호접란 바이러스 저항성 품종 육성을 위한 유전자 클론닝 및 형질전환

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Objectives

난바이러스 저항성 유전자 분석과 형질전환 벡터 제작 및 검정기술 개발과 A*grobacterium*과 Gene gun을 이용한 호접란 바이러스 저항성 품종 육성

Materials and Methods

1. Materials : · Cultivars - T101, 168, Cho
· Strain - LBA4404
· Genes - Odontoglossum ring spot virus (ORSV) coat protein
Cymbidium mosaic virus (CymMV) coat protein

2. Methods : · ORSV와 CymMV의 coat protein을 분리하기 위해 virus에 infection된 호접란으로부터 호접란 및 바이러스의 total RNA를 Proteinase K/SDS method를 이용하여 분리
· RT-PCR 결과 477bp의 ORSV와 651bp의 CymMV의 유전자를 클로닝
· RT-PCR product는 selection marker로 hygromycin과 spectinomycin을 가지고 있는 pMJ102 binary vector에 subcloning
· Transformation by *Agrobacterium* (LBA4404) strain and gene gun (Biolistic PDS-1000)
· Selection by hygromycin
· Analysis by GUS, PCR, RT-PCR and Southern blot

Results and Discussion

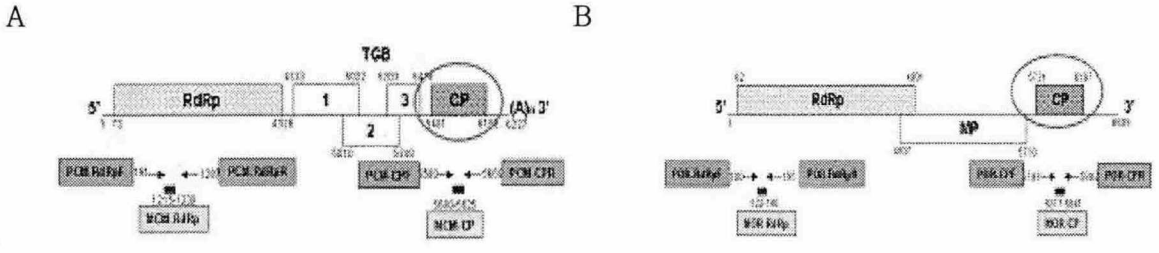

Fig 1. The full-length cDNA of CymMV gene(A) and ORSV gene(B)

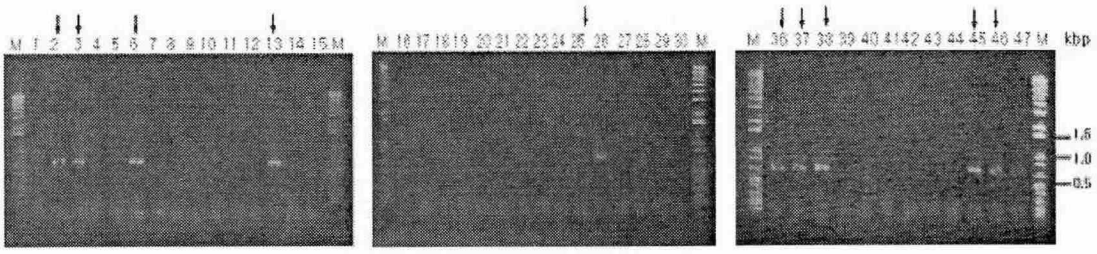

Fig 2. RT-PCR detection of CymMV gene in *Phalaenopsis*

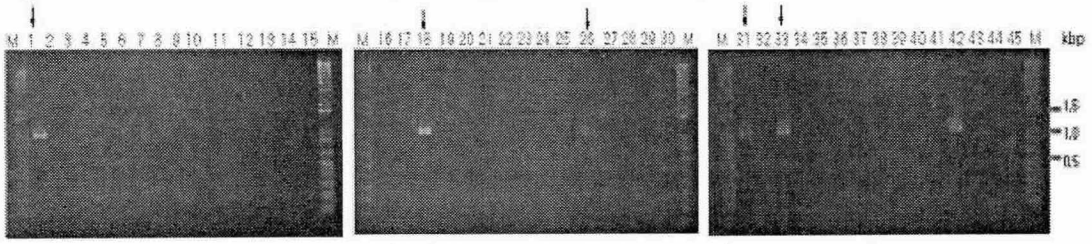

Fig 3. RT-PCR detection of ORSV gene in *Phalaenopsis*

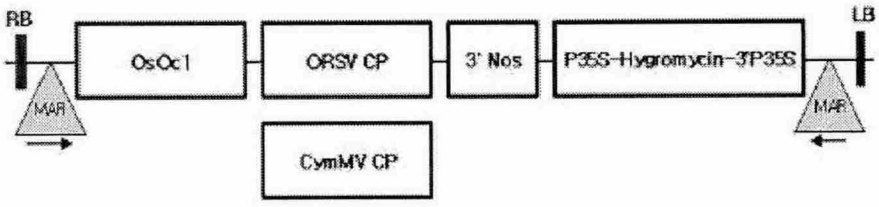

Fig 4. Structure of the vector with CymMV and ORSV coat protein genes. OsCc1;O.sativa cytochrome C promoter, att ;attR cassette of gateway system, MAR; 5'-Matrix Attachment Region of chicken lysozyme gene

Table 1. Effect of pre-culture time on transformation effciency in *Phalaenopsis.* (cv. T101)

	Pre-culture time (Day)					
	0	1	2	3	4	5
Regeneration rate (%)	13.6	20.3	38.3	59.3	72	60
Mean No. of regenerated PLBs	13	27	38	28	22	21
Transformation rate (%)	23	27	31	28	22	24

Table 2. Effect of infection time on transformation effciency in *Phalaenopsis.* (cv. T101)

	Infection time (Min)			
	5	15	30	45
Regeneration rate (%)	39	54	50	46
Mean No. of regenerated PLBs	5.3	6.1	5.7	5.8
Transformation rate (%)	23.2	34	27.8	26.3

Table 3. Effect of distance of gene gun on transformation in *Phalaenopsis.* (cv. T101)

Distance(cm)	Regeneration rate (%)	Mean No. of regenerated PLBs	Transformation rate (%)
3	73	2.3	26.5
6	76	2.7	30.8
9	57	2.7	28.2
12	52	3.0	27.8

Table 4. Effect of helium gas of gene gun on transformation in *Phalaenopsis.* (cv. T101)

Tungten particle size (micron)	Regeneration rate (%)	Mean No. of regenerated PLBs	Transformation rate (%)
1.1	76	2.7	30.8
0.7	31	2.3	27

Table 5. Effect of helium gas of gene gun on transformation in *Phalaenopsis.* (cv. T101)

Helium gas pressure(psi)	Regeneration rate (%)	Mean No. of regenerated PLBs	Transformation rate (%)
1350	52.5	1.9	28.2
1100	76	2.7	30.8
900	47.5	1.2	27.2

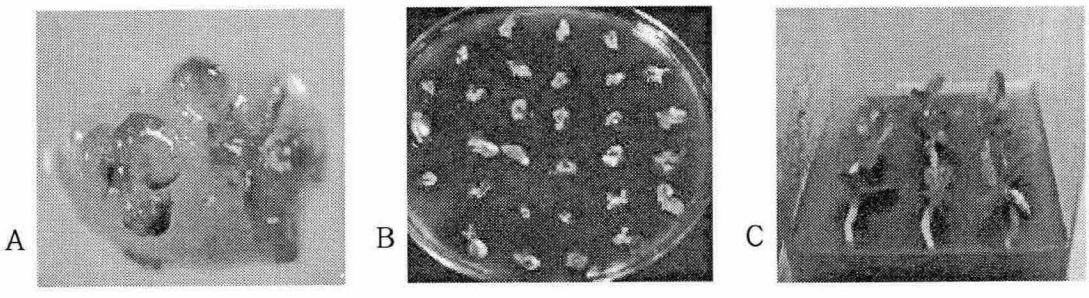

Fig 5. A. GUS assay of PLB after co-cultivation B. The selection of transgenic plants in hygromycin medium. C. The putative transgenic plants

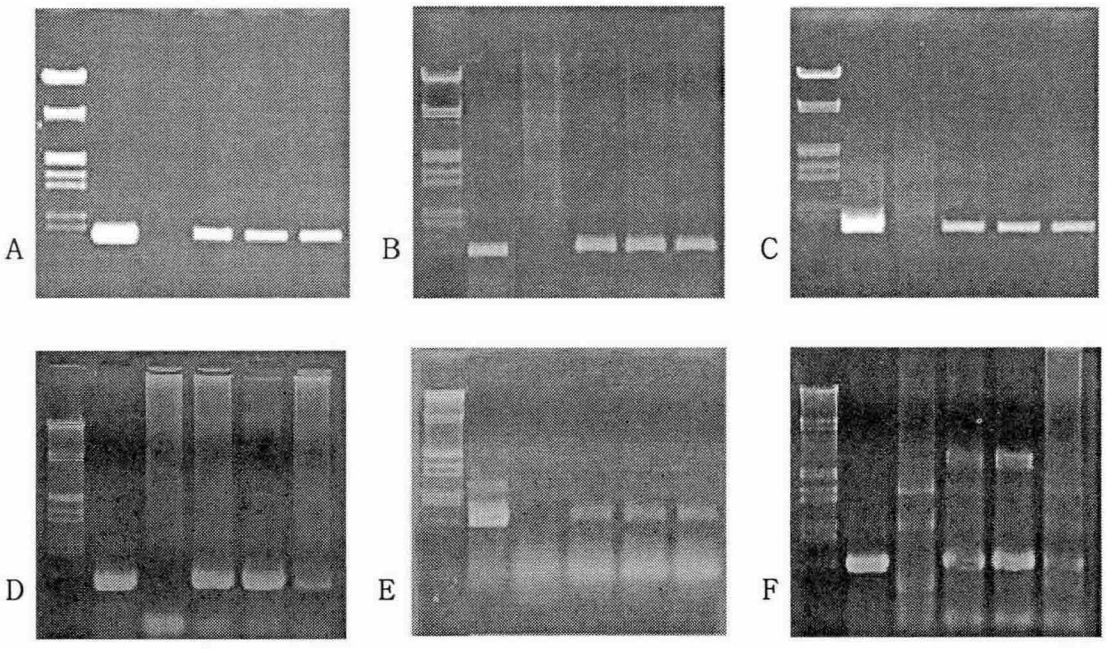

Fig. 6. PCR and RT PCR analysis of transgenic plants with CymMV(A-C) and ORSV(D-F) genes. A, D. PCR amplification of CymMV(A;667bp) and ORSV(D;493bp) genes specific primers in transgenic plants, M: marker, +:positive control, -:negative control, 1-3:transgenic plants. B, E. PCR amplification(564bp) of hygromycin primers in transgenic plants. C, F. RT PCR amplification of CymMV(C;667bp) and ORSV(F;493bp) genes specific primers in transgenic plants.

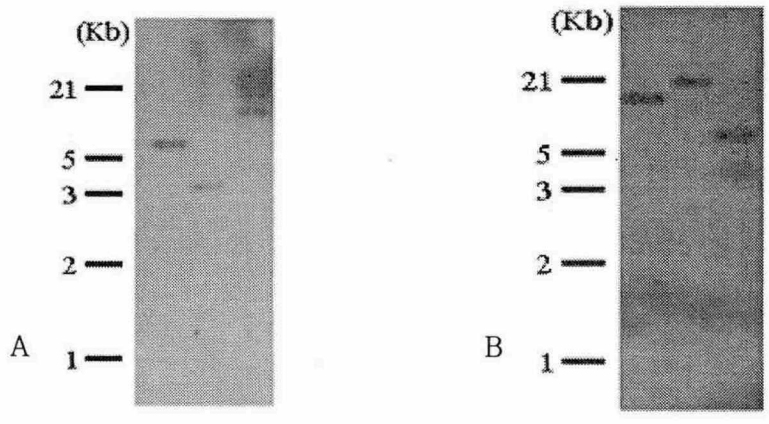

Fig. 7. Southern hybridization analysis of transgenic plants with CymMV gene(A) and ORSV gene(B)