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Study on Host Inoculation Methods

Inoculation: Introduction or artificial penetration of pathogenic inoculum into the host is called inoculation
Inoculum (pl. Inocula): The inoculum is any part of the pathogen that initiates infection. Thus, in fungi the inoculum may be spores, sclerotia or fragments of mycelium. In bacteria, protozoa, viruses, and viroids the inoculum is always whole individuals of them. In nematodes, the inoculum may be adult, juvenile or eggs of nematodes. In parasitic higher plants inoculum may be plant fragments or seeds.

Objectives:

- To prove parasitism of an organism
- To study the life cycle of an organism
- To study the pathogenic variation or specialization of pathogens
- To study varietal resistance in the host
- To determine the effect of environmental conditions on disease development

Methods:
Plant can be inoculated by various way. Some of these methods of inoculation are described below:
Soil Inoculation Method: In this method inocula are prepared on a suitable medium and transfer to the soil before planting seeds or seedlings. Root invading pathogens such as bacteria, fungi, nematodes can be mixed with potting soil where seeds or transplants are placed. Suspension of bacterial cells, fungal spores or nematodes maybe used. Fungi maybe grown in sterilized cereal grain which can be added to the soil directly.

Soil drenching method: In the soil drenching method ~ 5.0 ml of bacterial suspension is used for inoculation to each of the seedlings by drenching the soil around the root zone with the help of micro pipette. Before inoculation, the roots are slightly severed by inserting a sharp knife 1.0 cm away from the main stem. Root severing is done to ensure bacterial penetration through roots.

Spraying method: In this method standard suspension of fungus or bacteria is prepared and spread on the plant parts. A specific amount of inoculum (spores/ml) is sprayed. Spray should be done at afternoon for better infection and inoculated plant covered with a moist transparent polythene sheet for 24 hrs.

Dusting Method: Generally spores of smuts, rusts and powdery mildews are collected from the infected plants or its part and then directly can be dusted to the susceptible host in the favorable environmental conditions.

Puncturing Method: In puncturing method the inoculation is carried out with sterile dissection needle, by dipping the needle with the bacterial suspension and inserting at the axil of the leaf along with a drop of inoculum and gently pressing to ensure the inoculum reached the vascular tissues.

Leaf clipping method: Leaf clipping method is also carried out by dipping sterile scissors in the bacterial suspension and clipping the leaves. Three to four leaves are clipped per seedling by giving horizontal cut.

Rubbing Method: The leaf is rubbed lightly then distributing the inoculum over the entire surface. Immediately following inoculation, the leaves should be rinsed with water, which can be done by placing the leaves under the tap. A common mistake is to apply too much pressure when rubbing the leaf, which will damage cells and produce large, irregularly shaped lesions. Before inoculation the host plant is dusted finely with carborundum powder. There should be just sufficient carborundum to puncture the epidermal cells. This aids the immediate entry of the pathogens to the host tissues. But too much carborundum causes excessive damage to the plant tissues.

Inoculation of rice plant with fungus (*Rhizoctonia solani)*

Procedure:

1. Collect a pure culture of *Rhizoctonia solani* from the laboratory i. e. PDA media bearing *Rhizoctonia solani*.
2. Then, select healthy rice hills
3. Cut into small pieces (mycelia ball) by borer tool of the 3-days old cultures
4. Inoculate with *R. solani* by placing a mycelia ball beneath the leaf sheath.
5. Cover the inoculated sheath immediately with aluminum foil/wet cotton pad.
6. When typical lesions of sheath blight appear after 3 days the aluminum foil should be removed.
7. Seven days after inoculation, the lesion length on the sheath of the inoculated plants will be measured.

R. solani infected plants should be kept in a humidity chamber made of clear plastic for 5-7 days to allow for disease development. Plants allow to grow at 28°C under 14-h days in both the greenhouse and the humidity chamber. The humidity should be maintained between 80-100% in humidity chambers from the time of inoculation to disease evaluation.

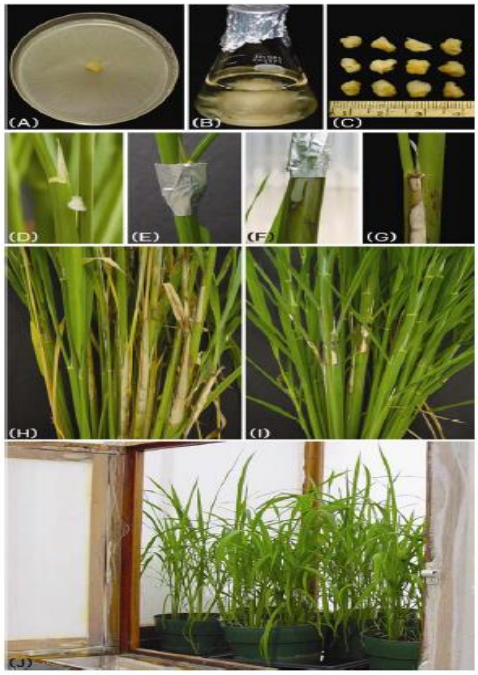

Figure 1. Schematic representation of sheath blight inoculation: A, 3 days after inoculation of *R. solani* on potato dextrose agar; B, 7 days after inoculation in potato dextrose broth; C, mycelia balls harvested 3 days after inoculation; D, inoculation of mycelia ball beneath leaf sheath; E, inoculated sheath covered with aluminum foil; F, appearance of disease symptoms 5-7 days after inoculation; G, removal of aluminum foil; H and I, *R. solani*-infected plants showing different level of infection between susceptible and resistant cultivars, respectively; and J, humidity chamber used to grow infected plants and maintain humidity between 80-100%.

Inoculation of rice plant with bacteria *(Xanthomonas oryzae* pv. *oryzae)*

Prepare a bacterial suspension as inocula:

- Take bacterial colonies in a test tube or conical flask with sterile water from the Nutrient Agar plate by scrapping with scalpel.
- Admixture by proper shaking
- Count the number of bacterial colony (Standard 10^8 cfu/ml) with the help of haemocytometer following dilution method

Inoculation Procedure:

1. Dip a pair of sterilized scissors in the bacterial suspension
2. Grip by hand and Cut the tip (1-3 inches) of the leaves (10-20) with the scissors.
3. Then cover the inoculated plants with polythene bay and incubate at 30^0c temp with 12/12 hrs light cycle.
4. After 10 days of incubation the typical characteristics symptom will be observed of bacterial leaf blight in the inoculated plant.

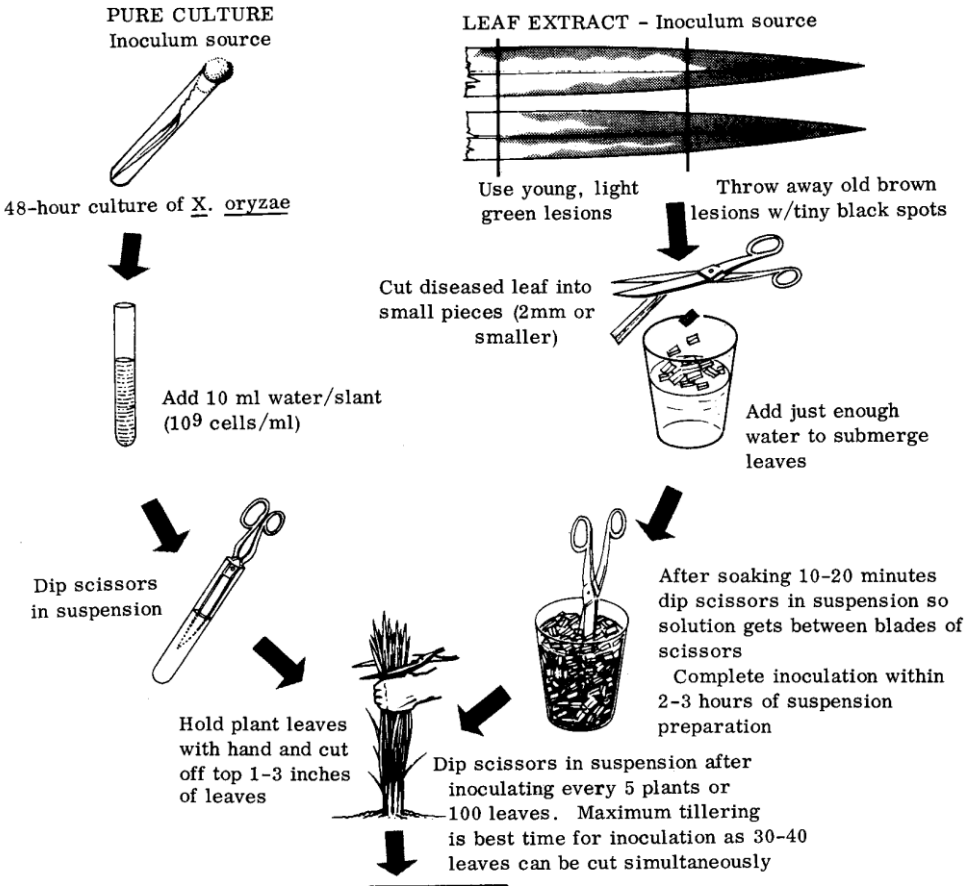

Figure 2. Schematic representation of Bacterial Leaf blight inoculation by clipping method

Inoculation of tomato plant with nematode *(Meloidogyne javanica)*

1. Collect the egg masses of nematode from root knot disease symptom of tomato plant by standard method and prepare the nematode culture medium
2. Transfer the nematode culture medium into healthy tomato plant
3. Mix the soil around the rhizosphere zone of the healthy plant
4. After few days the root knot symptom will be appeared

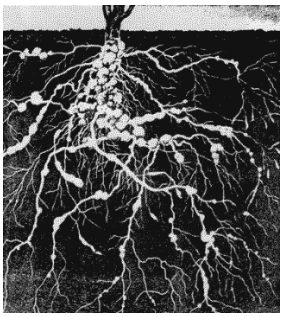

Figure 3. Root knot disease symptom

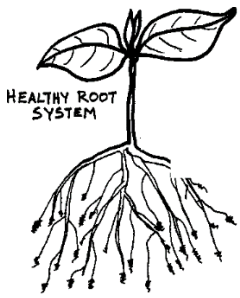

Figure 4. Healthy tomato plant

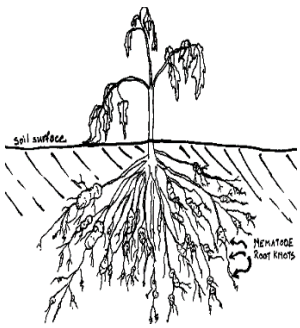

Figure 5. Root knot disease symptom of the tomato plant

Mechanical transmission or sap inoculation of plant virus

- Materials
- Pestles and mortars
- Muslin cloth
- Carborundum
- Chemicals for appropriate buffers

Procedure

1. Dust abrasive (corundum or carborundum or celite) sparingly on leaves of the test plants to be inoculated.
2. Use disposable gloves to inoculate plants. If not, wash hands thoroughly with soap and then rinse with water.
3. Support the leaf to be inoculated with one hand and apply inoculum on the leaf with fingers of other hand or muslin cloth or thick end of a pestle or with cotton swab.
4. Inoculate at least 5 plants per each treatment. Label the pots containing the test plants or plants individually with date and inoculum details (virus inoculated, dilution of the buffer and other details if necessary for the experiment).
5. Rinse the inoculated leaves with tap water and cover the plants with sheets of paper (old newspapers) overnight.
6. Wash hands thoroughly with soap (or with trisodium phosphate when highly infectious are handled) and then with water.
7. Monitor test plants regularly and record time and appearance of first symptoms and the symptom type (mosaic, ring spots, necrosis, chlorosis etc.).

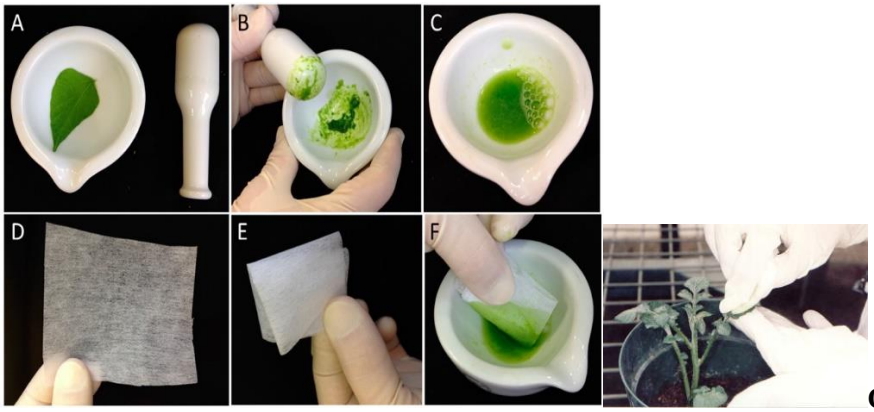

Figure 6. Mechanical transmission or sap inoculation of plant virus. A-C. Preparation of infected leaf sap by grinding an infected leaflet with a mortar and pestle. D-E. The Muslin cloth piece is folded in four. F. The folded muslin cloth piece is soaked in the leaf sap. G. Apply inoculum on the leaf with muslin cloth.