Molecular Breeding R.G., BioGreen 21 Program, RDA (Ramada Plaza Jeju, November 2-3, 2005)

과제 일련번호: 6

# Generation of virus-resistant *Phalaenopsis via Agrobacterium* and biolistic bombardment-mediated transformation

Young Jin Jeon[1], Kyung Jin Jung[1], Jong Hoon Kim[2], Doo Hwan Kim[1*]

[1]Department of Molecular and Biotechnology, Konkuk University; [2]Biotechnology & Breeding Research Institute, KV bio Inc., Seoul, 143-701, Korea
(*kimdh@konkuk.ac.kr)

In Korea, *phalaenopsis* is becoming more and more popular in parallel with its cultivation area being expanded. Yet, only very few technological efforts have been made for quality trait improvement including disease resistance. ORSV (Odontoglossum ringspot virus) and CymMV (Cymbidium mosaic virus) are very well known to inflict a serious damage to *phalaenopsis*. In order to obtain a new cultivar resistant to these viruses, we introduced ORSV and CymMV genes into *phalaenopsis* PLB cells using either *Agrobacterium* or particle bombardment-mediated transformation methods. PLBs used for the transformation were derived from several excellent cultivars in KV Bio. Inc. For *Agrobacterium*-mediated transformation, different pre-culture (0, 1, 2, 3, 4, 5 day) and co-culture (5, 15, 30, 45 min) times were tested for transformation efficiency. For biolistic bombardment, different particle sizes (0.7, 1.0m), projectile distances (3, 6, 9, 12cm), and helium pressures (900, 1100, 1350psi)were tested. Two-day pre-culture plus 15-min co-culture time led to the highest *Agrobacterium*-mediated transformation efficiency. As for biolistic bombardment, the best response was obtained at 1100 psi of helium pressure along with 1.0 m of tungsten particle size and 6 cm of target distance. Three months after culturing in hygromycin selection media, three putative plants transgenic for each gene construct were obtained by *Agrobacterium*-mediated transformation. The status of transformation and transgene expression was further confirmed by PCR and RT-PCR, and Southern blot analysis showed the presence of a single copy transgene in all the three transgenic plants. All these plants were transferred to pots in the greenhouse for investigation of environment adaptation, mutation and growth status through special seedling control. Using particle-mediated gene delivery system, three putative transformants for ORSV and one for CymMV were obtained and analyzed by PCR and RT-PCR. All of these plants were now being grown in the greenhouse.

---

† 주관과제명 (과제책임자): 호접란 내병성 품종 육성을 위한 유전자 클론닝 및 형질전환 (건국대학교 김두환)

‡ 총연구기간 (년차): 2003년 - 2006년 (3년차)