# Tobacco Mosaic Virus (TMV) Control

## Tomato Seed Processing Protocol

1. Don safety equipment, since this procedure uses acid and base solutions.
2. Manually extract seed/gel from tomatoes, placing seed in appropriate sized plastic cup for seed volume. Write and attach identification tag to the cup. The tag must remain with the seed through all steps, to prevent loss or misidentification.
3. Extract seed into 6 to 10 cups for acid/TSP treatment before proceeding if treating small seed volumes. For large seed volume, process 1 or 2 seed lots at a time. The goal is to avoid leaving seed in acid for more than *ca.* 30 minutes.
4. Carefully fill each cup with 50% v/v HCl acid until seeds are completely covered, set the timer for 20 minutes, starting the timer after all cups have been filled.
5. After 20 minutes has expired, carefully pour acid out of one cup through a strainer into an empty collection container. Seed should be in strainer now. If all of the seed is not in strainer use a small amount of water in the cup to rinse seed into strainer.
6. Rinse seed in strainer well with running water in sink. Rinse empty cup with water, and replace seed into the empty cup.
7. Repeat steps 5 and 6 for all cups in the tray.
8. Fill each of the cups with seed with 10% solution of trisodium phosphate (TSP) until seeds are completely covered. Set timer for 20 minutes and start after all cups have been filled.
9. Place paper towels on table to hold seed after processing.
10. After 20 minutes has expired, pour TSP out of one cup through a strainer into an empty collection container. Seed should be in strainer now. If all of the seed is not in strainer use a small amount of water in the cup to rinse seed into strainer
11. Rinse seed in strainer well with running water in sink. Empty seed out of strainer onto empty paper towel and place identification tag on paper towel.
12. Rinse empty cup with water and place on drying rack.
13. Repeat steps 10 through 12 for all cups in the tray.
14. For each seed lot, fold paper towels to hold seed, clip tag on outside of paper towel, and place folded pack in tray.
15. Place tray with set seed packs in seed dryer.
16. Verify dryer is turned on and is set at $75^{o}$ to $80^{o}$ F.
17. Let seed dry overnight or until paper towels are dry. Do not leave in dryer longer than required for seed to dry.
18. Label appropriate sized seed envelope with seed ID, place seed and ID tag into the seed envelope. Inventory seed including weight or number of seeds produced, depending on seed numbers.

## Hydrochloric Acid Preparation Protocol

The following is prepared into a container labeled:
**"Hydrochloric Acid diluted 50% v/v with H20"**

1. Turn on exhaust fan and put on lab coat, gloves and goggles.
2. Use an empty beaker and measure 1 L of cold water.
3. Using a funnel pour 1 L of cold water into the container.
4. Carefully open the bottle containing Hydrochloric acid 36.5 - 38.0 % and pour into the container to the full line (full line equals two liters in container).
5. Close the bottle of Hydrochloric acid and then close the container.
6. Return the bottle of Hydrochloric acid to storage.
7. Shake the container ten times. Verify cap is on secure before shaking.
8. Rinse funnel and replace to storage.
9. Place the container in storage.

## Tri Sodium Phosphate preparation protocol

The following is prepared into the container labeled:
**"Tri Sodium Phosphate 10% solution (100 grams / one Liter)"**

1. Put on lab coat, gloves and goggles.
2. Use an empty beaker and measure 1 L of hot water.
3. Using a funnel pour 1 L of hot water into the container.
4. Using a scale that is properly leveled, measure 200 grams of Tri Sodium Phosphate.
5. Using a funnel add 200 grams of Tri sodium Phosphate to the container.
6. Use hot water to help dissolve Tri Sodium Phosphate through funnel.
7. Fill the container to the full line. (Full line equals two liters in the container.)
8. Remove funnel, rinse and replace to storage.
9. Close container and shake vigorously.
10. Always shake before use.

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