

*

SILENCING OF RNA DIRECTED RNA POLYMERASE 1 GENE OF TOBACO LEADS TO ENHANCED RESISTANCE TO SOME PLANT VIRUSES

**

(/ / : / / :)

(*Nicotiana tabacum* cv. Samsun NN)
RDR1 . *RDR1*
Tobravirus (TRV) . *Tombusvirus* (TBSV) . (TMV)
.
RDR1

(RNA induced silencing complex, RISC)

(Baulcombe 2004)

RNA dependent RNA)

(RNA directed (polymerase, RdRp
RNA polymerase, RDR)

(Xie *et al.* 2004)

(Astier-Manificier & Cornuet 1971)

RDR

RDR6

RDR

(Schwach *et al.* 2005)

(RdRp)

(Potato virus X, PVX)

Zabel *et al.*

(*al.* 1974; Dorssers *et al.* 1984

DCL

Arabidopsis thaliana

(RISC)

RDR

(Dalmay *et al.* 2000)

RDR1

AtRDR1-AtRDR6

Zabel *et al.* 1974; Dorssers *et al.*

(Khan *et al.* 1986)

(*al.* 1982

(Xie *et al.* 2001)

RDR6

RDR6 RDR1

(Wassenegger & Krczal 2006)

(Schwach *et al.* 2005)

(dsRNA)

RDR6

RNase III

(Dicer like, DCL)

Small)

(PVX)

(interfering RNA, siRNA

...

:

| | | |
|---|---------------------------------------|--------------------------------------|
| (<i>Nicotiana tabacum</i> cv. Samsun NN) (TMV) | RDR6 | (Plum pox virus, PPV) |
| | RDR6 | (Vaislij & Jones 2009) |
| | <i>Nicotiana tabacum</i> | <i>NtRDR1</i> |
| T-46 (Tomato bushy stunt virus, TBSV) | | (Tobacco mosaic virus, TMV) |
| | (Xie <i>et al.</i> 2001) | (PVX) |
| U ₁ (Mowat, 1972) (TMV-U ₁) | <i>AtRDR1</i> | <i>NtRDR1</i> |
| (Canto and (TMV-GFP) (TRV) Palukaitis 2002) | | (<i>A. thaliana</i>) |
| W260 (Liu <i>et al.</i> 2002) (Cauliflower mosaic) J.E. Schoelz virus, CaMV) | | (TMV) (Tobacco rattle virus, TRV) |
| (Red clover necrotic mosaic virus, RCNMV) () S.A. Lommel | | (Yu <i>et al.</i> 2003) |
| (<i>Brassica rapa</i> cv. Just Right) (CaMV) <i>N. clevelandii</i> | | |
| | | <i>N. benthamiana</i> |
| N <i>NtRDR1</i> | <i>N. tabacum</i> cv. Samsun (TMV) | N |
| | | (Maule <i>et al.</i> 2007) |
| | <i>RDR1</i> | |
| (R-5) | | N (<i>NtRDR1</i>) |

/ / /) (R-14)
 (/ Na₂EDTA R-14 R-5
 .(Rakhshandehroo *et al.* 2009)

Mini Trans-Blot® Electrophoretic
 Transfer Cell (Bio-Rad)
 (Hybond N⁺ Amersham) R-14 R-5
NtRDR1
 (Rakhshandehroo *et al.* 2009) °C °C
 °C TMV-GFP
 TMV-GFP
 UV Small) *NtRDR1*
 (Canto & Palukaitis 2002) **interfering RNA blotting (Si-Blotting)**
 (RCNMV) R-14 R-5
 (TBSV) %
 Murshige-Skoog (MS)

N. clevelandii
N. benthamiana
 PCR (CaMV)
RDR-1
 Si-Blotting
 (Vanitharani *et al.* 2003)
 (PCR) R-14)
 5'-GAGAACATAGAAAACTCCTCAT-3' () (R-5)
 5'-GGATTGAAGTTCAACCTATCTG-3' (Canto *et al.* 2002)
 TBE % /

...

:

(CaMV)

°C

°C

/

°C

°C

°C

.() /

TMV-GFP

/

PCR

°C

TBE

N

N

(RDRI)

GeneRuler™100bp

N

°C

(DNA ladder, Fermentas)

(TMV)

TMV-GFP

NtRDRI

R-14

R-5

(*N. tabacum* cv. Samsun NN)

N

.()

N

MS

(TMV)

(Xie *et al.* 2001)

(R-5 R-14)

NtRDRI

NtRDRI *N. tabacum*

(TMV)

.()

.(Xie *et al.* 2001)

R-14 R-5

(Yu *et al.* 2003)

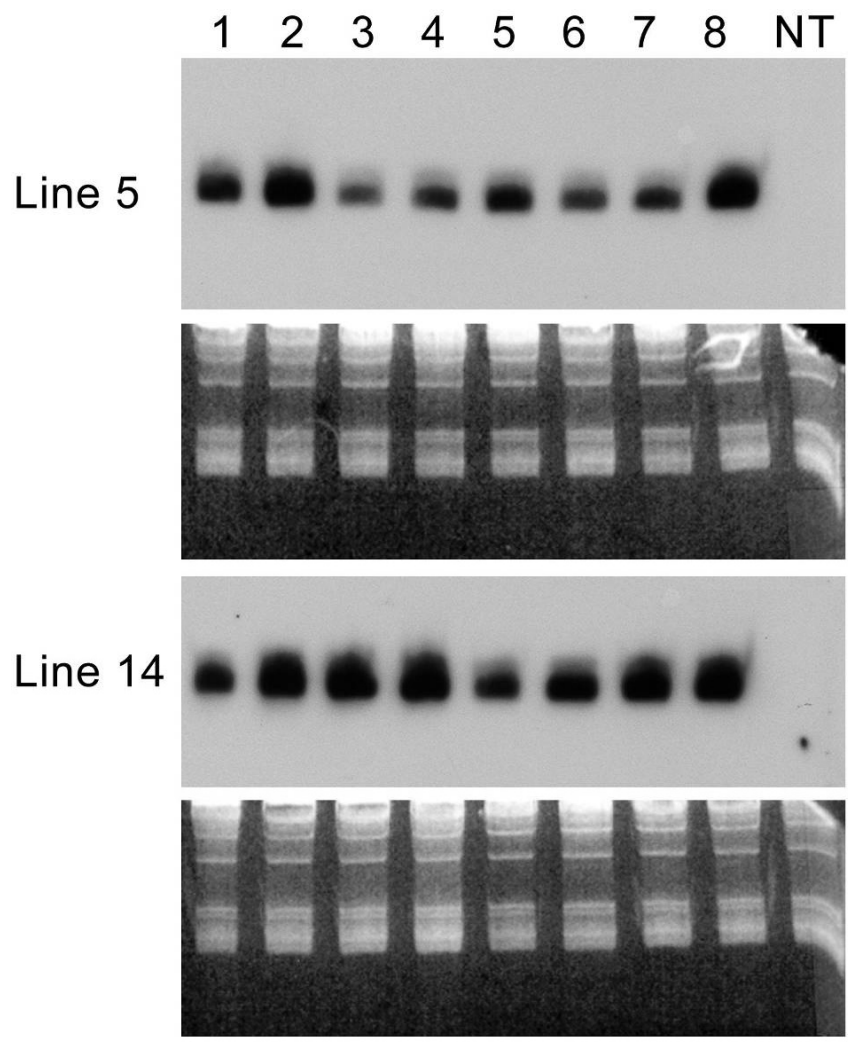
°C

AtRDRI

A. thaliana

(TMV)

(TMV)



() R-5 R-14 si-blotting RNA
 NtRDR1 (*Nicotiana tabacum* cv. Samsun NN) (NT)

Fig. 1. Detection of small RNAs using si-blotting technique in several transgenic R-14 and R-5 plants (1-8) and wild type (NT) tobacco (*Nicotiana tabacum* cv. Samsun NN) by specific NtRDR1 probe.

(*Chenopodium amaranticolor*)
 (TRV) (TRV) R-14 R-5
 (TBSV) () ()
 (back inoculation)



- (*Nicotiana tabacum* cv. Samsun NN) () () R-5
 . °C (TMV)

Fig. 2. Comparison of transgenic line R-5 (left) and wild type (right) tobacco (*Nicotiana tabacum* cv. Samsun NN) reactions to TMV inoculation and incubation at 25 °C.



R-14 (*Nicotiana tabacum* cv. Samsun NN)
 .(TMV-GFP)

Fig. 3. Reaction of transgenic line R-14 (*Nicotiana tabacum* cv. Samsun NN) to Tobacco mosaic virus-GFP.



() () R-5 (*Nicotiana tabacum* cv. Samsun NN)

.(TRV)

Fig. 4. Reaction of transgenic line R-5 (right) and wild type (left) tobacco (*Nicotiana tabacum* cv. Samsun NN) to Tobacco rattle virus (genus *Tobravirus*).

(Yang *et al.* 2004)

RDR1

()

NtRDR1

(CMV)

N. benthamiana

(PVX)

(PPV)

.(Ying *et al.* 2010)

(TRV)

R-14 R-5

(TBSV)

()

(CaMV)

(PVY)

(Arabis mosaic virus, ArMV)

()

N. benthamiana



() () R-5 (*Nicotiana tabacum* cv. Samsun NN)
(TBSV)

Fig. 5. Reaction of transgenic line R-5 (left) and wild type (right) tobaccos (*Nicotiana tabacum* cv. Samsun NN) to Tomato bushy stunt virus (genus *Tombusvirus*).

(RCNMV)

RDR1

(RCNMV)

(CaMV)

RDR1

RDR1

N. tabacum

RDR1

Arabidopsis

RDR1

RDR1

Xie *et al.*)

NtRDR1

RDR-1

(2001)

(*AtRdRP1*)

(Tobacco vine-clearing virus, TVCV) TMV

(Schwach *et al.* 2005) TRV
RDR1 RDR6 (Yu *et al.* 2003)
N. benthamiana
RDR1
N. benthamiana NbRDR1
(TMV) *Medicago truncatula* *MtRDR1*
TVCV TMV *benthamiana*
(TRV) (Yang *et al.*) Sunn hemp mosaic virus
(TBSV) *al.* 2004
NbRDR6
RDR1 (RDR6i) RDR6
PVX, PVY, TRV, TMV, TCV
(Y) CMV
TCV
Y CMV TMV TRV
(NT) RDR6i
(Ying *et al.* 2010) (Y) CMV PVY PVX
RDR6i
(NT)
(Mourrain *et al.*) PVX
(Prof. P. Palukaitis) (2000; Dalmay *et al.* 2000)
RDR6 abRNA
N. benthamiana