

Supplementary Materials

Table S1. Primer sequences used for synthesizing gRNA spacers and genotyping CRISPR-edited mutants.

Name	Sequence 5'→3'	Purpose
gRNAs- <i>qRA2</i>	gtcgctgtgccgctccaccaagg	CRISPR/CAS9
<i>qRA2</i> -F1	CCACAGATCTTCTTCCTTCC	Screening of lines
<i>qRA2</i> -R1	GGTATCCGAGATAGCTGATGG	Screening of lines
<i>qRA2</i> cDS-R	ATGGGTCTCTGGGAGCAG	Gene amplification
<i>qRA2</i> cDS-F	CTACGACTCTTTGGCCAGGA	Gene amplification
<i>qRA2</i> -qRT-PCR-F	CCGTCACTACCGCCTCCTCA	RT-qPCR
<i>qRA2</i> -qRT-PCR-R	CGGCGTGTCAAGGTATCCATTC	RT-qPCR
<i>UBIQUITIN</i> -F	AACCAGCTGAGGCCCAAGA	Reference gene
<i>UBIQUITIN</i> -R	ACGATTGATTAAACCAGTCCATGA	Reference gene

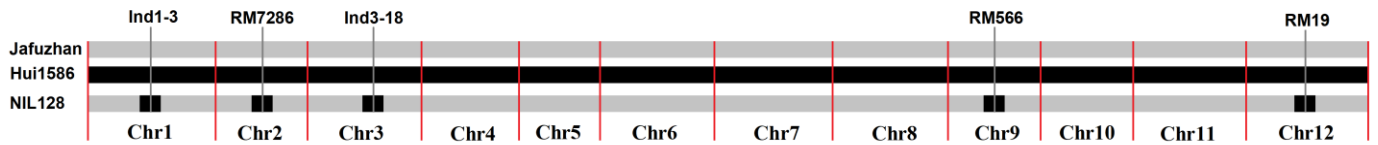


Figure S1. Analysis of the genetic background of NIL128. NIL128 basically recovered the genetic background of the recipient parents except for 5 loci.

Nip	ATGGGTTCTCTGGGAGCAGCAGGTAAGCCGCACGCCGTGTGCATGCCGTACCCGGCGCAGGGGCACATACCCCGATGCTGAACGTGGCGAAGCTGCTCCACGCCCGCGGCTTCGACGTC	120
qRA2	ATGGGTTCTCTGGGAGCAGCAGGTAAGCCGCACGCCGTGTGCATGCCGTACCCGGCGCAGGGGCACATACCCCGATGCTGAACGTGGCGAAGCTGCTCCACGCCCGCGGCTTCGACGTC	120
qpa2	ATGGGTTCTCTGGGAGCAGCAGGTAAGCCGCACGCCGTGTGCATGCCGTACCCGGCGCAGGGGCACATACCCCGATGCTGAACGTGGCGAAGCTGCTCCACGCCCGCGGCTTCGACGTC	120
Consensatgggttctctgggagcagcaggttaagccgcacgccgtgtgcatacccgcgccaggggacacatcccccgatgctgaacgtggcgagctgctccacgcccgcggttcgacgctc		
Nip	ACGTTTCGTGAACACCGAGTACAACCCACGCCCGCTCGTCCGCACCCCGCGCGAGGCCGCGGTGGCGGGCGCGCCGGGCTTCGGTTTCGCCACCATCCCCGACGGCTGCCGCCGTGCGAC	240
qRA2	ACGTTTCGTGAACACCGAGTACAACCCACGCCCGCTCGTCCGCACCCCGCGCGAGGCCGCGGTGGCGGGCGCGCCGGGCTTCGGTTTCGCCACCATCCCCGACGGCTGCCGCCGTGCGAC	240
qpa2	ACGTTTCGTGAACACCGAGTACAACCCACGCCCGCTCGTCCGCACCCCGCGCGAGGCCGCGGTGGCGGGCGCGCCGGGCTTCGGTTTCGCCACCATCCCCGACGGCTGCCGCCGTGCGAC	240
Consensacgttcgtgaacacccagtagacaacacgcccgccctcgctccgcaccccgcgcgaggcgcggtggcgggcgcgccgggcttcgggttcgccaccatccccgacggcctgcccgcgtcgagac		
Nip	GACGACGAGCTCAGCAGGACATCCCGTCGTGTGCCGTCCACCAAGGAGACCTGCCCTTGCCCCCTTCGCCCGCTCTCTGCCACAGCTGAATGACCCGCCACGGGCCACCCGCCCGTC	360
qRA2	GACGACGAGCTCAGCAGGACATCCCGTCGTGTGCCGTCCACCAAGGAGACCTGCCCTTGCCCCCTTCGCCCGCTCTCTGCCACAGCTGAATGACCCGCCACGGGCCACCCGCCCGTC	360
qpa2	GACGACGAGCTCAGCAGGACATCCCGTCGTGTGCCGTCCACCAAGGAGACCTGCCCTTGCCCCCTTCGCCCGCTCTCTGCCACAGCTGAATGACCCGCCACGGGCCACCCGCCCGTC	360
Consensgacgacgagctcagcaggacatcccgctcgctgtgcccgtccaccaaggagacctgaccttgccccttcggcgccctcctcgccagctgaatgacccgccacggggcaccgcccgctc		
Nip	ACCTGCGTCTCTCCGACGTCGTATGGGTTTTCATGGCTGCCGCCAAGGAGCTCGGCCCTCCCTACGTCCAGCTCTGGACAGCCAGCTCCATCAGTATCTCGGATACCGTCACTAC	480
qRA2	ACCTGCGTCTCTCCGACGTCGTATGGGTTTTCATGGCTGCCGCCAAGGAGCTCGGCCCTCCCTACGTCCAGCTCTGGACAGCCAGCTCCATCAGTATCTCGGATACCGTCACTAC	480
qra2	ACCTGCGTCTCTCCGACGTCGTATGGGTTTTCATGGCTGCCGCCAAGGAGCTCGGCCCTCCCTACGTCCAGCTCTGGACAGCCAGCTCCATCAGTATCTCGGATACCGTCACTAC	480
Consensacgttcgtcgtcgtccgagctcgctcatgggtttctccatggctgcccgaaggagctcgctcgcccttcggcgccctcctcgccagctcgtgacagccagctccatcagctatctcggataccgtcactac		
Nip	CGCCTCCTCATGAAACGTGGCCTTGCCCCACTCAAGATGTGCATCAGCTGACGAATGGATACCTTGACACGCCGGTGGAGAACGTGCCGGGGCTGAGGAACATGAGGATCAAGGACTTC	600
qRA2	CGCCTCCTCATGAAACGTGGCCTTGCCCCACTCAAGATGTGCATCAGCTGACGAATGGATACCTTGACACGCCGGTGGAGAACGTGCCGGGGCTGAGGAACATGAGGATCAAGGACTTC	600
qra2	CGCCTCCTCATGAAACGTGGCCTTGCCCCACTCAAGATGTGCATCAGCTGACGAATGGATACCTTGACACGCCGGTGGAGAACGTGCCGGGGCTGAGGAACATGAGGATCAAGGACTTC	600
Consenscgctcctcatg aacgtggccttgcccactcaagatgtcgatcagctgacgaatggataccttgacacgcgcggtggaagacgtgcccggggctgaggaacatgaggatcaaggacttc		
Nip	CCGAGCTTCATACACACGACGAACCCGGAGGAGTACATGGTAGGCTACGTCATCGAGGAGACGGAGCGCTGCAAGGATGCCTCGGCCATCATCGTCAACTCCTTCGGCGACCTCGAGGGT	720
qRA2	CCGAGCTTCATACACACGACGAACCCGGAGGAGTACATGGTAGGCTACGTCATCGAGGAGACGGAGCGCTGCAAGGATGCCTCGGCCATCATCGTCAACTCCTTCGGCGACCTCGAGGGT	720
qra2	CCGAGCTTCATACACACGACGAACCCGGAGGAGTACATGGTAGGCTACGTCATCGAGGAGACGGAGCGCTGCAAGGATGCCTCGGCCATCATCGTCAACTCCTTCGGCGACCTCGAGGGT	720
Consenscggagcttcatacacacgacgaacccggaggagtacatggtaggctacgtcatcgaggagacggagcgctgcaaggatgctcgcccatcatcgtaactccttcggcgacactcgagggt		
Nip	GAGGCGGTGGCGGCCATGGAGGGCGCTCGGCTTGCCCAAGGTGTACACGCTCGGGCCGCTGCCGCTGGTGGCGCGCAAGGACCCGCCCTCGCCGCGCGCTCGTCCATCAGGCTCAGCCTG	840
qRA2	GAGGCGGTGGCGGCCATGGAGGGCGCTCGGCTTGCCCAAGGTGTACACGCTCGGGCCGCTGCCGCTGGTGGCGCGCAAGGACCCGCCCTCGCCGCGCGCTCGTCCATCAGGCTCAGCCTG	840
qra2	GAGGCGGTGGCGGCCATGGAGGGCGCTCGGCTTGCCCAAGGTGTACACGCTCGGGCCGCTGCCGCTGGTGGCGCGCAAGGACCCGCCCTCGCCGCGCGCTCGTCCATCAGGCTCAGCCTG	840
Consensgaggcggtggcgccatggaggcgctcggttgcccaagggtgtacacgctcgggccgctgcccgtggtggcgcgcaaggacccgccctcgccgcgcgctcgctccatcaggctcagcctg		
Nip	TGGAAGGAGCAGGAGGAGTGCTTCAGTGGCTCGACGGCAAGGAGGCTGGCTCCGTCGTGTACGTCAACTTCGGCAGCATACCGTCATGACCAACGAGCAGCTGGTGGAGTTTCGCGTGG	960
qRA2	TGGAAGGAGCAGGAGGAGTGCTTCAGTGGCTCGACGGCAAGGAGGCTGGCTCCGTCGTGTACGTCAACTTCGGCAGCATACCGTCATGACCAACGAGCAGCTGGTGGAGTTTCGCGTGG	960
qra2	TGGAAGGAGCAGGAGGAGTGCTTCAGTGGCTCGACGGCAAGGAGGCTGGCTCCGTCGTGTACGTCAACTTCGGCAGCATACCGTCATGACCAACGAGCAGCTGGTGGAGTTTCGCGTGG	960
ConsensTggaaggagcaggaggagtgcttcagtggtctgacggcaaggaggctggctccgtcgtgtacgtcaacttcggcagcatcacgctcatgaccaacgagcagctggaggagttcgcggtg		
Nip	GGGCTGGCCAAACAGCGGCAGGGAGTTCTGTGGATCGTCCGGCGCGACCTCGTCAAGGGCGACACGGCCGTGCTGCCGCCGGAGTTCTGGCCGAGACGGCGGAGCGCGGCTCATGGCG	1080
qRA2	GGGCTGGCCAAACAGCGGCAGGGAGTTCTGTGGATCGTCCGGCGCGACCTCGTCAAGGGCGACACGGCCGTGCTGCCGCCGGAGTTCTGGCCGAGACGGCGGAGCGCGGCTCATGGCG	1080
qpa2	GGGCTGGCCAAACAGCGGCAGGGAGTTCTGTGGATCGTCCGGCGCGACCTCGTCAAGGGCGACACGGCCGTGCTGCCGCCGGAGTTCTGGCCGAGACGGCGGAGCGCGGCTCATGGCG	1080
Consensgggtggcccaacagcggcaggagttcctgtggatcgctcgggcgacctcgctcaaggcgacacggcgctgctgcccggaggttccttgcccgagacggcgaggcgccctcatggcg		
Nip	TCCTGGTGGCCGACGACGAGCTGCTGAACCAACCCCGCGTGGGCGCGTTCTTGACGCACAGCGGCTGGAACCTCGACGCTGGAGAGCTTGGCCGCGCGGCTGCCCGTCATCAGCTGGCCA	1200
qRA2	TCCTGGTGGCCGACGACGAGCTGCTGAACCAACCCCGCGTGGGCGCGTTCTTGACGCACAGCGGCTGGAACCTCGACGCTGGAGAGCTTGGCCGCGCGGCTGCCCGTCATCAGCTGGCCA	1200
qra2	TCCTGGTGGCCGACGACGAGCTGCTGAACCAACCCCGCGTGGGCGCGTTCTTGACGCACAGCGGCTGGAACCTCGACGCTGGAGAGCTTGGCCGCGCGGCTGCCCGTCATCAGCTGGCCA	1200
ConsensTcctggtgccccgacgaggacgtgctgaaccaccccgcggtggggcgcttcctgaacgcacagcggtggaactcgacgctggagagcttggcccgccggcgctgcccgctcatcagctggcca		
Nip	TTCTTCGCCGACACGACGAGCAACTGCCGGTACCAAGTGAACGAGTGGGGCGTCGGCATGGAGATCGACAGCAACGCTCAAGCGCGGCGCCGCTCGCGTGCCTCATCGCTGAGCTCATGGAA	1320
qRA2	TTCTTCGCCGACACGACGAGCAACTGCCGGTACCAAGTGAACGAGTGGGGCGTCGGCATGGAGATCGACAGCAACGCTCAAGCGCGGCGCCGCTCGCGTGCCTCATCGCTGAGCTCATGGAA	1320
qra2	TTCTTCGCCGACACGACGAGCAACTGCCGGTACCAAGTGAACGAGTGGGGCGTCGGCATGGAGATCGACAGCAACGCTCAAGCGCGGCGCCGCTCGCGTGCCTCATCGCTGAGCTCATGGAA	1320
ConsensTtcttcgccgaccagcagcgaactgccggtaccagtgaacgagtgggcgctcgccatggagatcgacagcaacgctcaagcgcgcgccgctcgcg gcctcatcgctgagctcatggaa		
Nip	GGTCAGAAAGGGGAAGGAGATGAGGAGGAAGCGGAGGAGTGGAGGGAGAAGCGCATCAGGGCGGCTAAGCCCGCGCGCTCGTCTCACCGCAACTTCGAAGAGCTGGTCCGCCACGTGCTC	1440
qRA2	GGTCAGAAAGGGGAAGGAGATGAGGAGGAAGCGGAGGAGTGGAGGGAGAAGCGCATCAGGGCGGCTAAGCCCGCGCGCTCGTCTCACCGCAACTTCGAAGAGCTGGTCCGCCACGTGCTC	1440
qra2	GGTCAGAAAGGGGAAGGAGATGAGGAGGAAGCGGAGGAGTGGAGGGAGAAGCGCATCAGGGCGGCTAAGCCCGCGCGCTCGTCTCACCGCAACTTCGAAGAGCTGGTCCGCCACGTGCTC	1440
Consensggtcagaaggggaaggagatgaggaggaaggcgaggagtgaggaggagaaggcgatcaggcgcgcttaagcccgcgcgctcgtctaccgcgaacttcgaagagctgggtccgcacgctgctc		
Nip	CTGGCCAAAGAGTCGTA	1457
qRA2	CTGGCCAAAGAGTCGTA	1457
qra2	CTGGCCAAAGAGTCGTA	1457
Consensctggccaaagagtcgta		

Figure S2. Multiple alignments of nucleic acid sequences. The nucleic acid sequences of Nip, *qRA2* and *qra2* in the LOC_Os02g51930 locus are displayed.

Nip	MGSLGAAGKPHAVCMFYPAQGHITPMLNVAKLLHARGFDVTFVNTEYNHARLVRTGEAAVAGAPGFRFATIPDGLPPSDDDDVTQDIPSLCRSTKETCLAPFRLLAQLNDPATGHFPV	120
qRA2	MGSLGAAGKPHAVCMFYPAQGHITPMLNVAKLLHARGFDVTFVNTEYNHARLVRTGEAAVAGAPGFRFATIPDGLPPSDDDDVTQDIPSLCRSTKETCLAPFRLLAQLNDPATGHFPV	120
qra2	MGSLGAAGKPHAVCMFYPAQGHITPMLNVAKLLHARGFDVTFVNTEYNHARLVRTGEAAVAGAPGFRFATIPDGLPPSDDDDVTQDIPSLCRSTKETCLAPFRLLAQLNDPATGHFPV	120
Consens	mgslgaagkphavcmfypaqghitpmlnvakllhargfdvtfvnteynharlvrtgeaavagapgfrfatipdglppsddddvtqdipsldcrstketclapfrllaqldndpatghppv	
Nip	TCVVSDEVVMGFSMAAAKELGLPYVQLWTASSISYLGVRHYRLLMERGLAPLKDVDQLTNGYLDTPVEDVPGLRNMRIKDFPSFIHTTNPEEYMGVYVIEETERCKDASAIIVNSFGDLEG	240
qRA2	TCVVSDEVVMGFSMAAAKELGLPYVQLWTASSISYLGVRHYRLLMERGLAPLKDVDQLTNGYLDTPVEDVPGLRNMRIKDFPSFIHTTNPEEYMGVYVIEETERCKDASAIIVNSFGDLEG	240
qra2	TCVVSDEVVMGFSMAAAKELGLPYVQLWTASSISYLGVRHYRLLMRGLAPLKDVDQLTNGYLDTPVEDVPGLRNMRIKDFPSFIHTTNPEEYMGVYVIEETERCKDASAIIVNSFGDLEG	239
Consens	tcvvsdevvmgfsmaaaakelglpyvqlwtassisylygrhyrllm glaplkdvdqltngyldtpvedvpglrnmrikdfpsfihttnpeeymvgvyieeterckdasaiivnsfgdleg	
Nip	EAVAAMEALGLPKVYTLGFLPLVARKDPPSPRRSSIRLSLWKEQEELQWLDGKEAGSVVYVNFSGSITVMTNEQLVEFAWGLANSGREFLWIVRRDLVKGDTAVLPPEFLAETAERGLMA	360
qRA2	EAVAAMEALGLPKVYTLGFLPLVARKDPPSPRRSSIRLSLWKEQEELQWLDGKEAGSVVYVNFSGSITVMTNEQLVEFAWGLANSGREFLWIVRRDLVKGDTAVLPPEFLAETAERGLMA	360
qra2	EAVAAMEALGLEKVYTLGFLPLVARKDPPSPRRSSIRLSLWKEQEELQWLDGKEAGSVVYVNFSGSITVMTNEQLVEFAWGLANSGREFLWIVRRDLVKGDTAVLPPEFLAETAERGLMA	359
Consens	eavaamealglpkvtytlgplplvarkdppsrrssirslswkeqeelqwidgkeagsvvynfgsitvmtneqlvefawglansgreflwivrrdlvkgdtavlppeflaetaerglma	
Nip	SWCPQQDVLNHPAVGAFLTHSGWNSTLESAAAGVFPVISWPFFADQQTNCRYQCNEWGVGMEIDSNVVRGAVAGLIAELMEGQKQKEMRRKAEENREKAIRAAKPGGSSHRNFEELVRHVL	480
qRA2	SWCPQQDVLNHPAVGAFLTHSGWNSTLESAAAGVFPVISWPFFADQQTNCRYQCNEWGVGMEIDSNVVRGAVAGLIAELMEGQKQKEMRRKAEENREKAIRAAKPGGSSHRNFEELVRHVL	480
qra2	SWCPQQDVLNHPAVGAFLTHSGWNSTLESAAAGVFPVISWPFFADQQTNCRYQCNEWGVGMEIDSNVVRGAVAGLIAELMEGQKQKEMRRKAEENREKAIRAAKPGGSSHRNFEELVRHVL	479
Consens	swcpqqdvlnhpavgaflthsgwnstleslaagvpviswpffadqqtncryqcnewgvvgmeidsnvkrava liaelmegqkgkemrrkaeeenrekairaaakpggsshrnfeelvrhvl	
Nip	LAKE	484
qRA2	LAKE	484
qra2	LAKE	483
Consens	lake	

Figure S3. Multiple alignments of amino acid sequences. Amino acid sequences of Nip, *qRA2* and *qra2* in the LOC_Os02g51930 locus are displayed.