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Full Length Article

Molecular Characterization of 60S Ribosomal Protein Gene L3-1 (*Rpl3-1*) from *Arachis hypogaea*

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Abstract

The ribosomal proteins have important functions for plant resistance. In this study, we analyzed an *Arachis hypogaea* 60S ribosomal protein L3-1 (*Rpl3-1*) gene which was isolated by Genefishing technology. The sequence of *Rpl3-1* cDNA contained an 1170 bp ORF (open reading frame) which can encode 389 amino acids. The genomic DNA contained 2543 bp including five exons and four introns. The *Rpl3-1* transcripts were expressed higher in roots than stems. The *Rpl3-1* mRNA transcripts was increase sharply 12 hours after *Ralstonia solanacearum* stress and was 3.19 times greater than control plants. The proteins phylogenetic analysis showed that *Rpl3-1* was very conservative in dicotyledon and monocotyledon species.

Key words: Peanut; Ribosomal protein; *Rpl3-1*; *Peanut* bacterial wilt; Resistance; *Ralstonia solanacearum*

Introduction

Ribosome is a very crucial organelle in all living organisms that can synthesize proteins by translating the genetic information of mRNA (Hogues *et al.* 2008). Ribosomal proteins (RPs) are important for regulation of peptidyl transfer (Powers and Walter 1999; Warner 1999; Gasch *et al.* 2001; Hu *et al.* 2014; Sun *et al.* 2017), ribosome subunits assembly and transport (Nika *et al.* 1997; Dresios *et al.* 2006; Bu *et al.* 2015; Xu *et al.* 2018) and DNA repair (Kim *et al.* 1995; Akanuma *et al.* 2012). Mutants of RPs produce incorrectly assembled ribosome that lead to the decrease in protein-biosynthesis and abnormal development (Wang *et al.* 2015).

In many plants, *Rpl3* gene plays a prominent role in biogenesis of ribosome and regulation of cell division (Popescu and Tumer 2010). Ribosomal proteins L3 have been found to be related to resistance to deoxynivalenol (DON) in tobacco (Kant *et al.* 2012). Also it is vitally important to the function of eIF5B (García-Gómez *et al.* 2014) and translational elongation fidelity (Al-Hadid *et al.* 2016). Some studies revealed that RPL3 is important in yeast translation and resistance (Noller 1997). Mutations in *Rpl3* gene affect the resistance to peptidyltransferase inhibitors (Fried and Wamer 1981).

Peanut (*Arachis hypogaea* L.) is a very important oil legume and nutritious food. However, little is known about *Rpl3* gene in peanut. Whether *Rpl3* gene is associated with bacterial wilt resistance has not been reported. But our previous experiments have isolated the gene from the bacterial wilt stress group by Genefishing technology. To consider the function of *Rpl3-1* gene in peanut bacterial wilt (BW) resistance, we study the molecular characteristic and expression level in peanut by *Ralstonia solanacearum* challenge from one peanut cultivar Ri Hua 1. According to homology sequences from other species, specific primers of *Rpl3* gene were designed. Phylogenetic analysis and quantitative real-time PCR analysis were also performed. This study will help us to study the mechanism of ribosome gene in peanut resistance.

Materials and Methods

Experimental details and treatments

Experimental materials: The cultivated *A. hypogaea* variety Ri Hua 1, a Virginia type cultivar with a high resistance to bacterial wilt (BW) both in the field and the laboratory was used. Peanut kernels were pre-germinated and planted in growth chamber at 28°C and 12 h photophase (16,000 lx). *R. solanacearum* RZ strain was isolated from Rizhao city, Shandong province by TZC screen plate (with 0.05% tetrazolium chloride) at 28°C for 48 h. Single clone was inoculated into 1 mL liquid YGPA culture medium (glucose 10 mg, yeast extract 5 mg, peptone 5 mg, pH = 7.2) and shook at 28°C for 48 h until the cell concentration reach OD_{600} = 0.6. The species identification of clone was conducted by a pair of *R. solanacearum* 16S specific primer (RS-F and RS-S, Table 1). Total bacterial DNA was isolated by Genomic DNA Purification Kit (Tiangen, China).

Treatments: For the *R. solanacearum* RZ strain challenge experiment, 48 one-month old plants were randomly divided into two groups. Every plant root from the treated group was incised 3mm and then dipped into bacterial liquid (OD_{600} = 0.6). The control group was treated with sterile water. After infecting, three plants were randomly sampled from every group at 0, 0.5, 1, 3, 6, 12, 24 and 48 h, respectively. Roots were cut and quickly frozen in liquid nitrogen and grounded for DNA and RNA extraction.

Isolation of *Rpl3-1* EST

Rpl3-1 EST was found by Genefishing™ DEG Premix Kit (Seegene, Korea) from bacterial suspension treated seeds. The RNAs were isolated from the samples of treated group (bacterial suspension) and control group (PCR-grade water) respectively by Trizol Reagent (Invitrogen, USA). RNA concentration and its integrity were tested by spectrophotometry and relative intensity of fluorescent dyes GelRed (US Everbright Inc, USA). The reverse transcription reaction mixture consisted of 3 μg RNA isolated from two groups, 5 μL RT buffer, 0.5 μL RNase inhibitor (40 U), 2 μL dNTP mix (10 m*M*), 1 μL dT-ACP1 (10 μM), 15.5 μL PCR-grade water (Tiangen, China) and 1 μL M-MLV reverse transcriptase (200 U) (Promega, USA). RT reaction mixture was carried out at 42ºC 90 min, then incubated at 70ºC 15 min to stop the reaction. The products were diluted by 80 μL of double distilled water for subsequent PCR. The 20 μL mixture contained 2×SeeAmp ACP Master-mix, 50 ng cDNA, 0.5 μM dT-ACP2 and 0.5 µM arbitrary ACP. It was conducted at 94ºC 5 min, 50ºC 3 min and 72ºC 1 min, then by 40 cycles of 94ºC 40 sec, 65ºC 40 sec and 72ºC 40 sec, and last at 72ºC for 5 min. The products were electrophoresis on a 2% agarose and 0.005% Gelred (US Everbright Inc, USA) gel then visualized under UV light. The products were ligated with the vector pMD18-T (Takara, Japan) and sequenced on an ABI3730 Automated Sequencer (Applied Biosystems, USA) by a pair of primers M13-47 and RV-M (Table 1).

cDNA cloning

Roots RNA was extracted by Trizol Reagent (Invitrogen, USA). M-MLV reverse transcriptase was used for cDNA synthesis (Promega USA). SMART-RACE methods were used to clone 3' and 5' ends of cDNA (Clontech, USA). Rpl3-1-F1 and Rpl3-1-F2 were used in the first round PCR and the second round PCR, respectively for the 3' end. Primers Rpl3-1-R1 and Rpl3-1-R2 were used in the first round PCR and the second round PCR, respectively for 5' end (Table 1). A 25 μL PCR reaction contained 1 μL of template mix, 1 μL of each primer (10 μmol/L), 12.5 μL of 2×PCR buffer (Tiangen, China), and 9.5 μL PCR grade water in a Thermal Cycler. The PCR was conducted at 94°C 5 min, then by 30 cycles (94°C 40s, 60°C 40s, 72°C 2 min, and the last step at 72°C 10 min for extension. The complete *Rpl3-1* gene ORF region was amplified by gene specific primers Rpl3-1-F and Rpl3-1-R (Table 1). PCR products were cloned into the vector pMD18-T (Takara, Japan) and sequenced on an ABI3730 Automated Sequencer (Applied Biosystems, USA).

Genomic sequence of *Rpl3-1*

Roots genomic DNA was got by Genomic DNA Kit (Tiangen, China). Rpl3-1-F/R (Table 1) were used to amplify the DNA sequence of *A. hypogaea Rpl3-1* gene. PCR products were cloned and sequenced in both the directions.

Alignment and phylogenetic analysis

The homology sequences were searched by BLAST at NCBI (http://www.ncbi.nlm.gov/blast). BioEdit 7.0.9.0 software (Hall 1999) and used to analyzed the nucleotide and deduced amino acid sequence. Kyte and Doolittle (1982) method was used to generate mean hydrophobicity. ClustalX 1.83 program (Thompson *et al.* 1997) was used to perform multiple sequence alignment. MEGA 5.2 (Tamura *et al.* 2011) were used to construct phylogenetic Maximum likelihood (ML) tree. Protein MolWt & AA Composition Calculator (http://www.proteomics.com.cn/proteomics/pi_tool.asp) were used to predicate molecular mass and the theoretical isoelectric point. SMART software (http://smart.embl-heidelberg.de/) was used to search motif.

Quantitative real-time PCR analysis of *Rpl3-1*

The *Rpl3-1* mRNA transcripts were analyzed by quantitative real-time PCR (qRT-PCR). Three samples were taken at 50 days after seeding to identify the expression patterns. The roots, leaves and stems of each sample were got and snapped into liquid nitrogen immediately and last stored at -80°C. Total RNA was extracted followed by Trizol protocol (Invitrogen). RNA concentration and RNA integrity were determined by running on a 1.2% agarose gel stained with GelRed (US Everbright Inc., USA). RNase-free DNase (TaKaRa) were used to remove DNA contamination. MMLV reverse transcriptase (Promega, WI, USA) was used to synthesize cDNA. Then the reaction was conducted at 42°C 1 h and the mix was stored at -80°C.

The qRT-PCR was performed in a Roche light cycle 2.0. A 20 μL volume contained 50ng cDNA template, 10 μL of 2×SYBR Green Master Mix (Takara), 0.4 μL of each of primers (10 μmol/L). The products of qRpl3-1-f/r, actin-f/r and TUA-f/r were 122 bp, 195 bp and 94 bp, respectively.

The qRT-PCR program was 95°C 30s, 45 cycles of 95°C 5s, 60°C 20s and 72°C 15s. Every sample was run in three wells and accompanied with the internal control. Melting curve analysis was used to confirm the uniqueness of PCR product. The relative expression level of *Rpl3-1 was* analyzed by comparative C_t method (Livak and Schmittgen

Table 1: Primers in the study

Primers	Sequence (5'-3')	Application
Rpl3-1-F1	GGTCAGAACGGATACCACCACAG	3' RACE
Rpl3-1-F2	TTCTTACATTGCGCCAATCCCTC	3' RACE
Rpl3-1-R1	CCTTCTTCTGCTTCAAACCCTTC	5' RACE
Rpl3-1-R2	ACCCACAACTCCGACGATAACCA	5' RACE
M13-47	CGCCAGGGTTTTCCCAGTCACGAC	vector universal primers
R-VM	GAGCGGATAACAATTTCACACAGG	vector universal primers
UPM	Long: ctaatacgactcactatagggcAAGCAGTGGTATCAACGCAGAGT Short: ctaatacgactcactatagggc	3' 5' RACE Universal Primer
NUP	AAGCAGTGGTATCAACGCAGAGT	3', 5' RACE Nested Universal Primer
Rpl3-1-F	ATGTCTCACAGGAAGTTCGAGCACC	Rpl3-1 cdna amplify
Rpl3-1-R	TTATGCCTTGAGGCGTCCAAAGAAC	Rpl3-1 cdna amplify
qRpl3-1-f	ATTTCTCCCGAGGAAGCGTG	Rpl3-1 qRT-PCR
qRpl3-1-r	TGTGGGTCATACCAGCCTTG	Rpl3-1 qRT-PCR
actin-f	TTGGAATGGGTCAGAAGGATGC	avtin qRT-PCR
actin-r	AGTGGTGCCTCAGTAAGAAGC	actin qRT-PCR
TUA-f	CTGATGTCGCTGTGCTCTTGG	TUA qRT-PCR
TUA-r	CTGTTGAGGTTGGTGTAGGTAGG	TUA qRT-PCR
RS-F	GTCGCCGTCAACTCACTTTC	*R. solanacearum* 16S specific primer
RS-R	GTCGCCGTCAGCAATGCGGAATCG	*R. solanacearum* 16S specific primer

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ATGTCTCACAGGAAGTTCGAGCACCCAAGACACGGTTCTCTGGGATTTCTCCCGAGGAAGCGTGCTGCTCGTCACAGAGGAAAAGTGAAG
M  S  H  R  K  F  E  H  P  R  H  G  S  L  G  F  L  P  R  K  R  A  A  R  H  R  G  K  V  K
GCATTCCCCAAGGATGATCCATCAAAATCACCCAAGCTTACTGCTTTCTTGGGTTACAAGGCTGGTATGACCCACATTGTTCGAGAGGTC
A  F  P  K  D  D  P  S  K  S  P  K  L  T  A  F  L  G  Y  K  A  G  M  T  H  I  V  R  E  V
GAGAAACCAGGATCCAAGCTTCACAAGAAGGAGACTTGTGAGCCGGTTACAATTATCGAGACCCCTCCAATGGTTATCGTCGGAGTTGTG
E  K  P  G  S  K  L  H  K  K  E  T  C  E  P  V  T  I  I  E  T  P  P  M  V  I  V  G  V  V
GGTTATGTGAAAACTCCAAGGGGTCTGAGGACCTTGAACACTGTATGGGCTCAGCATTTGAGTGAGGAGATCAAGCGTAGGTTTTACAAG
G  Y  V  K  T  P  R  G  L  R  T  L  N  T  V  W  A  Q  H  L  S  E  E  I  K  R  R  F  Y  K
AACTGGTGCAAGTCCAAGAAGAAGGCATTCACCAAGTACTCAAAGCAGTATGAATCTGAAGATGGAAAGAAGAGCATTGAATCACAGCTT
N  W  C  K  S  K  K  K  A  F  T  K  Y  S  K  Q  Y  E  S  E  D  G  K  K  S  I  E  S  Q  L
GAGAAGATCAAGAAATATGCAACCGTAGTCCGTGTTTTGGCTCACACTCAGATCAGAAAAATGAAGGGTTTGAAGCAGAAGAAGGCCCAT
E  K  I  K  K  Y  A  T  V  V  R  V  L  A  H  T  Q  I  R  K  M  K  G  L  K  Q  K  K  A  H
ATCATGGAGATCCAAGTCAACGGTGGCACTATTGCCCAGAAGGTGGACTTTGCCCATGGTTTCTTTGAGAAACAGGTTCCTATTGATGCT
I  M  E  I  Q  V  N  G  G  T  I  A  Q  K  V  D  F  A  H  G  F  F  E  K  Q  V  P  I  D  A
GTGTTCCAGAAGGATGAGATGATTGACATCATTGGTGTCACAAAAGGTAAGGGTTATGAAGGTGTTGTGACCCGTTGGGGTGTGACTCGT
V  F  Q  K  D  E  M  I  D  I  I  G  V  T  K  G  K  G  Y  E  G  V  V  T  R  W  G  V  T  R
CTTCCCCGCAAGACTCACAGGGGTTTGAGGAAGGTGGCTTGTATTGGTGCTTGGCATCCTGCTAGGGTATCCTTCACTGTTGCCAGGGCT
L  P  R  K  T  H  R  G  L  R  K  V  A  C  I  G  A  W  H  P  A  R  V  S  F  T  V  A  R  A
GGTCAGAACGGATACCACCACAGAACTGAGTTGAACAAGAAGATTTACAAGCTTGGCAAAGCTGGAACTGAGTCTCACACTGGTGATACC
G  Q  N  G  Y  H  H  R  T  E  L  N  K  K  I  Y  K  L  G  K  A  G  T  E  S  H  T  G  D  T
GAGTTTGACAGAACTGATAAGGACATTACCCCCATGGGTGGCTTCCCGCACTACGGTATTGTTAAGGATGATTATCTCATGGTCAAGGGT
E  F  D  R  T  D  K  D  I  T  P  M  G  G  F  P  H  Y  G  I  V  K  D  D  Y  L  M  V  K  G
TGCACAGTTGGTCCAAAAAAAAGGGTTCTTACATTGCGCCAATCCCTCCTCAAGCAGACTTCCCGTGTTGCCCTGGAGGAGATCAAGCTC
C  T  V  G  P  K  K  R  V  L  T  L  R  Q  S  L  L  K  Q  T  S  R  V  A  L  E  E  I  K  L
AAGTTCATCGACACCTCCTCAAAGTTTGGACATGGTCGCTTCCAGACAACACAAGAGAAGCAAAAGTTCTTTGGACGCCTCAAGGCATAA
K  F  I  D  T  S  S  K  F  G  H  G  R  F  Q  T  T  Q  E  K  Q  K  F  F  G  R  L  K  A  *
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Fig. 1: Sequence of peanut *Rpl3-1*

2001). The Ct values of target *Rpl3-1* and the internal control, *β*-actin, were used to determine the expression pattern at different development stages. The Ct values of *Rpl3-1* and TUA was used to determine for samples challenged by *R. solanacearum*.

Results

Molecular characterization of *Rpl3-1* cDNA

The *Rpl3-1* cDNA from *A. hypogaea* (GenBank accession No. JX424588) was 1170 bp ORF encoding 389 amino acids (Fig. 1). The predicted mature RPL3-1 was 44335.73 Da and the theoretical isoelectric point was 10.20. The RPL3-1 contained a typical ribosomal_L3 domain (region: from M1 to G370). Several species of RPL3-1 were downloaded from GenBank (Table 2). The multiple alignments of RPL3-1 sequences is shown in Fig. 2. The protein sequence of peanut contained 54 K residues (13.88%); 34 G residues (8.74%) and 29 T residues (7.46%). A conservative remarkable successive three-K (location from site 126 to 128); a couple of R (116–117); and four pairs of K (143–144, 154–155, 177–178, 283–284) have been found both in plants and animals. The ratios of structure random coil (c), extended strand (e), alpha helix (h) and beta turn (t) were 44.73, 25.96, 24.68 and 4.63%, respectively (Fig. 3). Peanut RPL3-1 3D model was got using the SWISS-MODEL Protein Modelling Server (Fig. 3). Mean Hydrophobicity profiles are shown in Fig. 3 also. Values of most positions are lower than one, especially in the location of four successive K, which are almost reaching to the lowest point.

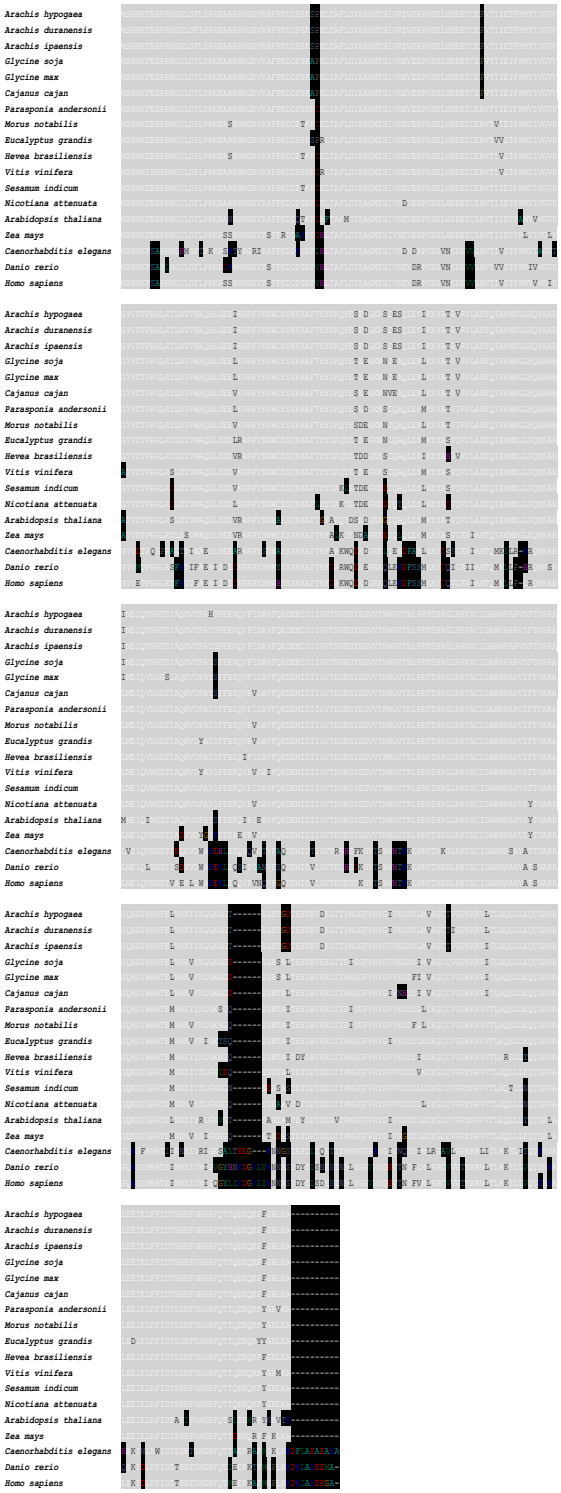

Fig. 2: Multiple alignment of RPL3-1 from eighteen species

The protein sequences used for analysis were as follows: *A. hypogaea* (JX424588), *A. duranensis* (XP_015942528.1), *A. ipaensis* (XP_016175365.1), *Glycine soja* (KHN46406.1), *G. max* (XP_003536417.1), *Cajanus cajan* (XP_020232111.1), *Parasponia andersonii* (PON32234.1), *Morus notabilis* (EXC12323.1), *Eucalyptus grandis* (XP_010063844.1), *Hevea brasiliensis*

Table 2: The species in this study

Species	Lineage	Accession number
Arachis hypogaea Ri Hua 1	dicotyledon	JX424588
A. duranensis	dicotyledon	XP_015942528.1
A. ipaensis	dicotyledon	XP_016175365.1
Glycine soja	dicotyledon	KHN46406.1
G. max	dicotyledon	XP_003536417.1
Cajanus cajan	dicotyledon	XP_020232111.1
Parasponia andersonii	dicotyledon	PON32234.1
Morus notabilis	dicotyledon	EXC12323.1
Eucalyptus grandis	dicotyledon	XP_010063844.1
Hevea brasiliensis	dicotyledon	XP_021681318.1
Vitis vinifera	dicotyledon	CBI18223.3
Sesamum indicum	dicotyledon	XP_011092385.1
Nicotiana attenuata	dicotyledon	XP_019249790.1
Arabidopsis thaliana	dicotyledon	NP_175009.1
Zea mays	monocotyledon	NP_001131208.1
Caenorhabditis elegans	invertebrate	NP_001021254.1
Danio rerio	Vertebrata	NP_001001590.1
Homo sapiens	Hominoid Mammalia Vertebrata	NP_000958.1

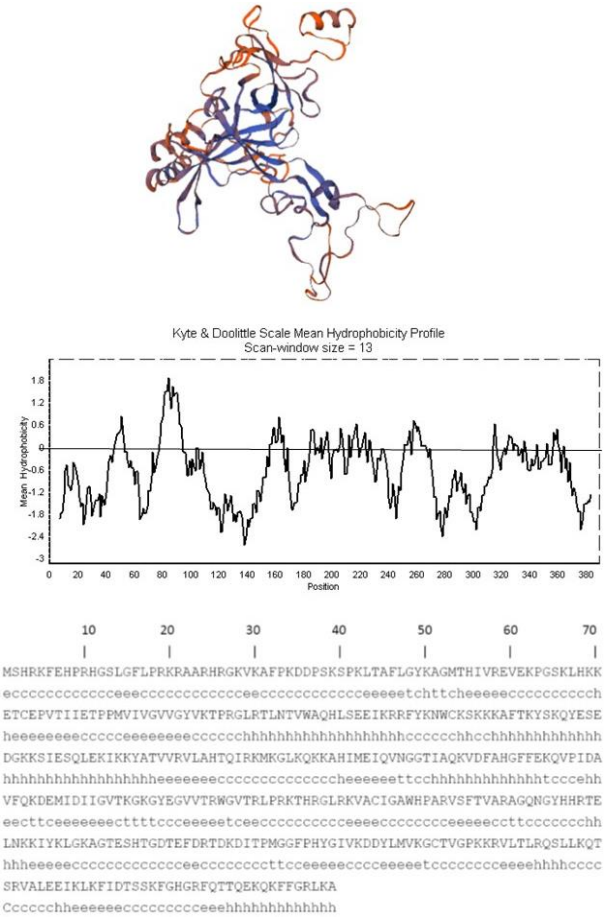

Fig. 3: The prediction of *Rpl3-1* secondary and 3D structure from *Arachis hypogaea*. e: extended strand; h: alpha helix; c: random coil; t: beta turn

(XP_021681318.1), *Vitis vinifera* (CBI18223.3), *Sesamum indicum* (XP_011092385.1), *Nicotiana attenuata* (XP_019249790.1), *Arabidopsis thaliana* (NP_175009.1), *Zea mays* (NP_001131208.1), *Caenorhabditis elegans* (NP_001021254.1), *Homo sapiens* (NP_000958.1) and *Danio rerio* (NP_001001590.1).

Genomic sequences of *Rpl3-1*

A 2543 bp sequence (accession number JX424600) has been submitted to the GenBank. The genomic *Rpl3-1* was made up of five exons and four introns (Fig. 1). The intron was located within the whole ORF. All exon-intron junctions follow the consensus rule of AG/GT.

Sequence alignment and phylogenetic analysis

The result showed that the protein sequences of *A. hypogaea* RPL3-1 shared 99.4 to 63.7% identity in the deduced protein sequence (Table 2), such as 99% identity with *A. duranensis* (XP_015942528.1) and *A. ipaensis* (XP_016175365.1), 94% with *G. soja* (KHN46406.1), *G. max* (XP_003536417.1), *C. cajan* (XP_020232111.1) and *P. andersonii* (PON32234.1), 92% to 90% with *M. notabilis* (EXC12323.1), *E. grandis* (XP_010063844.1), *H. brasiliensis* (XP_021681318.1), *V. vinifera* (CBI18223.3), *S. indicum* (XP_011092385.1) and *N. attenuata* (XP_019249790.1), 86% with *A. thaliana* (NP_175009.1), 85% with *Z. mays* (NP_001131208.1), 63% with *C. elegans* (NP_001021254.1), 64% with *D. rerio* (NP_001001590.1) and 67% with *H. sapiens* (NP_000958.1). The Maximum Likelihood (ML) phylogenic tree constructed based on RPL3-1 sequences is shown in Fig. 4. The tree is made up of one plant clade and one animal clade. The phylogenetic analysis showed conservation among species. This conservation plays a crucial role for basal cell activity in organisms.

Distribution of *Rpl3-1* transcripts

The qRT-PCR was used to analyze the distribution of *Rpl3-1* in roots, stems and leaves. The expression level of *Rpl3-1* transcripts in the roots after bacterial challenge was quantified using TUA as an internal control. The melting curve of *Rpl3-1*, *β*-actin and TUA had only one peak indicating that the amplification was specific. The relative expression level of *Rpl3-1* mRNA was higher in roots than that in leaves and stems (Fig. 5). The expression level of *Rpl3-1* transcripts was increase sharply 12 h after bacterial challenge and was 3.19 times more than control. Expression fell back to normal 48 h after bacterial challenge (Fig. 6).

Discussion

This is the first report of *Rpl3-1* gene of peanut. The peanut *Rpl3-1* cDNA contained an 1170 bp ORF encoding 389 amino acids and is highly conserved with other organisms

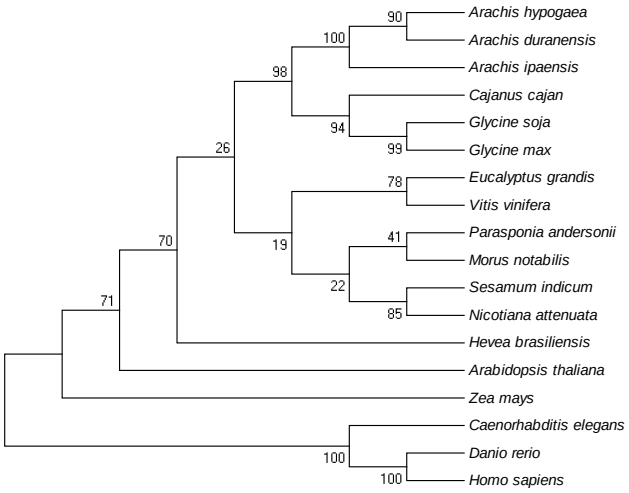

Fig. 4: The Maximum Likelihood (ML) phylogenic tree of RPL3-1 from eighteen species. The numbers represent the percentage of 1000 bootstrap replications

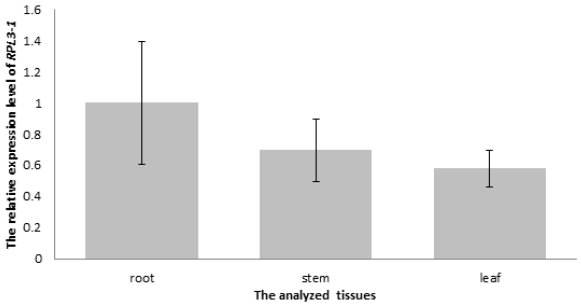

Fig. 5: The expression of the *Rpl3-1* transcripts measured by qRT-PCR. Vertical bars represent mean±S.D

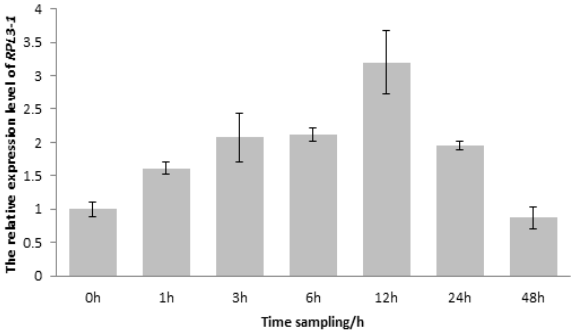

Fig. 6: The expression level of the *Rpl3-1* transcripts after *R. solanacearum* challenge. Each bar represents the mean from three determinations ± SD

(Fig. 2). The genomic DNA consists of 2543 bp including five exons and four introns. The *Rpl3-1* mRNA transcripts were mainly expressed in roots and leaves. *Rpl3-1* EST was identified by Genefishing^{TM} DEG Premix Kit (Seegene, Korea) from *R. solanacearum* bacterial suspension treated seeds. Then we identified *Rpl3-1* gene that is related to BW resistance in *A. hypogaea* with evidence of the increased

transcripts level in response to *R. solanacearum* challenge. RPL3 protein is highly conserved and its methylation is important to peptide bond forming (Schultz and Friesen 1983; Arif *et al.* 2019). Single amino acid changes of RPL3 protein was previously reported that can increase the deoxynivalenol (DON) tolerance in transgenic tobacco and yeast (Mitterbauer *et al.* 2004). Transgenic *Rpl3* gene corn plants had higher disease resistance to ear infections (Kant *et al.* 2012). Evidence of target site alteration of RPL3 protein gives rise of cultivar specific resistance to Fusarium head blight (FHB) in wheat (Miller and Ewen 1997). The bacterial wilt (BW) disease is a severe and devastating plant disease. BW caused by *R. solanacearum*, is reported to be one of the major serious bacterial diseases of peanut affecting peanut cultivation and causing high yield losses (Smith *et al.* 1995). RPL3 protein located at the peptidyltransferase center and it is related with protein translation, resistance and ribosome biogenesis (Bu *et al.* 2015; Sun *et al.* 2017; Xu *et al.* 2018). So it suggests that the enhanced expression level of *Rpl3* gene after BW infection may help plant to immune the pathogen by increasing ribosome biogenesis. In this study, *Rpl3-1* is related to peanut BW resistance for its increased transcripts after *R. solanacearum* challenge.

Multiple alignment of 18 RPL3-1 sequences showed that this ribosomal protein is highly conserved. The function of RPL3 is vital for ribosomal assembly through its high affinity to 23S rRNA (Speirer and Zimmermann 1976; Nowotny and Nierhaus 1982) and peptidyltransferase center formation (Khaitovich *et al.* 1999). The sequence of *A. hypogaea* deduced amino acid showed 99% identity to two progenitors of *A. duranensis* (XP_015942528.1) and *A. ipaensis* (XP_016175365.1). Sequence diversity might lead to different resistance (Lucyshyn *et al.* 2007). H199 of *A. hypogaea* is different from Y199 of *A. duranensis* and *A. duranensis*. For the vital function of protein histidine methylation (Al-Hadid *et al.* 2016), a comparison of H199 of peanut cultivars should be made in further research. Single nucleotide polymorphisms (SNPs) among peanut cultivar and two progenitors are also needed to be identified through experiment.

Conclusion

A novel *Rpl3-1* gene was isolated from peanut. This gene is conservative and related to *R. solanacearum* resistance in peanut. More and more studies have shown that ribosomal proteins are related to plant resistance. With the continuous development of high throughput sequencing technology, more in-depth studies of ribosomal protein genes, disease resistance genes and downstream disease resistance-related genes can be carried out from the perspective of transcriptomics and proteomics. Further studies will identify key SNPs that is important for function. These findings contribute to the study of ribosome proteins on regulation of disease resistance in plants.

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