RrGT2, A Key Gene Associated with Anthocyanin Biosynthesis in *Rosa Rugosa*, Was Identified Via Virus-Induced Gene Silencing and Overexpression

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Table S1. Primers used in the present study.

Primer Name	Sequence(5′-3′)	Description
3′*RrGT2*-F-outer	GCAGAGAGGAACTAGAAGAGCTTGGG	
3′*RrGT2*-F-inner	GCCAAGTTGATAGAGGACATGTGG	
B26	GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT	3′RACE for *RrGT2*
RrGT2-F	ATGACGCAACACCGCTTC	
RrGT2-R	CTAGGCTGGCAAACAGCCA	Full-length cDNA for *RrGT2*
p-*RrGT2*-F	ACTAGTATGACGCAACACCGCTTC	
p-*RrGT2*-R	GGTGACCCTAGGCTGGCAAACAGCCA	Generation for *pCAMBIA1304-RrGT2*
CpR-F	ATGGTAGATCTGACTAGTATGACGCAA	
CpR-R	TTCGAGCTGGTCACCCTAGG	Confirmation for *pCAMBIA1304-RrGT2*
TGR-F	GCTCTAGAATCAATCCTTCCCTGCAACTTG	
TGR-R	CCCTCGAGGATCTTTGTAGCCGTTGAAGTAG	Generation for *pTRV2-GFP- RrGT2*
TRV1-F	TTACAGGTTATTTGGGCTAG	
TRV1-R	CCGGGTTCAATTCCTTATC	RT-PCR for *TRV1*
TRV2-F	TGGGAGATGATACGCTGTT	Confirmation for recombinant silencing vector and RT-PCR for *TRV2*
TRV2-R	CCTAAAACTTCAGACACG	
GFP-F	ATGGTGAGCAAGGGCGAGGA	
GFP-R	CTTGTACAGCTCGTCCATGCC	RT-PCR for *GFP*
RrGAPDH-F	TTCTGCCTGCTCTCAATG	
RrGAPDH-R	TGCCTTCTTCTCAAGTCTG	qRT-PCR for *RrGAPDH*
q*RrGT2*-F	GTTGTTGGATTCCGGACGTC	
q*RrGT2*-R	CAAGCTCTTCTAGTTCCTCTCTGC	qRT-PCR for *RrGT2*
q*RrCHS*-F	ATGGTGACCGTCGAGGAAGTC	
q*RrCHS*-R	GCCTTGTGCTCGCTCTTAGTG	qRT-PCR for *RrCHS*
q*RrCHI*-F	ATGGCTCAATCAGTTACCGGA	
q*RrCHI*-R	GCACGGCCTTATCCTCCAAGT	qRT-PCR for *RrCHI*
q*RrF3H*-F	GAGATGTGACCAAGCAGTACA	
q*RrF3H*-R	TCAACGCCTCCTTCTCTAAAC	qRT-PCR for *RrF3H*
q*RrF3′H*-F	CTTGGTCATGGCTATTCCTTC	
q*RrF3′H*-R	CCATTGATGAGGTTGAGGTT	qRT-PCR for *RrF3′H*
q*RrDFR*-F	GTGAAGATGACTGGTTGGATG	
q*RrDFR*-R	CGAGAGTTGGGATAATGGTGA	qRT-PCR for *RrDFR*
q*RrANS*-F	CCAACTCCATCGTCATGCAC	
q*RrANS*-R	CAGCGGCTTGAGGATGATCTT	qRT-PCR for *RrANS*
NtACT-F	AGGGTTTGCTGGAGATGATG	
NtACT-R	CGGGTTAAGAGGTGCTTCAG	qRT-PCR for *NtACT*

* Restriction enzyme site are underlined.

Table S2. Anthocyanin contents in the flowers of *R. rugosa* 'Zizhi' and *R. davurica* subjected to different VIGS treatments (μg·g^{-1} FW).

Name	treatments	Cy3G	Cy3G5G	Pn3G	Pn3G5G	Pg3G	Pg3G5G	Dp3G	Dp3G5G
R. rugosa 'Zizhi'	Control	13.05 ± 0.04A	298.38 ± 3.85A	17.63 ± 0.12A	1773.93 ± 4.31A	5.04 ± 0.09A	52.88 ± 0.45A	-	-
	TRV-GFP	11.86 ± 0.70A	301.14 ± 1.57A	17.33 ± 0.07A	1660.26 ± 13.07B	4.82 ± 0.13A	45.32 ± 1.23B	-	-
	TRV-GFP-*RrGT2*	3.29 ± 0.22B	59.92 ± 0.28B	6.71± 0.20B	189.41 ± 0.54C	-	2.28 ± 0.23C	-	-
R. davurica	Control	56.72 ± 0.20A	2590.18 ± 2.45A	2.55 ± 0.03A	40.28 ± 0.09A	7.20 ± 0.01A	25.7 ± 0.36B	-	-
	TRV-GFP	55.59 ± 0.26B	2565.85 ± 3.26B	2.50 ± 0.01A	40.36 ± 0.11A	7.19 ± 0.01A	28.13 ± 0.27A	-	-
	TRV-GFP-*RrGT2*	16.28 ± 0.13C	425.77 ± 3.86C	-	1.79 ± 0.03B	0.68 ± 0.02B	7.23 ± 0.04C	-	-

* Data are the mean values ± SE of three independent replicates.Different upper case letters represent significant difference which is calculated using LSD analysis at the level of $P < 0.01$. '-' means that no corresponding anthocyanin was detected.

Table S3. Anthocyanin contents in the flowers of transgenic tobacco (μg·g^{-1} FW).

Name	treatments	Cy3G	Cy3G5G	Pn3G	Pn3G5G	Pg3G	Pg3G5G	Dp3G	Dp3G5G
	Control	28.6367 ± 0.024D	-	-	-	-	-	-	-
	P	28.3533 ± 0.029D	-	-	-	-	-	-	-
OE-*RrGT2* in Tobacco	T1	54.47 ± 0.036B	-	-	-	-	-	-	-
	T3	67.7667 ± 0.038A	-	-	-	-	-	-	-
	T6	51.6667 ± 0.353C	-	-	-	-	-	-	-

* Data are the mean values ± SE of three independent replicates.Different upper case letters represent significant difference which is calculated using LSD analysis at the level of $P < 0.01$. '-' means that no corresponding anthocyanin was detected.

Table S4. Anthocyanin contents in the flowers of transgenic tobacco subjected to different VIGS treatments (μg·g^{-1} FW).

Name	treatments	Cy3G	Cy3G5G	Pn3G	Pn3G5G	Pg3G	Pg3G5G	Dp3G	Dp3G5G
	Control	67.7667 ± 0.038A	-	-	-	-	-	-	-
VIGS of OE-*RrGT 2* Tobacco Plants	TRV-GFP	65.2267 ± 0.89B	-	-	-	-	-	-	-
	V1	11.71 ± 0.032D	-	-	-	-	-	-	-
	V2	15.3533 ± 0.029C	-	-	-	-	-	-	-
	V3	7.77 ± 0.064E	-	-	-	-	-	-	-

* Data are the mean values ± SE of three independent replicates.Different upper case letters represent significant difference which is calculated using LSD analysis at the level of $P < 0.01$. '-' means that no corresponding anthocyanin was detected.

Table S5. Silencing efficiency of VIGS in *R. rugosa* 'Zizhi' and *R. davurica.*

Name		TRV-GFP	TRV-GFP-*RrGT2*
R. rugosa 'Zizhi'	Total	20	20
	GFP positive	15	13
	RrGT2 silencing	-	13
	GSEI	-	65%
R. davurica	Total	20	20
	GFP positive	19	17
	RrGT2 silencing	-	17
	GSEI	-	85%

* GSEI, gene silencing efficiency of all infected plants.

2.S1 Construction and Verification of Recombinant Virus Vector pTRV2-GFP-RrGT2

The virus vector pTRV2-GFP and the specific fragment of the *RrGT2* gene were respectively digested (Figure S1B) and then were combined together , according to the preset viral vector recombination technology route (Figure S1A). Then the PCR (Figure S1C)was verified by specific primers (Table S1), and the base mutation and deletion were verified by double enzyme digestion (Figure S1D)and sequencing. All of the results above indicated that the recombinant virus vector pTRV2-GFP-*RrGT2* was successfully constructed.

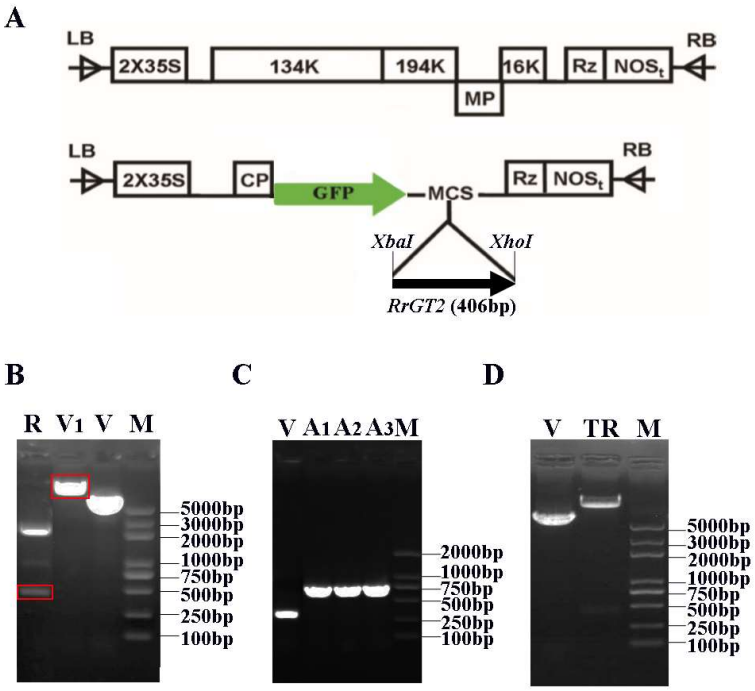

Figure S1. Construction and validation of the recombinant virus vector TRV-GFP-*RrGT2*. **(A)** Schematic diagram of the construction of TRV-GFP-*RrGT2*. **(B)** The virus vector and target gene fragment were digested separately. M: Marker; V: The plasmid of the empty virus vector was used as the control; V1: The empty virus vector was digested by double enzymes; R: The plasmid carrying the target gene fragment was digested by two enzymes. The red box indicates the portion that needs to be retained for subsequent operations. **(C)** The result of PCR verification. Three repeated PCR assays were performed on the recombinant virus vector and the results showed positive. M: Marker; V: The empty virus vector was used as the control; A1,A2,A3 was the three repetitions which were larger than the empty virus vector due to the

inserted *RrGT2* fragment. **(D)** Double enzyme digestion was used to verify the plasmid of the recombinant vector. M: Marker; V: The plasmid of the empty virus vector was used as the control; TR: The plasmid of the recombinant vector was digested into two correct parts.

2.S2 Construction and Verification of Recombinant Expression Vector pCAMBIA1304-RrGT2

Similar to the construction process of the recombinant virus vector , the empty vector and target gene were digested separately (Figure S2B) and linked together according to the pre-designed vector recombination technique route (Figure S2A). In order to verify whether the recombination was successful, we designed a pair of specific primers (Table S1) to detect the transformation results by PCR, and the results showed positive (Figure S2C). In order to eliminate the possibility of false positivity and to further verify whether the target gene had been mutated or deleted during the recombination, we identified the target gene by double enzyme digestion and sequencing. The results of double enzyme digestion (Figure S2D) showed that the recombinant vector was cut into two distinct fragments: one part of the original vector and the other part of the target gene. The plasmid of the empty vector was used as the control. The sequencing results also showed that there was no base mutation and deletion in the target gene fragment of the embedded vector. The results above showed that the recombinant expression vector *pCAMBIA1304-RrGT2* was constructed successfully.

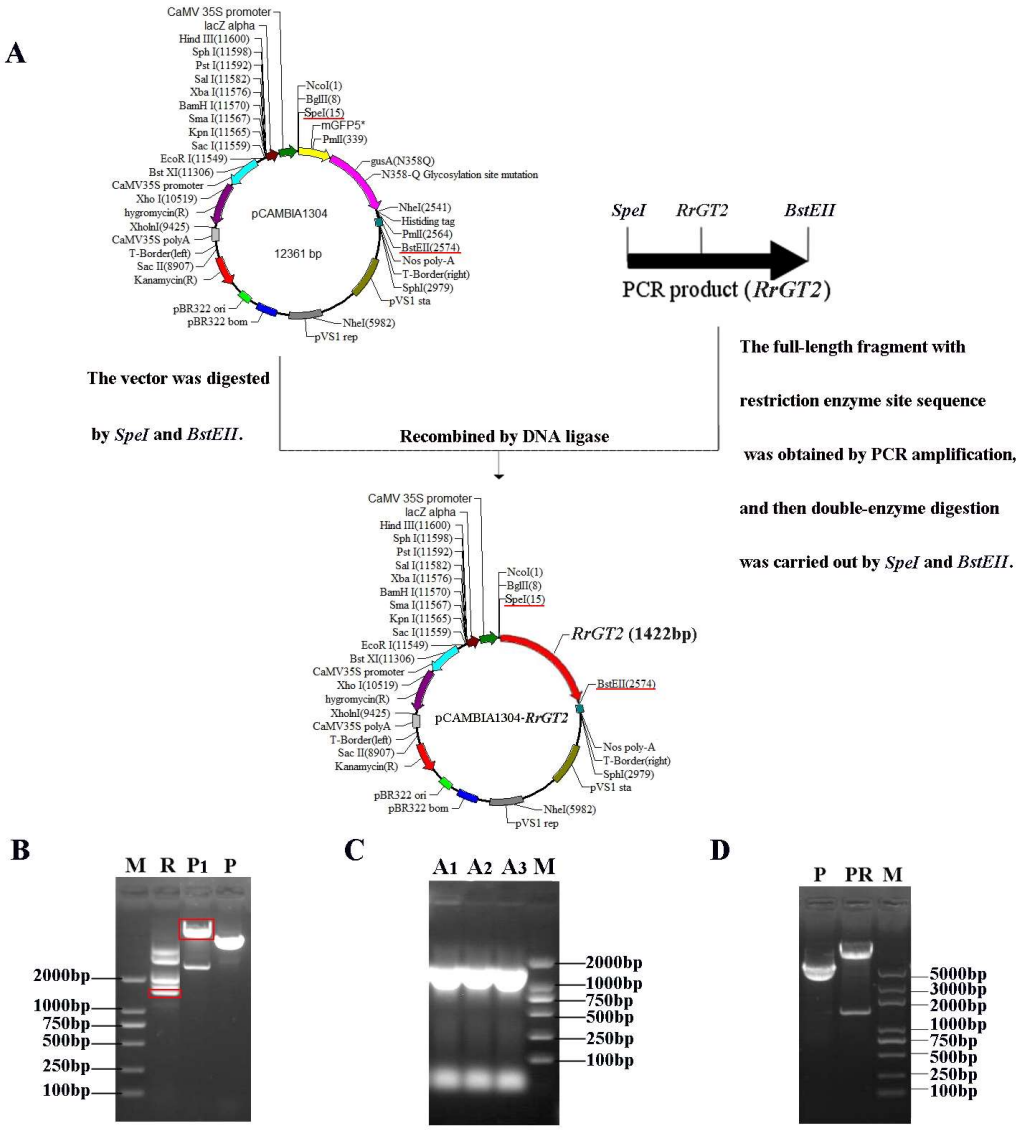

Figure S2. Construction and validation of the recombinant expression vector pCAMBIA1304-*RrGT2*. **(A)** Schematic diagram of the construction of pCAMBIA1304-*RrGT2*. **(B)** The vector and target gene were digested separately. M: Marker; P: The plasmid of the empty vector pCAMBIA1304 was used as the control; P1: The empty vector was digested by double enzymes; R: The plasmid carrying the target gene was digested by two enzymes. The red box indicates the portion that needs to be retained for subsequent operations. **(C)** The result of PCR verification. Three repeated PCR assays were performed on the recombinant vector and the results showed positive. M: Marker; A1,A2,A3 was the three repetitions. **(D)** Double enzyme digestion was used to verify the plasmid of the recombinant vector. M: Marker; P: The plasmid of the empty vector pCAMBIA1304 was used as the control; PR: The plasmid of the recombinant vector was digested into two correct parts.