



Tobacco Mosaic Virus (TMV) Control

Pepper Seed Sanitizing Protocol

1. Set up a dedicated, duplicate set of supplies in a room for the process from start to finish. The room should not need to be used by others working on other plants that are susceptible to the disease. Ex PMMoV must be isolated from only peppers, while TMV must be isolated from all Solanaceae. To avoid waste, estimate the amount of supplies that must be taken into the quarantine space because they cannot be removed for general circulation.

Supplies:

- a. Sharpies
 - b. Paper towels
 - c. Strainers
 - d. Rinse aid
 - e. Soil
 - f. Stakes
 - g. Flats 1
 - h. 0% TSP
 - i. Small cups
 - j. Light source and shelves
 - k. Several boxes of gloves
 - l. Watering can
 - m. Bench cover if desired S
 - n. oap
 - o. Trash can and liner
2. Clearly mark the space, supplies such as Sharpies that are tempting to borrow from the space and make it known to other users the significance of the markings.
 3. Fill flats with soil if not done already. Label stakes and insert into flat. Skip one or two rows between accessions so emerging seedlings from different accessions done do not contact one another.
 4. Wear gloves to avoid spreading TMV residue after you are done.
 5. Place the seed to be treated into a small cup such as a yogurt cup, label, and add 10% trisodium phosphate to cover the seed. Ensure all seed are in contact with the liquid by swirling and poke seed down into solution from the side of cup with a pipette tip. Instead of cups, 2ml eppendorf tubes may be used for large numbers of seed lots. If in doubt about whether the seeds are getting adequately contacted on all surfaces, use a lab shaker. Otherwise, agitate the seed periodically.
 6. After 2 hours begin the rinse step. Dump an accession of seed and used TSP into a strainer, rinse the cup into the strainer if the cup is to be reused. Rinse the seed under running water for about 1 minute. To ensure all seed is rinsed well, we place the strainer on a bucket with a notch cut into the side to allow the incoming water to drain. While some protocols

advocate the use of mesh bags to allow all the seed to be batch treated and rinsed, we find that this is antithetical to the goal of the process.

7. Plant the seed into a flat with soil and consider your gloves potentially contaminated at this point. Change gloves before grabbing more soil to cover the seed or moving on to the next seed lot.
8. Repeat 6 & 7 for each accession. To speed the process you may start rinsing the next accession while planting the previous. An assistant will also speed the process greatly.
9. Dispose of unwanted supplies and bench cover, clean the area.
10. Water and germinate normally, but in quarantine. Use trash bag liners under flats.
11. When seedlings emerge, sample when there are sufficient true leaves to sacrifice some from all accessions for testing. Cut open the tops of immunostrip buffer/grinding packs and label for the next steps.
12. While wearing gloves, collect a small leaf from all seedlings in an accession and place them between the grinding mesh, up to 10 seedlings per test. The total surface area collected should be the equivalent of a 1 inch x 1 inch square leaf. Partial leaves may be used to avoid overloading the buffer, or during grinding, the leaves may be only ground partially while ensuring each leaf is ground. The key to this step is to sample all the seedlings and have them contribute equally to the lysate while not overloading the buffer.
13. Change gloves and repeat step 12.
14. While wearing a new pair of gloves, grind the leaves as described in 12 and insert an immunostrip to the indicated depth and stand the mesh bag upright to develop. Repeat for all samples. It speeds up steps 12-14 greatly to have an assistant.
15. Once strips have developed check for positive results and lift all strips just out of the liquid and allow them to continue to develop.
16. In the case of a positive result consider adjacent accessions that tested negative potentially inoculated by step 12. They must be held in quarantine and retested in about 2 weeks. Positive samples should either be discarded in entirety or individual samples must be retested to identify the positive sample. The negative seedlings in this accession must then be retested in about 2 weeks as they were potentially inoculated during step 12. The seedlings that are downstream of the sampling in step 12 in the accession should be considered inoculated and discarded.
17. Except for those accessions that tested positive or ones noted in step 16 as needing further quarantine, the seedlings are transplanted into new containers and the old flats and soil discarded in the trash.