University of Tsukuba
Plant Transgenic Design Initiative
49th PTraD Research Seminar
T-PIRC Research Seminar

Date and Time: 2019/9/3 (Thu) 14:15 –15:00
Place: Gene Research Center, Seminar Room (211)

Studies on Genetic Engineering of *Oncidesa* Plants for Virus Resistance and Prolonged Shelf-life

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In this talk I will review firstly the establishment of a transformation system for *Oncidesa* using embryogenic callus. The embryogenic callus was induced from shoot apex tissues of *Oncidesa* ‘Gower Ramsey’, and the derived callus cultures maintained more than 10 years were viable in growth and exhibited high regeneration capability to develop PLB. Histological observations showed a unicellular origin for these PLBs. Cymbidium mosaic virus (CymMV) and Odontoglossum ringspot virus (ORSV) are the most common and serious diseases affecting the development of the orchid. To enhance the resistance of orchids against CymMV and ORSV, RNA interference (RNAi)-mediated resistance was employed. Highly conserved regions of 21 or 25 nt derived from the coat protein (CP) gene of CymMV and ORSV were chosen to construct the inverted repeat region that may form a hairpin structure. Various hairpin-type small interfering RNA expression RNAi vectors were constructed to facilitate CP mRNA degradation and subsequent loss of target CP expression. To test the efficiency of these constructs, reporter plasmids containing the CP gene of CymMV or ORSV were used. A fast and reliable quantitative analysis for gene silencing could be achieved by attaching the target CP gene to the gene encoding green fluorescent protein (GFP) and assaying the inactivation of GFP expression. Transient expression of RNAi vectors was performed in *Arabidopsis* protoplasts by polyethylene glycol (PEG) transformation and analyzed by flow cytometry. The silencing efficiency for these vectors ranged from 60% to 85% as determined by flow cytometric analysis. *OgEIN3* RNA-mediated silencing construct was transformed into *Oncidesa* by *Agrobacterium tumefaciens*. Confirmation of integration of T-DNA into leave was performed by positive GUS staining. The transgenic lines were confirmed by PCR and Southern analysis. Transgenic plant *EIN3* gene expression detected by qPCR was lower than untrasformant. In the transgenic plants the longevity was approximately 24 days longer for individual cut flowers and 33 days longer for flowers within inflorescences compared with those of the non-transgenic plants.

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