# Bacterial Wilt management in Tomato

## Training course guide

AVRDC
The World Vegetable Center

AVRDC.org

# ACKNOWLEDGEMENTS

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Additional information and images have been collected from acknowledged websites.

# CONTENTS

# INTRODUCTION

India is one among the world's largest major tomato growing countries. Tomato is best adapted to warm, dry environments and during the hot-wet season yields are low due to poor fruit-setting caused by very high temperatures, as well as many severe disease problems.

Among diseases, bacterial wilt (BW) is usually the most damaging. In India, a study showed 10 to 100 per cent incidence of BW during the summer. [1]

Some commercial bactericides are available in India although they have limited effectiveness.

The main control strategy has been the use of resistant varieties. However, the stability of BW resistance in tomato is highly affected by the pathogen density, pathogen strains, temperature, soil moisture, and the presence of root-knot nematode.

Private seed companies, AVRDC, and national agricultural research systems have worked on the development of BW-resistant tomato varieties, but there are still only a few varieties showing stable resistance [1].

Plants affected by Bacterial Wilt

# A FRAMEWORK FOR TRAINING:

## Purpose of this training package:
To provide practical technical skills and support for teaching by extension workers and community workers.

## Participants for the training course:
Agriculture extension workers, field staff, seed company staff, NGOs and community development workers, who propose to train farmers.

## Resource personnel:
Scientists from national and international research and extension institutes, leading seed companies, progressive and innovative farmers, who can apply the principles of adult learning.

## Training approaches to be used:
The sessions should include both classroom and field oriented hands-on sessions. Group work and case studies are integral parts of this training program. The manual has suggestions and directions on how to conduct each session. If the disease doesn't exist in the visited fields, classes need to be conducted using infected plant specimens brought from elsewhere.

## Duration of the training course:
Ideally a full working day will be required with a field visit to nearby fields for on-the-ground experience. However, the time needed may vary based on need.

## Materials needed:
This training package includes relevant technical bulletins, pamphlets, formats and survey sheets to be used by the trainers. This package will help the trainees when they actually go to villages for training farmers. Ideally the trainees should develop complementary training packages in local languages as part of this training program.

## Monitoring and Feedback mechanism:
Administering of structured surveys to the participants will enable the trainer to get meaningful feedback to review the performance of programs.

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# BACTERIAL WILT AND ITS CAUSAL ORGANISM

Tomato bacterial wilt is caused by a bacterium *Ralstonia solanacearum*, formerly known as *Pseudomonas solanacearum*. The pathogen has different races, each of them unique and each of them attacking different plants. Tomato bacterial wilt is mostly caused by the race 1 strain, which has a wide host range and can survive in the soil for a long period of time. Race 1 strains are highly variable in their genotype and aggressiveness on tomato. Some highly aggressive strains can cause severe symptoms, even on "resistant" varieties. Fortunately, such strains are not predominant. [1]

Bacterial wilt is a devastating disease causing tomato to wilt and die suddenly and with very little warning. It is nearly impossible to treat, but there are steps that can be taken to prevent its spread.

Bacterial wilt often happens where plants have been cut, injured or weakened by insects or simply by cultivation. This bacterium lives in the soil and will work its way quickly through the roots and up the stem of the plants, preventing water and nutrients from reaching the leaves causing the death of the plant.

Death of plants due to BW

# PATHOLOGY OF THE DISEASE

## Symptoms, identification and Diagnosis

• Mature, fruit-bearing plants are affected in mid-summer. The first symptoms are wilting of the youngest leaves, usually during the hottest part of the day. This can easily go unnoticed because the leaves stay green but eventually the entire plant wilts and dies.

• These dramatic symptoms occur when the weather is hot (over 30 degrees C), the humidity is high and lots of rainfall has left the ground wet. It's also more common in sandy soil with a high pH [4].

• Under less conducive conditions, wilt and decline will be slower, and numerous adventitious roots often form on the lower stems. In both cases, a brownish discoloration is present, first in the vascular system, and in advanced cases, spreading into the pith and cortex. The roots will exhibit varying degrees of decay [3].

• Recently wilted plants are green—this is a distinct symptom compared to other vascular diseases like Fusarium wilt, which develop yellowing of leaves [1].

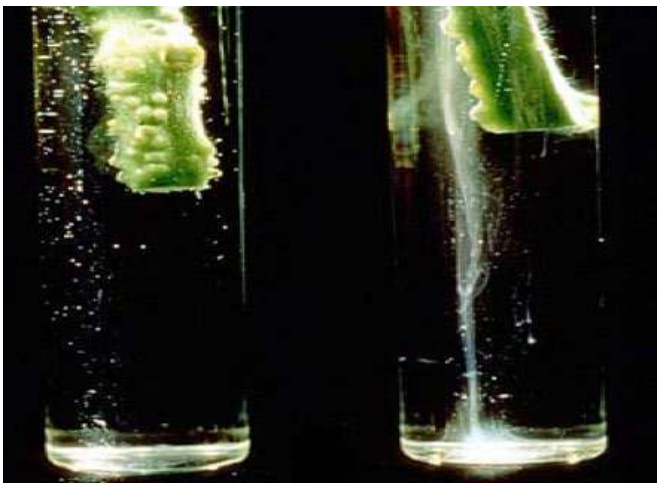
• After wilting, a vascular discoloration can be observed, sometimes with browning or maceration of the pith. A whitish bacterial mass can be seen when pressing an exposed stem slice.

## Bacterial Ooze test for diagnosis of the disease:

• Cut a portion of an infected stem at or near ground level, split it vertically and look for any discoloration inside. Also place the cut stem vertically in a glass of clear water, piercing it with a toothpick at the end to support it on the top of the glass. Look for the milky ooze running out of the cut stem end, like smoke.

• When the plant is infected, streaming will be seen from the stem.

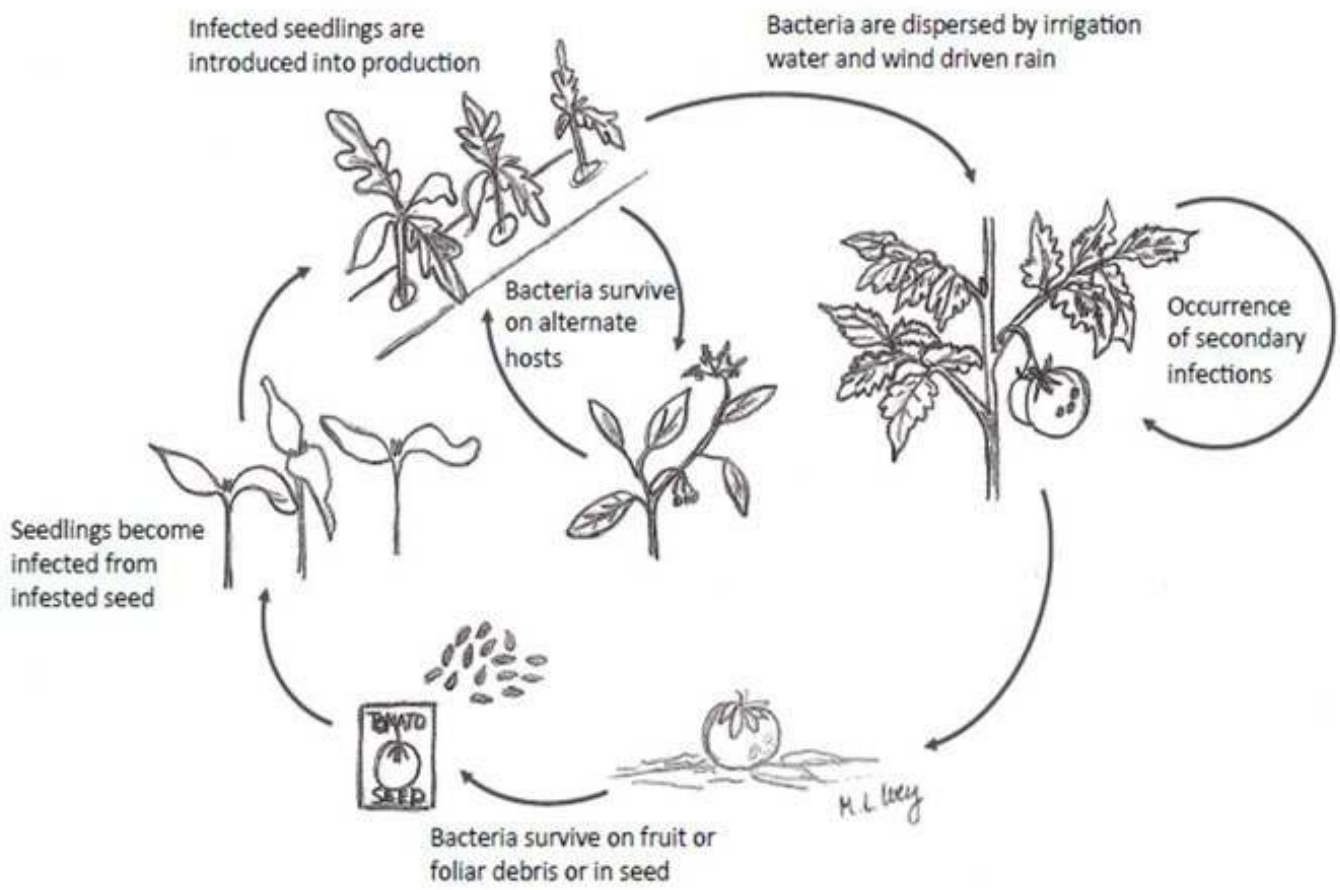
Browning of the pith in the affected plant



Diagnosis: Uninfected vs Infected

## Conditions for Disease Development

• *Ralstonia solanacearum* can overwinter in plant debris or diseased plants and wild hosts. The bacteria can survive for a long time in water (up to 40 years at 20–25 °C in pure water) and the bacterial population is reduced in extreme conditions ( eg. temperature, pH, salts.) [2].
• A large number of *R. solanacearum* bacteria can be shed from roots of symptomatic and non-symptomatic plants. Colonization of weeds by *R. solanacearum* affects the degree of carryover of inoculum. Many weeds may harbour the bacteria in the roots yet show no symptoms [9].
• Besides that, bacterial ooze (which is usually used as a sign for detection) on plant surfaces, can enter the surrounding soil or water, contaminating farming equipment or may be acquired by insect vectors [2].
• The bacterium usually enters the plant via a wound. Natural wounds (created by excision of flowers, genesis of lateral roots) as well as unnatural ones (by agricultural practices or nematodes and xylem-feeding bugs attack) can become entry sites.
• After invading a susceptible host, the bacterium multiplies and moves systematically within the plant before bacterial wilt symptoms occur.
•Wilting occurs with a high level of bacterial population in the xylem and is partially due to vascular blockages so that water cannot effectively reach the leaves.




Disease cycle of *R. solanacearum*

# RECOMMENDATIONS

No single control method will provide good and sustainable management of the disease, so integrating different methods is a must.

## Choose a clean field where-

- There is no history of bacterial wilt in the field or in neighbouring fields.
- The previous crop grown was paddy or another non-host crop
- The land is flat with good drainage
- The field is free from cross-flows of water from fields with the disease
- There is no root knot nematode problem

## Suppress the pathogen in the infected field

- Rotate with non-host crops like paddy or green manures, beans, corn, and cabbage.
- Avoid planting other Solanaceous crops (potato, pepper, and eggplant) in the same area.
- Flood the field 1 to 3 weeks before planting tomato.
- If the soil has a high pH, apply lime (Calcium carbonate) @ one tonne/ha to balance the soil pH. However it may take two years to lower it sufficiently.[6]
- Allow additional spacing between plants for air to circulate freely.
- Plant the seedlings in raised beds to improve drainage around the roots
- Reduce root knot nematodes in the field which aggravate bacterial wilt problems

## Use resistant varieties and clean seedlings

- Select BW-resistant tomato varieties, which have been tested locally.
- Use pathogen-free seedbeds to produce clean transplants. This can be assisted by fumigating the seedbeds.

During fumigation the seedbed is tightly covered with an airtight tarp or plastic. A registered fumigant (eg: Methyl bromide) is then injected under the cover and the seedbed is left undisturbed for three days. The cover is then removed and the soil is allowed to aerate for seven days before planting. A properly applied fumigant penetrates the soil and kills most existing viable seeds and pathogens.

- Use of resistant tomato or eggplant varieties as rootstocks can increase the resistance level of the scions.

## Prevent the spread of disease in the field

- Remove and destroy infected plants
- Reduce irrigation frequency and the quantity of water applied. If flood or sprinkler irrigation is used, restrict irrigations to every 7 to 10 days, depending on weather conditions. If the crop is drip irrigated, apply water every 4-5 days in cool months and every 2-3 days in hot months. [8]
- Prevent water flowing from infected fields to other field
- Drain the field quickly after rain
- Disinfect pruning tools either by-

1) Soaking them for at least 5 minutes in any disinfectant solution such as household bleach, Pine oil cleaner, Lysol or Dettol and rinse them with clean water and allow them to air dry before use, or
2) Wash with water or bleach or sterilize by putting them into a flame. [10]

- Avoid injury to roots during intercultural operations
- Remove and destroy affected plants at the end of the season.

## Using bio agents or bactericides:

- Spray the crop with Streptocycline (0.3g/L) 2-3 times at 10 day intervals after transplanting. [10]

# PREVENTIVE TECHNIQUES

## Grafting and its advantage over traditional methods

Grafting is a horticultural technique whereby tissues from one plant are inserted into those of another so as to join the two sets of vascular tissues. In most cases, one plant is selected for its roots and this is called the stock or rootstock. The other plant is selected for its stems, leaves, flowers, or fruits and is called the scion.

## Why to graft?

To reduce bacterial wilt disease and other diseases caused by fungal wilt, root knot nematodes and death of the crop due to temporary waterlogging in tomatoes.

## Advantages of grafting:

- Management of root-borne diseases is easy through planting of grafted seedlings.
- Plants grow healthy and can withstand initial infestations.
- Plants can survive waterlogged condition.
- Organic growers in particular can gain from grafting because growing tomatoes in soil and compost rather than in sterile media often leads to problems with weak roots, as a result of soil-borne pathogens.

## Selecting a Scion:

This is the fruiting part of the grafted plant. Any popular and widely grown tomato hybrid/ variety that has the desired fruit characteristics can be used to produce scions for grafting. Indeterminate varieties should be used in green houses or net houses and determinate or semi indeterminate varieties in open fields.

## Selecting Rootstock:

Select the rootstock based on its potential for resisting the soil borne diseases.
To choose the right rootstock, the first step is identifying prevalent pathogens in the area.

Fields where solanaceous crops (tomatoes, potatoes, and peppers) have often been grown have recurring problems such as bacterial wilt, verticillium wilt and root-knot nematodes. Disease resistant eggplants are the best source of rootstock and if the seeds are not available, it is suggested to use Solanum torvum as a rootstock, which is available locally. Solanum sisymbriifolium also proved to be a good rootstock.

## Raising Rootstock and scion:

Both scion and rootstock should have same stem thickness (diameter) at the time of grafting.

To ensure this, the rootstock has to be sown little earlier than the tomato scion. If wild solaneceous plants like *S. torvum* or *S. sisymbriifolium* are used, they have to sown first as they often have low germination rates and slow growth.

## Preparation for grafting:

- A nursery of eggplant rootstocks should be started earlier than the nursery for tomato scions.
- For the nursery media prepare a mixture of cocopeat and vermicompost in a 1:1 ratio.
- Avoid using soil for the eggplant rootstock nursery to prevent soil borne diseases.
- Select pro trays with 50/100 holes and fill them with the nursery mixture.
- Sow the eggplant seeds first.
- Slowly irrigate the seedling trays with a watering can or mug.
- When the germination of eggplant seed is completed, start sowing of tomato seeds in different trays in the same manner as the eggplant seed was sown.

The seeds for grafting need to be sown two weeks before typical, non-grafted transplanting. This allows the newly grafted seedlings to spend up to one week in the chamber followed by one week under shade or in the greenhouse to re-acclimatize to normal light conditions before they are put into the field.

As germination varies with the variety, sowing time may be altered to grow different cultivars to the same size. In many cases, rootstock varieties take two to five days longer to germinate than the scion.

Before getting into the grafting process, the healing chamber should be constructed and kept ready so that the grafted seedlings can be protected in the chamber without much delay.

## Grafting methods and steps in the process:

Tomato seedlings are almost always grafted using the 'tube grafting' method in commercial propagation.

**Tube Grafting Method (Method 1)**

Tube Grafting or Japanese Top-Grafting is carried out when the plants are very small and the rootstock and scion can be held together with a 1.5–2 mm tube. This is also called the 'Japanese Grafting Method'. The diameter of the scion and rootstock must be matching. The advantages of this method of grafting is:

- Very good graft union
- Seedlings produced in a short period
- More seedlings can be produced as it requires only local materials.

The disadvantage is:
- The height of the rootstock above the soil is short, so earthing up of the seedlings in the field is not possible as the soil will touch the scion

**Materials**
- Scion seedlings at a two-true-leaf stage. Target diameter of the grafting site is 1.5 mm.
- Rootstock seedlings at the same leaf stage. Target diameter of grafting site is 1.5 mm.
- Razor blade (used for shaving) works best for this grafting method.
- Grafting tubes/Cycle valve tubes.
- Scissors to cut the valve tubes to the desired length.


https://goo.gl/UGNKYP

## Steps

i. Initially, cut the cycle valve tube into 1.5-2 mm long pieces at an angle.

ii. Cut the rootstock under the cotyledons at a 30-45 degree or sharper angle. Cutting the rootstock above the cotyledons is not advised as axillary buds may grow out when vigorous rootstocks are used.

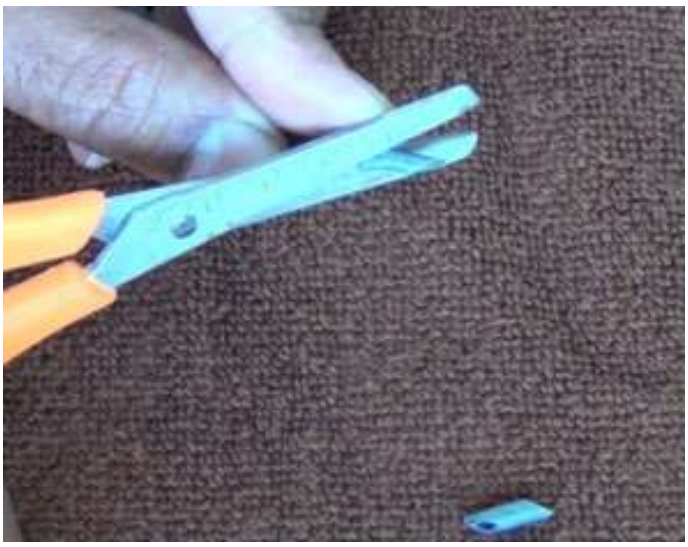
iii. Prepare the scion with matching stem width cut in the same angle at about 5-10 mm below the cotyledons.

iv. Place one tube a half way down on top of the cut end of the scion.

v. Insert the scion and grafting tube onto the rootstock so that the cut surfaces align perfectly.

**Transplanting and care of grafted seedlings in the open field**
- The tube need not be taken off as the growing stem applies pressure on it so that it breaks off on its own.
- Plant the grafted plant so that the graft union remains at least 2.5 cm above the soil. This will prevent root formation from the scion.
- Suckers usually develop from the rootstock after grafting and become obvious 6-8 days after grafting. These suckers should be removed as soon as they emerge.
- Staking of the grafted plants is essential as the earthing up is not possible because of the need to avoid soil contact above the grafted joint to assure protection from soil borne diseases.

**Tips for success:**
For proper healing to take place, the vascular tissue in the rootstock and scion must align so that their tissues can easily grow together, forming a strong union for water and nutrient uptake. An essential component for grafting success is to use rootstock and scion plants that have similar stem diameters. Grafting should take place when the plants are not water stressed. Early in the morning or just after dark are excellent times to graft as transpiration has typically slowed down. Grafting should be

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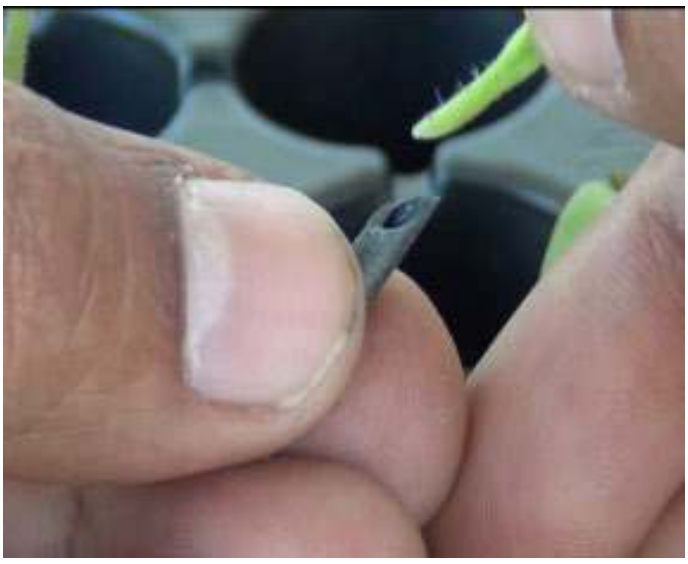

done indoors or under some sort of shading. If daytime grafting is essential due to timing and labour concerns, move the plants to a shady area in the morning before transpiration increases to prevent unwanted water stress during the process.

**Cleft (v-shaped) grafting method (Method 2)**

This technique can be easily practised. This grafting method also has some amount of flexibility in terms of matching the size of the scion and rootstock. As the rootstock shoots will be completely removed, this method has less chance of developing rootstock shoots in the field.

**Materials**
- Scion seedlings at a two or three-true leaf stage.
- Rootstock seedlings at a two or three-true leaf stage.
- Razor (shaving) blade works the best for this grafting method.
- Grafting clips. Size needs to be selected for matching seedlings.

**Steps**

i. Using a sharp clean blade, decapitate the rootstock seedling with a horizontal cut approximately at a height of 3-5cm.

ii. The decapitated rootstock seedling without any leaves is shown. The foliage of the rootstock seedling is discarded.

iii. Bisect the truncated rootstock stem at its widest diameter to a depth of 4 mm.

iv. Remove the roots of the scion seedlings with a horizontal cut approximately 5 mm above the cotyledons.

v. Trim the cut surface of the scion seedling to the shape of a wedge with sides approximately 4 mm long. (Alternatively, in the two cut process, two diagonal cuts at an angle of 65 degrees are made to separate the scion from its roots and to form the wedge).

vi. Insert the trimmed scion into the vertical slit of the rootstock.

vii. Secure the graft with a clip.

**Tips:**
- Selecting matching sizes of scion and rootstock is important in this grafting procedure.
- Trimming leaves from the scion seedling can be beneficial as this can reduce water loss in grafted seedlings. The trimming can be done two to three days before grafting process.
- Grafting clips should also be selected according to the stem size. Clips that are too big cannot hold the grafted union and clips that are too small apply too much pressure and may deform the union.

## HEALING CHAMBER

The purpose of the healing chamber is to protect the scion from water stress. This can be accomplished by slowing the transpiration stream—the movement of water from inside the plant tissue into the atmosphere. The best way to do this is to increase the humidity, decrease light and temperature.

The seedlings resulted from joining a rootstock and scion must reconnect vascular tissues so that water and nutrients can be supplied to the scion from the rootstock. This process occurs in a chamber where humidity, light and temperature can be regulated.

Construction is relatively simple and inexpensive, and selecting a proper place in the farm to locate the chamber plays a critical role. While the grafts are healing in the chamber, they must receive 80 to 95 per cent humidity, minimal
direct sunlight and a temperature between 24° C and 30° C. The daily temperature variation must remain low, as additional stress can decrease grafting success. The best place for a healing chamber is indoors.

Healing chambers can also be maintained inside a greenhouse so long as there is sufficient

shading to keep the grafts from being exposed to excessive heat inside the chamber.
A simple healing chamber consists of a frame covered by polyethylene sheet, which keeps the humidity level high during the healing process. The floor of the chamber should hold water. During the first few days after grafting, an opaque /black polythene covering is used to keep all light out of the chamber.

**Materials required:**
• PVC pipes, clamps, or wooden sticks or any other suitable material to make the frame can also be used
• Transparent polythene
• Opaque/Black polythene
• Wooden stick
• Binder clips
• Water

Construct a frame ideally using 2.5 cm polyvinyl chloride (PVC) piping (or any other available size) as illustrated in Figure. The height of the frame should not be so low that it touches the plants or too high to reduce the humidity.

Cover the PVC frame with a layer of clear plastic so that the sides and ends can be easily pulled up to check on the grafts during healing.

Use a wooden stick at the base to make a shallow pool of water which does not touch the seedling tray.

## Healing of graft union

Move the tray filled with grafted plants for healing for up to 7 days.

Pour water into the healing chamber. Keep the seedling tray on the wooden sticks above the water. Care should be taken to ensure that the tray should not directly touch the water as it will promote damping off diseases of the seedlings.

Use black plastic to block all available sunlight from entering the chamber until the leaves of the newly grafted transplants attain normal turgor levels and they no longer show signs of moisture stress.

Then cover the chamber with black polythene to prevent light penetration. This will help the scion to maintain its stored energy reserves until healing is completed.

After 24-48 hours, remove the black polythene and allow limited indirect light until the healing process is completed. in two more days.

Four days after grafting, open the transparent polythene for the seedlings to acclimatize to the external environment.

Two days after this, move the seedlings outside, but keep them under shade for two to three more days and before transplanting in the main field.

# SUGGESTED SESSION PLAN

• It is best to run the training sessions close to an infected field. If this is not possible, live infected plant specimens need to be brought to the classroom for demonstration.
• Hands-on training in grafting with actual seedlings is also essential. Make sure the materials for building a healing chamber are collected and kept ready in advance.

| | Topic | Duration | Resource person /facilitator |
|---|---|---|---|
| 1 | Introduction to Bacterial wilt- symptoms and diagnosis | 60 min | Pathologist/Plant protection expert |
| 2 | Learning to manage the disease | 60 min | Pathologist/Plant protection expert/ innovative farmer |
| 4 | How to graft Tomato seedlings | 90 min | Agronomist/ innovative farmer/ nursery man/ grafting Expert |
| 5 | Constructing a Healing chamber | 90 min | Agronomist/ innovative farmer/ nursery man/ grafting Expert |

# PRACTICAL EXERCISES

## Diagnosis of Bacterial Wilt:

**Purpose:** To provide skills in identifying the symptoms and diagnosing the disease

**Activities:** Field visit, observation and diagnosis of symptoms

- Participants will divide into two mixed teams of experienced and beginners.
- Two teams will check for disease in two different plots and tag the suspected plants.
- After 15 minutes, all the members will reassemble near the suspected plants and conduct an ooze test to diagnose the disease.
- The trainer will demonstrate the technique with one sample and the trainees will do one test each with different plants.
- After that they will explain how they could suspect the infected plants and then confirm this with a test.

## Managing the disease:

**Purpose:** To bring out the trainees' existing knowledge and to incorporate additional methods/practices that can be adopted for effective management of the problem.

**Activities:** Field visit, sharing of experiences and group discussion

- Participants will form into two or three groups of no more than 12 each, and share their experiences on how they manage the problem in their own fields and what they have observed their neighbours doing.
- One of the group members will be noting down the points of the discussion on a board.
- After the specified time period, the team leader will summarise the discussions to come up with the most effective way of controlling the pathogen based on group experiences.

## Grafting of seedlings and post grafting care

**Purpose:** To provide hands-on practice with two different methods of grafting. To deliver the techniques/tips for a successful graft union

**Activities:** A video on grafting will be played, stopping at crucial points to interact with the trainees.

**Hands-on-practice:** The participants will then be supplied with raw materials to do grafting by themselves, with support from the facilitator as needed.

After learning to graft, trainees will practise the right way of protecting newly grafted plants in the healing chamber.

### Constructing a healing chamber to protect grafted seedlings

**Purpose:** To help the participants learn how to protect a graft union.

**Activities:** Participants will make a healing chamber by themselves under the guidance of the trainer.

- During the process, they will also discuss the possibility of using cheaper alternative materials that may be available in their areas, and come up with suggestions to share with each other.

## COMMUNICATION MATERIALS

Hand-outs /technical manual
Management of bacterial wilt

Powerpoints on
Bacterial wilt symptom and diagnosis
Grafting

Videos of AVRDC
Grafting
Healing chamber

Videos from YouTube
Grafting vegetables
Grafting Tomatoes-Healing chamber

## QUIZ (PRE AND POST-TEST)

Bacterial wilt is a disease caused by bacterium that results in wilting of the plants.
**a. Yes** **b. No** **c. Don't know**

Bacterial wilt is more rapid in hot weather and high humidity
**a. Yes** **b. No** **c. Don't know**

Streaming of fluid from a cut stem when placed in clear water indicates the presence of Bacterial wilt.
**a. True** **b. False** **c. No idea**

Grafting offers protection from Bacterial wilt
**a. Agree** **b. Disagree** **c. No idea**

Grafted seedlings can be directly transplanted into the field
**a. Strongly agree** **b. Agree** **c. Disagree**

A healing chamber initially protects the grafted seedlings
**a. True** **b. False** **c. No idea**

Bacterial wilt can be controlled by hanging sticky traps in the field
**a. True** **b. False** **c. No idea**

Resistant varieties can be grown to control the disease to certain extent
**a. Yes** **b. No** **c. Can't say**

The disease does not persist in a field after harvesting of the crop
**a. Yes** **b. No** **c. Don't know**

## GLOSSARY

| | |
|---|---|
| BW | Bacterial Wilt |
| R.solanacearum | Ralstania solanacearum |
| g | Grams |
| l | Litres |
| ml | Millilitres |
| Eg | Example |
| Min | Minutes |
| $m^2$ | Square meters |
| Degrees C | Degrees Celsius |
| Ha | Hectare |

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