Abstract

Orchid, amongst the most popular flowers in the world, play a major role in the economy of many Asian countries. However, detrimental effects by various viruses can reduce the economic value of this horticultural crop. There have been a number of viruses known to occur in orchids globally with one of the most common being Cymbidium mosaic virus (CymMV). Plant genetic engineering approaches have been successful in producing crops with viral resistance, however there has been a concern expressed that resistance resulting from single gene constructs might be rather narrow (specific to only certain virus sub-type) could easily break down. Thus this research aimed to develop a multiple strategy approach towards the control of CymMV in orchids by combining the concepts of pathogen derived resistance and antibody mediated resistance. A plant transformation vector, pCAMBIA 1304-CymMV-fwd was constructed to contain coat protein sequences from the CymMV. The construct was successfully transformed into explant samples and RT-PCR analysis showed that the pathogen derived resistance sequence was successfully expressed in plants. In a second part to the study anti-CymMV was isolated from a *Toxoplasma gondii*-immunized single-chain variable fragment (ScFv) antibody phage-display library. Four antibodies were isolated and analyzed through BLASTX and nucleotide BLAST but only one provided significant similarity to PDPK1 3-phosphoinositide dependent protein kinase-1 [Homo sapiens]. The two genes have the potential to be pyramided together in a single plant transformation vector, or to be introduced into plants by successive transformation, in order to develop orchids with longer term viral resistance.

Abstrak

Bunga orkid adalah antara bunga yang paling popular di dunia dan memainkan peranan besar dalam ekonomi banyak negara-negara Asia. Akan tetapi, orkid yang dijangkiti virus akan merosakkan bunga-bunganya dan sekaligus mengganggu hasil penjualan bunga-bunga ini. Terdapat pelbagai virus yang dikenal pasti merosakkan orkid dan yang paling membinasakan bagi orkid iaitu Cymbidium mosaic virus (CymMV). Kini terdapat semakin banyak penyelidikan dalam bidang genetic molekul tumbuhan yang dijalankan untuk menghasilkan tumbuhan yang resistan terhadao virus-virus yang membinasakan ini melalui teknik "gene silencing". Walau bagaimanapun adalah menjadi kebimbangan sesetengah pihak bahawa melalui model "gene silencing" ini hanya mampu menghalang sejenis virus sahaja dari terus menular dan hasilnya adalah sangat spesifik terhadap sejenis virus sahaja serta mudah untuk dibinasakan oleh virus. Oleh itu dalam penyelidikan ini, kami bercadang untuk menggunakan beberapa strategi genetic molecular dalam membina vector transformasi tumbuhan (plant transformation vector) yang mampu mengawal penyebaran virus ini. Kami telah menggunakan konsep "pathogen derived resistance" dan antibody mediated resistance" dalam penyelidikan kami. Kami telah Berjaya membina vector transfomrasi tumbuhan pCAMBIA 1304-CymMV-fwd dengan menggunakan kot protin CymMV. Konstruk ini telah Berjaya ditransfomrasikan ke dalam sampel tumbuhan kami dan analisis melalui RT-PCR menunjukkan jujukan "pathogen derived resistance" Berjaya diekspreskan ke dalam tumbuhan. Untuk bahagian kedua penyelidikan ini, anti-CymMV telah Berjaya diisolasi daripada "toxoplasma gondii-immunized single-chain variable fragment (ScFv) antibody phage display library". Empat antibody dianalisa bagaimanapun hanya satu memberikan kepututsan yang signifikan apabila dibandingkan dengan menggunakan BLAST nukleotida dan BLASTX. Kedua-dua konstruk mempunyai potensi untuk

digabungkan bersama membentuk vector pyramid dalam vector transformasi tumbuhan untuk digunakan dalam membentuk orkid yang mempunyai jangka resistan yang lebih panjang.

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Abbreviation

A	Adenine
ATCC	America Type Culture Collection
BSA	Bovine Serum Albumin
C	Cytosine
Cm	centimeter
°C	degree Centigrade/Celcius
CMV	Cucumber mosaic virus
CP	coat protein
CPMP	coat protein-mediated protection
CymMV	Cymbidium mosaic potexvirus
DCL	dicer like
dsRNA	double stranded RNA
E.coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
e.g.	example
ELISA	Enzyme-linked immunosorbent assay
G	Guanine
g	gram
GFP	green fluorescent protein
GUS	beta-glucuronidase
HCl	hydrochloric acid
IPTG	Isopropyl-β-D—Thiogalactosidase
kb	kilobase
K_2HPO_4	potassium phosphate dibasic
L	litre
LB	Luria Bertani
M	Molar
mg	milligram
mL	mililitre
mm	milimetre
mM	milimolar
mRNA	messenger RNA
mg	microgram
µL	microliter
NaCl	sodium azide
nm	nanometer
OD	optical density
OPD	O-phenylene diamine
ORSV	Odontoglossum Ringspot Tobamovirus
PBS	phosphate buffered saline
PBST	phosphate buffered saline- Tween 20
PCR	polymerase chain reaction
PDR	Pathogen derived resistance
PEG	Polyethylene glycol
pfu	plaque forming unit
pg	pictogram
PTGS	post transcriptional gene silencing
QCM	Quartz crystal microbalance
RdRp	RNA-dependent RNA polymerase

RFLP	Restriction fragment length polymorphism
RISC	RNA-inducing silencing complex
RNA	ribonucleic acid
RPAS	Recombinant Phage Antibody System
RT-PCR	Reverse transcriptase polymerase chain reaction
scFv	single-chain variable fragment
shRNA	small hairpin RNA
T	Thymine
TBE	Tris base
TGS	Transcriptional gene silencing
TMV	tobacco mosaic virus
VIGS	virus induced gene silencing
vsiRNA	virus-derived small interference RNA
v/v	volume / volume
w/v	weight / volume