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Characterization of *Agrobacterium* strains from agricultural soils of Bandarawela, Sri Lanka

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Agrobacterium is a Gram negative, rod shaped, aerobic and motile soil inhabiting bacterium of the family Rhizobiaceae. It is well known as the causative agent of crown gall disease of many plant species around the world. However, not all the *Agrobacterium* strains are pathogenic and can cause galls. Only the virulent strains cause crown gall disease on number of plant species and are found only in contaminated soils. These virulent strains of *A. tumefaciens* harbor Ti plasmids with transfer DNA (T-DNA) region and virulence (*vir*) genes that are responsible for the pathogenicity. *virD2* gene codes for virD2 protein and the endonuclease domain of the virD2 protein cleaves T-DNA border sequences. The *ipt* gene is the T-DNA borne cytokinin synthesis gene. Therefore, the presence of *virD2* gene and *ipt* gene are useful in identifying pathogenic strains of *Agrobacterium*. The major objective of this research was to determine whether agricultural soils of Bandarawela were contaminated with virulence strains of *A. tumefaciens*. Soil samples were collected and bacteria were isolated using soil dilution method, and cultured on Yeast Mannitol Agar supplemented with Congo red and on Yeast Extract Peptone Agar. Five pure cultures of putatively *Agrobacterium* were further characterized using morphological and biochemical tests including Gram staining, catalase test, citrate utilization test, sugar fermentation test and 3-ketolactose test. These testes were often used for the species level identification of *A. tumefaciens*. Out of five isolates four were rod shaped with rounded ends and were either single or in pairs. However, the other isolate was in chains and long rod shaped. Interestingly, all the isolates were positive for all the biochemical tests. However, these tests do not help differentiating the virulence strains. Molecular characterization of all the soil isolates were carried out using universal 16s rRNA primers and *Agrobacterium* specific primers targeting *virD2* and *ipt* genes. PCR amplification with virD2 primers successfully amplified the targeted band of 224 bp in all five isolates while ipt produced the expected fragment of about 427 bp in three of the isolates. *virD2* gene sequences of selected soil isolates were 100-99% similar to the *A. tumefaciens* of the GenBank accession CP032925 and CP032929 reported from Taiwan. According to morphological, biochemical, and molecular characterization using *virD2* and *ipt* genes it was confirmed that the soil in the inspected field of Bandarawela is contaminated with pathogenic strains of *A. tumefaciens*. Therefore, farmers should maintain awareness when cultivating susceptible plant varieties in these fields.

Keywords: pathogenic, Ti plasmid, virD2 gene