

# Expression of *ripAA* gene in a soil-born *Pseudomonas mosselii* can promote the control efficacy against tobacco bacterial wilt

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**Table S1.** Primers used for molecular cloning and RT-PCR analysis in this study

Primer pair	Sequence(5'-3')	Cutting sites	Description or purpose
<b>Primers used for molecular cloning</b>			
Hy1.F / Hy1.R	TCGGATCCCTACGAGCTGAGCA TCGTCGACCTGAATTCAGGAAGTAGTAG TTGCCGTCGTA	<i>Bam</i> HI/ <i>Sal</i> I	A 604-bp DNA fragment containing 5' terminus of a hypothetical gene in <i>Pseudomonas mosselii</i> A1

Hy2.F / Hy2.R	TCGAATTCTCAAGCTTCCAACAACCTGCG CGGTG TCGTCGACCTGCTTGATATTGGCTTCC	<i>EcoRI/SalI</i>	A 677-bp DNA fragment of 3' terminus of a hypothetical gene in <i>Pseudomonas mosselli</i> A1
RipAA.F / RipAA.R	TCGAATTCGCTCGGGCCAAAACGCTT TCAAGCTTACAAGTCCTCTTCAGAAATG AGCTTTTGCTCGCCGTCGCTATCGCTATC G	<i>EcoRI/HindIII</i>	A 1100-bp DNA fragment containing <i>RipAA</i> gene and its native promoter
PRipAA.F/PRipAA.R	ACGGTACCCGCACGAGGAT TGCTCGAGCTCAATCTCCTGAGTTTCAA ATAG	<i>KpnI/XhoI</i>	A 276-bp <i>RipAA</i> gene promoter cloned to fuse with <i>lacZ</i>

#### Primers used for RT-PCR analysis

16S rRNA.F/ 16S rRNA.R	GTAAAGCGCGCGTAGGTGGT CGAAGGCACCAATCCATCTCT	464-bp
RipAA.F/ RipAA.R	TGACGACGGGAGGGTGACATAT CATGCACGCAGTTGCTGTATT	366-bp
Nt-EF1 $\alpha$ -F/ Nb-EF1 $\alpha$ -R	TGAGATGCACCACGAAGCTC CCAACATTGTCACCAGGAAGTG	51-bp [1]
qNt1-F/ qNt1-R	TGACAGCCTTTCTGCACTTGAT CTGAGAATCCGTAGAACCACCA	133-bp, detection for gene Nitab4.5_0011671g0010.1
qNt2-F/ qNt2-R	TAGATCAGGCTAAACAAAGGAT TAGTCGCACTGGGTATGGTATT	128-bp, detection for gene Nitab4.5_0000273g0080.1
qNt3-F/ qNt3-R	AATCTGACAGCAGGAAGGAATC TGTACGTGGTTAGTCACTGGAT	119-bp, detection for gene Nitab4.5_0002130g0010.1
qNt4-F/ qNt4-R	CCCACCTTATTGTGATCCTGAA AGGCACTGACAAGCCATCCACT	91-bp, detection for gene Nitab4.5_0000274g0040.1

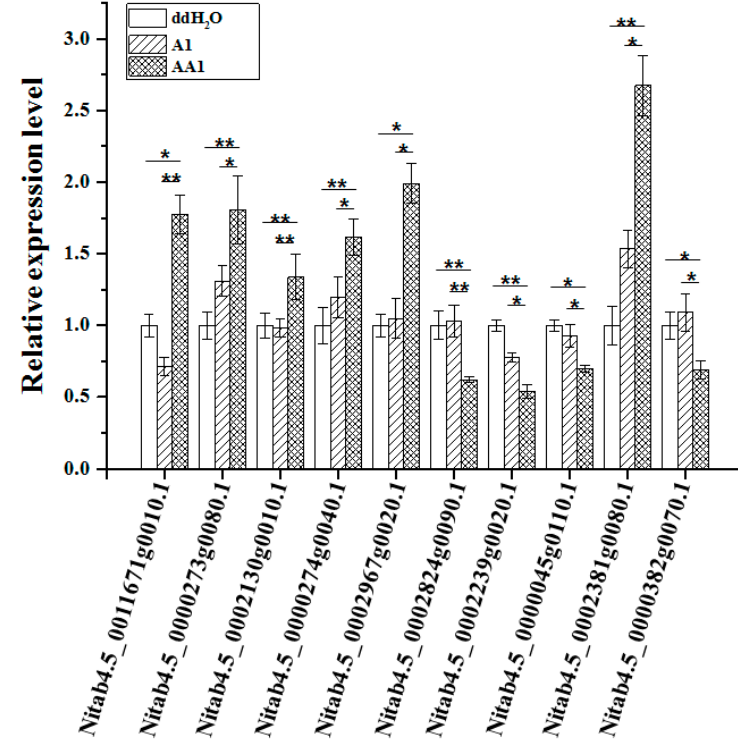
qNt5-F/ qNt5-R	CTCCAAACAGCCTCATCCTC CATGACAGACATGCCACAGC	138-bp, detection for gene Nitab4.5_0002967g0020.1
qNt6-F/ qNt6-R	GCGAAATCTGTGGATAAGGGTG CTGATAGCACAAAGTTGGAGCA	105-bp, detection for gene Nitab4.5_0002824g0090.1
qNt7-F/ qNt7-R	CGGTTTAAGCCATGAGTATGA AATGCTTGCCTTGTGACCTCT	150-bp, detection for gene Nitab4.5_0002239g0020.1
qNt8-F/ qNt8-R	TGAACTGTTTGAGATGGCTGAG TGACCATAAACCACCACTCCTG	147-bp, detection for gene Nitab4.5_0000045g0110.1
qNt9-F/ qNt9-R	GAATAACATTTGAGCCAGAACG TCAAATCCTGGATAATAGGGTG	129-bp, detection for gene Nitab4.5_0002381g0080.1
qNt10-F/ qNt10-R	TATGGGTCGGTGAGAAGAGTTT GGATTTGACTTCATTTGGAGGG	108-bp, detection for gene Nitab4.5_0000382g0070.1

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The 5' end of each primer contains a restriction enzyme site for cloning into the expression plasmids.

## References

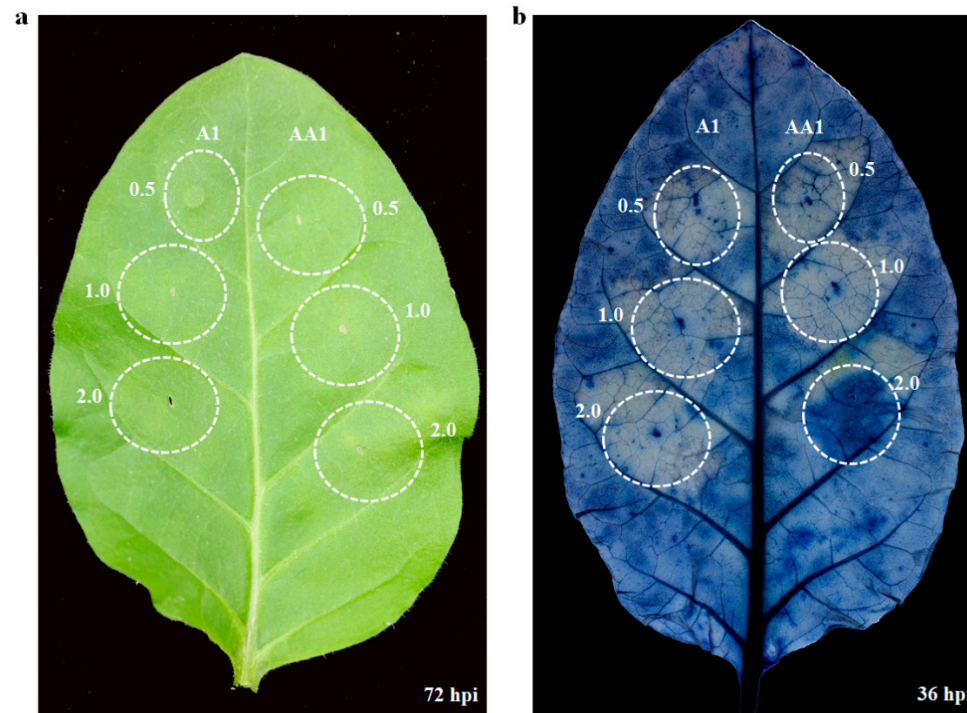
1. Schmidt, G.W.; Delaney, S.K. Stable internal reference genes for normalization of real-time RT-PCR in tobacco (*Nicotiana tabacum*) during development and abiotic stress. *Mol Genet Genomics*. **2010**, *283*, 233-241.



**Supplementary Figure S1.** qRT-PCR analysis of ten genes from 1525 differentially expressed genes in *N. tabacum* roots for different treatments. RNA was extracted from roots collecting from 4-week-old *N. tabacum* NC89 plants at 2 days after inoculation with ddH<sub>2</sub>O, 5 mL of a  $1 \times 10^8$  CFU/mL cell suspension of *P. mosselii* A1 or AA1. The blank column represents the sample treated with ddH<sub>2</sub>O, the column filled with diagonal rod represents the sample treated with *P. mosselii* A1, and the column filled with grid represents the sample treated with *P. mosselii* AA1. The sequence and annotation of all the tested genes were referred to the database ([https://solgenomics.net/organism/Nicotiana\\_benthamiana/genome](https://solgenomics.net/organism/Nicotiana_benthamiana/genome)). Error bars represented the standard deviation from three independent experiments. Differences were evaluated using Student's t-tests (\*P < 0.05 and \*\*P < 0.01).



**Supplementary Figure S2.** The plant growth phenotype among *N. tabacum* by three different treatments. The germinated tobacco seedlings were plant in the soil inoculated with ddH<sub>2</sub>O, 5 mL of a  $1 \times 10^8$  CFU/mL cell suspension of *P. mosselii* A1 or AA1. And the phenotype was recorded at 6 weeks after inoculation.



**Supplementary Figure S3.** Cell death in *N. tabacum* leaves was induced by high concentration of *P. mosselii* AA1: (a) no obvious phenotype in tobacco leaves after inoculation with *P. mosselii* A1 or AA1 strain. The dotted circles indicated the inoculated areas. The different inoculation concentrations of *P. mosselii* A1 or AA1 strain were OD600=0.5, 1.0 and 2.0, respectively. Photographs were taken at 3 days postinfiltration. (b) detection of plant cell death. Tobacco leaves (36 h postinfiltration) were tested by trypan blue staining. The infiltrated leaves were collected and boiled in a 1:1 mixture containing 96% ethanol and staining solution (10 mL lactic acid, 10 mL phenol, 10 mL glycerol, 10 mL H<sub>2</sub>O and 10 mg trypan blue) for approximately 5 min until its green colour has vanished completely visible to the naked eye. The leaves were then detained in detaining solution (dissolve 250 g chloral hydrate into 100 mL H<sub>2</sub>O) overnight.