

## **RAPID ASSESSMENT OF PLANT PATHOGENIC NATURE OF BACTERIAL ISOLATES**

Once the bacterium is isolated from the diseased plant material, it is necessary to know whether the isolated bacteria is the actual cause of the disease and is pathogenic. The determination of pathogenic nature of the isolated bacteria on its natural host generally takes 4–15 days or even longer, depending on the types of symptoms they induce on their natural host plant; per se, induction of water-soaking symptoms in natural host plants requires 4–8 days, induction of wilt symptoms requires 8–15 days, and induction of a tumor requires approximately a month or more. Due to this, a rapid assessment method to determine the pathogenic nature of bacterial isolates is an important aspect.

### **Material Required:**

Test bacterial culture, tobacco plant, brinjal plant, stramonium plant, cowpea plant, hypodermal syringe, distilled sterile water, label tags, incubation humid chamber, and so on.

### **BY HYPERSENSITIVE REACTION ON A TOBACCO PLANT**

The method is based on the fact that only plant pathogenic bacteria are able to induce a hypersensitive reaction (HR) in tobacco. Saprophytic bacteria do not induce HRs in tobacco. Diluted bacterial culture suspension is inoculated by the injection infiltration method in tobacco leaves with a hypodermic syringe. Different cultures may be inoculated in different interveinal sections of the same tobacco leaf. The cultures, which are plant pathogenic, produce quick necrosis (HR) within 24 hours. Sometimes the HR is produced as early as 8 hours (Borkar, 1982). Those isolates that give an HR in tobacco plants are surely plant pathogenic bacterial isolates. If several different types of colonies are encountered in isolation plates, this method may be used to select pathogenic ones. The test is useful particularly in differentiating phytopathogenic xanthomonads and pseudomonads from saprophytic ones.

### **BY HYPERSENSITIVE REACTION ON A NON-HOST PLANT**

Most of the plant pathogenic bacteria produce hypersensitive browning reactions on tobacco plants. However, in most of the places and laboratories, the tobacco plant may not be available. In such cases, other non-host plants can be used to check the HR. Brinjal or eggplant and stramonium

plant are the most suitable for this purpose (Borkar, 1982). Inoculate a bacterial suspension (0.1 OD or 10<sup>7</sup> cfu/ml) in the dorsal side of the leaf of test plant by the syringe infiltration technique and keep the plant at 28 ± 2°C with more than 85 percent atmospheric humidity. Observe the HR reaction which develops within 18–24 hours on brinjal/stramonium leaves.

### **BY REACTION OF BACTERIA ON SOFT FRUITS**

The reaction of plant pathogenic bacteria, particularly *Erwinia*, on soft pear fruit is very quick (Nagarale, 2010). The inoculation of bacteria into pear fruit produced soft rot of tissue and light to dark-brown coloration of rotted tissues within 24 hours of bacterial inoculation. Infiltrate the bacterial suspension (0.2 ml) beneath the skin of the pear fruit or banana fruit and incubate at room temperature (at 27°C). Observe the development of soft rot in the fruit due to bacterium.