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Cloning and Expression Characteristics Analysis of *HcNPR1* Gene Related to Root-knot Nematode in Kenaf

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Abstract In order to excavate the related resistant genes in kenaf to root-knot nematodes and explore the molecular mechanism of the interaction between kenaf and root-knot nematodes, based on the obtained gene *NPR1* related to root-knot nematode from kenaf by the transcriptome sequencing, the PCR and RACE technology were used to clone the full length of gene *NPR1* in kenaf, and the gene was named as *HcNPR1* in this study. The cDNA of resistant gene *HcNPR1* to root-knot nematode has a full length of 2 058 bp, and its gene open reading frame (ORF) is 1 776 bp (Chr: 164-1939), which encodes a protein of 591 amino acids, with an isoelectric point of 6.02 and a molecular weight of 65.321 ku. This gene has 4 conserved domains of *NPR1* gene shared by the plants. Real-time RT-PCR results showed that the expression of *HcNPR1* gene changed significantly in kenaf after stress of 1 mmol/L jasmonic acid (JA), 1 mmol/L salicylic acid (SA) and 2 mmol/L ethylene (ET). The gene *HcNPR1* expression induced by JA and ET was significantly stronger than that by SA. The expression of *HcNPR1* gene reached the maximum when it was treated by SA for 12 hours, and the time for the strongest induction response of JA and ET was 6 hours. The induction trend of the three hormones increased rapidly first and then decreased. Therefore, it is speculated that the *HcNPR1* gene in kenaf plays a role on the resistance to root-knot nematodes. The results of this study can provide a theoretical basis for the genetic improvement of kenaf varieties resistant to root-knot nematode and the prevention and control of kenaf root-knot nematode disease in the future.

Keywords Kenaf (*Hibiscus cannabinus* L.); Root-knot nematode; *HcNPR1*; Cloning and expression

Kenaf (*Hibiscus cannabinus* L.) is an annual herbaceous fiber crop. As the Malvaceae fiber plant with the largest planting area in the world (Liao et al., 2018), it is often used to develop adsorbents, high-biomass ropes, textiles, fibers in recycled plastics, and livestock feed (Ashori et al., 2006; Saba et al., 2015). Compared with other hemp varieties, kenaf can adapt to multiple ecological environments and soil types, and has a wide planting range (Niu et al., 2018). Root-knot nematodes (*Meloidogyne* SPP.) have a wide host range and have been found to involve more than 5 500 host crops, especially in tropical and temperate regions, causing serious damage to crops (Jaouannet et al., 2012). In China, root-knot nematode disease in kenaf producing areas has seriously affected the yield and quality of kenaf. It parasitized on the roots of kenaf, produced rootnodule, and caused root rot, leading to serious symptoms of stunted growth or accelerated plant death (Tian et al., 2018).

In the process of pathogenic microorganisms infecting plants, plants will secrete R protein encoded by the resistance gene (R gene), which will trigger a series of defense responses of plants, effectively inhibiting the invasion of pathogenic microorganisms (Zheng et al., 2018). With the development of bioinformatics, 112 known R genes and 104 335 predicted R genes have been found in 241 plant species (Hermoso et al., 2013). In 1997, *HS1-pro-1*, the first gene resistant to *Heterodera Schachtii* Schmidt, was cloned (Cai et al., 1997). Subsequently, several anti-nematode genes such as *Cre3*, *Mi*, *Gpa2*, *Hero*, *Gro1-4* and *Ma* were cloned and validated (Cai et al., 1997; Claverie et al., 2011), but there are few reports on cloning, isolation and identification of kenaf root-knot nematode genes. In the early stage of our laboratory, the root-knot nematode resistant kenaf 'Roselle 4391' was used as the test material. After inoculation with *Meloidogyne enterolobii*, the resistant kenaf plants were screened

out, and the plant total RNA was extracted by Trizol method. The gene *NPR1* associated with root knot resistance was screened by transcriptome sequencing (unpublished). In this study, primers were designed to clone *NPR1* gene with reference to kenaf genome sequence and nucleic acid structure and sequence characteristics of various root-knot nematodes genes. The 3' and 5' ends of *NPR1* gene were amplified by RACE method, and the full length of *NPR1* gene was obtained after splicing. Based on jasmonic acid (JA), salicylic acid (SA) and ethylene (ET), which are important signal molecules of disease resistance signaling pathway in plants, the response patterns of the genes to JA, SA and ET were analyzed according to bioinformatics annotation. It is important to explore the genes of kenaf root-knot nematodes and breed kenaf root-knot nematodes.

1 Results and Analysis

1.1 Total RNA extraction and identification of kenaf

The total RNA of kenaf was extracted, and whether the total RNA of kenaf was qualified was tested. The OD_{260}/OD_{280} of the compound was 2.07, indicating that the total RNA extracted from kenaf samples was qualified. The total kenaf sample extracted from the test sample is RNA, and two clear bands can be seen (Figure 1), without obvious dragging, indicating that the total kenaf RNA in the sample has not been degraded significantly, and the extraction is successful, which can be applied in subsequent experiments.

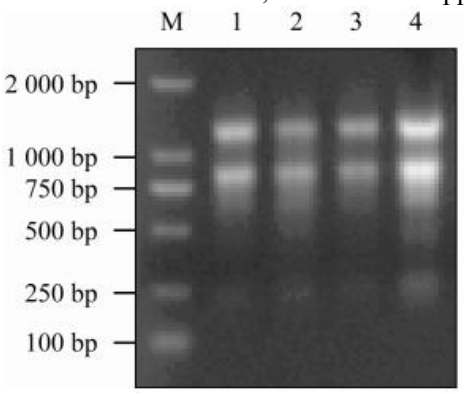

Figure 1 Electrophoresis identification results of kenaf RNA

Note: M: DL2000 DNA Marker;1, 2, 3, and 4 are the four repeats for of total RNA of kenaf

1.2 RACE amplification of 3' end and 5' end of root-knot nematode *HcNPR1* gene in kenaf

According to the known kenaf gene fragments, 3’ end primers and 5' end primers (RC564-NPR1-RT1/ RC564-NPR1-RT2) were designed for amplification (RACE technique). Kenaf stem and leaf RNA was used as template to perform PCR twice, and finally the corresponding instruments were used for detection. Analysis of the resulting images showed that RACE amplification at the 3' end of *HcNPR1* gene yielded a band of about 930 bp, and RACE amplification at the 5' end yielded a band of about 820 bp (Figure 2).

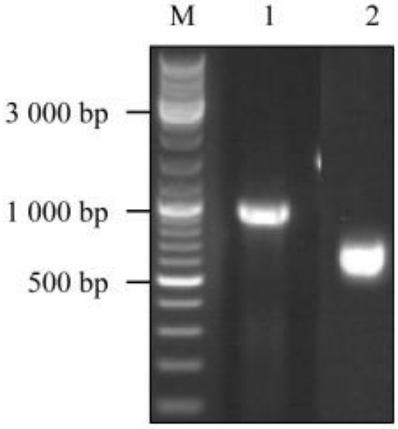

Figure 2 Results of *HcNPR1*-3’RACE and 5’RACE product by agarose electrophoresis

Note: M: DL10000 DNA Marker; 1 is the result of 3’RACE agarose electrophoresis; 2 is the result of 5’RACE agarose electrophoresis

1.3 Amino acid sequence analysis of kenaf root-knot nematode *HcNPR1* gene and its encoding

The sequencing results of 5'RACE and 3'RACE sequences were spliced with DNAman software, and the full-length *NPR1* gene sequence was obtained by removing the vector sequence. The total length of *HcNPR1* sequence was 2 058 bp, the open reading frame was 1 776 bp (Chr: 164-1939), the total length of *HcNPR1* sequence was 591 amino acids, its isoelectric point was 6.02, and its molecular weight was 65.321 Ku. The usage of four bases was as follows: A=26.35% [468], T=25.62% [455], C=22.58% [401], G=24.45% [452], uncertain base = 0.00% [0], (A+T)=51.97% [923], (C+G)=48.03% [853]. Kenaf *HcNPR1* gene encodes 19 amino acids (colorless amino acids). The highest repetition frequency was leucine (73), followed by alanine (54) and aspartic acid (48). The lowest repetition frequency was glusteine (13) and methionine (12). By type, there were 279 non-polar amino acids (47.21%), 134 uncharged amino acids (22.67%), 91 positively charged amino acids (15.40%) and 87 negatively charged amino acids (14.72%). According to this judgment, the number of non-polar amino acids in HcNPR1 numbered protein is much higher than that of polar amino acids, so it is speculated that the hydrophobicity of HcNPR1 protein is stronger than that of hydrophilicity (Figure 3).

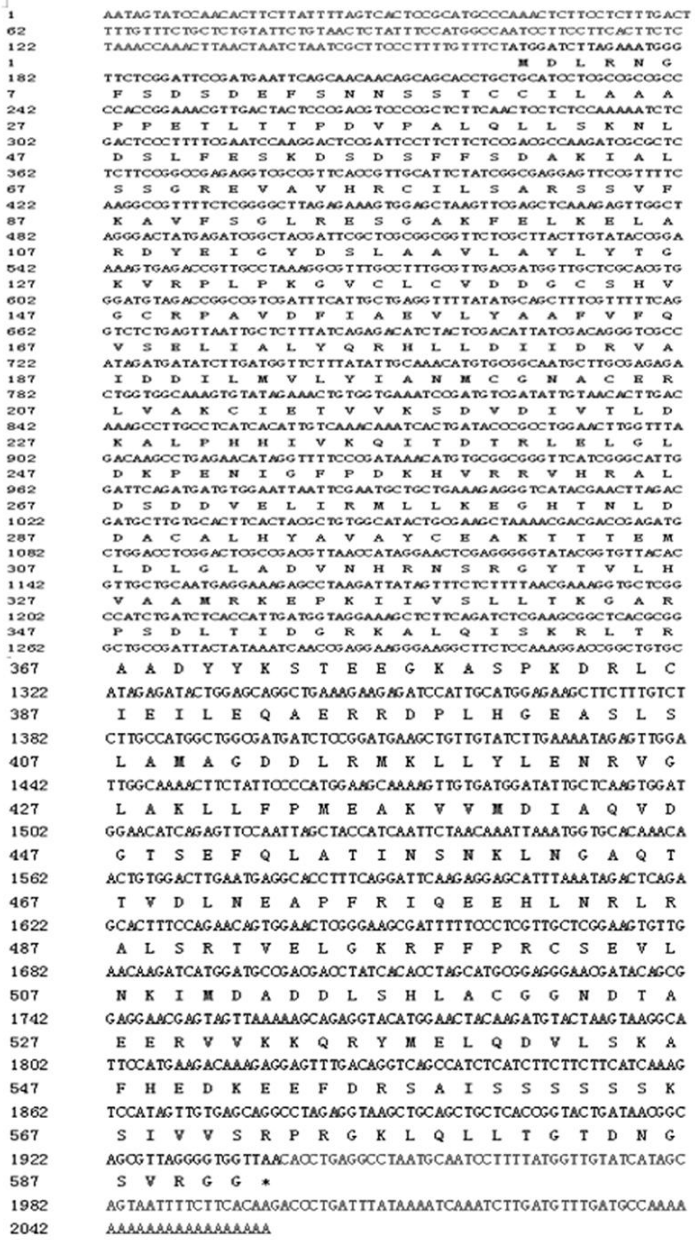

Figure 3 cDNA nucleotide sequence and deduced amino acid sequence of *HcNPR1* gene

Note: Numbers at left mean sequence of nucleotide and amino acid; The red part of the sequence indicates the non-coding region of the gene

1.4 Secondary structure and domain of kenaf HcNPR1 protein

The secondary structure of HcNPR1 protein was predicted by GOVIV online program. The results showed that the secondary structure of HcNPR1 protein was mainly composed of α-helical structure, random coil and folded extension chain. Among them, α-helix accounted for 49.58%, random coil accounted for 39.76%, and extended band accounted for 10.66%. Therefore, the most important secondary structures of kenaf HcNPR1 were α-helix and random coil (Figure 4). NCBI analysis of the conserved domains of HcNPR1 protein showed that the gene contained the conserved domains common to plant NPR1 proteins such as ANK (ankyrin repeat), BTB_POZ_NPR_plant domain, DUF and NPR1-like C (Figure 5).

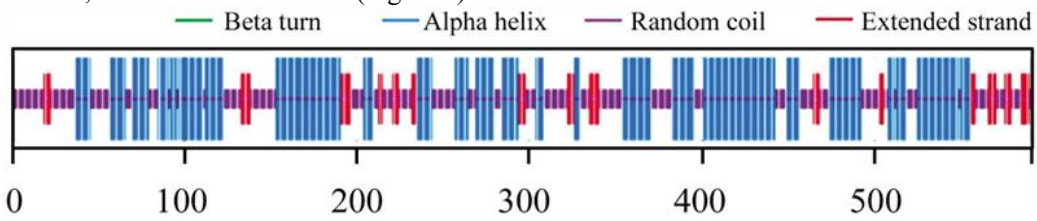

Figure 4 Secondary structure of kenaf HcNPR1 protein

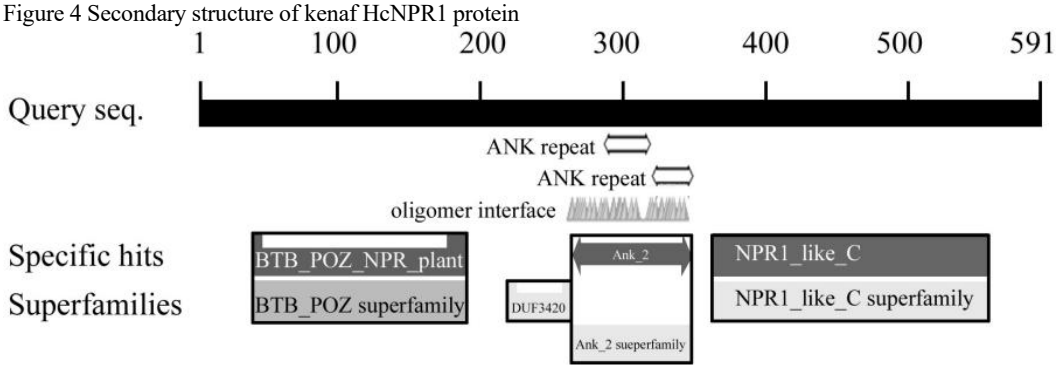

Figure 5 Conserved domain structure prediction of HcNPR1

1.5 Comparison of amino acid homology and phylogenetic analysis of kenaf *HcNPR1* gene

The amino acid sequence of the cloned kenaf root-knot nematode *HcNPR1* gene was compared with that of other crops obtained from NCBI website (Figure 6). It was found that kenaf HcNPR1 protein and NPR1 protein of most species shared BTB/POZ domain and NPR1-like C domain. Some sequences in the BTB/POZ domain of most plant species are relatively conserved and contain several identical amino acid sequences.

The NCBI database was searched, kenaf HcNPR1 protein and other 30 species NPR1 protein were phylogenetic analyzed and the evolutionary tree was constructed (Figure 7). The results showed that all the proteins were roughly divided into five groups. Kenaf HcNPR1 was grouped with *Hibiscus Syriacus*, *Gossypium raimondii* and *Gossypium australe*. The phylogenetic relationship between kenaf HcNPR1 protein and other species showed that kenaf HcNPR1 was more closely related to *Hibiscus syriacus*.

1.6 Response pattern of kenaf root-knot nematode *HcNPR1* gene to hormone

The RNA of kenaf seedlings treated with three hormones was extracted at each time point and reverse-transcribed into cDNA. Real-time PCR was used to analyze the response level of *HcNPR1* gene to the three hormones. The results showed that the expression of *HcNPR1* gene in kenaf was significantly changed after jasmonic acid (JA), salicylic acid (SA) and ethylene (ET) stress, and the effect of jasmonic acid and ethylene on *HcNPR1* gene expression was significantly stronger than that of salicylic acid (Figure 8). The expression of HcNPR1 induced by ethylene and jasmonic acid was significantly higher than that of salicylic acid. The change trend of NPR1 gene expression after treatment with the three hormones was basically the same, which rapidly increased to the maximum value within 6~12 h and then decreased. At 12 h of salicylic acid treatment, the maximum expression of HcNPR1 gene reached 20 times of that at 12 h of control (Figure 8C), while the most intense response time of jasmonic acid and ethylene induction was at 6 h, which were 150 times and 580 times of that at 6 h of control, respectively (Figure

8A; Figure 8 B). All three hormones can induce the expression of *HCNPR1* gene, and the response of *HCNPR1* gene to the three hormones is obvious in the early stage of induction.

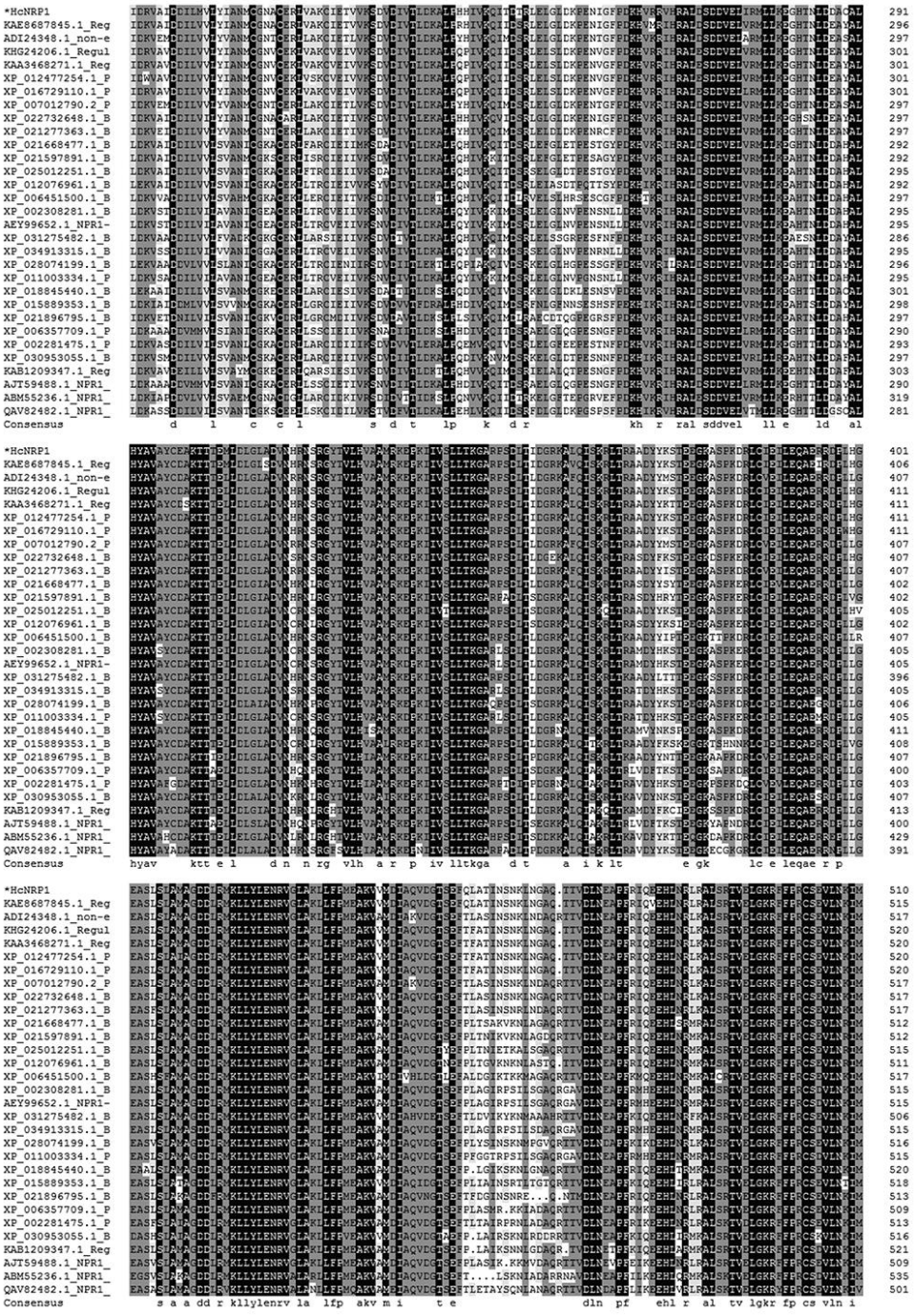

Figure 6 Comparison of the homology between the deduced amino acid sequence of Kenaf *HcNPR1* and other plant *NPR1*

Note: *Hibiscus syriacus* (KAE8687845); *Theobroma cacao* (ADI24348.1, XP_007012790.2); *Beta vulgaris* (ABM55236.1); *Artemisia_annua* (QAV82482.1); *Gossypiumaustrale* (KAA3468271.1); *Gossypium raimondii* (XP_012477254.1); *Gossypium hirsutum* (XP_016729110.1); *Durio zibethinus* (XP_022732648.1); *Herrania umbratica* (XP_021277363.1); *Hevea brasiliensis* (XP_021668477.1); *Manihot esculenta* (XP_021597891.1); *Ricinus communis* (XP_025012251.1); *Populus alba* (XP_034913315.1); *Jatropha curcas* (XP_012076961.1); *Citrus clementina* (XP_006451500.1); *Populus trichocarpa* (XP_002308281.1); *Populus deltoides* (AEY99652.1); *Pistacia vera* (XP_031275482.1); *Populus euphratica* (XP_011003334.1); *Juglans regia* (XP_018845440.1); *Ziziphus jujuba* (XP_015889353.1); *Carica papaya* (XP_021896795.1); *Solanum tuberosum* (XP_006357709.1); *Vitis vinifera* L. (XP_002281475.1); *Quercus lobata* (XP_030953055.1); *Morella rubra* (KAB1209347.1). The same as below

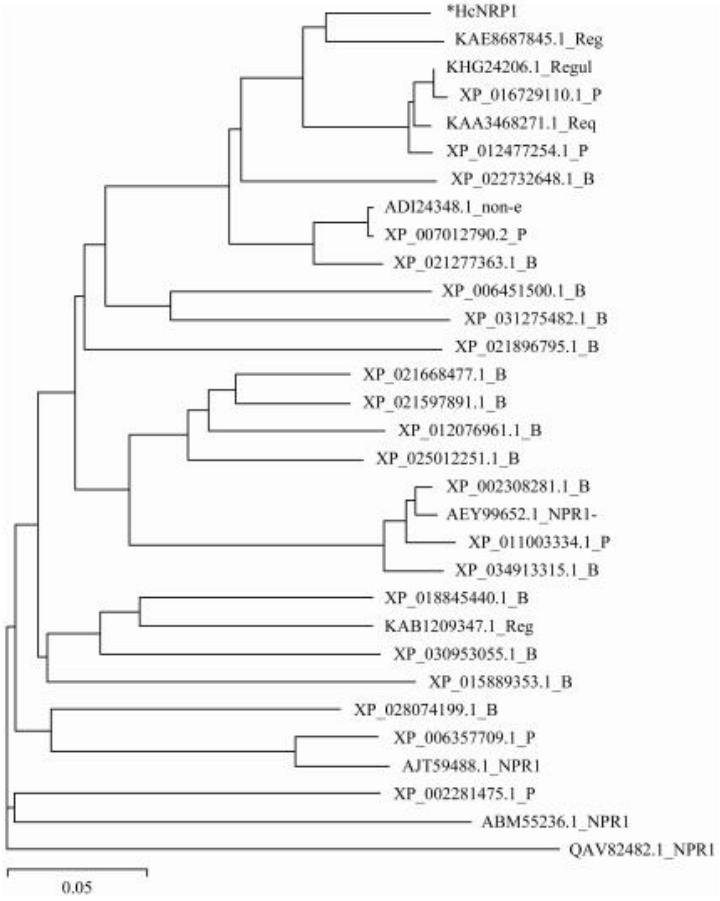

Figure 7 Phylogenetic tree of kenaf HcNPR1 protein and other plants NPR1

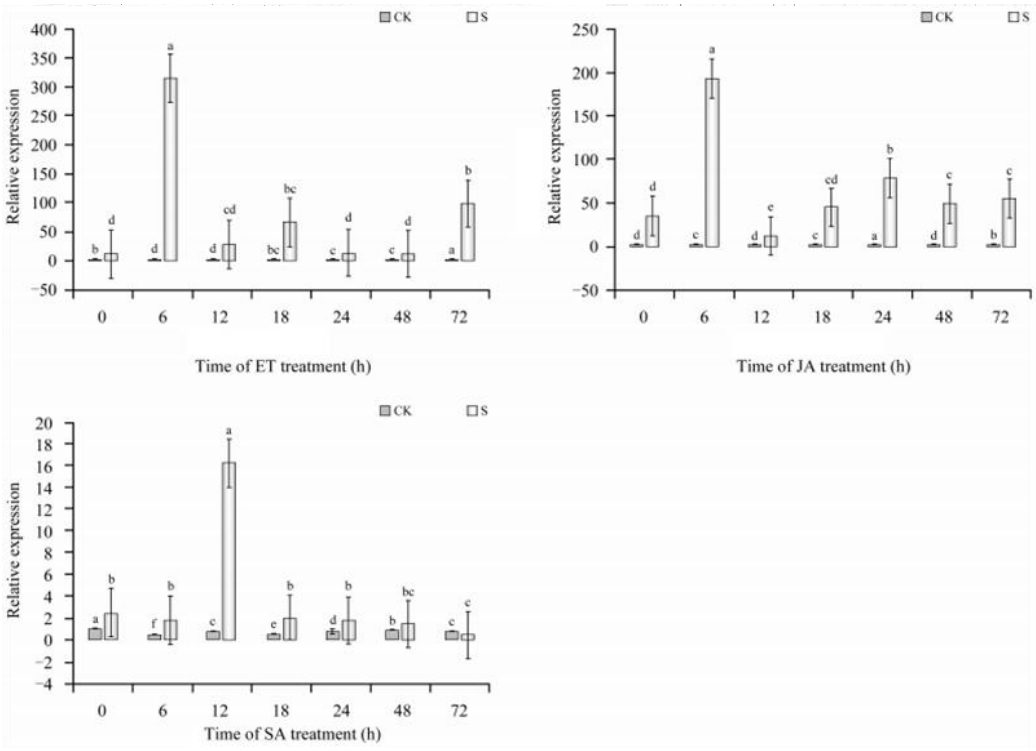

Figure 8 Expression characteristics of *HcNPR1* gene under different stress treatments

Note: The small letters in the figure indicate significant differences at the $p \leq 0.01$ level; the red font indicates a value whose relative expression is less than 0

2 Discussion

NPR1 (*Nonexpressor of pathogenesisrelated gene 1*), also known as *NIM1*, plays a central regulatory role in plant disease resistance and is the key in plant disease resistance signaling network. *NPR1* gene overexpression has been found to improve disease resistance in a variety of plants (Cao et al., 1994). *NPR1* gene also plays a regulatory role in many plants, such as *Theobroma cacao*, wheat (*Triticum aestivum* L.) and cotton (*Gossypium SPP*) (Shi et al., 2010; Zhang et al., 2013), but there are few studies on cloning, isolation and identification of kenaf resistance genes. In this study, according to kenaf genome sequence, primers were designed to clone NPR1 gene, and the full length of NPR1 gene was cloned by RACE method and named *HCNPR1*. *HCNPR1* gene has both conserved domains common to plant NPR1, and ANK (ankyrin repeat) sequence and BTB_POZ_NPR_plant domain play an important role in the function of *NPR1* gene (Michacly and Bennett, 1992). By analyzing the *NPR1* gene in other species, the function of kenaf *HcNPR1* gene can be predicted. For example, the *NPR1* gene in *Theobroma cacao* also has 591 coding amino acids, which can participate in SA-JA signal crosstalk, proving that JA responds to *TcNPR1* gene expression (Shi et al., 2010). In *Cabernet Sauvignon Grapevine*, *NPR1* gene has 584 coding amino acids. In terms of the regulation of defense related genes, overexpression of *NPR1* gene improved salt tolerance, but had no effect on drought tolerance (Zhang et al., 2013). Can be predicted accordingly, through kenaf *HCNPR1* genes may affect a certain signaling pathways, thereby indirectly defense conditions outside the damage, it is also possible under the stress of the outside environment, through regulating its trace chemical activation HCNPR1 gene expression, to enhance their defense system directly and reducing plant injury. These predictions are expected to be confirmed in subsequent studies.

In the process of evolution, plants have formed a set of defense systems that activate the expression of defense genes in vivo through various signaling molecules, thus showing resistance to adverse environment. The signaling molecules related to this mainly include salicylic acid, jasmonic acid, ethylene and abscisic acid (Wang et al., 2011). Based on the fact that jasmonic acid (JA), salicylic acid (SA) and ethylene (ET) are important signal molecules of disease resistance signaling pathway in plants, this study analyzed the response pattern of this gene to JA, SA and ET. The results showed that the expression of *HcNPR1* gene was significantly up-regulated in kenaf after jasmonic acid (JA), salicylic acid (SA) and ethylene (ET) stress. But the gene's response in the three kinds of hormone, jasmonic acid and ethylene induced response the strongest for 6 h, and salicylic acid in response to the most intense time in 12 h, in three kinds of hormone regulation pathway *HCNPR1* genes play an important role, can through the complexity of network signaling pathways involved in biological stress signal conduction.

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3 Materials and Methods

3.1 Experimental materials and reagents

Kenaf 'Roselle 4391' was donated by the Institute of Hemp, Chinese Academy of Agricultural Sciences. RevertAid Premium Reverse Transcriptase was purchased from Thermo Fisher; LA *Taq* was purchased from TaKaRa; SuperReal PreMix Plus SYBR Green was purchased from Tiangen Biotech Co., Ltd. Trizol extraction

kit and column DNA glue recovery kit were purchased from Sangon Biotech (Shanghai) Co., Ltd. All three phytohormones were purchased from Shanghai Yuanye Biotech Co., Ltd.

3.2 Hormone induction treatment

The experiment was carried out in the nursery of Plant Protection Institute of Hainan Academy of Agricultural Sciences. The cultured soil (red soil: compound fertilizer: coconut bran=1:1:1) was sterilized at 120℃ for 2 h, and kenaf seeds were watered in time after sowing. When the leaves grew to 4~5 true leaves, they were transferred to culture bottles and soaked in 200 mL of 1 mmol/L jasmonic acid medium, 1 mmol/L salicylic acid medium and 2 mmol/L ethephon medium, respectively. After 0, 6, 12, 18, 24, 48 and 72 h, samples were taken and frozen in liquid nitrogen (-80℃).

3.3 Identification of *HcNPR1* gene in kenaf root-knot nematodes

Based on the previous transcriptome data and disease resistance expression profile data, the Unigene sequences associated with kenaf resistance to *Meloidogyne enterolobii* were screened from the resistant/susceptible differentially expressed genes, and the annotation results were Contig sequences of *NPR1* gene. Primers were designed based on the Contig sequence (Table 1), and the full-length *NPR1* gene was cloned by RACE method.

Table 1 Primers used for cloning *HcNPR1* gene

Primer name	Sequence	Remarks
5'adaptor	GCTGTCAACGATACGCTACGTAACGGCATGACAGTGGGIIGGGIIGGGIIG	Joint primer
3'adaptor	GCTGTCAACGATACGCTACGTAACGGCATGACAGTGTTTTTTTTTTTTTTTT	
5.3'outer	GCTGTCAACGATACGCTACGTAAC	
5.3'inner	GCTACGTAACGGCATGACAGTG	
NPR1-F1	GAGGGGGTATACGGTGTTACACGTTGCT	3'RACE specific primer
NPR1-F2	GCTGCAATGAGGAAAGAGCCTAAGATTATAGTT	
NPR1-R2	AGGCAACGGTCTCACTTTTCCGGTAT	5'RACE specific primer
NPR1-R1	CAACCATCGTCAACGCAAAGGCAA	
NPR1-RT2	CCACATCATCTGAATCCAAT	
NPR1-RT1	ACCCTCTTTCAGCAGCA	

3.4 Total RNA extraction from kenaf

Total kenaf RNA was extracted with Trizol extraction kit of Sangon Biotech (Shanghai) Co., Ltd. For details, please refer to the instructions. Genomic DNA was removed with DNase I for use.

3.5 First-strand cDNA synthesis

Reagents were added to 0.2 mL PCR tube: 5 μL total RNA, 1 μL random primer, 1 μL ddH_2O, and warm bath at 70℃ for 5 min. Ice bath for 2 min; Centrifuge and add reagents: 2.0 μL 5X First-Strand Buffer, 0.5 μL 10 mmol dNTP, 0.25 μL Rnase inhibitor, 0.25 μL Reverse Transcriptase and 10.0 μL Total volume: 42℃ for 60 min, 72℃ for 10 min.

3.6 3'RACE amplification of kenaf root-knot nematode *HcNPR1* gene cDNA sequence

Nested PCR amplification was carried out using 3'adaptor as reverse primer and cDNA as template. The reaction process consisted of two rounds. The first round of reaction system was as follows: 12.5 μL 2X GC Buffer I, 0.5 μL 10 μM RC564-NPR1-F1, 0.5 μL 10 μM 5.3'outer, 4 μL 2.5 mM dNTP, 1 μL cDNA, 0.2 μL 5 U/μL *Taq* enzyme, 6.3 μL ddH_20. The reaction procedure was as follows: pre-denaturation at 95℃ for 3 min, denaturation at 94℃ for 30 s, annealing at 58℃ for 30 s, extension at 72℃ for 60 s, 33 cycles, and repair and extension at 72℃ for 7 min.

The second reaction system is as follows: 25 μL 2X GC BufferI, 1 μL 10 μM RC564-NPR1-F2, 1 μL 10 μM 5.3'inner, 8 μL 2.5 mM dNTP, 1 μL first-round PCR dilution product, 0.5 μL 5 U/μL Taq enzyme, 12.5 uL ddH_2O. The reaction procedure is the same as the first round.

The PCR products were recovered by cutting glue, connected to the pMD18-T vector, and transformed into competent cells (SK2301) by heat shock. The positive clones were selected and sequenced by Sangon.

3.7 5'RACE amplification of the cDNA sequence of kenaf root-knot nematode *HcNPR1* gene

Using specific primers RC564-NPR1-RT1/ RC564-NPR1-RT2, the cDNA was obtained by terminal C method. After RNase H and TdT treatment, the cDNA was amplified by nested PCR.

There are two rounds in the reaction process. The reaction system of the first round is as follows: 12.5 μL 2X GC Buffer I, 0.5 μL 10 μM 5'adaptor, 0.5 μL 10 μM NPR1-RT1, 4 μL 2.5 mM dNTP, 1 μL cDNA, 0.2 μL 5 U/μL Taq enzyme, 6.3 μL ddH_20. The reaction procedure was as follows: pre-denaturation at 95℃ for 3 min, denaturation at 94℃ for 30 s, annealing at 68℃ for 30 s, extension at 72℃ for 60 s, 33 cycles, and repair and extension at 72℃ for 7 min.

The second reaction system is as follows: 25 μL 2X GC Buffer I, 1 μL10 μM 5.3'outer, 1 μL 10 μM NPR1-RT2, 8 μL 2.5 mM dNTP, 1 μL first-round PCR dilution product, 0.5 μL 5 U/μL *Taq* enzyme, 12.5 μL ddH_20. The reaction procedure was the same as in the first round.

The PCR products were recovered by cutting glue, connected to the pMD18-T vector, and transformed into competent cells (SK2301) by heat shock. The positive clones were selected and sequenced by Sangon.

3.8 Full-length splicing and sequence analysis of *HcNPR1* gene in kenaf root-knot nematodes

The 5'RACE sequence and 3'RACE sequence were spliced by DNAman software, and the full-length *NPR1* gene sequence was obtained by removing the vector sequence. DNAstar software was used to analyze the full-length sequence of *NPR1* gene. Then use NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) online software to retrieve the sequencing results, use in the NCBI site BLASTX to compare HcNPR1 protein sequences. Protein sequences with >90% similarity was downloaded for phylogenetic analysis. The secondary structure of NPR1 protein was predicted by the online program GOVIV (http://npsa-prabi.ibcp.fr), and the conserved domain of NPR1 protein was analyzed by NCBI. The homology and phylogenetic tree were constructed by DNAman software.

3.9 Quantitative PCR analysis of *HcNPR1* gene of kenaf root-knot nematodes

The total RNA extracted from kenaf was transcribed, and the reaction procedure of 20 μL reverse transcription system was as follows: 85℃ for 5 s, 42℃ for 30 min, and 4℃ for termination. Fluorescence was quantified using a CFX-96 Touch instrument. Using the above reverse transcription cDNA as template, reaction system was 20 μL, upstream and downstream primer sequences were 0.7 μL each (Table 2), cDNA template 2 μL, RNase-free ddH_2O 6.6 μL, 2XSuper RealpreMixplus 10 μL. Each treatment was repeated three times. The reaction conditions included 40 cycles of pre-denaturation at 95°C for 15 min, denaturation at 95°C for 10 s, denaturation at 60°C for 20 s, and extension at 72°C for 20 s. The melting curve was generated from 65℃ to 95℃. The CT value was obtained. The relative gene expression differences were analyzed by $2^{-\triangle\triangle CT}$ method, and the results were mean ±SD. SPSS 23.0 statistical analysis software was used for data processing, and t-test was used for quantitative comparison. $p<0.05$ was considered statistically significant.

Table 2 The primers used in this paper

Primer name	Sequence	Application
N-f3	TACGGTGTTACACGTTGCTGC	NPR1 qPCR target gene primer
N-r3	CTTCCCTTCCTCGGTTGATTTA	NPR1 qPCR target gene primer
A-F1	GATATTCAACCCCTTGTCTGTG	qPCR internal reference primer
A-R1	CTTCTGACCCATCCCAACCAT	qPCR internal reference primer

Authors' Contributions

WXW is the experimental designer and the executor of the study. WXW and WHF completed the data analysis and wrote the first draft of the paper. RK and WTQ participated in the experimental design and analysis of the experimental results; CMC and WHF were the architects and principals of the project, and supervised the experimental design, data analysis, and paper writing and

revision. All authors read and approved the final manuscript.

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Reference

Ashori A., Harun J., Raverty W.D., and Yusoff M.N.M., 2006, Chemical and morphological characteristics of Malaysian cultivated kenaf (*Hibiscus cannabinus*) fiber, Polym-Plast. Technol., 45(1): 131-134
https://doi.org/10.1080/03602550500373782

Cai D., Kleine M., Kifle S., Harloff H.J., Sandal N.N., Marcker K.A., Klein-Lankhorst R.M., Salentijn E.M., Lange W.J., Stiekema W.J., Wyss U., Grundler F.M., and Jung C., 1997, Positional cloning of a gene for nematode resistance in sugar beet, Science, 275(5301): 832-834
https://doi.org/10.1126/science.275.5301.832

Cao H., Bowling S.A., Gordon A.S., and Dong X., 1994, Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance, Plant Cell, 6(11): 1583-1592
https://doi.org/10.2307/3869945

Claverie M., Dirlewanger E., Bosselut N., Van Ghelder C., Voisin R., Kleinhentz M., Lafargue B., Abad P., Rosso M.N., Chalhoub B., and Esmenjaud D., 2011, The *Ma* gene for complete-spectrum resistance to *Meloidogyne* species in *Prunus* is a TNL with a huge repeated C-terminal post-LRR region, Plant Physiol., 156(2): 779-792
https://doi.org/10.1104/pp.111.176230

Hermoso A., Vlasova A.V., Sanseverino W., D'Alessandro R., Andolfo G., Frusciante L., Roma1 G., Ercolano M., and Lowy E., eds., 2013, The plant resistance gene database (PRGdb): a Wiki-based system for the annotation of R-gene, IWBBIO, Granada, Spain, pp.711-712

Jaouannet M., Perfus-Barbeoch L., Deleury E., Magliano M., Engler G., Vieira P., Danchin E.G.J., Rocha M.D., Coquillard P., Abad P., and Rosso M.N., 2012, A root-knot nematode-secreted protein is injected into giant cells and targeted to the nuclei, New Phytol., 194(4): 924-931
https://doi.org/10.1111/j.1469-8137.2012.04164.x

Liao X.F., Zhao Y.H., Kong X.J., Khan A., Zhou B.J., Liu D.M., Kashif M.H., Chen P., Wang H., and Zhou R.Y., 2018, Complete sequence of kenaf (*Hibiscus cannabinus*) mitochondrial genome and comparative analysis with the mitochondrial genomes of other plants, Sci. Rep., 8(1): 12714
https://doi.org/10.1038/s41598-018-30297-w

Michacly P., and Bennett V., 1992, The ANK repeat: a ubiquitous motif involved in macromolecular recognition, Trends Cell Biol., 2(5): 127-129
https://doi.org/10.1016/0962-8924(92)90084-Z

Niu L.M., Cao R., Kang J.Q., Zhang X., and Lv J.Y., 2018, Ascorbate-glutathione cycle and ultrastructural analyses of two kenaf cμLtivars (*Hibiscus cannabinus* L.) under chromium stress, Int. J. Environ. Res. Public Health, 15(7): 1467
https://doi.org/10.3390/ijerph15071467

Saba N., Jawaid M., Hakeem K.R., Paridah M.T., Khalina A., and Alothman O.Y., 2015, Potential of bioenergy production from industrial kenaf (*Hibiscus cannabinus* L.) based on Malaysian perspective, Renew. Sust. Energ. Rev., 42: 446-459
https://doi.org/10.1016/j.rser.2014.10.029

Shi Z., Maximova S.N., Liu Y., Verica J., and Guiltinan M.J., 2010, Functional analysis of the *Theobroma cacao NPR1* gene in Arabidopsis, BMC Plant Biol., 10: 248
https://doi.org/10.1186/1471-2229-10-248

Tian W., Wang H.F., Chen H., Rui K., Su J.G., and Chen M.C., 2018, Control effects of 6 chemicals on root knof nematode of kenaf, Nongyao (Agrochemicals), 57(3): 212-214

Wang L., Gao X.Q., Zhu L.H., Zhou Y.L., and Li Z.K., 2011, Advances in research on function of WRKY transcription factor genes in plant resistance, Zhiwu Yichuang Ziyuan Xuebao (Journal of Plant Genetic Resources), 12(1): 80-85

Zhang Y.M., Ni X.L., Ma H.Q., and Qiu W.P., 2013, Characterization of *NPR1* genes from Norton and Cabernet Sauvignon grapevine, J. Integr. Agric., 12(7): 1152-1161
https://doi.org/10.1016/S2095-3119(13)60432-3

Zheng Y.G., Wu Y.F., Li J., Zhang T., and Wang X.D., 2018, Identification of NBS-LRR-like disease-resistant genes in *Cinnamomum camphora* and clone of two *CcRNL* Genes, Shengwu Jishu Tongbao (Biotechnology Bulletin), 34(2): 142-149