

Supplementary Material

Differential suppression of *Nicotiana benthamiana* innate immune responses by transiently expressed *Pseudomonas syringae* type III effectors

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						- Section 1
	(1) 1	,10	20	30		48
HopO1-1 Pta 11528	(1) MGN	ICGTSGSNHV	YSPPISPQ	HASGSSTPVE	SASGTMLS	LSHEQII
HopO1-1 Pto DC3000	(1) MGN	ICGTSGSNHV	YSPPISPQ	HASGSSTPVE	SASGTMLS	LSHEQII
Consensus	(1) MGN	ICGTSGSNHV	YSPPISPQ	HASGSSTPVE	SASGTMLS	SLSHEQII
			10.007F	Sec. 0.23		- Section 2
	(47) 47		60	70	80	92
HopO1-1 Pta 11528	(47) <mark>SQN</mark>	YASNIKGKYF	TNPRKGPS	PRLSDTLMK	ALSSVIT	EKKRLKS
HopO1-1 Pto DC3000	(47) <mark>SQN</mark>	YASNIKGKYF	TNPRKGPS	PRLSDTLMK	ALSSVIT	EKKRLKS
Consensus	(47) SQN	YASNIKGKYF	TNPRKGPS	PRLSDTLMKC	ALSSVIT	EKKRLKS
		100	440	100		- Section 3
U	(93) 93	,100	,110	,120		130
HopO1-1 Pta 11528	(93) QPR	SIAQDIQPPN	ISMIKNALD	EKDSHPFGDC	FSDDEFLA	THLALSC
HopO1-1 Pto DC3000	(93) QPR	SIAQDIQPPN	SMIKNALD	EKDSHPFGDC	FSDDEFLA	THEYTSC
Consensus	(93) QPK	SIAQDIQPPI	SMIKNALD	EKDSHPFGDC	FSDDEFLA	Contion 4
	(120) 139	15	1	160	170	
HonO1-1 Pta 11528	(139) 1.70	PINHHLEVAL	KNDUADUU	FAMESGLART	AOVPDVOI	ISCOLHRO
HopO1-1 Pto DC3000	(139) LYB	PINHHLBYAR	KNDVAPUU	EAMNSGLART	AOYPDYOU	ISGOLHRG
Consensus	(139) LYB	PINHHLRYAE	KNDVAPVV	EAM SGLAKI	AOYPDYO	SGOLHRG
						- Section 5
	(185) 185	190	200	210	220	230
HopO1-1 Pta 11528	(185) TKC	KMDDGEVMSF	FREGNTYR	DDAFMSTSTR	MDVTEEFT	SDVTLHI
HopO1-1 Pto DC3000	(185) IKC	KMDDGEVMSF	FREGNTYR	DDAFMSTSTF	MDVTEEFT	SDVTLHI
Consensus	(185) IKC	KMDDGEVMSF	FKPGNTYR	DDAFMSTSTF	MDVTEEFT	SDVTLHI
						- Section 6
	(231) 231	240	250	260)	278
HopO1-1 Pta 11528	(231) 088	SAVNIGPESE	NPYEDEAL.	IPPLTPFKVI	SLHKQDDF	WHVHLNE
HopO1-1 Pto DC3000	(231) QSS	SAVNIGPESE	NPYEDEAL	IPPLTPFKVI	GLHKQDDF	WHVHLNE
Consensus	(231) QSS	SAVNIGPFSF	NPYEDEAL	IPPLTPFKVI	LHKQDDF	WHVHLNE
	-	2010/141				— Section 7
	(277) 277	284				
HopO1-1 Pta 11528	(277) <mark>IAE</mark>	SSDE-				
HopO1-1 Pto DC3000	(277) <mark>IAE</mark>	SSDE-				
Consensus	(277) TAE	SSDE				

Figure S1. Sequence alignment of HopO1-1_{Pta11528}.

Amino acid alignment of HopO1- $1_{Pta11528}$ and HopO1- $1_{PtoDC3000}$. Identical residues are labelled in yellow.

						-Section 1
	(1) 1	10	20		30	4
HopT1-1 Pta 11528	(1) MKTV3	SNHSIPSTNI	VVDAGAE1	LAQKSQP	VS <mark>SEIQRNS</mark>	KIEKAVIE
HopT1-1 Pto DC3000	(1) MKTVS	SNHSIPSTNI	VVDAGTE1	SAQKSQP	VCSEIQRNS	KIEKAVIE
Consensus	(1) MKTVS	SNHSIPSTNI	VVDAG ET	ACKSOP	V SEIQRNS	KIEKAVIE
	(47) 47	F	0	70	ອາ	Section 2
HopT1-1 Pta 11528	(47) HT ADI	UDAAUMTTCI	LUDTLTDU	RUDANCE	UNCHARTUO	AUGDBUDG
HopT1-1 Pto DC3000	(47) HIADI	HPAAKMTIS	LVDTLTDV	FURAHGE	VKGWABIVQ	AVSRPHDS
Consensus	(47) HIADE	HPAAKMTISA	LVDTLTDV	FVRAHGE	VKGWAEIVQ	AVSRPHDS
						— Section 3
	(93) 93	100	110	,120		138
Hop 11-1 Pta 11528	(93) NRHGS	SGVLSPRFDU	MGSVGWNA	AAIRATS	RVGTLREKG	TLFTNLML
Consensus	(93) NRHG	SGVLSPRFDU	MGSVGWNA	AAIRATS	RVGTLREKG	TLFTNLML
oonoonouo	(35) NRHG:	SGVLSPRFD	MGSVGWNA	CAAL RATS	RVGILREKG	Section 4
	(139) 139	150	1	60	170	184
HopT1-1 Pta 11528	(139) SNNFI	KHLLKRVVSI	PALOOKLE	GGVDLNY	LKACEGDLY	VMSGWAAR
HopT1-1 Pto DC3000	(139) SNNFF	KHL LKRVVNI	PALQOKLD	GGLDLNY	LKACEGDLY	VMSGWAAR
Consensus	(139) SNNFE	KHLLKRVV I	PALQQKLD	GGLDLNY	LKACEGD LY	VMSGWAAP
		100	000	010		
HopT1_1 Dta 11528	(185) 185	,190	200	210	220	23.
HopT1-1 Pto DC3000	(185) ASES	REQIGRARY	TASNLSQI	LISAREL.	AFHRHNPVN	HPSAQTKV
Consensus	(185) ASEST	REGIGKARY	TASNLSOT	LISAREL	AFHRHNPUN	HPSAOTK
	(DICTION		-Section 6
	(231) 231	240	250		260	276
HopT1-1 Pta 11528	(231) GFDK(GLPEESDLQV	LRGHGS SV	WSVRPGS	D FAKRAEVS	GKPIIAGE
Hop I 1-1 Pto DC3000	(231) GFDK(GLPEESDLQ\	/LRGHGSSV	WSVRPGS	DFAKRAEVS	GKPIIAGE
Consensus	(231) GFDR(GLPEESDLQ	LRGHGSSV	WSVRPGS	DFAKRAEVS	GKPIIAGE
	(277) 277	2	90	300	310	
HopT1-1 Pta 11528	(277) SCTM	SDMUAUADRI	ADACINST	GTRSROM	LERLUDYAC	VAVRODS
HopT1-1 Pto DC3000	(277) SGTA	SRMVAVARFI	APACLESI	GIESEON	LKELVRYAC	YAYFGODS
Consensus	(277) SGTAS	SRMVAVARFI	APACLESI	GIESEQN	LKELVRYAC	YAYFGQDS
						— Section 8
	(323) 323	330	340	350		366
Hop 11-1 Pta 11528	(323) HHSMI	LEVNLGVASH	IGMPEQUDD	TLYNEPF	SNSIKGRGF	GIDNLAQF
Consensus	(323) HHSMI	LEVNLOVASI	IGMP EQUDE	TIVNEPF	SNSIKGRGF	GIDNLAH
	(50) HISH	DEVINDOVASI	IGHP & QWDL	TEINEFF	SNSINGKGF	-Section 9
	(369) 369	379				
HopT1-1 Pta 11528	(369) QVVR	QAAQKS-				
HopT1-1 Pto DC3000	(369) QVVR	QAAQKS-				
Consensus	(369) QVVR(QAAQKS				

Figure S2. Sequence alignment of HopT1-1_{*Pta*11528}.

Amino acid alignment of HopT1- $1_{Pta11528}$ and HopT1- $1_{PtoDC3000}$. Identical residues are labelled in yellow and conservative substitutions in green.

													Sectio	m 1
	(1)	1		10			20		-	30				45
AvrPtoB Pta 11528	(1)	MVG	INRAG	PSGI	AYIA	HTD	PEP	ASGR	ARE	sss	GASS	SNS	PQV	PP-
AvrPtoB Pto DC3000	(1)	MAG	INRAG	PSG	YFV	GHTD	PEP	VSGQ	AHG	SGS(DASS	SNS	PQV	Q P R
Consensus	(1)	MG	INRAG	PSG	AY (GHTD	PEP	SG	A :	S S(GASSS	SNS	PQV	P
		100			-		_	-	_	_			Sectio	n 2
	(46)	46	_	_	60		_	70	_	_	90		_	90
AvrPtoB Pta 11528	(45)		- PPSE	ASAS	SQARI	RRE	RL L	RSRP	LSR	ETRI	BULE	QGM	PPT.	A EA
AvrPtoB Pto DC3000	(46)	PSN	r <mark>p p s</mark> n	APAI	PPPT	GR-E	RLS	RSTA	LSR	QTRI	BWLE	QGM	PTAI	EDA
Consensus	(46)		PPS	A A		RE	RL	RS	LSR	TRI	EM L E(QCM	P Cardia	DA
	(01)	01		100			110			120			Secu	125
Aug Dto D Dto 11509	(91)	PUD	DBWC	AD	AADT	TADA	PTD	DDD-		- 01	TA CIT	T A 11	ARTON	THO
AvrPtoB Pto DC3000	(90)	SUDI		TAD	AAP		DDT	DRAT	ana	SADI	DDCM	T A H	ANG.	TVO
Consensus	(91)	VR	RP	SAD	AA D	A A		P	ava	one	G	TAH	ANS	TVO
				01001				•			· · ·		Sectio	m 4
	(136)	136			15	0		160)		170			180
AvrPtoB Pta 11528	(125)	QLVS	AGAD	LAHI	RTM	RNI	MRG	EEMA	LSR.	A EQ S	ILRI	EHF	PNM	IAT
AvrPtoB Pto DC3000	(135)	QLVS	BEGAD	ISHI	[RNM]	LRNA	MNG	DAVA	FSR	VEQI	J <mark>IFR</mark>	OHF	PNM	PMH
Consensus	(136)	QLVS	5 GAD	IAHI	TR MI	LRN	MG	D MA	SR	EQ	IR	HF	PNM	
	-	1000		1.4.4.4		2			83	1.4			Sectio	m 5
	(181)	181		190			200		-	210		-	-	225
AvrPtoB Pta 11528	(170)	CIN1	PHSEL	AIEI	LRHAI	RRA	DRQ	QAAS	T PA	RTA	RPP.	AAR	TPT	QSS
AVIPTOB Pto DC3000	(180)	GISI	RDSEL	AIEI	LRGA	RRA	VHQ	QAAS	APV	RSP	PTP.	AS-	- PA	ASS
00113611303	(101)	61	SEL	AIRI	LR AI	L R RA	, u	UAAS	Υ.	RS .	1 12	8.A	Sectio	55
	(226)	226			24	0	_	250)		260	3	2001	270
AvrPtoB Pta 11528	(215)	ggg	SOPSI	RCDI	PADIA	TPD	OGP	SSMA	STS.	0 7 0 1	TO P SI	DDD	UNO	TPT
AvrPtoB Pto DC3000	(223)	SGS	SORSL	FGRI	FARL	APN	OGR	SSNT	AAS	OTPI	DRSI	PPR	VNO	RPI
Consensus	(226)	S SS	QRSL	FGRI	FARLI	I P	QGR	SSN	A SI	OTPU	DRSI	PPR	VNQ	PI
	-									-			Sectio	m 7
	(271)	271		280	_		290		1	300			_	315
AvrPtoB Pta 11528	(260)	RPDI	RAAMR	NRGI	IN Q A.	AAL	QGL	VQQG	VNL	EDLI	RTAL	BRH	L L RI	HQP
AvrPtoB Pto DC3000	(268)	RVD	RAAMR	NRGI	DEAL	AAL	RGL	Adde	VNL	EHLI	RTAL	BRH	VMQ:	RLP
Consensus	(2/1)	R DI	RAAMR	NRGI	A	AAL	GL	Aððe	VNL.	ELI	RTAL	ERH	LL	P
	(210)	316			33	0	_	340	1		350	1	Section	360
Aur Dte D. Dte 11529	(316)	7.07	T	TOCT	TO T D		-	CT 110	CONT				T CD	0.00
AvrPtoB Pto DC3000	(313)	TPLI	TCSA	LONU	CIN	STD	LOR	SLVO	HDL	ML	JUAL		LCL	DDS
Consensus	(316)	IPLI	DIA A	LO	GI I	PSID	AE	SLV	PL	LL	VAL	RM	LG	RP
				-									Sectio	m 9
	(361)	361		370			380		-	390				405
AvrPtoB Pta 11528	(349)	VSAL	PPRPA	VPVI	H <mark>P</mark> P A	ASRR	PD G	ARSS	ALR	V <mark>I</mark> PI	ERED	YEN	NVA	Y GM
AvrPtoB Pto DC3000	(358)	AER	PRPA	V PV	APAT	ASRR	PDG	TRAT	RLR	VMPI	ERED	YEN	NVA	YCV
Consensus	(361)		PRPA	VPV	P	ASRR	PDG	RAS	LR	VIPI	ERED	YEN	NVA	YCM
	-											<u> </u>	ection	n 10
	(406)	406			42	0		430)	_	440		_	460
AvrPtoB Pta 11528	(394)	RLLI	ILNPG	VGVI	RRV <mark>V.</mark>	AAFI	TD P	AD RP	AVVI	DDII	RA A RI	DPI	TSQI	FNQ
AvrPtoB Pto DC3000	(403)	RLLI	ILNPG	VGVI	RQAV	AFV	TDR	AERP	AVV.	ANI	RAALI	DPI	ASQ	FSQ
Consensus	(406)	RLLI	NLNPG	VGVI	R VA	AAFI	TD	AD RP	AVV	I	RAA I	DPI	SQ	FQ
	(451)	451		460			470			480			ecuor	495
AverDtoB Dto 11528	(451)	LDT	CV AD	UPC	NDD	2 D D A	PUL	WDDD	A TH	CLR	PDI	CT.P	NDD	0.017
AvrPtoB Pto DC3000	(448)	LRT	SKAD	ARSI	RRLC	REDA	ADH	HTDD	VTH	CLF	GRL	SLS	NPD	nov
Consensus	(451)	LRT	ISKA	ES	1	RDA	Н	H DD	TH	CLF	L	SL	NPD	voc
										1			ection	12
	(496)	496			51	0		520)		530			540
AvrPtoB Pta 11528	(484)	IGL	GNPT	DISI	EL YS(QGN	KD L	VFMD	MKK	LAQI	LAG	KPE	HPMI	N RQ
AvrPtoB Pto DC3000	(493)	IGL	GNPT	DTS	DP <mark>YS(</mark>	EGN	KD L	AFMD	MKK	LAQI	LAG	KPE	HPM	TRE
Consensus	(496)	IGL	AGNPT	DTS	YS	CN	KD L	FMD	MKK	LAQI	FLAGE	KPE	HPM	R
	10.41	E44			EF							— s	ection	n 13
AurDiaD Dia 11500	(541)	041	DTT	1112.	00	-								
AvrPtoB Pta 11528	(523)	TIN	RNTA	LA LA	PDTU									
Consensus	(541)	I-	A TA	YAT	FRTU	p								
	·- ·-)					21								

Figure S3. Sequence alignment of AvrPtoB_{Pta11528}.

Amino acid alignment of $AvrPtoB_{Pta11528}$ and $AvrPtoB_{PtoDC3000}$. Identical residues are labelled in yellow and conservative substitutions in green.



Figure S4. Sequence alignment of HopX1_{Pta11528}.

Amino acid alignment of HopX1_{*Pta*11528} and HopX1_{*Pto*DC3000}. Identical residues are labelled in yellow and conservative substitutions in green.



Figure S5. Sequence alignment of AvrPto_{Pta11528}.

Amino acid alignment of $AvrPto_{Pta11528}$ and $AvrPto_{PtoDC3000}$. Identical residues are labelled in yellow and conservative substitutions in green.



Figure S6. Sequence alignment of HopI1_{Pta11528}.

Amino acid alignment of HopI1_{*Pta*11528} and HopI1_{*Pto*DC3000}. Identical residues are labelled in yellow and conservative substitutions in green.



Figure S7. Sequence alignment of HopF1_{Pta11528}.

Amino acid alignment of HopF1_{*Pta*11528} and HopF2_{*Pto*DC3000}. Identical residues are labelled in yellow and conservative substitutions in green.



Figure S8. Accumulation of Pto DC3000 and Pta 11528 effector proteins in N. benthamiana.

(A) Western blot showing accumulation of *Pto* DC3000 effector proteins in leaf tissue after *A. tumefaciens* mediated transient expression in *N. benthamiana* for two days. A Coomassie brilliant blue loading control is shown in the lower panel.

(B) Western blot showing accumulation of all *Pta* 11528 effector proteins in leaf tissue after *A*. *tumefaciens* mediated transient expression in *N*. *benthamiana* for two days. A Coomassie brilliant blue loading control is shown in the lower panel.





Suppression of $hopM1_{PtoDC3000}$ and $hopAD1_{PtoDC3000}$ induced cell death by *Pto* DC3000 effectors. The effector $hopM1_{PtoDC3000}$ or $hopAD1_{PtoDC3000}$ was co-expressed in *N. benthamiana* leaves with each effector in the *Pto* DC3000 repertoire, or an *EV* control, as depicted in the upper schematic diagram. Each of the two *A. tumefaciens* strains containing the respective effector gene or an EV control were prepared at OD₆₀₀=0.5 then mixed in equal volumes. Cell death induced by the effectors was scored 6 days after infiltration. Similar results were obtained in two independent experiments.



Figure S10. *Pta* 11528 AvrPtoB can interfere with *hopAD1*_{PtoDC3000} or *hopM1*_{PtoDC3000}-induced cell death.

Suppression of $hopM1_{PtoDC3000}$ and $hopAD1_{PtoDC3000}$ induced cell death by the *Pta* 11528 effector AvrPtoB. The effector $hopM1_{PtoDC3000}$ or $hopAD1_{PtoDC3000}$ was co-expressed in *N. benthamiana* leaves with $avrPtoB_{Pta11528}$ or an *EV* control, as depicted in the upper schematic diagram. Each of the two *A. tumefaciens* strains containing the respective effector gene or an EV control were prepared at OD₆₀₀=0.5 then mixed in equal volumes. Cell death induced by the effectors was scored 6 days after infiltration. Similar results were obtained in two independent experiments.



Figure S11. *Pto* DC3000 effectors cannot collectively suppress the induction of the hypersensitive response (HR) triggered upon recognition of $AvrPto_{PtoDC3000}$ and/or $AvrPtoB_{PtoDC3000}$ in transgenic *N. benthamiana* plants expressing the tomato *Pto* and *Prf* genes (*N. benthamiana* R411A).

Transgenic *N. benthamiana* plants were infiltrated with a high dose $(5x10^7 \text{ cfu/ml})$ of *Pto* DC3000 (DC3000), *Pto* DC3000 *hrcC* (*hrcC*), *Pto* DC3000 $\Delta avrPto$ ($\Delta avrPto$), *Pto* DC3000 $\Delta avrPtoB$ ($\Delta avrPtoB$) and *Pto* DC3000 $\Delta avrPto\Delta avrPtoB$ ($\Delta avrPtoB$). The HR was visible 24-48 h after infiltration, and photographed at 3 days post infiltration. Similar results were obtained in two independent experiments.