CLONE AND CHARACTERISTICS OF A NOVEL SOYBEAN RECEPTOR – LIKE PROTEIN KINASE *rlpk* 2 GENE*

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Abstract Receptor – like protein kinases (RLKs) play major roles in plant cellular processes and stress responses. Here we reported rapid amplification of cDNA ends (RACE) of a novel soybean receptor – like kinase gene *rlpk* 2. Full length *rlpk* 2 cDNA was cloned according to the results of RACE. Details of PCR condition were discussed. The resultant cDNA sequence has been submitted into the GenBank database and assigned the accession No. AY687391.

Key words Soybean; Terminal deoxynucleotidyl Transferase (TdT); Receptor – like protein kinase; Rapid Amplification of a cDNA Ends (RACE)
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Receptor protein kinases comprise multigene families in animals. They are plasma membrane – bound and play an important role in the perception and transmittance of external signals[1-3]. Many signals are perceived by the extracellular domains of receptor protein kinases and are transduced by activation of intracellular kinase domains. Receptor protein kinases in animals can autophosphorylate either on tyrosine residues or on serine and/or threonine residues. Most plant counterparts, receptor – like protein kinases (RLKs), have topological features of the tyrosine receptor protein kinases but contain sequence motifs characteristic of serine/threonine kinases. Plant RLK gene families participate in wide spectra of cellular responses[4]. We previously isolated partial cDNA of four novel soybean (*Glycine max*) receptor – like protein kinase genes(*rlpk*1, *rlpk*2, *rlpk*3, *rlpk*4)[5]. In this study, We used Rapid Amplification of Cdna Ends (RACE) method to clone the 5′and 3′termini of *rlpk*2 cDNA. Based on the results of RACE, We cloned the full – length cDNA sequence of *rlpk*2 gene.

1 Materials and methods

1.1 Organisms and growth conditions

Seeds of soybean variety Kefeng 34 (obtained from Tianjin Agriculture Science Institute) were soaked with water and germinated at 24 ℃ in the dark for 48h. Radicles were collected for RNA extraction. Escherchia coli strain DH5a was grown at 37 ℃ in Lura – Bertani medium(LB) supplemented with relative antibiotics.

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1.2 Enzymes and chemicals

Reverse transcriptase AMV was obtained from Promega. Restriction endonucleases, TdT and ExTaq were obtained from TaKaRa (Bio. Co. Ltd. Dalian. China) Other chemicals were obtained from Shanghai Sangon Biological Engineering Technology & Service Co., Ltd.

1.3 Total RNA preparation

The extraction of total RNA was done according to the method of molecular cloning manual[6] based on guanidinium thiocyanate method and stored at −70℃ for future use.

1.4 Primers preparation

In this paper, all the primers used were synthesized by Shanghai Sangon Biological Engineering Technology & Service Co., Ltd. They are listed in the talbe 1.

Table 1 Sequences of primers used in this study

Primer name	Sequence
3R−2:	5′−TAATGTGTTGCTTGATCAAGATCTCAATC−3′
n3R−2:	5′−CCGAATAGCTGGGACATTTGGATAT−3′
P2853:	5′−GCGAATTCd(T)$_{17}$−3′
5R−2:	5′−GTAACCATGCATGGCATATTCTGGAG−3′
n5R−2:	5′−ATATCCAAATGTCCCAGCTATTCGG−3′
AGP	5′−GGCCACGCGTCGACTAGTAC(G)$_{16}$−3′
UGP	5′−GGCCACGCGTCGACTAGTAC−3′
2−ful−F	5′−GGATCCCATGAGATCAGTAAGTTCCCCTGG−3′
2−ful−R	5′−GGTCACCTTAATCTCTCTTCTCCAAGTAAG−3′

1.5 Cloning and sequencing of PCR product

All the PCR products were cloned into pMD−18T (TaKaRa Bio. Dalian. China Co. Ltd) and transformed into DH5a. DNA sequencing was done by Shanghai Sangon Biological Engineering Technology & Service Co., Ltd.

1.6 Amplification of 3′−end cDNA fragment

According to the known partial cDNA fragment of *rlpk* 2, an outer specific primer 3R−2 and a nested specific primer n3R−2 was designed. 2μg total RNA was reversely transcribed into cDNA by the reverse transcriptase AMV with Oligo(dT) primer P2853. 3′−end fragment of *rlpk*2 cDNA was amplified using the primer pairs 3R−2 and P2853 in 50μl system by 1.25U ExTaq. The PCR mixture was denatured by heating at 94℃ for 5min. 30 cycles of amplification were performed under the condition of 94℃ for 30s, 50℃ for 30s, 72℃ for 90s following an elongation of 72℃ for 10min. The primary PCR product was diluted 100 times with dd−H_2O and 5μl of the dilution was applied as the template of second PCR. The second PCR was performed using the primer pair n3R−2 and P2853 under the similar condition of primary PCR. The specific fragment of the secondary PCR was then cloned into TA vector and sequenced.

1.7 Amplification of 5′−end cDNA fragment

2.0μg of total RNA was reversely transcribed into cDNA using the outer gene specific primer 5R−2 by the reverse transcriptase AMV. An Oligo(dC) tail was added to the 3′end of the purified cDNA using TdT following the instruction of the supplier(TaKaRa Bio. Dalian. China Co. Ltd). The products were diluted to 500μl with dd−H_2O and 5μl of which was applied as the primary PCR template. The primary amplification was performed using the primer pairs 5R−2 and AGP in a 50ul system by 1.25U ExTaq. The PCR mixture was denatured by heating at 94℃ for 5min. 30 cycles of amplification were performed under the condition of 94℃ for 30s, 58℃ for 30s, 72℃ for 180s, following an elongation of 72℃ for 10min. The primary PCR product was diluted 100 times with dd−H_2O and 5μl of which was applied as the template of secondary PCR. The secondary PCR was performed using the primer pair n5R−2 and UGP under the very same condition of primary PCR. The specific fragment of secondary PCR was then cloned and sequenced.

1.8 Amplification of full−length *rlpk* 2 cDNA

A pair of specific primer 2−ful−F and 2−ful−R was designed according to the RACE results. 2μg of total RNA was reversely transcribed into cDNA by the reverse transcriptase AMV with the Oligo(dT) primer P2853. Full−length cDNA of *rlpk* 2 was amplified with 2−ful−F and 2−ful−R in 50μl system by 1.25U ExTaq. The PCR mixture was denatured by heating at 94℃ for 3min. 35

cycles of amplification were performed under the condition of 94 ℃ for 50s, 55 ℃ for 90s, 72 ℃ for 180s and at last an elongation of 72 ℃ for 10min. The specific PCR product was then cloned into pMD – 18T (TaKaRa Bio. Dalian. China Co. Ltd) and transformed into E. coli DH5a. Analysis of the sequence was done Shanghai Sangon Biological Engineering Technology & Service Co., Ltd. The resultant cDNA sequence and the deduced amino acid sequence have been submitted into the GenBank database and assigned the Accession No. AY687391.

1.9 Prediction of *rlpk*2 using Scansite software

Use the protein analysis software NCBI Conserved Domain Search online (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) to predict *rlpk* 2 gene structure.

2 Results

2.1 Rapid amplification of 5′and 3′*rlpk*2 cDNA

For rapid amplification of 3 termini cDNA, the gene specific primer 3R – 2 and the primer P2853 were used in the primary PCR. The secondary PCR was performed using the primer pair n3R – 2 and P2853. The RACE of 5′termini of *rlpk*2 cDNA was done using Terminal deoxynucleotidyl Transferase (TdT) following the instruction of the supplier. For the primary PCR, the gene specific primer 5R – 2 and primer UAP were used. The nested PCR was done using primer AGP and the nested primer n5R – 2 (Fig. 1, Fig. 5). Both specific fragments of the secondary PCR for3′– and 5′ – RACE of *rlpk* 2 gene were then cloned and sequenced.

Fig. 1　Sketch of RACE of *rlpk*2

2.2 Identify of TA Clone of full length of rlpk2cDNA and its sequencing

A pair of primer 2 – ful – F and 2 – ful – R was designed according to the RACE results. Full length of *rlpk*2 cDNA amplification by RT – PCR method with 2 – ful – F and 2 – ful – R as primers was done (Fig. 2). The specific product was purified using TaKaRa purified Kit and directly cloned into pMD – 18T vector. The PCR method was used to identify positive clones. Plasmid of positive clone (pMD – *rlpk*2) was digested with HindIII and EcoRI to confirm the PCR results (Fig. 3). Several confirmed clones were sent to Shanghai Sangon Biological Engineering Technology & Service Co., Ltd for sequencing. The sequencing results showed all the clones had identical DNA insert fragment. The DNA sequence and its deduced amino acid are showed as Fig. 4.

The preliminary structural analysis of *rlpk* 2 protein was done by software NCBI Conserved Domain Search online (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) (Fig. 6).

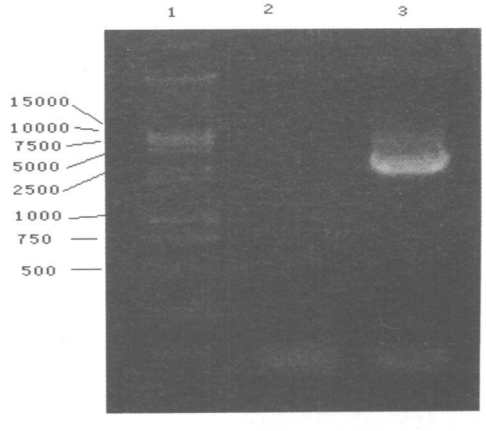

Fig. 2　Electrophoresis of PCR amplification of full length *rlpk*2 cDNA

1: DNA marker; 2: PCR negative control with no DNA template; 3: PCR product with cDNA as template

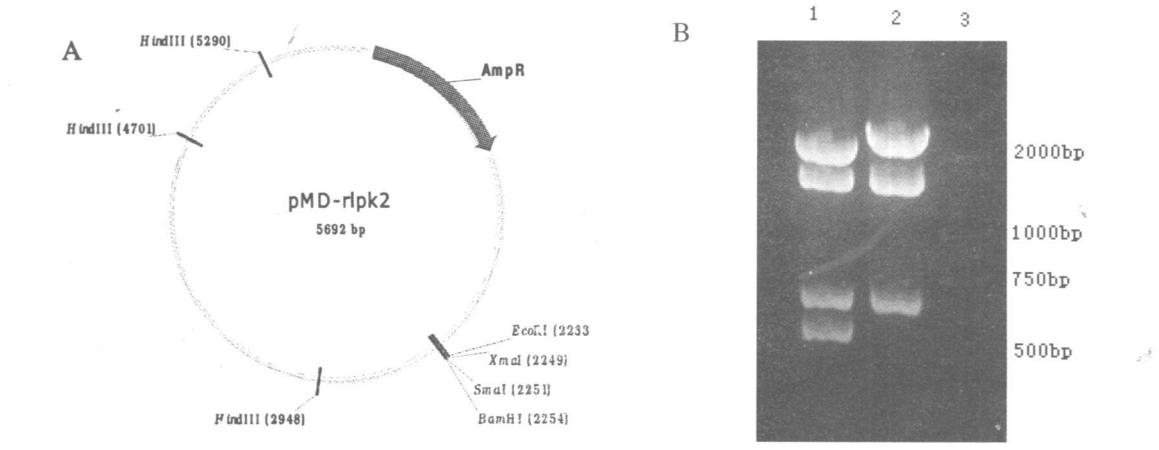

Fig. 3 Identification of the vector pMD – *rlpk*2

A: Diagram of the vector pMD – rlpk2 with restriction enzyme sites; B: Electrophoresis of the vector pMD – *rlpk*2 digested with HindIII / EcoRI or HindIII. 1: HindIII and EcoRI digestion; 2: HindIII digestion; 3: DNA Marker

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gtggttcgtgtgaactttcacttattcaatttctcaccatacaaattaaagtcatcttctgcgttatatctttggctgcc      80
attaatgtttccccaccaactctttcatgccatatagaatgatagctacttaattatatctttctccgcagctacttaat     160
ttcttctcataaattctgaggtttaatagtttagtcatcatcaaaacagcaagagcaagcATGAGATCAGTAAGTTCCCC     240
                                                        M  R  S  V  S  S  P       7

TGGTTTCTTCTTCCTATTGTTTCTTGCTGCCTTTAACTTTGCCAACTTTGCTTCTGGAGCCACTCTGCTTCAAGATGAAG     320
 G  F  F  F  L  L  F  L  A  A  F  N  F  A  N  F  A  S  G  A  T  L  L  Q  D  E  V      34
TGAAAGCTCTGGAAGATATATCCAAGACACTTGGGAAGAAGGACTGGGATTTCAACGTAAATCCGTGCAGTGGACAACGT     400
  K  A  L  E  D  I  S  K  T  L  G  K  K  D  W  D  F  N  V  N  P  C  S  G  Q  R       60
AATTGGACTTCTGCGGTTCAAGTGAAAGGGAGTGAAAACAATGTCACATGTGATTGTACCTTTGCCAATGGCACCGTCTG     480
N  W  T  S  A  V  Q  V  K  G  S  E  N  N  V  T  C  D  C  T  F  A  N  G  T  V  C       87
CCATGTCACCAACATACTTTTGAAATCACAAAAACTCCCTGGCACTCTCCCTCGGGATTTGTTCAGGTTGCCTTTCCTTC     560
 H  V  T  N  I  L  L  K  S  Q  K  L  P  G  T  L  P  R  D  L  F  R  L  P  F  L  Q     114
AAGAAATTGACCTTACTCGCAACTACCTAAACGGTACAATTCCTAAAGAATGGGGCTCCACGAAACTTGCCATAATTTCC     640
  E  I  D  L  T  R  N  Y  L  N  G  T  I  P  K  E  W  G  S  T  K  L  A  I  I  S      140
CTCCTAGGAAATCGATTAATAGGTTCAATACCAATAGAGATAGCAAACATATCCACTCTTCAAAGTTTGGTCTTGGAGGG     720
L  L  G  N  R  L  I  G  S  I  P  I  E  I  A  N  I  S  T  L  Q  S  L  V  L  E  G     167
CAATCAACTGTCTGGAAATCTTCCTCCTGAGCTTGGGAATCTAACCCAAATTCAAAAACTGCTACTTTCCTCCAACAATT     800
 N  Q  L  S  G  N  L  P  P  E  L  G  N  L  T  Q  I  Q  K  L  L  L  S  S  N  N  F     194
TTATTGGAGAATTACCAGTAACATTGGTCAAGCTCACTACGTTGCAGGATATTCGAATTGGGGATAATCAATTCTCTGGG     880
  I  G  E  L  P  V  T  L  V  K  L  T  T  L  Q  D  I  R  I  G  D  N  Q  F  S  G      220
AAGATACCTAATTTTATTCAAAGCTTGACAAGTCTCCAAAAACTAGTGATTCAAGGGAGTGGATTAAGCGGGCCAATTCC     960
K  I  P  N  F  I  Q  S  L  T  S  L  Q  K  L  V  I  Q  G  S  G  L  S  G  P  I  P     247
GTCTGGAATTTCATTTCTAGAAAACTTAACGGACTTGAGAATTAGTGATCTGAATGGATCTGAACATTCTCTTTTCCCAC    1040
 S  G  I  S  F  L  E  N  L  T  D  L  R  I  S  D  L  N  G  S  E  H  S  L  F  P  Q     274
AACTTAATCAGATGAAGAACTTGAAATATCTGATTCTAAGGAATTGCAACATCAATGGAACACTACCTCCATATCTTGGG    1120
  L  N  Q  M  K  N  L  K  Y  L  I  L  R  N  C  N  I  N  G  T  L  P  P  Y  L  G      300
AATATGACAACTTTAAAAAACTTAGACCTTAGCTTTAACAAATTGACTGGACCAATTCCAAGTACCTATGATGCCCTAAG    1200
N  M  T  T  L  K  N  L  D  L  S  F  N  K  L  T  G  P  I  P  S  T  Y  D  A  L  R     327
AAAAGTGGATTACATATATTTAACTGGGAATCTTCTCAATGGACAAGTACCTGCATGGACAGAGAAAAGTGACAATGTGG    1280
 K  V  D  Y  I  Y  L  T  G  N  L  L  N  G  Q  V  P  A  W  T  E  K  S  D  N  V  D     354
ATATTTCATTTAACAACTTCAGCGTCACAAGCCAAGGGAGTACATGTCAAATTGGAAATGTGAACTTGTTTGCTAGCTCT    1360
  I  S  F  N  N  F  S  V  T  S  Q  G  S  T  C  Q  I  G  N  V  N  L  F  A  S  S      380
ATGACGCACAATGACTCAGGAACAGTTGCATGCTTAGGAAGCAGTGTCTGTCAAGAAACGTTATATTCTCTTCATATAAA    1440
M  T  H  N  D  S  G  T  V  A  C  L  G  S  S  V  C  Q  E  T  L  Y  S  L  H  I  N     407
TTGTGGTGGAAAGATAGTAACAGACAATGGAAGCACATATGATGATGATTCAGATACTGGAGGTCCAGCAAGATTTCACC    1520
 C  G  G  K  I  V  T  D  N  G  S  T  Y  D  D  D  S  D  T  G  G  P  A  R  F  H  R     434
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GAAGTGGCACAAAAAATTGGGCATATATCAATACTGGTAACTTTATGGATAATGACGCTGGCGCCTATTATATCGTGCAA   1600
  S  G  T  K  N  W  A  Y  I  N  T  G  N  F  M  D  N  D  A  G  A  Y  Y  I  V  Q     460
AATAAAACTTTGCTTTCTACGGACAATGTTGACCTATACATGGATGCTCGAGTTTCTCCCATTTCTCTTACTTACTATGG   1680
N  K  T  L  L  S  T  D  N  V  D  L  Y  M  D  A  R  V  S  P  I  S  L  T  Y  Y  G     487
ATTTTGCCTGGGAAATGGAAACTACACGGTTAATCTACATTTTGCGGAAATCATGTTCATTGATGATCAAACATTTAACA   1760
 F  C  L  G  N  G  N  Y  T  V  N  L  H  F  A  E  I  M  F  I  D  D  Q  T  F  N  S   514
GCTTAGGAAGACGAGTATTTGACATCTATATTCAGGGAGCGCTAGTGAAGAAGGACTTTGATATTGTAGAAGAAGCAGGA   1840
  L  G  R  R  V  F  D  I  Y  I  Q  G  A  L  V  K  K  D  F  D  I  V  E  E  A  G     540
GGAATTGGTAAGGCAGTCATCACATCATTCACTGCTGTTGTGACTAGTAATACCTTGGAGATCCGCTTGTATTGGGCTGG   1920
G  I  G  K  A  V  I  T  S  F  T  A  V  V  T  S  N  T  L  E  I  R  L  Y  W  A  G     567
AAAGGGAACAACCAGTCTTCCATTTAGATCAGTATATGGTCCTCTTATATCAGCTATATCTGTGGAACCTGATTTTACAC   2000
 K  G  T  T  S  L  P  F  R  S  V  Y  G  P  L  I  S  A  I  S  V  E  P  D  F  T  P   594
CTCCTTCGAAAAACAAAAGTAGCATATCTGTAGGAGTTGTGGTTGGAGTTGTGGCAGCAGGAGCAGTTGTTATCATCCTT   2080
  P  S  K  N  K  S  S  I  S  V  G  V  V  V  G  V  V  A  A  G  A  V  V  I  I  L     620
GTACTTGGTATACTTTGGTGGAAAGGCTGCTTCGGAAAGAAAAGTTCTTTAGAGAGAGAGCTACAAGGTTTAGACCTGCG   2160
V  L  G  I  L  W  W  K  G  C  F  G  K  K  S  S  L  E  R  E  L  Q  G  L  D  L  R     647
AACGGGTTTGTTTACTTTAAGACAAATCAAAGCAGCGACAAACAACTTTGATGTTGCCAATAAGATTGGAGAAGGAGGTT   2240
 T  G  L  F  T  L  R  Q  I  K  A  A  T  N  N  F  D  V  A  N  K  I  G  E  G  G  F   674
TTGGTCCTGTGTACAAGGGATGTTTCTCAGATGGAACACTAATAGCTGTCAAACAACTTTCTTCTAAATCAAGGCAAGGG   2320
  G  P  V  Y  K  G  C  F  S  D  G  T  L  I  A  V  K  Q  L  S  S  K  S  R  Q  G     700
AATCGTGAGTTTTTAAATGAGATAGGCATGATTTCTGCTCTGCAGCATCCTCATCTTGTTAAACTCTATGGATGTTGTGT   2400
N  R  E  F  L  N  E  I  G  M  I  S  A  L  Q  H  P  H  L  V  K  L  Y  G  C  C  V     727
GGAGGGAGATCAACTATTGTTGGTATATGAATATATGGAAAACAATAGTCTTGCTCGCGCTCTATTTGGCGCCGAAGAAC   2480
 E  G  D  Q  L  L  L  V  Y  E  Y  M  E  N  N  S  L  A  R  A  L  F  G  A  E  E  H   754
ACCAAATAAAATTGGACTGGACAACAAGATACAAGATTTGTGTTGGTATTGCTAGAGGTTTGGCATACCTCCATGAAGAA   2560
  Q  I  K  L  D  W  T  T  R  Y  K  I  C  V  G  I  A  R  G  L  A  Y  L  H  E  E     780
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3R-2

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TCAAGATTGAAGATTGTTCATAGGGACATTAAGGCAACTAATGTGTTGCTTGATCAAGATCTCAATCCAAAAATATCAGA   2640
S  R  L  K  I  V  H  R  D  I  K  A  T  N  V  L  L  D  Q  D  L  N  P  K  I  S  D     807
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n3R-2

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CTTTGGTTTGGCCAAGCTTGATGAAGAGGACAACACTCATATTAGCACCCGAATAGCTGGGACATTTGGATATATGGCTC   2720
 F  G  L  A  K  L  D  E  E  D  N  T  H  I  S  T  R  I  A  G  T  F  G  Y  M  A  P   834
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5R-2　　n5R-2

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CAGAATATGCCATGCATGGTTACTTGACTGATAAAGCAGACGTCTATAGCTTTGGAATTGTTGCTTTGGAAATCATAAAC   2800
  E  Y  A  M  H  G  Y  L  T  D  K  A  D  V  Y  S  F  G  I  V  A  L  E  I  I  N     860
GGAAGGAGCAATACTATTCACCGACAAAAAGAAGAATCATTCTCTGTTCTTGAATGGGCACATCTGTTGAGAGAGAAAGG   2880
G  R  S  N  T  I  H  R  Q  K  E  E  S  F  S  V  L  E  W  A  H  L  L  R  E  K  G     887
AGATATAATGGATCTAGTTGATAGAAGATTAGGTTTAGAGTTCAACAAAGAGGAGGCGCTAGTGATGATCAAGGTAGCTC   2960
 D  I  M  D  L  V  D  R  R  L  G  L  E  F  N  K  E  E  A  L  V  M  I  K  V  A  L   914
TCTTATGCACTAATGTGACTGCAGCACTTAGGCCAACCATGTCATCGGTGGTCAGCATGCTAGAAGGAAAAATTGTTGTT   3040
  L  C  T  N  V  T  A  A  L  R  P  T  M  S  S  V  V  S  M  L  E  G  K  I  V  V     940
GATGAAGAGTTTTCTGGAGAGACAACTGAGGTATTGGATGAGAAGAAAATGGAGAAAATGAGATTGTATTACCAAGAGCT   3120
D  E  E  F  S  G  E  T  T  E  V  L  D  E  K  K  M  E  K  M  R  L  Y  Y  Q  E  L     967
GAGTAACTCAAAGGAAGAACCATGGACAGCTTCATCTACTTCTGTAGCAGATCTTTATCCTGTGGGCCTAGATTCTTCTT   3200
 S  N  S  K  E  E  P  W  T  A  S  S  T  S  V  A  D  L  Y  P  V  G  L  D  S  S  Y   994
ACTTGGAGAAGAGAGATTAAagactttggagaaaagttgctttaattttgtattaattattttgttgtgagtttatgtgc   3280
  L  E  K  R  D                                                                    999
aaaattcatttctttcttgtaaatctatttgtgattgtttgaacgtgtgcaaatgtatgcatcagtagctttggtttttt   3360
aaaaaaaaaaaaaaaaaa                                                                 3378
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Fig. 4　DNA and deduced amino acid sequence of *rlpk* 2 cDNA. The cDNA was cloned as described in the Materials and Methods. Amino acid residues are designated by single letter code below the nucleotide sequence. The 5′ and 3′ untranslated sequences are indicated by lower case letters and the coding region is shown in upper case letters. The start codon and the stop codon were underlined. Positions of primers and their orientation are shown.

3 Discussion

There were many methods to amplify 5 termini of a gene. Terminal deoxynucleotidyl Transferase (TdT) was a traditional method to rapidly amplify 5′termini of cDNA(Fig. 5). To successfully amplify cDNA ends of a unknown gene, especially to amplify the 5 termini, PCR condition was very important using TdT method [7,8]. In order to optimize the nested PCR condition, different concentration of [Mg^{2+}] and template was tested. Finally 1mM[Mg^{2+}] and 5ml of 100′diluted product of primary PCR were used in the RACE reaction.

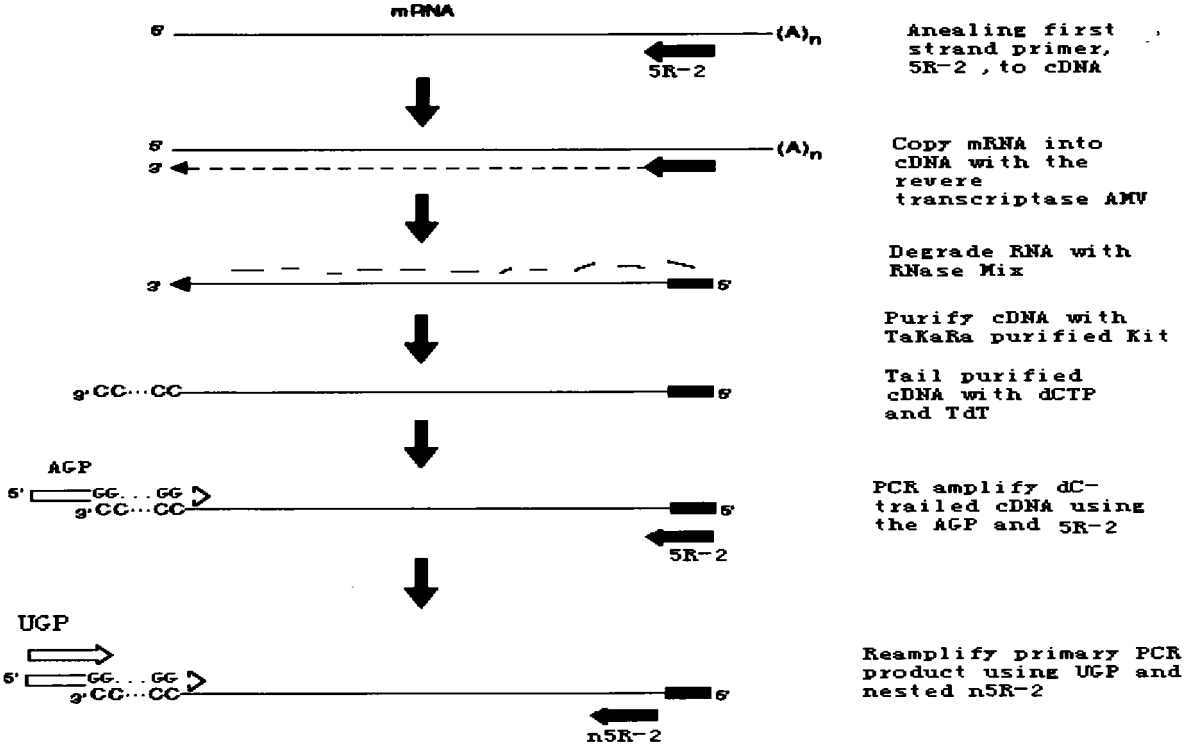

Fig. 5 Overview of the 5′RACE procedure

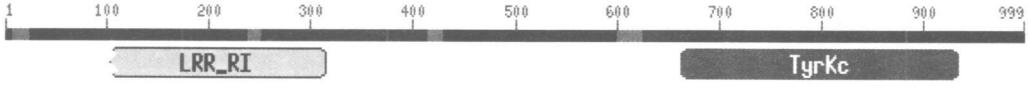

Fig. 6 Putative LRR and kinase domains in *rlpk* 2 protein predicted by NCBI Conserved Domain Search . 1 – 999: amino acids of *rlpk* 2 protein; 111 – 357: plant specific LRR profile; 661 – 935: tyrosine kinase (RTK) catalytic domain

A pair of primers 2 – ful – F and 2 – ful – R was designed according to the RACE results to amplify the full length *rlpk* 2 cDNA (Fig. 1). Sequenced TA clone vector pMD – *rlpk* 2 confirmed the RACE results.

The full length of *rlpk*2 cDNA sequence revealed an open reading frame of 999 amino acids. Structural prediction by NCBI Conserved Domain Search (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) suggested that *rlpk* 2 harbored typical conserved domains of leucine – rich repeats (LRRs) and tyrosine kinase (RTK) catalytic domain (Fig. 6)[9,10]. Database searches revealed that *rlpk* 2 shows 54% of identity and 68% of sequence similarity with an *Arabidopsis* receptor – like serine/threonine kinase gene (GenBank Accession No. BAB02650) on the amino acid level. All these information implied that RLPK2 was a novel soybean LRR receptor – like protein kinase.

The *rlpk*2 gene was found to be associated with the soybean leaves senescence in our previous work[5]. To elucidate the function of *rlpk*2 gene, we have constructed *rlpk*2 – RNAi binary vector to

knockout *rlpk*2 in soybean by agrobacterium – mediated transformation method. Several T_0 transgenic plants have been obtained and further study is in process.

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一个新的大豆类受体蛋白激酶基因 *rlpk*2 的克隆及其结构特征

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摘要　植物类受体蛋白激酶在细胞生长及对环境胁迫反应方面起着非常重要的作用。本文报告用 RACE 方法(快速扩增 cDNA 末端)扩增一个新的大豆类受体蛋白激酶基因 *rlpk*2 的全长 cDNA。根据 RACE 的结果,我们克隆了 *rlpk*2 的 cDNA,其序列信息已经提交到 GenBank (Accession No. AY687391)。经初步分析, *rlpk* 2 编码产物为 LRR 型类受体蛋白激酶。

关键词　大豆;　末端转移酶;　类受体蛋白激酶;　快速扩增 cDNA 末端