35

Preparation of Coat Protein-Containing Binary Vectors for Use in Agrobacterium-Mediated Transformation

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1. Introduction

Transgenic plants expressing the coat protein (CP), and/or the RNA transcript of the CP gene of many RNA viruses, may be protected against the virus from which the CP gene was derived, and, in some cases, against related viruses (for further details, *see* Chapter 3). Since it is not possible to predict from the literature when protein expression is not required, the procedure described in this chapter will be designed to obtain CP expression. The initiation codon of the CP may be deleted or mutated to prevent production of the protein.

To date, the majority of CP-mediated resistant transgenic plants have been dicots, since the majority of species are more amenable to transformation by the naturally occurring soil bacterium *Agrobacterium tumefaciens* and are more easily regenerated to whole plants than monocots. However, recent improved regeneration efficiency of cereals (especially rice and maize) and the demonstration that rice may be transformed by *Agrobacterium* ***(1)*** are likely to lead to the extension of the technique to the economically important cereal crops.

Wild-type *Agrobacterium* strains produce tumors on host-plant tissues, which prevent subsequent regeneration of plants; therefore, disarmed strains, in which a portion of the Ti (tumor-inducing) plasmid has been deleted, have been produced, e.g., LBA4404, pGV3850 ***(2,3)***. These strains are nononcogenic, but still possess the virulence (*vir*) genes necessary for the transfer of foreign genetic material to the plant.

For *Agrobacterium*-mediated gene transfer, the foreign DNA must be present between the left (LB) and right (RB) borders of the Ti plasmid. The large size of the Ti plasmid (wild-type plasmids are approx 200 kb) precludes its direct

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