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Full Length Article

Structural and Functional Analysis of RPL16 a Large Ribosomal Subunit Protein of Plastid Translational Machinery in Plants

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Abstract

Plastids have their own complex gene expression machinery. Components of this machinery are of *E. coli* type with exceptions, as most of the genes are plastid encoded while others are nuclear encoded. One of the components of the machinery is ribosomal proteins, large and small in size. Of these proteins, a large subunit protein 16 is not structurally and functionally characterized yet. We report here structural and functional analysis of the protein by employing various bioinformatics tools. Complete *rpl16* sequence analysis coupled with phylogenetic analysis, and protein-protein interaction revealed that the RPL16 is thermo-stable, acidic and hydrophilic in nature. This 134 amino acids long protein, consists of two alpha helices and seven beta sheets with a molecular weight of 15.21 kDa. The 3D structure of RPL16 was predicted with SWISS-MODEL and was analyzed with Ramachandran (RMSD of 0.20Å) on-line plot to access the suitability of various amino acid residues in the favored region. The predicted counterparts of RPL16 are RPL1, RPL2, RPL2.1, RPL2.2, RPL13, Rpl18/15, emb1473 and MRPL11. Among these RPL2.2, RPL2.1, Rpl18/15, RPL13, emb1473, RPL4/1, RPL11 and MRPL11 have still not been explored for their function even in *Arabidopsis* whereas rpl2 and rpl4 have functionally been analyzed. Hence, we report that RPL16 has essential role in plastids.

Keywords: RPL16; Computational biology; Protein-protein interaction; Phylogenetic analysis

Introduction

During evolution though most of the genes from plastid genome were transferred to the nucleus yet several ancestral genes are retained by plastids. The plastids not only retained its genetic material but also kept intact mechanisms of gene expression and heritability. Plastome of size 120-160 kb encodes~120 proteins, which play critical role in photosynthesis and in organellar genetic system, including transcriptional and translational machinery (Bock, 2007; Krech *et al*., 2012). Plastid translation proceeds in prokaryotic fashion and components of this machinery are: rRNA and the ribosomal proteins that consist of large and small subunit proteins. The large subunit (50S) harbors three rRNAs: 23S, 4.5S and 5S whereas small ribosomal subunit (30S) a single rRNA molecule *i.e*., 16S rRNA (Schwarz and Kossel, 1980). The similarity of chlororibosome to bacterial counterpart is thought to be the first molecular evidence for endosymbiosis (Schwarz and Kossel, 1980).

The ribosomes are encoded by a single operon, but mature proteins accumulated as a result of splicing process (Bollenbach *et al*., 2007). The assembly of ribosomes depends on the proteins, which help ribosomal RNA to fold and associate with ribosomal proteins (Fristedt *et al*., 2014). Genetic system related genes include complete sets of tRNAs and ribosomal proteins. Ribosomal proteins of plastid are homologues in *E. coli* except five proteins which are only encoded by plastid (plastid-specific ribosomal proteins PSRP) genome (Yamaguchi *et al*., 2000). In case of 30S subunit, 21 out of 24, 31 out of 33 proteins (for 50S ribosomal subunit) have *E. coli* homologues. Plastid specific ribosomal proteins (PSRPs): PSRP2, PSRP3 and PSRP4 of small subunits and PSRP5 and PSRP6 of larger subunit are plastid specific (Yamaguchi *et al*., 2000). Further, ribosomal proteins are classified as nuclear encoded and plastid encoded depending on the presence of their relevant genes location (Yamaguchi *et al*., 2000). Twelve out of the 24 small ribosomal subunit proteins are plastid encoded but 24 out of 33 large ribosomal subunit proteins are nucleus encoded, hence most of the genes of large subunit have migrated to nucleus during the course of evolution (Yamaguchi *et al*., 2000).

Targeted inactivation has been employed to reveal out function of plastid encoded genes in the process of translation. Various genes of ribosomal large subunit (rpl22, rpl32, rpl36 and rpl23) and ribosomal small subunit (rps3, rps15 and rps 16) were evaluated through reverse genetics to explore their role in cellular functions. Three of the genes of large subunit (rpl22, rpl32 and rpl23) were found to be essential whereas rpl36 was found to be non-essential.

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Likewise, two of the genes of ribosomal small subunit (rps3 and rps16) were found to be essential whereas rps15 was found to be non-essential (Fleischmann *et al*., 2011). The level of essentiality is variable depending upon the role of gene in cellular activities. In certain cases, a plant is not able to survive or grow whereas in other cases stunted growth with reduced photosynthetic performance is observed. Ribosomal proteins sequences are very similar in both bacteria and chloroplast. This similarity led to believe that ribosomal proteins playing role in one organism might be a representative of the other (Tiller and Bock, 2014).

A large number of genes have been studied through reverse genetics and have been evaluated for their function in the cell. Since the advent of computational biology, it has proved to be a good tool to functional genomics. The data retrieved through various bioinformatics tools not only helps to predict function of a gene but also facilitates in designing the knockout experiments. Considering the importance of computational biology in functional genomics, gene product of *rpl16* was characterized by using bioinformatics tools to evaluate its possible role in the process of translation.

Materials and Methods

Retrieval of rpl16 Sequences and their Characterization

The amino acids sequences of *Nicotiana tobaccum* (Accession no.: NP_054536.1) and five closely related *Nicotiana* species having greater than 90% sequence similarity [*N. sylestris* (Accession no.: YP_358715.1), *N. undulate* (Accession no.: YP_004891644.1), *N. tomentosiformis* (Accession no.: YP_398901.1), *N. otophora* (Accession no.: YP_009336420.1) and *N. attenuata* (Accession no.: MG182422)] were retrieved from NCBI using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). ProtParam tool of EXPASy server was used to find the physiochemical properties of the *rpl16* protein (https://web.expasy.org/cgi-bin/protparam/protparam; Gasteiger *et al*., 2003). ClustalX2 (Larkin *et al*., 2007) along with ESPript-3 (http://espript.ibcp.fr/ESPript/ESPript/; Gouet *et al*., 2003), and SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/secpred_sopma.pl;Geourjon and Deleage, 1995) were used to find the conserved residues, responsible for catalytic activity of the rpl16 protein product. The online server PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/; McGuffin *et al*., 2000) was used for the prediction of rpl16 secondary structure.

Structural Characterization

SWISS-MODEL, an online server (https://swissmodel.expasy.org/) was used to predict 3D structure of rpl16 protein of *N. tobaccum* (Accession no.: NP_054536.1). The predicted structure was evaluated by Verify 3D (http://servicesn.mbi.ucla.edu/Verify3D/; Liithy *et al*., 1992), ERRAT(http://servicesn.mbi.ucla.edu/ERRAT/; Colovos and Yeates, 1993), PROCHECK (http://servicesn.mbi.ucla.edu/PROCHECK/; Laskowski *et al*., 1993) and QMEAN score. Global and per-residue model quality has been evaluated by using the QMEAN (Benkert *et al.,* 2009) scoring function. In order to attain improved performance, the weights of individual QMEAN terms have specifically been trained for SWISS-MODEL (Guex *et al*., 2009). Further FATCAT (Yuzhen and Godzika, 2004) and Chimera (Pettersen *et al*., 2004) were used for the superimposition of the predicted protein structure with template.

Protein-proteins Interaction

Computational tools are very useful to find interaction of proteins with each other as well as with their counterparts involved in any metabolic or biosynthesis pathway. For this, STRING (https://string-db.org/) online interaction database was used. The online server still lacks information about *N. tabaccum* in its data, so the *rpl16* gene of *Arabidopsis thaliana* was used to blast and to find its interacting partners during the translation process occurring in the plastid of cruciferous model plant.

Results

Sequence Characterization of *rpl16*

Retrieved amino acid sequence of *rpl16* of *N. tabaccum* (Accession no.: NP_054536.1) was characterized using ProtParam tool of EXPASY (Gasteiger *et al*., 2003). Physically, *rpl16* derived protein consists of 134 amino acids with a molecular weight of 15213.87 Daltons and theoretical pI 11.88. Values for aliphatic index and instability complex were 74.33 and 58.41, respectively which indicate level of stability of the protein. It has an estimated half-life of 30 h in mammalian reticulocytes under *in vitro* conditions whereas >20 h in yeast and >10 h in *E. coli* under *in vivo* conditions. Similarly, extinction coefficient (20970) is related to its relation concentration in the solution (Qamar and Khan, 2017). Grand average of hydropathicity (GRAVY) index is an indicator of protein solubility and its negative value (-0.520) revealed that RPL16 is hydrophilic in nature (Atsushi, 1998). Amino acids composition of the protein under investigation is given in Table 1.

Functional and Structural Characterization

Multiple sequence alignment was performed to identify similarity among homolog sequences (Fig. 1). The *rpl16* sequences from *N. tabaccum* (Accession no.: NP_054536.1) and five closely related *Nicotiana* species having greater than 90% sequence similarity (*N. sylestris* (Accession no.: YP_358715.1), *N. undulate* (Accession no.: YP_004891644.1), *N. tomentosiformis* (Accession no.: YP_398901.1), *N. otophora* (Accession no.: YP_009336420.1) and *N. attenuata* (Accession no.: MG182422) showed significant level of sequence conserveness among each other.

Table 1: Amino acids composition of RPL16 protein

Amino Acids	Total number	Percentage composition
Ala (A)	12	9.0%
Arg (R)	19	14.2%
Asn (N)	3	2.2%
Asp (D)	1	0.7%
Cys (C)	0	0.0%
Gln (Q)	4	3.0%
Glu (E)	6	4.5%
Gly (G)	13	9.7%
His (H)	3	2.2%
Ile (I)	12	9.0%
Leu (L)	6	4.5%
Lys (K)	9	6.7%
Met (M)	6	4.5%
Phe (F)	4	3.0%
Pro (P)	8	6.0%
Ser (S)	9	6.7%
Thr (T)	7	5.2%
Trp (W)	3	2.2%
Tyr (Y)	3	2.2%
Val (V)	6	4.5%
Pyl (O)	0	0.0%
Sec (U)	0	0.0%

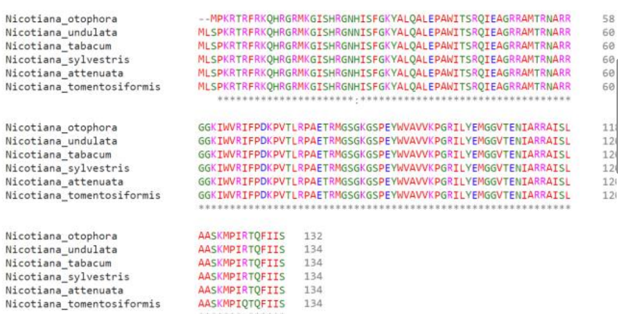

Fig. 1: Multiple sequence alignment of *rpl16* protein from *N. tobaccum* (Accession no.: NP_054536.1) and five closely related *Nicotiana* species (*N. sylestris* (Accession no.: YP_358715.1), *N. undulate* (Accession no.: YP_004891644.1), *N. tomentosiformis* (Accession no.: YP_398901.1), *N. otophora* (Accession no.: YP_009336420.1) and *N. attenuata* (Accession no.: MG182422)

Protein secondary structure is the three dimensional form of local segments of proteins (Perticaroli *et al*., 2014). The secondary structure arises from the hydrogen bonds formed between atoms of the polypeptide backbone (Rehman and Botelho, 2017). Secondary protein has crucial role in various signaling pathways for organism growth and development. Any mutational or conformational change in protein secondary structure greatly suppresses the signaling process in *Escherichia coli* (Emr and Silhavy, 1983), may result in sickle cell disease in human (Rehman and Botelho, 2017). Functional annotation is an important step in computational biology for comparative analysis of various genes/proteins and to predict their particular function. A number of tools are available online freely and/or system installed, to predict secondary structure of the proteins with a degree of accuracy from 56 to 70% (Qamar and Khan, 2017). We selected SOPMA and PSIPRED servers owing to their superior features. They have gained additional 4% prediction power and have attained 73.2% success rates compared to other currently used tools (Geourjon and Gilber, 1995; McGuffin *et al*., 2000). According to the predicted results, RPL16 contains 26.87% alpha helix followed by 23.88% extended strand, 9.70% beta turns and major share of 39.55% random coil in its secondary structure (Fig. 2).

Phylogenetic analysis also helps to predict conservancy and prevalence of any gene within related plant species. PHYLIP package (Felsenstein, 1995) is a comprehensive collection of software tools that implement various algorithms for the creation of phylogenetic trees. Four of the most prominent algorithms of PHYLIP are: Unweighted Pair Group Method with Arithmetic Mean (UPGMA), Maximum parsimony (MP), Neighbour-joining (NJ) and Maximum-likelihood (ML) (Felsenstein, 1978). Phylogenetic dendogram was constructed using neighbor joining method based on multiple sequence alignment of *rpl16*, with other closely related species and was analyzed (Fig. 3).

The SWISS-MODEL first searched different templates. We selected a suitable template having significant identity with query and proceeded for meddling. Predicted structure was evaluated by Varify3D. At least 80% of the amino acids have scored >= 0.2 in the 3D/1D profile. In our predicted structure, 83.58% of amino acid residues have averaged 3D-1D score >= 0.2 which estimates the matching of 3D model with sequence analysis by ERRAT and shows the quality factor of protein 90 which indicates that this structure has average quality. Ramachandran plot shows that 93.7% residues fall in favorable region and 5.4% in additional allowed region. Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor were not greater than 20%. A good quality model would be expected to have over 90% in the most favored regions. So, our predicted structure has good quality (Fig. 4).

Protein-protein Interaction

Availability of complete information about plastid and nuclear genomes provide basic data for the development of bioinformatical databases and tools which help to find and predict the nuts and bolts of various cellular pathways (Mehmood *et al*., 2014). These online databases are continually updated depending upon the data available through reverse genetic or other advanced omics approaches. STRING is one of the valid online databases that facilitate researchers to design their experiments with more precision and accuracy (Pieper *et al*., 2014). It provides live interaction of protein within an organism and helps to design primers for possible targets in Northern blot analysis (Fig. 5 and Table 2). *Rpl16* gene product of model plant (*Arabidopsis thaliana*) was found to interact with the RPL2, RPL2.2, RPL2.1, Rpl18/15 (L18/L15: AT5G64670), RPL13 (L13: AT3G01790), emb1473, RPL4/1 (L4/L1: AT2G20060), RPL4, RPL1 (AT5G51610) and MRPL11.

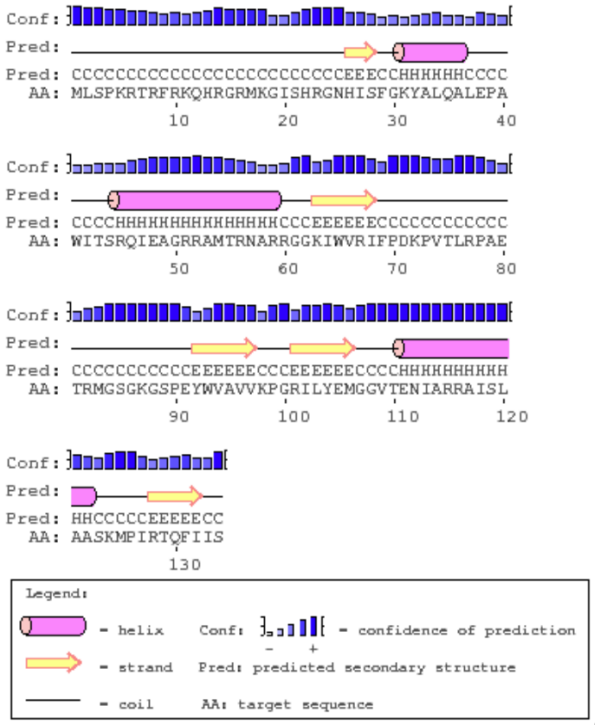

Fig. 2: Secondary structure prediction of rpl16 of *N. tobaccum* (Accession no.: NP_054536.1) using PSIPRED

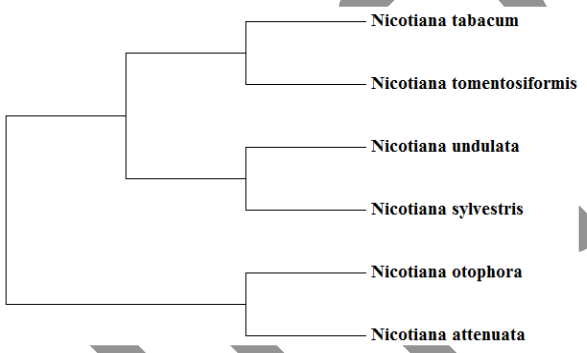

Fig. 3: Phylogenetic tree of *rpl16* protein from *N. tobaccum* (Accession no.: NP_054536.1) and five closely related *Nicotiana* species (*N. sylestris* (Accession no.: YP_358715.1), *N. undulate* (Accession no.: YP_004891644.1), *N. tomentosiformis* (Accession no.: YP_398901.1), *N. otophora* (Accession no.: YP_009336420.1) and *N. attenuata* (Accession no.: MG182422) developed using neighbor joining method based on multiple sequence alignment

Out of these, RPL2.2, RPL2.1, Rpl18/15 (L18/L15: AT5G64670), RPL13 (L13: AT3G01790), emb1473, RPL4/1 (L4/L1: AT2G20060), RPL11 (AT5G51610) and MRPL11 are still with their unknown function within the host organism. *RPL4* is an essential gene and is required for basal ribosomal activity, which is crucial at the

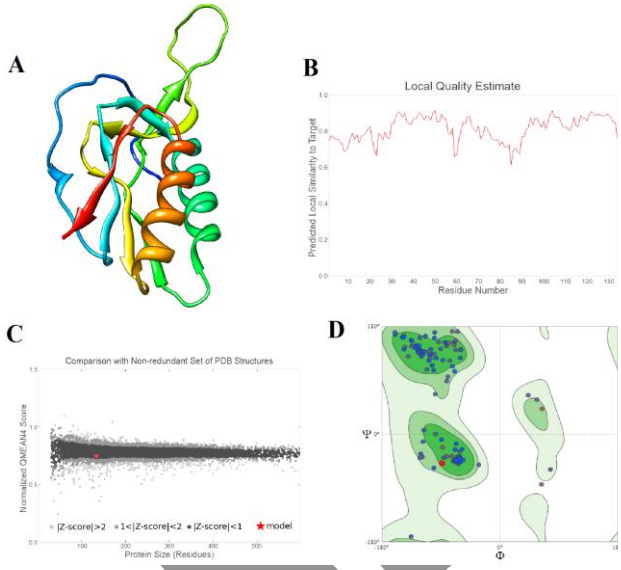

Fig. 4: (A) Predicted 3D model of RPL16 protein by SWISS-MODEL; (B) Graph give local quality estimate for the total number of residues in the favour regions. 98.5% (130/132); (C) plot C give Z score value for the model quality and suitability; (D) Ramachandran plot showing amino acids in allowed region

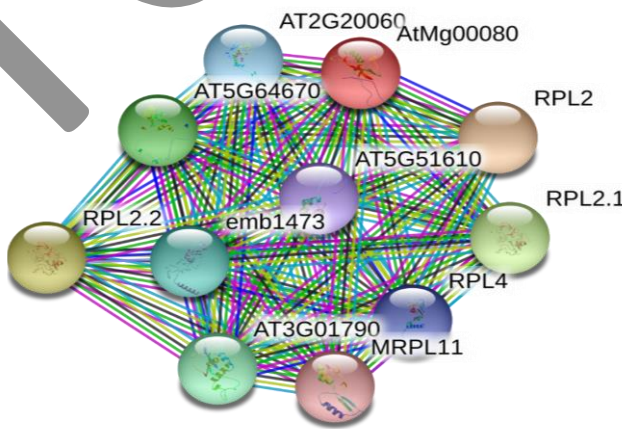

Fig. 5: Protein-protein interaction of *rpl16* plastid gene product of *Arabidopsis thaliana* with other plastidic, mitochondrial and nuclear gene products using online STRING database

globular stage and during the transition from globular to the heart stage of embryogenesis along with PRPS20, -L1, -L27 and -L35 (Romani *et al*., 2012). The protein components of mRpl11 are the structural component of 39S mitochondrial ribosome. It is a nuclear encoded gene having two pseudo-genes on the chromosome number 5 and 12 in the *Arabidopsis thaliana*. In addition to biogenesis and maintenance, it is also predicted to be involved in mitochondrial translation.

Table 2: The predicted protein partners of RPL16 in the plastid translation of *Arabidopsis thaliana* with known/ proposed functions

Predicted functional partner	Protein name, its function and its amino acids length
RPL2	*Nucleic acid-binding, OB-fold-like protein.[Source-TAIR;Acc-ATMG00560] (349 aa)*
RPL2.2	*Ribosomal protein L2 (274 aa)*
RPL2.1	*Ribosomal protein L2 (274 aa)*
Rpl18/15 (L18/L15: AT5G64670)	*Ribosomal protein L18e/L15 (281 aa)*
RPL13 (L13: AT3G01790)	*Large subunit ribosomal protein L13 (205 aa)*
emb1473	*Embryo defective 1473 (241 aa)*
RPL4/1 (L4/L1: AT2G20060)	*Ribosomal protein L4/L1 family (300 aa)*
RPL4	*Ribosomal protein L4; This protein binds directly and specifically to 23S rRNA (By similarity). May play a role in plastid transcriptional regulation (282 aa)*
RPL11 (AT5G51610)	*Ribosomal protein L11 (182 aa)*
MRPL11	*Mitochondrial ribosomal protein L11 (155 aa)*

Discussion

The computational information is of central importance in omics and functional genomics. Amino acid sequence of gene, its 3D protein model, physical and chemical properties, molecular weight, catalytic residues, and mutation rate during the course of evolution, its presence and evolutionary relationship with various species could be inferred with an acceptable index of accuracy. The findings of computational tools are now routinely incorporated with wet lab. Experiments to precisely define the role of any gene of unknown function revealed that most of the plastid genes have been explored so far, to find their role in cellular life through bioinformatics and reverse genetics but fewer of the genes are still to be explored. Reverse genetics approaches elaborate the exact role of a gene and its protein product in growth and development. Gene deletion studies led to find role of individual ribosomal protein in translation and ribonucleo protein complexes (Kaczanowska and Ryden-Aulin, 2007; Schmeing and Ramakrishnan, 2009).

These knockout studies reveled that ribosomal genes are of two types: essential and non-essential (Fleischmann *et al*., 2011). Even the non-essential genes have some role in cellular metabolism particularly in photosynthetic performance (Romani *et al*., 2012). Chloro-ribosome, the key constituent of plastidic translational machinery, consists of two multi-component subunits which are comprised of rRNA and certainproteins (Fristedt *et al*., 2014). To evaluate role of *rpl16* gene in cellular metabolism, conserved regions of *rpl16* gene were identified among the closely related *Nicotiana* species. Further, physical and chemical properties of protein product and their interaction with other proteins (involved in translational machinery) were also studied. STRING analysis led to find that RPL16 interacts with RPL2 (L2), RPL2.2, RPL2.1, RPL2.1, AT3G01790, emb1473, AT2G20060, RPL4, and MRPL11 AT5G51610 of *Arabidopsis thaliana*. This interaction was found to be operated at transcriptional, post-transcriptional, translational or post-translational level, which not only defines its role in basic cellular processes but also in growth and development of the organism.

Ribosomal proteins have some regulatory role in translation and their deletion may have adverse phenotypic effects, ranging from embryo lethality to vitality (photosynthetic lesions and decrease in the expression of plastid proteins) (Romani *et al*, 2012). Various *rpl* genes have been worked out to elucidate their function. Few of these have been evaluated to be essential *i.e*., rpl1, 10, 13, 18, 31 (Bryant *et al*., 2011), rpl20 (Rogalski *et al*., 2008), rpl22, 23, 32 (Fleischmann *et al.*, 2011), rpl14, 27, 28 (Romani *et al*., 2012) and rpl21 (Yin *et al*., 2012) whereas others have been evaluated to be non-essential ribosomal proteins. These include rpl11 (Pesaresi *et al*., 2001), rpl24 (Tiller *et al*., 2012), rpl33 (Rogalski *et al*., 2008), rpl35 (Romani *et al*., 2012), rpl36 (Fleischmann *et al*., 2011).

RPL2 has been found to be essential in the *E. coli* and mitochondria of seed plants whereas plastidic rpl2 has not yet been worked out (Adamo *et al*., 2008). Shoji *et al*. (2011) explored seven genes of ribosomal large subunit as non-essential. Since, *rpl16* is a novel gene of the plastome and has not yet been explored for its function in the plastidic translational machinery, we report here that RPL16 plays a crucial role in plastid translation which needs to be further validated through wet lab experimentation.

Conclusion

Plastid large subunit protein 16 is somehow thermally stable, acidic and hydrophilic in nature with molecular size of 15.21 kDa. It is conserved among a wide variety of plant species. It contained 26.87% alpha helices, 9.70% beta turns, 23.88% extended strands and 39.55% random coil in its secondary structure. It has overall negative charge with an isoelectric point of 11.88. Protein-protein interaction proposed that its transcriptional and translational product interacted with a number of nuclear, plastidic and mitochondrial genes and their products. This interaction analysis led to propose that *rpl16* gene is an essential component of plastid translation machinery and plays some key role in plant growth and development.

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