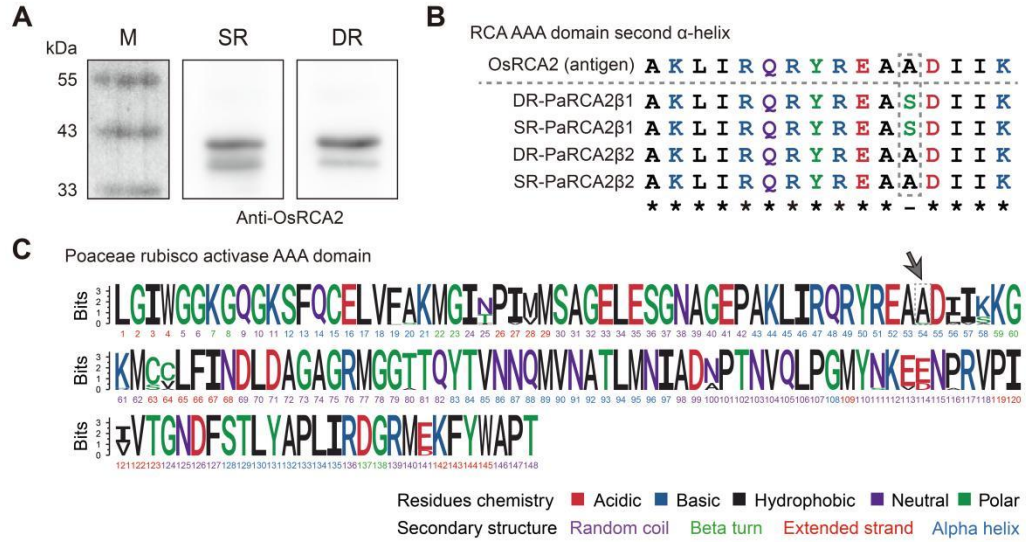


附图1 重组的*PaRCA*基因组PCR鉴定  
Appendix figure 1 Genome PCR identification of recombinant *PaRCA*



附图3 *PaRCA2- $\beta$* 基因的2个蛋白产物在芦苇中的突变位点  
Appendix figure 3 The mutation site between two proteins of *PaRCA2- $\beta$*  genes in *Phragmites australis*

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PaRCA_beta_1      1  -----CGTTC-----CGTTCAACCAAGAAGC AGC ---GCAGA
PaRCA_beta_2      1  GCCATCAC..A..ACTG TAAGTGC GCCTAGCAA.....C.....AGTA.....
PaRCA_beta_1r     1  -----
PaRCA_beta_2r     1  GCCATCAC..A..ACTG TAAGTGC GCCTAGCAA.....C.....AGTA.....
consensus         1  .....**..*****.*****.....*****

PaRCA_beta_1      30  GATGGCGGCCACCTTCTCCTCCACCGTCGGAGCCCCGGTGC GTGTGCTTCCATTTACATC
PaRCA_beta_2      61  .....G.....T.....
PaRCA_beta_1r     30  .....T.....
PaRCA_beta_2r     61  .....G.....T.....
consensus         61  *****.*****.*****.*****

PaRCA_beta_1      90  TCATGCTCAGCCACTTCTTCTTCTTGTTCAGATTAATTGATGGATGTGATATGAGTAT
PaRCA_beta_2     121  .....G..GC.....C.....C....
PaRCA_beta_1r     90  .....G..GC.....C.....C....
PaRCA_beta_2r     121  .....G..GC.....C.....C....
consensus         121  *****.*****.*****.*****

PaRCA_beta_1     150  TTGGCAGGCTCCACACCGACCAGCTTCCTCGGGAAGAAGCTCAAGAAGCAGGTGACCGC
PaRCA_beta_2     181  .....
PaRCA_beta_1r     150  .....
PaRCA_beta_2r     181  .....
consensus         181  *****

PaRCA_beta_1     210  GGCCGTGAACTACCATGGCAAGAGCTCCAGCGCCAACAGGTTCAAGGTCATGGCCAAGGA
PaRCA_beta_2     241  .....
PaRCA_beta_1r     210  .....
PaRCA_beta_2r     241  .....
consensus         241  *****

PaRCA_beta_1     270  GCTTGACGAGAGCAAGCAGACCGACCAGGACCGGTGGAAGGGCCTCGCCTACGACATCTC
PaRCA_beta_2     301  .....
PaRCA_beta_1r     270  .....
PaRCA_beta_2r     301  .....
consensus         301  *****

PaRCA_beta_1     330  CGACGACCAGCAGGACATCACCAGGGGCAAGGGCATCGTCTGACTCCCTCTTCCAGGCGCC
PaRCA_beta_2     361  .....
PaRCA_beta_1r     330  .....
PaRCA_beta_2r     361  .....
consensus         361  *****

PaRCA_beta_1     390  CATGGGTGACGGCACCCACGAGGCCGTGCTCAGCTCCTACGAGTACATCAGCCAGGGGCT
PaRCA_beta_2     421  .....
PaRCA_beta_1r     390  .....
PaRCA_beta_2r     421  .....
consensus         421  *****

PaRCA_beta_1     450  CAGACAGTAAGCACACTGCATCTGCATGCACCTTAATCATATTTAATTTAAGCATATAGA
PaRCA_beta_2     481  .....---A.....---
PaRCA_beta_1r     450  .....
PaRCA_beta_2r     481  .....---
consensus         481  *****.*****.*****

PaRCA_beta_1     510  GAGAGAGAGACGCATGCATGCTCAGCTCAATGTAACTCGATCAACTGAATC-ATTATGCC
PaRCA_beta_2     534  -.....T.....G.....G..C.C...
PaRCA_beta_1r     510  .....G.....-.....
PaRCA_beta_2r     538  -.....T.....G.....G..C.C...
consensus         541  .*****.*****.*****.*****.*****

PaRCA_beta_1     569  TTGTGTGCCTGCCTGCCTGCCTGCCTGCAGATTCAATTTGGACAACACCATGGACGGCTT
PaRCA_beta_2     593  ..T.-----.....G.....C.....
PaRCA_beta_1r     569  .....G.....
PaRCA_beta_2r     597  ..T.-----.....G.....C.....
consensus         601  **.*****.*****.*****

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PaRCA_beta_1 1259 GGATAACCCCCGCGTGCCCATCATCGTCACCGGTAACGACTTCTCCACCTCTACGCGCC
PaRCA_beta_2 1271 .....
PaRCA_beta_1r 1259 .....
PaRCA_beta_2r 1275 .....
consensus 1321 *****

PaRCA_beta_1 1319 ACTCATCCGCGACGGTCGTATGGAGAAGTTCTACTGGGCTCCCACCCGCGAGGACCGGAT
PaRCA_beta_2 1331 .....
PaRCA_beta_1r 1319 .....
PaRCA_beta_2r 1335 .....
consensus 1381 *****

PaRCA_beta_1 1379 CGGTGTCTCCAAGGGTATCTTCCGCTCCGACAACGTCCCCGATGACGCCATTGTCAAGAT
PaRCA_beta_2 1391 .....
PaRCA_beta_1r 1379 .....
PaRCA_beta_2r 1395 .....
consensus 1441 *****

PaRCA_beta_1 1439 CGTCGACACCTTCCCTGGGCAGTCCATCGACTTCTTCGGTGCCCTGCGTGCCCGGGTGTA
PaRCA_beta_2 1451 .....
PaRCA_beta_1r 1439 .....
PaRCA_beta_2r 1455 .....
consensus 1501 *****

PaRCA_beta_1 1499 CGACGACGAGGTGCGCAAATGGGTCCGAGAGACCGGAGTCGAGAACATCGGCAAGAGGCT
PaRCA_beta_2 1511 .....G.....
PaRCA_beta_1r 1499 .....G.....
PaRCA_beta_2r 1515 .....
consensus 1561 *****

PaRCA_beta_1 1559 TGTCAACTCCCGGGAGGGCCACCGACCTTCGAGCAGCCCAAGATGACGGTGGAGAAGCT
PaRCA_beta_2 1571 .....
PaRCA_beta_1r 1559 .....
PaRCA_beta_2r 1575 .....
consensus 1621 *****

PaRCA_beta_1 1619 CTTGGAGTACGGCCACATGCTGGTGCAGGAGCAGGAGAATGTCAAGCGTGTGCAGCTTGC
PaRCA_beta_2 1631 .....
PaRCA_beta_1r 1619 .....G.....
PaRCA_beta_2r 1635 .....G.....
consensus 1681 *****

PaRCA_beta_1 1679 TGACAAGTACCTCAGCGAGGCAGCTCTTGGTGCAGCTAACGACGATGCCATCAAGTCTGG
PaRCA_beta_2 1691 .....
PaRCA_beta_1r 1679 .....
PaRCA_beta_2r 1695 .....
consensus 1741 *****

PaRCA_beta_1 1739 TGCCTTCTACCAGCAGTAGAACATTATA -----ATAGGATC
PaRCA_beta_2 1751 .....TTGCTAGGCGCCGCGCCGCCTGC.....
PaRCA_beta_1r 1739 .....TTGCTAGG---CGCCGCGCCTGC.....
PaRCA_beta_2r 1755 .....-----.....
consensus 1801 *****

PaRCA_beta_1 1775 CAGGAGGTGAAT -----TTTAATTTTCG
PaRCA_beta_2 1811 .....TGCCTGCCCTCTTTCTACCTATTCAGTTAATTTCTGC.....T.
PaRCA_beta_1r 1795 .....TGCCTGCCCTCTTTCTACCTATTCAGTTAATTTCTGC.....T.
PaRCA_beta_2r 1791 .....-----.....
consensus 1861 *****

PaRCA_beta_1 1797 CACTCCTGCTACATACAGTATTCACTTCGATCTGTACTTTACTAAACTCA--ATTCTTGC
PaRCA_beta_2 1871 ---.TCAT..TTAGTT..TC.TCT.CA.T...C...C...GC...TGTT..G.A...
PaRCA_beta_1r 1855 ---.TCAT..TTAGTT..TC.TCT.CA.T...C...C...GC...TGTT..G.A...
PaRCA_beta_2r 1813 .....--.....
consensus 1921 ...**...**...**.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*

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PaRCA_beta_1 1855 --ATCTATAACTGCATACTTGCATATTTAATATCCAGTTTAA-----
PaRCA_beta_2 1928 AT..T.....T.----.....G..AGGATTGTTGAGTTATAT
PaRCA_beta_1r 1912 AT..T.....T.----.....G..AGGATTGTTGAGTTACAT
PaRCA_beta_2r 1871 --.....-----
consensus 1981 ..**.*.....**.....**.*.....

PaRCA_beta_1 1895 -AGGACATATATAA -----TTATCTTCTCACCAAAGTGTGCACATCATATTTTCAGGT
PaRCA_beta_2 1984 T.....C....CCGGCCGGCC.....T...T.A...A.C.....
PaRCA_beta_1r 1968 T.....C....CCGGCCGGCA.....T...T.A...A.C.....
PaRCA_beta_2r 1911 -.....-----
consensus 2041 .*****.**** .....*****.****.*.****.*.*****

PaRCA_beta_1 1946 AAAGCAGCGCAGCAAGTTAATGTTCTGTACCGGAAGGTTTCACTGACCCAACGGCGAGG
PaRCA_beta_2 2044 .....G.....-.....G...C...-----AG..
PaRCA_beta_1r 2028 .....G.....-.....G...C...-----AG..
PaRCA_beta_2r 1962 .....-----
consensus 2101 *****.*****.*****.***.***.....*..**

PaRCA_beta_1 2006 AGCGATCGATGCCAGCTGCCCTTACATCTTTCAAGC ---CTGCCATTTTTATTATTCCC
PaRCA_beta_2 2096 TA.C.A...C.G....T..T....A...T...TTAAT.....-...C....
PaRCA_beta_1r 2080 TA.C.A...C.G....T..T....A...T...TTAAT.....T...C....
PaRCA_beta_2r 2022 .....-----
consensus 2161 ..*.*.***.*.*****.***.*****.***.*** .....*****.***.***

PaRCA_beta_1 2062 CAT ---GTTTTACTCTTTGTACTAAAATACCGGATCCGGCCAAGCGATAGCTGGGCATG
PaRCA_beta_2 2155 G..GTTA.....
PaRCA_beta_1r 2140 G..GTTA.....
PaRCA_beta_2r 2078 .....-----
consensus 2221 ..*.....*****.....*****

PaRCA_beta_1 2118 AAACG
PaRCA_beta_2 2207 ----
PaRCA_beta_1r 2192 ----
PaRCA_beta_2r 2134 ....
consensus 2281 *.....

Primer used:
PaRCA_beta_1 β1_F : β1_R
PaRCA_beta_1r β1_F : β2_R
PaRCA_beta_2 β2_F : β2_R
PaRCA_beta_2r β2_F : β1_R

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**附图 2** 芦苇RCA2基因的代表性序列  
**Appendix figure 2** Representative genome sequence of RCA2 genes in *Phragmites australis*

附表 1 芦苇RCA转录本(LOC\_Os11g47970)

Appendix table 1 RCA transcripts of *Phragmites australis* (LOC\_Os11g47970)

No.	Path	FLC	Length (bp)	Intron	Score (bits)	E-value
PB.15448.1	12:81913(+)	2	1803	0	573	1.0E-161
PB.15449.1	17:11940(+)	4	1525	0	379	1.0E-103
PB.15449.2	17:32029(+)	53	1693	0	581	1.0E-164
PB.15449.3	17:32138(+)	3	1799	0	589	1.0E-166
PB.15449.4	17:31938(+)	11	1738	1	581	1.0E-164
PB.15449.5	17:31923(+)	2	1596	0	531	1.0E-149
PB.15449.6	17:31979(+)	3	1564	0	589	1.0E-166
PB.15449.7	17:31933(+)	3	1465	0	406	1.0E-111
PB.15449.8	17:31938(+)	6	1368	0	581	1.0E-164
PB.15449.9	17:72249(+)	2	1909	0	565	1.0E-159
PB.15449.10	17:81979(+)	2	1555	0	519	1.0E-145
PB.15449.11	17:111983(+)	2	1701	1	553	1.0E-156
PB.15449.12	17:6671980(+)	10	1210	0	589	1.0E-166
PB.15449.13	17:8781981(+)	6	1100	0	581	1.0E-164
PB.15450.1	19:31566(+)	2	1460	0	428	1.0E-118
PB.15450.2	19:9141566(+)	2	637	0	204	1.0E-50
PB.15451.1	22:32214(+)	4	2199	5	597	1.0E-169
PB.15451.2	22:32209(+)	3	1798	1	567	1.0E-160
PB.15451.3	22:32183(+)	2	1709	1	567	1.0E-160
PB.15451.4	22:32199(+)	6	1701	1	597	1.0E-169
PB.15451.5	22:32203(+)	5	1612	0	521	1.0E-146
PB.15451.6	22:31950(+)	14	1512	0	581	1.0E-164
PB.15451.7	22:82196(+)	2	712	0	297	9.0E-79

No.	Path	FLC	Length (bp)	Intron	Score (bits)	E-value
PB.15451.8	22:12662183(+)	3	754	0	254	1.0E-65
PB.15452.1	24:11582(+)	2	1582	0	377	1.0E-102
PB.15453.1	25:11586(+)	2	1586	0	575	1.0E-162
PB.15454.1	26:11667(+)	3	1667	0	589	1.0E-166
PB.15455.1	27:1734(+)	14	734	0	210	2.0E-52
PB.15456.1	28:1796(+)	2	796	0	220	2.0E-55
PB.15457.1	29:11719(+)	2	1719	0	404	1.0E-111
PB.15458.1	3:11912(+)	468	1694	0	589	1.0E-166
PB.15458.2	3:41849(+)	4	1562	0	422	1.0E-116
PB.15458.3	3:51818(+)	3	1712	1	599	1.0E-169
PB.15458.4	3:71766(+)	2	1556	0	589	1.0E-166
PB.15458.5	3:6281842(+)	7	1122	0	589	1.0E-166
PB.15458.6	3:9201893(+)	7	922	0	438	1.0E-121
PB.15459.1	4:12007(+)	2	1811	1	581	1.0E-164
PB.15459.2	4:61956(+)	2	1717	1	575	1.0E-162
PB.15459.3	4:2152009(+)	2	1691	1	591	1.0E-167
PB.15460.1	5:11863(+)	23	1668	0	589	1.0E-166
PB.15460.2	5:71819(+)	2	1441	0	377	1.0E-102
PB.15460.3	5:9821867(+)	13	863	0	383	1.0E-104

Consider each of the most complete transcripts as predicted gene that predicts the *PaRCA* gene sequence. FLC, Full Length Coverage counts.

**附表 2** 用于 *PaRCA* 分类的拟南芥和 19 个禾本科植物中的 *RCA* 基因

**Appendix table 2** *RCA* genes of *Arabidopsis thaliana* and 19 Poaceae plants used for *PaRCA* classification

Subfamily	Organism (species)	Gene No.	Annotation	Types
Panicoideae	<i>Panicum hallii</i>	<i>Pahal.H00088</i>	<i>PhRCA1</i>	β
		<i>Pahal.H00089</i>	<i>PhRCA2</i>	β
	<i>Panicum virgatum</i>	<i>Pavir.Ha00322</i>	<i>PvaRCA2</i>	β
		<i>Pavir.Hb00012</i>	<i>PvbRCA1</i>	α
		<i>Pavir.Hb00014</i>	<i>PvbRCA2</i>	β
	<i>Setaria italica</i>	<i>Seita.8G251300</i>	<i>SiRCA1</i>	α
		<i>Seita.8G251200</i>	<i>SiRCA2</i>	β
	<i>Sorghum bicolor</i>	<i>Sobic.005G231600</i>	<i>SbRCA1</i>	α
		<i>Sobic.005G231500</i>	<i>SbRCA2</i>	β
	<i>Zea mays PH207</i>	<i>Zm00008a014848_T01</i>	<i>ZmRCA1</i>	α
		<i>Zm00008a014849_T01</i>	<i>ZmRCA2</i>	β
	Pooideae	<i>Brachypodium distachyon</i>	<i>Bradi4g09120</i>	<i>BdRCA1</i>
<i>Bradi4g09125</i>			<i>BdRCA2</i>	α/β
<i>Brachypodium stacei</i>		<i>Brast10G200100</i>	<i>BsRCA1</i>	β
		<i>Brast10G200000</i>	<i>BsRCA2</i>	α/β
Oryzoideae	<i>Oryza sativa</i>	<i>LOC_Os11g47980 (Pseudogene)</i>	<i>OsRCA1</i>	NA
		<i>LOC_Os11g47970</i>	<i>OsRCA2</i>	α/β
Chloridoideae	<i>Oropetium thomaeum</i>	<i>Oropetium_20150105_26716A (front)</i>	<i>OtRCA1</i>	α
		<i>Oropetium_20150105_26716A (back)</i>	<i>OtRCA2</i>	β
Camelineae	<i>Arabidopsis thaliana columbia</i>	<i>AT2G39730</i>	<i>AtRCA</i>	α/β

All sequences data were obtained from Phytozome12 (JGI). Protein types were defined by CTE domain existence (α) or not (β) in products of each gene. α/β means both two products can be produced.



**附表 3** 利用MALDI-TOF/TOF鉴定 2 种生态型芦苇双向电泳凝胶中的RCA蛋白点

**Appendix table 3** Identification of RCA protein spots in 2-DE gels from two ecotypes of *Phragmites australis* by MALDI-TOF/T

Spot		Protein				Sequence-tag		MW (kDa) / pI		
No.	SR No.	DR No.	PM	SC (%)	Score	Sequence	Ions Score	Theoretical	Apparent	
396	PB.822.1	PB.1306.1	5	15.85	284	K.GLAYDISDDQQDITR.G	116	42.43/5.19	42.64/5.09	
						R.VPIIVTGNDFSTLYAPLIR.D	89			
						K.LVDTFPGQSIDFFGALR.A	104			
						R.VYDDEVR.R	26			
						R.EGPPTFEQPK.M	61			
298	PB.822.1	PB.1306.1	5	22.61	475	K.GLAYDISDDQQDITR.G	124	42.43/5.19	42.54/5.18	
						K.GIVDSLQAP(M)GDGTHEAVLSSYEYISQGLR.Q	97			
						K.(M)CALFINDLDAGAGR.M	122			
						R.VPIIVTGNDFSTLYAPLIR.D	129			
						K.IVDTFPGQSIDFFGALR.A	121			
103	PB.822.1	PB.1306.1	7	17.02	556	K.GLAYDISDDQQDITR.G	123	42.43/5.19	42.32/5.24	
						K.MCALFINDLDAGAGR.M	95			
						K.(M)CALFINDLDAGAGR.M	50			
						R.VPIIVTGNDFSTLYAPLIR.D	121			
						K.FYWAPTR.E	44			
						K.LVDTFPGQSIDFFGALR.A	129			
						R.VYDDEVR.R	44			

Spot		Protein			Sequence-tag			MW (kDa) / pI		
No.	SR No.	DR No.	PM	SC (%)	Score	Sequence	Ions	Score	Theoretical	Apparent
297	PB.822.1	PB.1306.1	5	15.85	327	K.GLAYDISDDQDITR.G	125		42.43/5.19	42.41/5.30
						R.VPIIVTGNDFSTLYAPLIR.D	112			
						K.IVDTFPGQSIDFFGALR.A	114			
						R.VYDDEV.R.K	37			
						R.EGPPTFEQPK.M	62			
176	PB.822.1	PB.1306.1	4	13.52	214	K.(M)CALFINDLDAGAGR.M	79		42.43/5.19	39.82/5.56
						R.VPIIVTGNDFSTLYAPLIR.D	71			
						K.LVDTFPGQSIDFFGALR.A	124			
						R.VYDDEV.R.R	34			
109	PB.822.1	PB.1306.1	8	22.61	363	K.SFQCELVFAK.M	64		42.43/5.19	39.89/5.77
						K.MCALFINDLDAGAGR.M	51			
						R.VPIIVTGNDFSTLYAPLIR.D	91			
						K.FYWAPTR.E	40			
						K.LVDTFPGQSIDFFGALR.A	110			
						R.VYDDEV.R.R	61			
						K.WVGETGVENIGK.R	96			
R.EGPPTFEQPK.M	71									

PM, Peptide Match; SC, Sequence Coverage (No signal peptide); Theoretical, calculated with predicted protein except signal peptide; Apparent, In-Gel measured. (M) means oxidation methionine residue.