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MOLECULAR CLONING AND SEQUENCE ANALYSIS OF RIBOSOMAL PROTEIN GENES IN MAIZE

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ABSTRACT

Ribosomal proteins are the essential components of ribosome, playing an important role in cell, involved protein synthesis and some other enzyme activities. In the current study, five ribosomal protein genes were successfully cloned from the maize (*Zea mays* L.) inbred line Southern 202 with reverse transcription polymerase chain reaction (RT-PCR) strategy. All of them were sequenced and analyzed preliminarily. The results showed that the complete coding regions of *RPS9*, *RPS10*, *RPS14*, RPS16 and *RPS18* genes encode mature proteins with 193, 179, 150, 148 and 152 amino acids (AA), respectively. Bioinformatics analysis using the related data within NCBI/GenBank indicated that these sequences share high similarities at both DNA and protein levels. But same gene not only can encode different AA in the different variety in same species, but the number of encoded AA residues is probably different. Whereas in animals especially mammalian, the DNA and protein sequences of ribosomal protein genes have higher coincidence, and the number of AA residues encoded is very stable. The knowledge stated here could be beneficial for maize breeding programs and studying the functions of ribosomal proteins.

Key words: Maize (*Zea mays* L.), Ribosomal protein gene, Cloning, Bioinformatics analysis

Abbreviations: AA – amino acids; EB - ethidium bromide; ORF - open reading frame ; PCR - polymerase chain reaction; pI - isoelectric point; RT-PCR - reverse transcription polymerase chain reaction; SNP single-nucleotide polymorphism

INTRODUCTION

Selecting an inbred line and utilizing its heterosis can greatly improve maize yield, which is also the basic principles for corn breeders in maize (*Zea mays* L.) breeding project. The maize breeding targets with high-stable yield, resistant diseases, high combining ability and good quality are controlled by the traits of parental inbred lines (When, 2002). Southern 202, bred by Nanchong Academy of Agricultural Sciences, Sichuan Province, China, is an elite inbred line with high general combining ability and special combining ability, comprehensively good agronomic traits, high disease and stress resistance. Currently, the inbred line has been widely used as a key donor line in maize breeding programs in China and several superior hybrids had been developed from the parental line. For example, in 2001, the hybrid Zhengtian 1 was obtained by the hybridization between inbred lines Southern 202 and Southern 637, which has highly resistant to *Exserohilum turcicum*, and moderate resistant to *Rhizoctonia solani* and *Bipolaris maydis*, high yielding potential, and in 2007 it has been validated through Sichuan Province and widely planted in China today.

Ribosome, the organelle that catalyzes protein synthesis, consists of a small 40S subunit and a large 60S subunit. The two subunits are composed of 4 rRNA and approximately 80 structurally distinct proteins. According to the order of binding to rRNA, the ribosomal proteins are divided into three groups, Primary binding protein, Subprime binding protein, and Late binding protein (Arnold and Reilly, 1999). In 30S small subunit assembling of *E. coli*, S4、S7、S8、S15、S17 and S20 that can directly bind to 16S rRNA are called Primary binding protein, the second groups protein including S5、S6、S9、S12、S13、S16、S18 and S19 that can bind to 16S rRNA are called Subprime binding protein, and the others including S2、S3、S10、S11、S14 and S21 are called Late binding protein (Hamacher et al., 2006).

Ribosomal proteins not only participate in balancing the synthesis of the RNA and protein components of the ribosome itself, but also perform other extraribosomal functions such as DNA replication, transcription and repair, RNA splicing and modification, cell growth and

proliferation, regulation of apoptosis and development, and cellular transformation, et al. (Wool, 1996; Lai and Xu, 2007; Warner and McIntosh, 2009). The previous studies on ribosomal proteins were focused mainly on *Escherichia coli*, many ribosomal proteins have been identified, and some of their functions have already been clarified. However, the reports on ribosomal proteins and their genes in plant are limited (Arnold and Reilly, 1999; Hamacher et al., 2006).

Therefore, five ribosomal proteins of 40S small subunit from maize inbred line Southern 202 were cloned in this present study, and their sequence characteristics are analyzed in detail. The knowledge stated here is beneficial for maize breeding and studding the function of ribosomal proteins.

MATERIALS AND METHODS

The young and fresh leaves of maize inbred line Southern 202 were friendly provided by Nanchong Academy of Agricultural Sciences, Sichuan Province, China. The tissues were frozen in liquid nitrogen and stored at -80 °C before processing.

Total RNA extraction

Total RNA was isolated from the leaves of maize inbred line Southern 202 with RNAiso Plus reagent (TAKARA Biotechnology (Dalian) Co., LTD., China). The purity and yield of total RNA were determined by monitoring the A260/A280 absorbance ratio with NanoDrop 2000 instrument. The sample integrality was tested by 1.0% agarose gel electrophoresis stained with ethidium bromide (EB). Finally, the RNA samples were diluted to 300 ng/μl.

Cloning of the cDNA encoding region

According to the manufacturer's protocol, cDNA was synthesized from total RNA with a reverse transcription kit named TaKaRa RNA PCR Kit (AMV) 3.0 (TAKARA Biotechnology (Dalian) Co., LTD., China). The 10 μl reaction system included AMV Reverse Transcriptase (5 U/μl) 1 μl, dNTP Mixture (10 mM) 1 μl, $MgCl_2$ (25 mM) 2 μl, 10 × RT Buffer 1 μl, RNase Inhibitor 0.25 μl, Oligo dT-Adaptor Primer (2.5 pmol/ μl) 0.5 μl, RNA sample (500 ng/μl) 1 μl, and finally added RNase Free dH_2O up to 10 μl. The reverse transcriptase reaction was carried out at 42 °C for 30 min, 99 °C for 5 min and 5 °C for 5 min.

The PCR (polymerase chain reaction) primers were designed by Primer Premier 5.0 software, according to the open reading frame (ORF) sequence of reported ribosomal protein genes. The sequences of the forward and reverse primes and other information were listed in Table 1.

The synthesized first-strand cDNA was used as a template. The total reaction volume for DNA amplification was 50 μl. Reaction mixtures contained 5 × PCR Buffer 10 μl, *TaKaRa Ex Taq* HS (5 U/ μl) 0.25 μl, Reverse transcriptase mixture 10 μl, the special primers (20 μM) (Table 1) each 0.5 μl, and added dH_2O up to 50 μl. The amplification procedure was as follows: 94 °C for 2 min, followed by 30 cycles of 94 °C for 30s, 46 °C for 30s, 72 °C for 1 min, and a final elongation step of 72 °C for 7 min.

Table 1. Sequences of primes used for RT-PCR amplification

Gene	Primer sequence (5'→3')		Reference sequences and GenBank accession number
RPS9	forward	ATGGTGCATGTTAACTTCTACC	*Zea mays*, EU964329.1
	reverse	TTACTCCTCGTCCTCCTCA	
RPS10	forward	ATGATCATCTCCAAGAAGAAC	*Zea mays*, EU977087.1
	reverse	TCACTCCATGGAAGATCCACTG	
RPS14	forward	ATGTCGAGGAGGAAGACCA	*Zea mays*, EU960087.1
	reverse	CTACAGCCTCCTTCCCCT	
RPS16	forward	ATGACCGTGCTGAGCC	*Zea mays*, EU963976.1
	reverse	TCAACGGTACGACTTCTGG	
RPS18	forward	ATGTCGCTGATCGCC	*Zea mays*, EU952700.1
	reverse	TTATCGCTTCTTGGAGACACC	

After amplification, PCR products were separated by electrophoresis on 1.5% agarose gel with 1 × TAE (Tris-acetate-EDTA) buffer, stained with EB and visualized under UV light. The expected fragments of PCR products were harvested and purified from gel using a DNA extraction kit (Sangon Biotech (Shanghai) Co., Ltd., China), and then ligated into a pUC18-T vector at 16°C for 12 h. The recombinant molecules were transformed into *E. coli* complete cells (JM109), and then spread onto the LB-plate containing 50 μg/ml ampicillin, 200 mg/ml IPTG, and 20 mg/ml X-gal. After 13 h, pick 3-5 single colonies as template for PCR identification. Finally, the positive cloning was sequenced by Sanger method at Sangon Biotech (Shanghai) Co., Ltd., China.

Sequence analysis

The sequences were analyzed using ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/orfig.cgi); BLAST algorithms (http://blast.ncbi.nlm.nih.gov/Blast.cgi) were used to search for sequence homology; multiple sequence alignment was performed by DNAMAN 6.0 software; theoretical pI and molecular weight of protein were

computed online (http://web.expasy.org/compute_pi/); the analysis of protein functional sites was performed online (https://www.predictprotein.org/).

RESULTS

Gene cloning

The target DNA fragments were obtained from the RT-PCR products of electrophoresis, and their sizes were approximately 700 bp, 700 bp, 500 bp, 500 bp, and 500 bp in length. The positive clones were screened out and sequenced, and the results indicated that all the five cloned genes contain a complete ORF, with 582 bp, 540 bp, 453 bp, 447 bp and 459 bp in length, respectively. According to the identity comparison results between our nucleotide sequences and the data in GenBank, it was concluded that the five cloned genes here should be *RPS9*, *RPS10*, *RPS14*, *RPS16* and *RPS18* of maize, and the DNA and encoded amino acids (AA) sequences were shown in Figure 1, Figure 2, Figure 3, Figure 4 and Figure 5, respectively.

```
1     ATGGTGCATGTTAACTTCTACCGCAACTATGGGAAGACTTTCAAGAAGCCAAGGCGGCCG
1      M  V  H  V  N  F  Y  R  N  Y  G  K  T  F  K  K  P  R  R  P
61    TATGAGAAGGAGCCGCCTAGATGCTGAGCTGAAGCTGGTTGGTGAGTATGGCCTGCGGTGC
21     Y  E  K  E  R  L  D  A  E  L  K  L  V  G  E  Y  G  L  R  C
121   AAGCGTGAGCTGTGGCGCGTGCAGTATGCCCTGAGCCGTATCAGGAATGCAGCCAGGGAG
41     K  R  E  L  W  R  V  Q  Y  A  L  S  R  I  R  N  A  A  R  E
181   TTGCTCACCCTGGATGAGAAGAACCCACGCCGTATCTTTGAGGGCGAGGCGCTCCTCCGT
61     L  L  T  L  D  E  K  N  P  R  R  I  F  E  G  E  A  L  L  R
241   CGCATGAACAGATATGGTCTTCTTGGCGAGGGACAGAACAAGCTTGATTACGTGCTTGCC
81     R  M  N  R  Y  G  L  L  G  E  G  Q  N  K  L  D  Y  V  L  A
301   CTCACTGTTGAGAACTTCCTCCAGCGCCGCCTCCAGACCATCGTCTTCAAGAATGGCATG
101    L  T  V  E  N  F  L  Q  R  R  L  Q  T  I  V  F  K  N  G  M
361   GCCAAGTCCATCCACCATGCTCGTGTCCTGATCAGGCAGCGCCACATCAGGGTGGGAAGG
121    A  K  S  I  H  H  A  R  V  L  I  R  Q  R  H  I  R  V  G  R
421   CAGCTCGTCAACATCCCGTCGTTCATGGTCAGGGTCGAATCAGAGAAGCACATCGACTTC
141    Q  L  V  N  I  P  S  F  M  V  R  V  E  S  E  K  H  I  D  F
481   TCCCTCACCAGCCCTCTGGGTGGTGGCCCCGCCGGAAGGGTGAAGCGGAAGAACCAGAAG
161    S  L  T  S  P  L  G  G  G  P  A  G  R  V  K  R  K  N  Q  K
541   AAGGCCTCAGGGGCGGCGACGCTGAGGAGGACGAGGAGTAA
181    K  A  S  G  G  G  D  A  E  E  D  E  E  *
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Figure 1. The nucleotide and deduced AA sequences of *RPS9* gene cloned in maize. With an ORF of 582 bp encoding 193 AA.

```
1     ATGATCATCTCCAAGAAGAACCGCCGCGAGATCTGCAAGTACATCTTCCATGAGGGGGTT
1      M  I  I  S  K  K  N  R  R  E  I  C  K  Y  I  F  H  E  G  V
61    CTATATGCCAAGAAGGACTACAACCTGGCCAAGCACCCCAAGCTTGACGTGCCCAACCTG
21     L  Y  A  K  K  D  Y  N  L  A  K  H  P  K  L  D  V  P  N  L
121   GAGGTGATTAAGCTCATGCAGAGCTTCAAGTCCAAGGAGTATGTCAGGGAGACCTTCTCC
41     E  V  I  K  L  M  Q  S  F  K  S  K  E  Y  V  R  E  T  F  S
181   TGGCAGTACTACTACTGGTACCTCACCAACGATGGCATTGAGCACCTCCGCAGCTTCCTC
61     W  Q  Y  Y  Y  W  Y  L  T  N  D  G  I  E  H  L  R  S  F  L
241   AACCTGCCGTCAGAGGTTGTGCCCAACACCCTCAAGAAGTCCTCCAAGCCCCCGTCCCGT
81     N  L  P  S  E  V  V  P  N  T  L  K  K  S  S  K  P  P  S  R
301   CCCTTTGGCTCTGGCCCACCGGGTGACCGCCCCAGGGGTCCCCCTCGCTTTGGGGAAGAC
101    P  F  G  S  G  P  P  G  D  R  P  R  G  P  P  R  F  G  E  D
361   AGACCTAGGTTTGGGGATAGGGATGGTTACAGAGGAGGTCCACGAGGTGCAATGGGTGAT
121    R  P  R  F  G  D  R  D  G  Y  R  G  G  P  R  G  A  M  G  D
421   TTTGGTGGTGAGAAGGGTAGTGCTCCTGCGGATTTCCAGCCATCTTTTAGGGGTAGCAGA
141    F  G  G  E  K  G  S  A  P  A  D  F  Q  P  S  F  R  G  S  R
481   CCTGGCTTCGGCCGTGGTGGCGGCAGCGCTTTTGGTGCCAGTGGATCTTCCATGGAGTGA
161    P  G  F  G  R  G  G  G  S  A  F  G  A  S  G  S  S  M  E  *
```

Figure 2. The nucleotide and deduced AA sequences of *RPS10* gene cloned in maize. With an ORF of 540 bp encoding 179 AA.

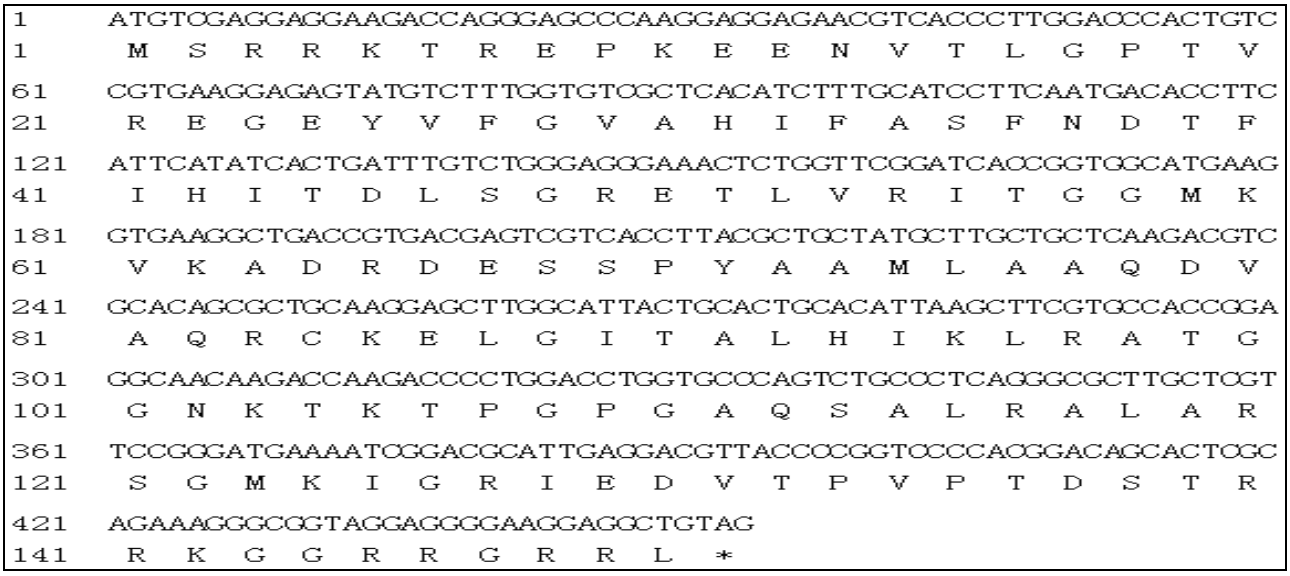

Figure 3. The nucleotide and deduced AA sequences of *RPS14* gene cloned in maize. With an ORF of 453 bp encoding 150 AA.

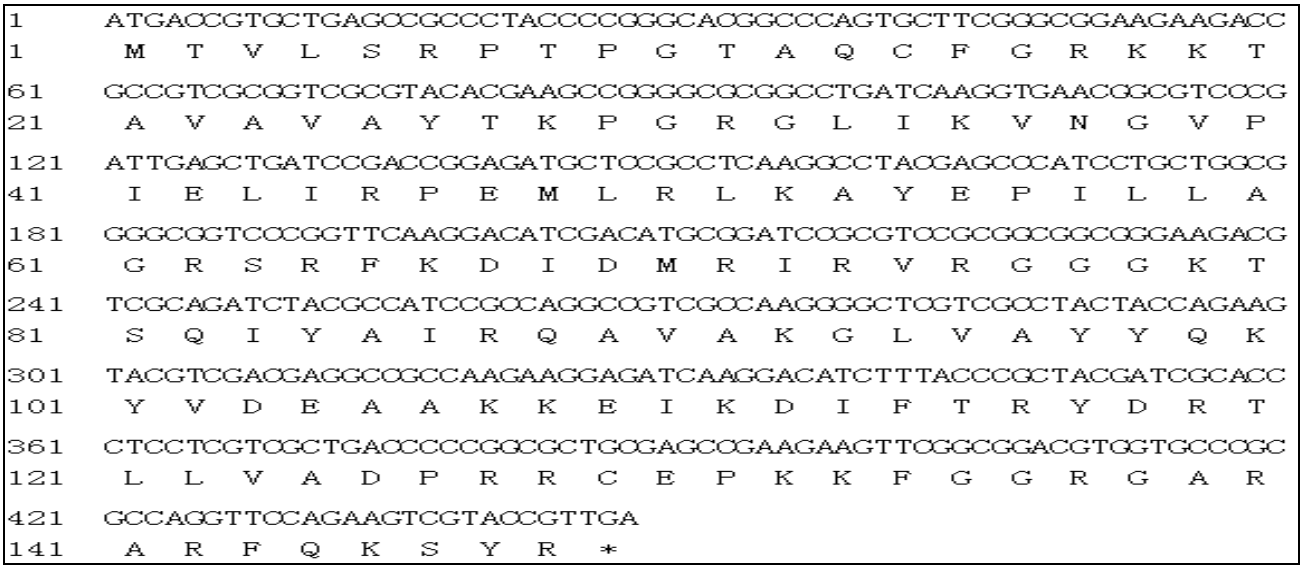

Figure 4. The nucleotide and deduced AA sequences of *RPS16* gene cloned in maize. With an ORF of 447 bp encoding 148 AA.

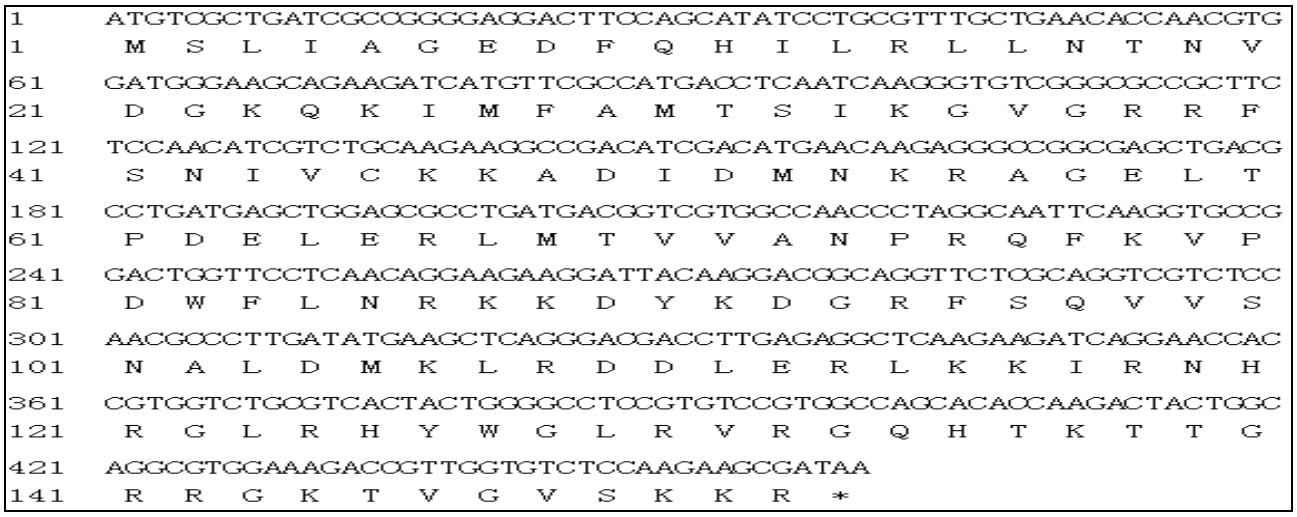

Figure 5. The nucleotide and deduced AA sequences of *RPS18* gene cloned in maize. With an ORF of 459 bp encoding 152 AA.

These genes have been submitted to GenBank, and the access number was also allocated. The statistic information on length of ORF, number of encoded AA, pI value, molecular weight and GenBank accession number for the five cloned genes was listed in Table 2. The longest gene is *RPS9*, with 582 bp coding 193 AA. Followed by *RPS10*, *RPS18* and *RPS14*, and the shortest gene is RPS16, only 447 bp in length, including an ORF of 148 AA. The pI values of the five protein deduced from the cloned genes ranged from 9.81 (*RPS10*) to 10.74 (*RPS18*). The calculated molecular weight of the five proteins are also different, *RPS9* had the largest value, up to 22.52 kD, followed by *RPS10*, *RPS18* and RPS16 in turn, the least molecular weight was found in *RPS14*, only 16.39 kD.

Table 2. The cloned ribosomal protein genes in this study

Genes	Length of ORF (bp)	Number of encoded AA	pI value of encoded protein	Molecular weight of encoded protein (kD)	GenBank accession number
RPS9	582	193	10.29	22.52	JX232263.1
RPS10	540	179	9.81	20.06	JX232264.1
RPS14	453	150	10.56	16.39	JX232265.1
RPS16	447	148	10.59	16.77	JX232266.1
RPS18	459	152	10.74	17.69	JX232267.1

Functional sites analysis of cloned genes

The cloned genes were further analyzed functional sites using their encoded proteins online, and the results indicated that *RPS9*, *RPS10*, *RPS14*, *RPS16* and *RPS18* genes had 4, 5, 7, 6 and 5 patterns, respectively (Table 3). There were total ten different patters found in the five proteins deduced from the cloned genes, including cAMP- and cGMP-dependent protein kinase phosphorylation site, protein kinase C phosphorylation site, casein kinase II phosphorylation site, N-myristoylation site, tyrosine kinase phosphorylation site, N-glycosylation site, amidation site, pibosomal protein S11 signature, ribosomal protein S9 signature and Ribosomal protein S13 signature. All the five encoded protein had cAMP- and cGMP-dependent protein kinase phosphorylation site, protein kinase C phosphorylation site and casein kinase II phosphorylation site, and N-myristoylation site was found in all of them but for *RPS10* gene. Whereas, Ribosomal protein S11, S9 and S13 signatures only occurred in *RPS14*, RPS16 and *RPS18* genes, respectively. The results revealed that all the sites were very important for their biological functions in cell.

DISCUSSION

As a part of ribosome, ribosomal proteins play a crucial role in catalyzing protein synthesis and other enzyme activities. In this paper, five ribosomal protein genes (*RPS9*, *RPS10*, *RPS14*, *RPS16*, *RPS18*) of 40S small subunit were successfully cloned from maize inbred line Southern 202. By analyzing these genes with the NCBI/GenBank data, these sequences share high similarity both at the nucleotide sequence and the protein levels. For the five genes cloned in this study, their experimental results were discussed one by one as follows.

Table 3. The functional sites of proteins deduced from cloned genes in this study

Functional patterns found	No.	Position
RPS9 gene		
cAMP- and cGMP-dependent protein kinase phosphorylation site	1	180 to 183 KKAS
Protein kinase C phosphorylation site	2	13 to 15 TFK; 154 to 156 SEK
Casein kinase II phosphorylation site	1	63 to 66 TLDE
N-myristoylation site	3	119 to 124 GMAKSI; 167 to 172 GGGPAG; 184 to 189 GGGDAE
RPS10 gene		
cAMP- and cGMP-dependent protein kinase phosphorylation site	1	92 to 95 KKSS
Protein kinase C phosphorylation site	5	4 to 6 SKK; 48 to 50 SFK; 90 to 92 TLK; 94 to 96 SSK; 155 to 157 SFR
Casein kinase II phosphorylation site	1	176 to 179 SSME
Tyrosine kinase phosphorylation site	2	6 to 14 KNRREICKY; 123 to 130 RFGDRDGY
N-myristoylation site	6	19 to 24 GVLYAK; 132 to 137 GGPRGA; 142 to 147 GGEKGS; 158 to 163 GSRPGF; 166 to 171 GGGSAF; 172 to 177 GASGSS
RPS14 gene		
N-glycosylation site	3	13 to 16 NVTL; 37 to 40 NDTF; 102 to 105 NKTK
cAMP- and cGMP-dependent protein kinase phosphorylation site	1	3 to 6 RRKT
Protein kinase C phosphorylation site	4	2 to 4 SRR; 19 to 21 TVR; 47 to 9 SGR; 138 to 140 STR
Casein kinase II phosphorylation site	3	19 to 22 TVRE; 35 to 38 SFND; 47 to 50 SGRE
N-myristoylation site	4	100 to 105 GGNKTK: 110 to 115 GAQSAL; 122 to 127 GMKIGR; 143 to 148 GGRRGR
Amidation site	1	143 to 146 GGRR
Ribosomal protein S11 signature	1	115 to 137 LRALARSGMKIGRIEDVTPVPTD
RPS16 gene		
cAMP- and cGMP-dependent protein kinase phosphorylation site	1	17 to 20 RKKT
Protein kinase C phosphorylation site	1	146 to 148 SYR
Casein kinase II phosphorylation site	1	115 to 118 TRYD
N-myristoylation site	4	10 to 15 GTAQCF; 76 to 81 GGGKTS; 135 to 140 GGRGAR
Amidation site	1	15 to 18 FGRK
Ribosomal protein S9 signature	1	76 to 94 GGGKTSQIYAIRQAVAKGL
RPS18 gene		
cAMP- and cGMP-dependent protein kinase phosphorylation site	1	38 to 41 RRFS
Protein kinase C phosphorylation site	3	32 to 34 SIK; 139 to 141 TGR; 149 to 151 SKK
Casein kinase II phosphorylation site	2	18 to 21 TNVD; 60 to 63 TPDE
Amidation site	2	36 to 39 VGRR; 139 to 142 TGRR
Ribosomal protein S13 signature	1	121 to 134 RGLRHYWGLRVRGQ

RPS9 gene

Sequence comparison through Blast Search (http://www.ncbi.nih.gov) using Genbank database showed that the *RPS9* gene cloned by us shares high homology with many other gramineous plants at both nucleic acid and protein levels. The protein sequence encoded by the gene shares 100% similarity with reported *RPS9* gene of maize (EU964329.1). At nucleic acid level, only eight SNPs were found between the *RPS9* sequence of inbred line South 202 and EU964329, one of them is transversion (G-C), others are T-C transition.

Previous studies displayed that altering the C terminus of yeast *RPS9* protein will lead to a significant change in ribosomal association with many mRNAs, *RPS9* may take part in the translation of many mRNAs (Pnueli and Arava, 2007). Lindström and Zhang (2008) thought *RPS9* is a novel B23/NPM-binding protein, which is required at the stage of normal cell proliferation. Subsequently, Lindström and Nistér (2010) found functional silence of ribosomal protein S9 will cause induce senescence or apoptosis of cancer cells. But the functional difference of *RPS9* protein in plants has not been studied. In our results, *RPS9* gene was successfully cloned from the maize inbred line Southern 202, encoding 193AA. According to the search results in GenBank database, there may be two different *RPS9* genes in different maize variety: one gene encodes 193 AA including our results; the other encodes 194 AA (NM_001155768.1). Comparison of functional sites indicated that N-myristoylation site is also different between the gene cloned by us (JX232263.1) and NM_001155768.1. On the contrary, in the animal kingdom, *RPS9* gene of fruit fly and other insects encodes 195 AA residues, and all vertebrates also encode 195 AA residues. These results indicated the *RPS9* gene shares high conservative, but unlike all vertebrates, encoded AA is stable in number. So the evolution process of *RPS9* gene is worth studying.

RPS10 gene

The sequence of cloned *RPS10* gene from maize inbred line South 202 shares high similarity with other reported genes in gramineous plants. In different varieties of maize, the sequence presents difference. At protein level, only the 125th AA residue (V-G) is different between the sequence we cloned and NP_001152734.1, which is caused by the 374th site base (T-G) change. Another single-nucleotide polymorphism (SNP) sit is found in the 487th site base, the change located in the third nucleotide of a codon, belonging to synonymous mutation.

For *RPS10* protein, it is general encoded in the mitochondrion, whereas in several other species it is encoded in nuclear and thus must be imported into the mitochondrial matrix to function (Knoop et al., 1995). Interestingly, in Lactuca sativa and Daucus carota, *RPS10* independently gained different N-terminal extensions from other genes, which are essential for mitochondrial import, and following transfer to the nucleus. However, in maize, *RPS10* has not acquired an extension upon transfer, but it can be readily imported into mitochondria (Murcha et al., 2005). In fact, the *RPS10* gene is absent from the mitochondrial genome of rice and has been transferred to the nucleus (Kubo et al., 2000). There are two *RPS10* genes in the rice nuclear genome and that their transcripts differ in abundance, the two *RPS10* genes were mapped on chromosomes 6 and 12 by RFLP (restriction fragment length polymorphism) markers. Majewski et al. (2009) found that *RPS10* takes part in photoperiod regulation, its express level gradually increase in vegetative phase in Arabidopsis. However, about the report on its function is fewer. Recently, Yazaki (2012) found some DBA patients with mutations in *RPS10*. In maize, there is at least another *RPS10* gene, which encodes 182 AA (NM_001155450) and shares high similarity with our gene encoding 179 AA (JX232263.1). In animal kingdom, the AA number encoded by *RPS10* gene is also different. The *RPS10* proteins of fruit fly and zebra fish encode 163, 166 AA, respectively; while mammalia it encodes 165 AA residues, and thus, the AA number of *RPS10* protein is quite different between animal and plant.

RPS14 gene

RPS14 protein is widespread in the lower and higher organisms. Previous research displayed that *RPS14* have transferred from the mitochondrion to the nucleus in grasses, and maize mitochondrial genome does not contain *RPS14*, nevertheless, in wheat there is a nonfunctional *RPS14* pseudogene in mitochondrial DNA of wheat, the functional *RPS14* gene is located in the nucleus (Sandoval, et al., 2004; Figueroa et al., 2000). As for its function, Zhou et al. (2013) thought *RPS14* takes part in regulation of MDM2-p53 feedback loop in response to ribosomal stress. It binds to the central acidic domain of MDM2 and inhibits MDM2 activity, elevating p53 level and activity. Oliva et al. (2010) found that its loss is a potential causal factor of 5q- syndrome in humans. In this study, we successfully cloned the coding region of *RPS14* gene, the length of cDNA fragment cloned is 453 bp in size, encoding 150 AA.

Alignment analysis indicated the nucleotide sequence and the deduced AA sequence showed high similarity with other species or variety studied. However, both the nucleotide sequence and the deduced AA sequence are different even in the same species. The *RPS14* gene in maize with accession number NM_001158971.1 encodes 160 AA residues, while the others encode 150 AA residues, including EU960087.1, NM_001158971, NM_001137768 and ours (JX232265.1). Additionally, the *RPS14* genes from sorphum (XM_002437295) and rice (NM_001052517) also encode 150 AA, but the *RPS14* genes encoding 151 AA were already found from fruit fly to higher mammalian. To be noticed, compared to ours (JX232265.1), the *RPS14* gene of NM_001158971 in maize has an additional Casein kinase II phosphorylation site, but missing an N-glycosylation site and Protein kinase C phosphorylation site. So, the function of *RPS14* gene needs to be further confirmed.

RPS16 gene

The study on *RPS16* gene is rather less, especially in plant. Wood et al. (2000) found that *RPS14* and *Rps16* performed high expression level in tortula ruralis gametophytes during a desiccation-rehydration cycle. Ajuh et al. (2000) found RPS16 protein has interaction with CDC5L, which act as a positive regulator of cell cycle G2/M progression. Here, we reported the RPS16 gene of the maize inbred line South 202, which contains an ORF of 447 bp encoding 148 AA.

The RPS16 proteins derived from maize and sorghum encode 148 and 149 AA, respectively, but they share over 92% similarity in AA sequence and the difference of N-terminal between them is obvious. To be noticed that RPS16 genes coding 148 and 146 AA was found in fruit fly and vertebrate, respectively. The knowledge here will be helpful for further study the function of *RPS16* gene.

RPS18 gene

RPS18 protein could be a novel substrate for CaMK II, providing a potential link between Ca^{2+}-mobilizing agents and protein translation (Mishra-Goruret et al., 2002), but subsequently, *RPS18* was identified as a cofilin-binding protein (Kusuiet et al., 2004). In *E. coli*, *RPS13* protein, the ortholog product of *RPS18* gene, is involved in the binding of fMet-tRNA and in the initiation of translation (Kenmochi et al., 1998). In this study, we successfully cloned the *RPS18* gene, which encode 152 AA, its nucleic acids sequences and protein sequence is highly conservative in evolution according to the data in GenBank.

The sequence cloned here (JX232267.1) is full accord with NM_001147782.1. Compared with NM_001158140.1 and EU952700.1, only an AA residue (the 62 site) was found, but at the nucleic acid level, Many SNPs sites are displayed between them. Unlike the *RPS9*, *RPS10*, *RPS14* and *RPS16* genes, the AA number of *RPS18* protein is identical between plant and animal,

which suggested that the *RPS18* shares high conservation. For its function, Maroniche et al. (2011) found *RPS18* gene exhibits a stable expression levels in virus-infected plant hoppers and can be used as a reference gene of real-time quantitative PCR.

CONCLUSION

We have successfully isolated five ribosomal protein genes from maize inbred line Southern 202, including *RPS9*, *RPS10*, *RPS14*, RPS16 and *RPS18* genes, these genes have high similarities to other related genes at both nucleic acids and protein sequences levels. In addition, the functional sites of the five genes were analyzed online. Compared to previous studies on ribosomal protein genes with out results, it was found that the number of AA residues encoded presents differences between same ribosomal protein genes in different species or varieties. These results indicated the conservation is lower in plant even the same species than higher animal. Comparatively, the ribosomal genes in animal, especially mammalian, are more conservative than those in plant. The knowledge stated here are helpful for future studying the physiological function of ribosomal proteins in plant.

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