ISOLATION AND PRELIMINARY IDENTIFICATION OF CAUSAL AGENT OF ROSE CROWN GALL DISEASE IN BADULLA DISTRICT

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ABSTRACT

Crown gall disease is an economically important bacterial disease in rose. *Agrobacterium tumefaciens* is a soil-borne bacterium that causes the crown gall disease, by forming crown gall tumors on dicots and some monocots. Crown gall disease affected rose plants were observed in Welimada and Neluwa (Atampitiya) areas in Badulla district in recent past of Sri Lanka. This study was focused to isolate pathogen from crown gall infected rose plants and characterize the pathogen by biochemical and pathogenicity tests. In addition, effect of selected antibiotics on the growth of causal agent of the disease was investigated. The crown gall samples were collected at floriculture nurseries in Neluwa and Haputale. The samples were isolated separately as Neluwa (Isolate 1) and Haputale (Isolate 2) at Regional Agricultural Research and Development Center, Bandarawela. The isolates were selected based on their morphological characteristics after 48 h at 28 °C on Yeast Mannitol Agar (YMA) medium. The selected colonies were transferred to Yeast Mannitol Agar (YMA) medium for purification. Colonies of isolate 1 and isolate 2 were translucent, mucoid, white to cream coloured, circular with entire edges and easily suspended in water. The optimum growth was between 25 °C and 27 °C. Potassium Hydroxide (KOH) solubility test, mobility test and catalase production test were conducted to characterize the isolates. The isolates were gram negative, and positive for mobility, catalase. The pathogenic nature of the organism was confirmed by a bioassay on carrot disks. Green colour tumor formation was observed on carrot disks by isolate 2 after 24 days of incubation and tumors were not formed by isolate 1. Test of determining growth inhibition ability of antibiotics and copper on isolate 2 was conducted using poison plate technique. The effects of four antibiotics (Neomycin Sulphate, Streptomycin, Rifampicin, and Ampicillin) and Copper on isolate 2 were evaluated. Neomycine Sulphate indicated the highest inhibitive activity. Rifampicin indicated the lowest inhibitive activity. According to the increment of concentration of antibiotic, inhibitive activity increased. Copper at 6 gL^{-1} indicated the highest inhibitive activity. Based on the results of biochemical tests and pathogenicity (tumor forming ability) tests, the isolate 2 was confirmed as *Agrobacterium tumefaciens*.

Keywords: *Agrobacterium tumefaciens*, Antibiotics, Crown gall, Pathogenecity test, Rose