

**DETECTION OF *Agrobacterium tumefaciens* IN
ARTIFICIALLY INOCULATED TOMATO PLANTS
USING POLYMERASE CHAIN REACTION**

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ABSTRACT

Detection of *Agrobacterium tumefaciens* in artificially inoculated tomato plants using Polymerase Chain Reaction.

Agrobacterium tumefaciens is used in plant biotechnology for its DNA transmission capabilities. It enables the insertion of foreign genes into plants, making it a widely used approach in genetic engineering producing genetically modified plants. This study aims to assess of the risk of infection posed by accidental release of the plant transgenic vector *Agrobacterium tumefaciens* LBA4404 into the soil inside a transgenic containment. A known F1 phenotype *Solanum lycopersicum* infected by 10^{-5} , 10^{-6} , 10^{-7} and 10^{-8} of dilution factor of *Agrobacterium tumefaciens* LBA4404 containing plasmid vector pCambia1303 was used for this purpose. The leaf samples of *Solanum lycopersicum* were collected on the thirtieth, sixtieth and ninetieth day after the transplantation. The DNA from the leaves of *Solanum lycopersicum* extracted by using CTAB DNA extraction and tested for the presence of the 35S DNA promoter by using the Polymerase Chain Reaction. Testing the presence of the 35S DNA promoter by PCR revealed that none of the leaf sample infected by either one of the dosages of *Agrobacterium tumefaciens*. Increasing the concentration and wounding the *Solanum lycopersicum* are the possible ways to enhance the transformation efficiency of *Agrobacterium tumefaciens*. This finding will be useful for the risk assessment of research that creating GM plant by using plant transgenic vector *Agrobacterium tumefaciens* LBA4404.



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ABSTRAK

Agrobacterium tumefaciens telah digunakan di dalam bidang bioteknologi kerana kebolehan dalam transmisi DNA. Ia membolehkan pemasukan DNA asing ke dalam tumbuhan, membuatkan ia digunakan secara meluas dalam kejuruteraan genetik untuk menghasilkan tumbuhan yang terubah-suai secara genetik. Kajian ini dijalankan bertujuan untuk mengkaji risiko infeksi yang disebabkan oleh penyebaran secara tidak sengaja vector transgenic *Agrobacterium tumefaciens* LBA4404 kedalam tanah di dalam bangunan transgenik. Generasi F1 bagi *Solanum lycopersicum* telah diinfeksi dengan 10^5 , 10^6 , 10^7 dan 10^8 dos *Agrobacterium tumefaciens* LBA4404 yang mengandungi plasmid vektor pCAMBIA1303, di dalam bangunan transgenik. Sampel daun *Solanum lycopersicum* diambil pada hari ketiga puluh, keenam puluh dan kesembilan puluh selepas pemindahan pokok. DNA daripada sampel daun *Solanum lycopersicum* diekstrak dengan menggunakan kaedah pengekstrakan CTAB DNA dan diuji untuk kehadiran 35S promoter dengan menggunakan Polymerase Chain Reaction. Hasil kajian menunjukkan bahawa tiada satu pun daripada tomato yang dijangkiti dari salah satu sukatan *Agrobacterium tumefaciens*. Meningkatkan kepekatan *Agrobacterium tumefaciens* and melakukan tumbuhan adalah cara-cara yang mungkin boleh meningkatkan kadar keberkesanan transformasi *Agrobacterium tumefaciens*. Penemuan daripada kajian ini berguna untuk kajian tentang risiko dalam penyelidikan yang menghasilkan tumbuhan GM dengan menggunakan *Agrobacterium tumefaciens* LBA4404 sebagai vektor.



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