements

We thank Dr. David Brenner of the USDA-ARS National Plant Germplasm System for initial guidance in developing this protocol.