TRANSFORMATION OF CLONED AND PARTIALLY MODIFIED COAT PROTEIN AND REPLICASE SEQUENCES OF BEET WESTERN YELLOWS VIRUS (BWYV) INTO *NICOTIANA BENTHAMIANA* AND *BRASSICA NAPUS*

G. LAUCKE, E. MAISS, J. SCHIEMANN

Federal Biological Research Centre, Institute for Biochemistry and Plant Virology, Messeweg 11/12, 38104 Braunschweig, Germany

ABSTRACT

Recent surveys have confirmed the widespread occurrence of the aphid-transmitted beet western yellows virus (BWYV) in winter rape. In order to increase the BWYV resistance in this important crop plant we have cloned and partially modified coat protein and replicase sequences of the virus for the transformation into *Nicotiana benthamiana* and *Brassica napus*. The different molecular approaches including translatable and non-translatable viral sequences are also considered under biosafety aspects.

EXPERIMENTAL

The sequences which have been transformed by Agrobacterium-mediated gene transfer into *Nicotiana benthamiana* and *Brassica napus* include:

a) the native coat protein gene (protein expression; CP),
b) the coat protein gene (protein expression) with a non-translatable internal "VPg" (CP-VPg),
c) the coat protein gene made non-translatable by the introduction of several stop-codons (NLATCP),
d) the 19kD "VpG" (protein expression; VPg),
e) the replicase gene (protein expression; REP),
f) different C- and N-terminal sequences of the replicase gene (ORF3^5', ORF3^3').

Until now, more than 50 *Nicotiana benthamiana* and 26 *Brassica napus* plants have been regenerated on kanamycin. The transformed oil seed rape (Tab. 1) and tobacco plants (Tab. 2) have been tested for GUS activity, NPT activity (NPT-ELISA), and presence of the nptII gene by PCR, northern and/or southern hybridization. The segregation of the tobacco R1 plants has been tested on kanamycin-containing medium. Tobacco R1 plants have been aphid-infected with BWYV. First results of virus resistance tests with transgenic tobacco and oil seed rape plants will be presented.

Table 1. Regenerated oil seed rape plants after agrobacterium transformation

No.	Construct	NPT-PCR	GUS-Assay	Northern (NPT)	NPT-ELISA	Vector
1	CP-VPg			positive	0.071	pGV3850
2	ORF3*5´	positive		positive	0.012	pGV3850
3	ORF3*5´			positive	0.092	pGV3850
4	ORF3*5´			positive	0.135	pGV3850
5	ORF3*3´			positive	0.000	pGV3850
6	CP-VPg			positive	0.063	pGV3850
7	CP-VPg			positive	0.156	pGV3850
8	ORF3*3´	positive		positive	0.086	pGV3850
9	ORF3*3´			positive	0.104	pGV3850
10	CP-VPg			positive	0.000	pGV3850
11	CP-VPg			positive	0.300	pGV3850
12	ORF3*5´			positive	0.088	pGV3850
13	CP-VPg			positive	0.011	pGV3850
14	CP-VPg			positive	0.091	pGV3850
15	CP-VPg			positive	0.028	pGV3850
16	ORF3*5´	positive	positive		3.000	EHA 101/pLX
17	ORF3*5´	positive	positive		0.052	EHA 101/pLX
18	ORF3*5´	positive	positive			EHA 101/pLX
19	CP-VPg					pGV3850
20	CP-VPg					pGV3850
21	CP-VPg					pGV3850
22	NLATCP		positive		0.025	EHA 101/pLX
23	NLATCP				0.029	EHA 101/pLX
24	ORF3*5´				3.000	EHA 101/pLX
25	ORF3*5´				2.001	EHA 101/pLX
26	ORF3*5´				3.000	EHA 101/pLX

Table 2. Regenerated *Nicotiana benthamiana* plants after agrobacterium transformation

No. & Construct	GUS-Assay	NPT-PCR	R1-seeds on kanamycin-plates	Southern
REP				
6	positive	positive	Segregation	n.t.
20	positive	positive	Segregation	n.t.
35	negative	negative	Segregation	n.t.
37	positive	n. t	Segregation	n.t.
67	n. t.	positive	Segregation	n.t.
CP				
30	positive	n.t.	Segregation	n. t.
31	positive	n.t.	Segregation	n. t.
32	positive	n.t.	Segregation	positive
CP-VPg				
62	negative	positive	Segregation	n.t.
63	positive	n. t.	Segregation	positive
82	n. t.	positive	Segregation	n.t.
101	n. t.	n.t.	Segregation	n.t.
NLATCP				
92	n.t.	positive	Segregation	n.t.
93	n.t.	positive	Segregation	n.t.
94	n.t.	positive	Segregation	n.t.
140	n.t.	negative	Segregation	n.t.
VPg				
103	n. t.	n.t.	Segregation	n.t.
130	n. t.	n.t.	Segregation	n.t.
131	n. t.	n.t.	Segregation	n.t.
133	n. t.	n.t.	Segregation	n.t.
ORF3*5`				
121	n. t	n.t.	Segregation	n.t.
ORF3*3`				
97	n. t	n. t	Segregation	n.t.
104	positive	n. t	Segregation	n.t.
105	n. t	n. t	Segregation	n.t.