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Production of full length infectious cDNA clones of *Strawberry mild yellow edge virus* (SMYEV) and transmission trials with the strawberry aphid (*Chaetosiphon fragaefolii*)

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Introduction: *Strawberry mild yellow edge virus* is a well characterized member of the Potexvirus genus. The virus is the causal agent of the economically important SMYE disease of strawberry. Its genome has been completely sequenced and infectious cDNA clones obtained (1, 2). The diversity of SMYEV has been investigated (4). Despite SMYEV variability, efficient detection assays are available. SMYEV can readily be detected using ELISA or molecular detection assays as well as traditionally used biological indexing in susceptible indicator plants. Surprisingly for a Potexvirus, which are not usually vector borne, SMYEV is transmitted by the strawberry aphid *Chaetosiphon fragaefolii* (Cockerell). In earlier experiments no vector transmission from strawberry plants was achieved after infection with a full length cDNA clone of isolate MY-18 (2). Since this isolate was kept for a longer period of time on *Rubus rosifolius* as experimental host a number of full length infectious cDNA clones were generated from seven strawberry isolates in this study. Transmission trials were initiated to investigate the unusual aphid borne transmission of the virus in nature.

primer names	Sequence	Tm
SYEpolyTB	TTTTTTTTTTTTTTTAAGGAAAAAGAAAAACAAAC	
SMYE_INf5	TTTCATTTGGAGAGGGAAAACATAACCAACCCACTCTAGA	63°C
SMYE_INf3	ATGCCATGCCGACCCAAGGAAAAAGAAAAACAAACCTATACTAGT	61

Table1: Primers used for RT and In-Fusion full length cDNA cloning; Virus seq.+15 bp vector nucleotides

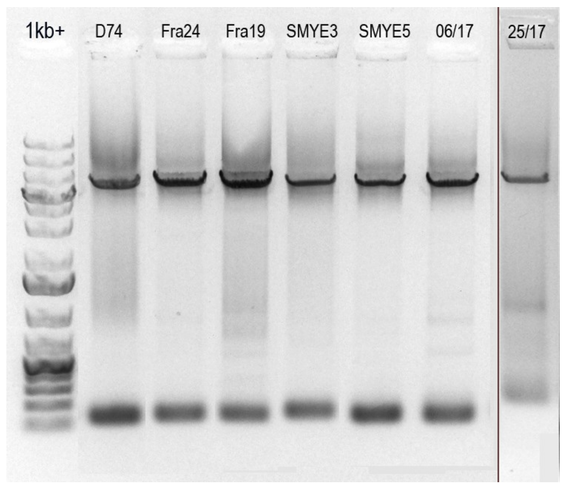

Fig. 1: 1: marker; 2-7 full length PCR fragments of SMYEV isolates (size 6 kb)

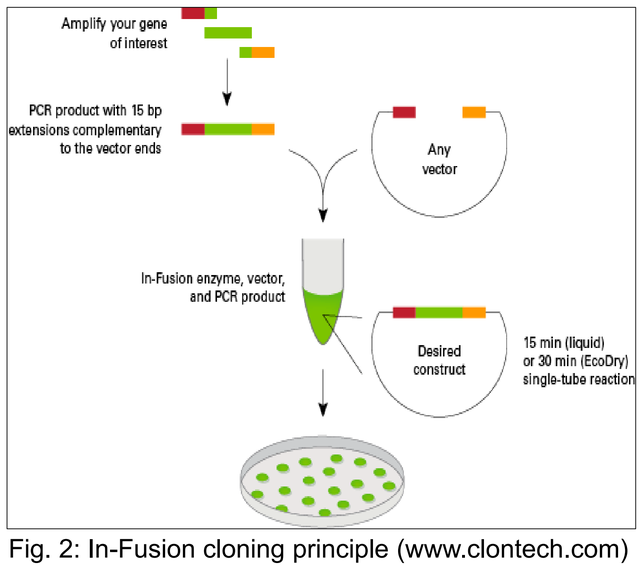

Fig. 2: In-Fusion cloning principle (www.clontech.com)

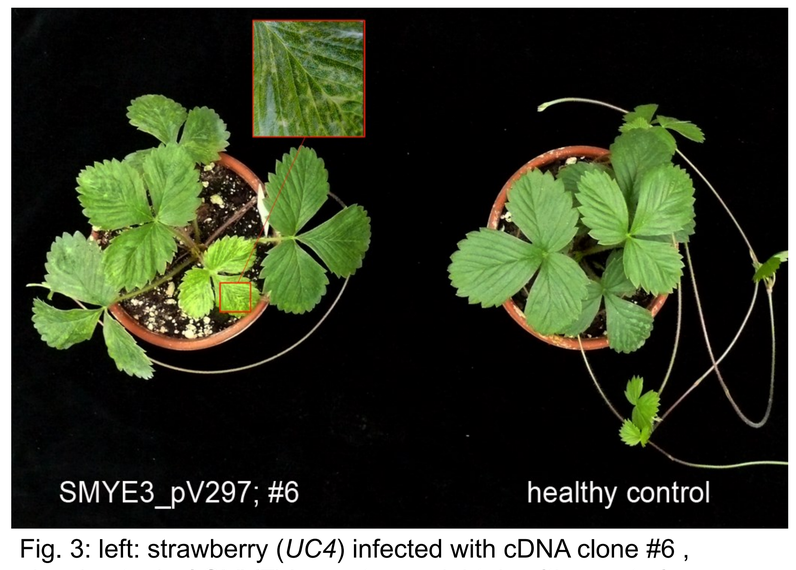

Fig. 3: left: strawberry (*UC4*) infected with cDNA clone #6 , showing typical SMYEV symptoms; right: healthy control

Materials and Methods: Seven different isolates of SMYEV, 3 greenhouse and 4 field-grown sources, were chosen for cDNA cloning. RNA was extracted with RNeasy Plant Mini Kit (Qiagen) from 50 mg infected strawberry leaf tissue. RT-PCR was performed with RevertAid Maxima Reverse Transcriptase (ThermoScientific) using specific primer SYEPolyTb (Tab. 1). The obtained cDNA was used for full length PCR with the aid of Precisor High-Fidelity DNA Polymerase (BioCat) and primers SMYE_Inf5 and SMYE_Inf3 (Tab. 1). PCR fragments (Fig. 1) were cleaned up with PCR Purification Kit (Qiagen) and mixed with pBin Vector pV297 obtained from E. Maiss, Hannover (3) and In-Fusion cloning enzyme mix (Takara Clontech Bio Europe) (Fig. 2). $CaCl_2$ competent cells (Stellar™, Takara Clontech Bio Europe) were transformed following providers instructions. Colonies were screened by PCR with detection primers SMYE F/R (5). Plasmids from overnight in LB(kan) grown cultures were prepared using Qiagen Miniprep Kit and subsequently digested with Bam HI (NEB). Agrobacteria (ATHV) were transformed with full length SMYEV cDNA clones and used for inoculation of *Fragaria vesca* UC4 or UC5 strawberry plants.

isolate	full length cDNA clones	infectious cDNA clones	pos. plants/ total no. of plants	efficiency	no. of plants for aphid transm. *(cDNA clone infected)*	transmitted	no. of plants for aphid transmission *(original source)*	transmitted
D74*	6	5	15 /41	37%	9	0	36	36 x SMYEV 36 x SPV1
			6 /10	60%				
			2 /3	67%				
			8 /20	40%				
			1 /3	33%				
fra24	2	1	18 /20	90%	6	0	not available	
fra19	1	1	3 /20	15%	9	0	not available	
SMYE3	22	2	5 /25	20%			18	0
			34 /46	74%	18	0		
SMYE5	1	1	23 /26	88%	9	0	18	0
06/17	4	2	19 /26	69%	32	0	not available	
			2 /4	50%				
25 /17**	5	4	1 /10	10%	9	0	40	0 x SMYEV 8 x SCV+
			11 /19	58%				
			2 /4	50%				
			2 /4	50%				

Table 2: Inoculation and aphid transmission experiments;

*original source D74 is infected with SMYEV and SPV1.

**original source 25/17 is infected with SMYEV and SCV.

Results and Conclusion: SMYEV full length infectious cDNA clones were obtained from seven different isolates (Tab. 2). 16 out of 41 plasmids were able to infect strawberry indicator plants by agroinculation. Typical induced symptoms are depicted in Fig. 3. Of the four original isolates (1 recently obtained from the field) available in the greenhouse aphid transmission was initiated. SMYEV could be transmitted from original mixed infection with SMYEV and SPV1 for isolate D74. Only *strawberry crinkle virus* (SCV) could be transmitted for mixed infection (SMYEV+SCV) isolate 25/17. Further aphid transmission experiments from original isolates and from agroinoculated UC4 and UC5 plants are carried out. Depending on the outcome of the aphid transmission trials, co-infection experiments with strawberry polerovirus 1 (SPV1) (6) are intended to evaluate a potential helper function of this virus in vector transmission.

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