

# Capsid Protein Gene-Mediated Resistance to Barely Yellow Mosaic Virus and Barely Mild Mosaic Virus in Transgenic Barely

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## Introduction

Barely yellow mosaic(BaYMV) and barely mild mosaic (BaMMV) bymoviruses are both transmitted by the soil-inhabiting fungus *Polymyxa graminis*, and are responsible for economic losses in barley crops in Asia and Europe. Because chemical control of the vector is ineffective, the losses can only be prevented by growing resistant barley cultivars. The objective of this study is to produce resistant barley plants by transformation with viral coat protein(cp) genes.

## Materials and Methods

Plant expression vectors, containing the BaYMV (strainHN) or BaMMV(strain Kor)CP genes downstream of modified 35S promoter(with 7 copies of enhancer) and intron 1 of phaseolin gene were used to transform immature embryos of barley cV. New Golden by particle bombardment. Regenerated plants possessing the CP gene were selected by PCR and Southern hybridization, and self-fertilized.

Seedlings of T1 or T2 lines were tested by mechanical inoculation for virus resistance, and examined by PCR and Western blot analysis for CP Virus infection was assessed by ELISA and RT-PCR.

**Table 1.** Numbers of barley plants(T<sub>0</sub>) transformed with BaYMV or BaMMV coat protein genes

Plasmid	BaYMV-CP			BaMMV-CP		
	Embryos	Hyg- Resist	CP (+)	Embryos	Hyg- Resist	CP (+)
A + B	1740	43	9	1590	67	19
C	1650	52	8	2250	78	46
Total	3390	95	17	3840	145	65

Transformation with CP genes was examined by PCR and Southern hybridization

Particle Bombardment Bio-rad pds-1000/He 1.6 Gold Particle Stopping plate to target:approx.9cm 30 Immature Embryos/plate 2 Shots/plate 1 Week culture without Hyg Selection

## Results and Discussion

Resistance tests of T<sub>1</sub> plants transformed with the BaYMV CP gene showed that at least four independent lines had clear resistance to BaYMV(i.e. no virus infection) but two other lines were highly susceptible with severe symptoms. The CP gene was detected in all resistant T<sub>1</sub> plants by genomic PCR. Most of T<sub>2</sub> progenies derived from the resistant T<sub>1</sub> lines also showed resistance. In contrast, only one out of 21 independent T<sub>2</sub> lines transformed with the BaMMV CP gene tested showed clear resistance to BaMMV, and others were very susceptible. Further analyses of resistance and CP gene expression are in progress.

Table 2. Mechanical inoculation tests of barley plants (T<sub>1</sub>) transformed with BaYMV CP gene

line no.	infected / tested*
636	0/1 ( 0%)
638	0/9 ( 0%)
749	1**/7 ( 14%)
750	0/13 ( 0%)
754	0/9 ( 0%)
non-transformed	20/20 ( 100%)
602	13/14 ( 93%)
603	14/15 ( 93%)
non-transformed	19/20 ( 93%)

\* Virus infection was assessed by ELISA and RT-PCR

\*\* trait mosaic

To plants	T <sub>1</sub> plants	T <sub>2</sub> plants
line no.	virus infection	infected / tested
636	→ -	→ 0 / 6
638	→ -	→ 0 / 9
	-	→ 1 / 4
	-	→ 1 / 6
749	→ +	→ 1 / 14
	-	→ 0 / 6
	-	→ 1 / 7
750	→ -	→ 2 / 9
	-	→ 0 / 7
	-	→ 0 / 9
754	→ -	→ 1 / 6
	-	→ 0 / 6

+ faint mosaic

Fig. 1. Resistance tests of BaYMV-CP T<sub>2</sub> progenies

Table 3. Mechanical inoculation tests of barley plants (T<sub>2</sub>) transformed with BaMMV CP gene

line no.	infected / tested*
1	4/5 ( 80%)
2	0/6 ( 0%)
3	6/12 ( 50%)
4	8/11 ( 73%)
5	0/9 ( 63%)
6	11/15 ( 73%)
non-transformed	20/20 ( 100%)
7	11/12 ( 92%)
8	3/5 ( 60%)
9	8/10 ( 80%)
10	11/14 ( 79%)
non-transformed	18/20 ( 90%)