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Spinach Germination Protocol V.1

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We use this protocol and it's working

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Abstract

This germination protocol was developed for spinach (*Spinacia oleracea L.*) to overcome difficulties associated with 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.

Materials

Bleach/Detergent Wash:
- Spinach seeds (~2 times as many as the final desired quantity of plants)
- Milli-Q water
- Tween 20 detergent
- 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
- Whatman No.1 Filter paper
- Milli-Q water
- Hydrogen peroxide (H2O2)
- 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 width x 15 inch length x 7 inch height)
- 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 Milli-Q 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 10 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 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 Milli-Q 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.

13 Repeat steps 9 through 12 six more times (for a total of 7 Milli-Q water rinses).

Osmopriming Protocol

14 Measure out and add 90 mL of Milli-Q water to the 100 mL beaker.

15 Measure out and add 10 mL of Hydrogen peroxide (H2O2) to the same beaker. Mix solution.

16 Lay out a petri dish and its lid.

17 Place one piece of filter paper into petri dish.

18 Cover filter paper with hydrogen peroxide solution until saturated, with no more than one 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 piece of filter paper.

21 Apply more solution until the second piece of filter paper is also saturated without leaving 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 3 through 10 for the other varieties of spinach if you have more.

25 Leave the petri dishes in the growth chamber (20/15 °C day/night) 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 3 through 9 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 m2 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 One at a time.

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.