

The construction of a fungal immunomodulatory protein LingZhi-8 by transgenic plant and viral vector

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ABSTRACT

The *Ganoderma lucidum* LZ-8 protein with immune regulatory capacity is a very valuable protein. In order to express the LZ-8 protein in plant system, lz-8 gene was constructed into a plant viral vector and a plant transformation vector, respectively. In the plant transgene approach, three constructs were obtained: (1) LZ-8: the lz-8 gene sequence was originally obtained from *G. lucidum*, (2) LZKD: the lz-8 gene was the same as that in (1) but with additional nucleotide sequences corresponding to KDEL signal peptide at the 3' end of the lz-8 gene, (3) synLZKD: the construction approach was the same as (2) except that the lz-8 gene sequence was modified according to the codon optimization for the express in tomato and squash. These three lz-8 gene were cloned into Ti vector pGA482G and introduced into *Nicotiana benthamiana* by *Agrobacterium*-mediated transformation. The transformed tobacco leaf discs were screened on 100 mg L⁻¹ kanamycin and 300 mg L⁻¹ carbenicillin medium. Eleven putative LZ-8 transgenic lines, 8 putative LZKD transgenic lines, and 16 putative synLZKD transgenic lines were obtained after antibiotic selection. Five LZ-8 transgenic lines, 2 LZKD transgenic lines, and 10 synLZKD transgenic lines were further confirmed by PCR amplification with primers specific to nptII and lz-8 genes, respectively. Additionally, two Zucchini yellow mosaic virus (ZYMV) vector with the lz-8 gene were constructed: (1) ZYLZ8: the lz-8 nt sequence was originally from *G. lucidum*, (2) ZYsynLZKD: lz-8 gene was modified using the optimize codon for the expression in tomato and squash. These two constructs were introduced in *Cucurbita moschata* by particle gun bombardment. The squash leaves showed systemic symptom with mosaic and leaf curl down at 10-14 d.p.i. Squash inoculated by ZYsynLZKD expressed more severe symptom than plants inoculated with ZYLZ8 and wild type ZYMV. The wild type ZYMV only showed mosaic symptom. Total RNA was extracted from the symptomatic upper leaves of the inoculated squash and the transcript of lz-8 gene was confirmed by RT-PCR amplification coupled with PvuII and XhoI digestion. In this study, the lz-8 gene was successfully constructed into transgenic tobacco and ZYMV viral vector. The plant express system of the LZ-8 protein will be further analyzed.

Keywords : Zucchini yellow mosaic virus (ZYMV)、*Agrobacterium*-mediated transformation、*Cucurbita moschata*、*Nicotiana benthamiana*

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