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REVIEW

Translation initiation in plants: roles and implications beyond protein synthesis

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Abstract

Protein synthesis is a ubiquitous and essential process in all organisms, including plants. It is primarily regulated at translation initiation stage which is mediated through a number of translation initiation factors (eIFs). It is now becoming more apparent that in addition to synthesis of proteins, eIFs also regulate various aspects of plant development and their interaction with environment. Translation initiation factors, such as eIF3, eIF4A, eIF4E, eIF4G, and eIF5A affect different processes during vegetative and reproductive growth like embryogenesis, xylogenesis, flowering, sporogenesis, pollen germination, *etc*. On the contrary, eIF1A, eIF2, eIF4, and eIF5A are associated with interaction of plants with different abiotic stresses, such as high temperature, salinity, oxidative stress, *etc*. Similarly, eIF4E and eIF4G have roles in interaction with many viruses. Therefore, the translation initiation factors are important candidates for improving plant performance and adaptation. A large number of genes encoding eIFs can functionally be validated and utilized through genetic engineering approaches for better adaptability and performance of plants by inhibiting/minimizing or increasing expression of desired eIF(s).

Additional key words: embryogenesis, germination, high temperature, oxidative stress, salinity, viral diseases, water stress.

Introduction

In eukaryotes, expression of genes is a complex process regulated at multiple levels, such as transcription, post-transcription, structure and stability of mRNA, protein synthesis, and protein degradation. The process of protein synthesis also known as translation is dynamic, universal, and essential for all organisms (Steitz 2008, Sonenberg and Hinnebusch 2009). It is divided into four phases: initiation, elongation, termination, and ribosome recycling (Pacheco and Martinez-Salas 2010). Of the four phases, initiation of translation is the most complex, main rate-limiting, and most regulated step. Translation initiation may be defined as process of assembly of elongation-competent 80S ribosomes in which the initiation codon is base-paired with the anticodon loop of initiator tRNA (Met-tRNA$_i$) in the ribosomal peptidyl (P)-site. In eukaryotes, initiation of translation involves participation of messenger RNA (mRNA) to be translated, ribosomal subunits, methionine, initiator transfer RNA (tRNA$_i$), enzymes, and associated components to activate and charge initiator tRNA with methionine, and a suite of eukaryotic translation initiation factors (eIFs).

In plants, the overall principles of protein synthesis are similar to those of other eukaryotes, but some differences do exist, suggesting plant specific regulation and implications of translation (Browning 2004). Further, apart from synthesis of proteins, translation initiation factors play various other important roles in plants. The

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Abbreviations: AA - amino acid; CITE - cap-independent translation element; Cys - cysteine; eIF - translation initiation factor; GEF - guanine nucleotide exchange factor; Met - methionine; mRNA - messenger RNA; ORF - open reading frame; PABP - poly(A)-binding protein; PIC - pre-initiation complex; P-site - peptidyl site; RRM - RNA recognition motif; tRNAi - initiator transfer RNA; UTR - untranslated region.
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eIF3, eIF4A, eIF4E, eIF4G, and eIF5A are involved in vegetative and reproductive growth processes like embryogenesis, xylogenesis, flowering, sporogenesis, and pollen grain germination. The eIF5A also plays a pivotal role in leaf senescence (Roy *et al*. 2011, Vain *et al*. 2011, Martinez-Silva 2012). In addition, the eIF1A, eIF5A, and eIF4E are entailed in abiotic stresses like high temperature, oxidative, osmotic, salt, and nutrient stress tolerance (Ma *et al*. 2010, Xu *et al*. 2011, Wang *et al*. 2012). The eIF4E, and eIF4G play significant roles against virus infection and are involved in resistance against potyviruses, maize rough dwarf disease, tungro spherical virus, and potato virus Y (Lee *et al*. 2010, Duan *et al*. 2012, Wang and Krishnaswamy 2012). Hence, the eIF4E and eIF4G are potential target genes in the development of genetic resistance to viruses for crop improvement. Recent literature has established strong platform for engineering translation initiation machinery for improving growth, development, and adaptation. In this review, we discuss literature available on the translation initiation in plants and its influence on growth, development, and interaction with environment.

Overview of translation initiation in eukaryotes

The process of protein synthesis can be subdivided into four major stages: initiation, elongation, termination, and ribosomes recycling. As this review is devoted to translation initiation, hence other three stages (elongation, termination, and recycling) will not be discussed here. A recent review (Dever and Green 2012) has extensively covered these three stages of protein synthesis. Also, a detailed mechanism and principles of translation initiation has been reviewed (Jackson *et al.* 2010, Malys and McCarthy 2011). Basically, translation initiation involves a set of reactions that place the start codon AUG of mRNA in the P-site of ribosome, basepaired with the anticodon UAC of Met-tRNA$_i$. Thus, it covers all steps between dissociation of ribosomal subunits upon termination in previous translation cycle, and assembly at mRNA start codon of a ribosome ready for elongation. The main steps of translation initiation in eukaryotes including plants are: *1*) formation of 43S pre-initiation complex (PIC), *2*) attachment of 43S PIC to mRNA, *3*) scanning of mRNA 5′-untranslated region (UTR) by 43S PIC, *4*) selection of initiator aa-tRNA, *5*) selection of correct translation start site, and *6*) joining of ribosomal subunits and release of factors. At the end of initiation phase, the next phase of translation, *i.e*., elongation phase, marked by formation of the first peptide bond upon accepting the first elongator tRNA becomes ready to proceed. In this section the six steps of translation initiation are discussed briefly. There are good reviews on these processes in eukaryotes (Pestova and Kalupaeva 2002, Marintchev and Wagner 2004). As a few eIFs play multiple roles in more than one step of translation initiation, these are briefly described in a separate section.

Formation of 43S preinitiation complex: Initiation of translation needs separated 40S and 60S ribosomal subunits and a pool of eIFs. The separated ribosomal subunits are derived from recycling post-termination ribosomal complexes which comprise an 80S ribosome still bound to mRNA, tRNA, and release factors. Post-termination ribosomal complexes are recycled by releasing these ligands and dissociating ribosomes into free 60S subunits, mRNA, and tRNA-bound 40S subunits. The eIFs known to mediate this dissociation process are listed in Table 1 Suppl. Further, for becoming amenable for initiating translation, the dissociated ribosomal subunits need to remain separated. This activity of remaining separated is known as anti-association. The main function of anti-association process is to provide a pool of ribosomal subunits for translation initiation and also to prevent a premature assembly of translationally inactive ribosomes during initiation as well as ribosome assembly at an incorrect site. The eIF3 carries the main subunit dissociation and anti-association function. However, other two eIFs, eIF1A and eIF2 have also been reported to be involved in dissociation of ribosomes (Pisarev *et al*. 2007). The eIF3, eIF1, and eIF1A remain associated with a recycled 40S subunit and prevent its re-association with a 60S subunit. Thus, the eIF3, eIF1, and eIF1A are recruited to a 40S subunit during recycling, whereas eIF2-GTP-Met-tRNA$_i$ subsequently attaches to the recycled 40S subunit, bound simultaneously to the eIF3, eIF1, and eIF1A to form 43S PIC. The ternary complex of eIF2 with GTP and Met-tRNA$_i$ stabilizes eIF3 binding and also provides a steric block against subunit joining. The structural elucidation of 43S PIC has yielded better insights into its spatial architecture (Yu *et al*. 2009). However, the complete understanding of the interaction and mechanism need spatio-temporal dynamic studies of complex formation and its function.

Attachment of 43S preinitiation complex to mRNA: Recruitment of mRNA to a eukaryotic ribosome begins with formation of a ternary complex eIF2-GTP-Met-tRNA$_i$. The ternary complex binds to a 40S ribosomal subunit aided by eIF1, eIF1A, and eIF3 to form 43S PIC which subsequently binds to mRNA on its 5′ UTR. The 5′-UTR contains secondary structures which need to be unwound for ribosomal attachment. The eIF4F is the main eIF complex involved in the binding of mRNA to 43S PIC. The eIF4F comprises eIF4A (DEAD-box RNA helicase), eIF4E (a cap-binding protein), and eIF4G. The eIF4G functions as a ‘scaffold’ that binds eIF4E, eIF4A, a poly(A)-binding protein (PABP), and eIF3. The eIF4B and eIF4H enhance eIF4A helicase activity (Von der Haar *et al*. 2004, Schütz *et al*. 2008, Marintchev *et al*. 2009).

Scanning of mRNA 5′-UTR by 43S preinitiation complex: After attachment, 43S PIC scans mRNA downstream of the cap to the initiation codon. The scanning consists of two linked processes: the unwinding of secondary structures in 5′-UTR and ribosomal movement along it. Recently, it was observed that there is a linear correlation between scanning time and 5'-UTRs, and the ribosome movement is a unidirectional motion with rate being virtually independent of a particular mRNA sequence and secondary structure (Vassilenko *et al*. 2011). It has been revealed that an increase in 5'-UTR length results in an increased translation time regardless of its primary and secondary structures. The eIF1 rejects non-AUG codons during scanning by blocking the release of Pi from partially hydrolyzed eIF2-GDP-Pi in the scanning of 43S PIC. The eIF3 has also been reported to be involved in the scanning process. The eIF3 interacts with mRNA upstream of the E-site forming an extension of mRNA-binding channel that might contribute to scanning (Pisarev *et al*. 2008). Also, in addition to promoting the attachment, eIF4A, eIF4G and eIF4B assist to 43S PIC during scanning.

Selection of initiator aa-tRNA: In eukaryotes, translation is initiated by a special Met-tRNA designated as Met-tRNA$_i$. Specificity of Met-tRNA$_i$ involves following activities: *1*) recognition of initiator tRNA by aminoacyl-tRNA synthetase, *2*) discrimination against initiator tRNA by elongation factors (EFs), *3*) discrimination against uncharged or mischarged initiator tRNA by eIFs, and *4*) discrimination against elongator tRNAs by eIFs. Aminoacyl tRNA synthetases catalyze ligation of amino acids to their cognate tRNAs. The overall reaction can be represented as:

$$AA + ATP + tRNA \rightarrow AA\text{-}tRNA + AMP + Pi.$$

Recognition of initiator tRNA by aminoacyl-tRNA synthetase involves interaction of aminoacyl-tRNA synthetase with the anticodon triplet on tRNA and the acceptor end of the tRNA. Initiator tRNA is specific for methionine, but distinct from methionine-specific elongator tRNA. The tRNA$_i$ is post-transcriptionally modified, which makes it to be able to be discriminated against an initiator tRNA by elongation factors. Further, initiator tRNA must be charged with the correct amino acid, methionine. The eIF2 is responsible for selection and recruitment of initiator tRNA to a ribosome. The release of eIF2 from Met-tRNA$_i$ is mediated by hydrolysis of GTP, which is catalyzed by eIF2.

Selection of correct translation start site: Components carrying out scanning of mRNA 5′-UTR possess discriminatory mechanisms that prevent a partial base-pairing of codon triplets in 5′-UTR with the Met-tRNA$_i$ anticodon and promote recognition of correct initiation codon. This property is known as fidelity of initiation. Factors regulating the selection of correct translation start site are: *1*) nucleotide context around AUG, *2*) length and presence or absence of secondary structure in 5'-UTR, *3*) 3'-poly-A tail, and *4*) sequences in 3'-UTR. In eukaryotes, majority of mRNAs contain only one open reading frame (ORF), their 5'-end is 'capped' with an m^{7}G-cap through a reverse 5'- 5' bond, and have a poly-A tail at their 3'-end. Both the 5'-cap and 3'-poly-A tail are important for an efficient translation. A 43S PIC is first recruited to a 5'-cap through interactions with eIF4F and a cap-binding complex. The 43S PIC then scans downstream along the mRNA to the initiation codon to form a 48S complex (Schmitt *et al*. 2010). The start codon is mostly the first AUG as a nucleotide context around AUG significantly influences initiation efficiency. An optimal sequence context for the AUG start codon is GCCA/GCCAUGG. Secondary structure in 5'-UTR greatly impacts translation efficiency. Also, sequences in 3'-UTR are reported to regulate translation through their involvement in providing binding sites for eIFs. The eIF1 empowers 43S PIC to discriminate against non-AUG triplets and AUG triplets that have a poor context or are located within eight nucleotides from the mRNA 5′-end, and also dissociates ribosomal complexes that aberrantly assemble at such triplets in its absence.

Joining of ribosomal subunits and release of factors: Joining of ribosomal subunits depends on a proper orientation of initiator aa-tRNA$_i$ and it is achieved upon correct start codon recognition. The 40S subunit with initiator aa-tRNA in the P-site base paired to the start AUG codon of mRNA binds to a 60S subunit and forms a translationally active ribosome. Subunit joining is promoted by eIF5B. The other factor, eIF1A, when bound alone to a 40S subunit, stimulates rates of both subunit joining and dissociation, and also stabilizes the binding of eIF5B to the small subunit. The two factors are coordinately released after subunit joining. An event that triggers factor release is start site recognition. The eIF1 binds to the interface surface of the small subunit and need to be displaced from there before subunit joining. Start site recognition also induces GTP hydrolysis by eIF2, and eIF2-GDP-eIF5B and eIF1A are coordinately released after subunit joining (Choi *et al*. 2000, Olsen *et al*. 2003). The release is triggered by GTP hydrolysis by eIF5B. The eIF5B.GDP has a lower affinity for a ribosome than eIF5B.GTP and dissociates quickly (Lee *et al*. 2002).

Translation initiation factors

In eukaryotes, a range of eIFs have been identified (Table 1 Suppl.), however, the list still seems to be incomplete and getting swollen with time. Some eIFs are conserved across all eukaryotes, however, variations do occur for some eIFs in various eukaryotic organisms including plants. Structural aspects of the translation

initiation system have been extensively described in the review of Marintchev and Wagner (2004). A generalized brief summary of eIFs is given below.

eIF1 and eIF1A: The eIF1 is highly conserved among all eukaryotes and is important for scanning and start codon selection. It cooperates with eIF3 in subunit dissociation/ antiassociation (Pestova and Kolupaeva 2002). Together with eIF1A, eIF1 is required for formation of a 43S mRNA complex that is competent for scanning and accurate recognition of the initiation codon. Both eIF1 and eIF1A are involved in distinguishing between start codons in a "good" context (*i.e.*, the Kozak consensus sequence surrounding many start codons) and a bad context. It was studied that in the absence of eIF1A, PICs are able to scan through good context AUG codons, and in the absence of eIF1, a PIC is more likely to stop at an AUG codon in a bad context (Mitchell and Lorsch 2008). The eIF1 and eIF1A act synergistically and facilitate a ternary complex binding to a 40S ribosome (Olsen *et al*. 2003). The eIF1A is essential for transferring initiator Met-$tRNA_i$ to a 40S ribosomal subunit (Huang *et al*. 2004). It also stabilizes the binding of eIF5B to the small ribosomal subunit (Marintchev and Wagner 2004).

eIF2 and eIF2B: The eIF2 is a heterotrimer composed of eIF2α, eIF2β, and eIF2γ. The β subunit promotes GTPase activity and modulates the initiator tRNA binding of eIF2γ. The γ subunit forms the structural core of eIF2 heterotrimeric protein but essential functions are performed by eIF2β (Hinnebusch 2000). In addition to their role in delivering Met-$tRNA_i$ to the ribosome, the three subunits of eIF2 also function in selecting the correct translation start site (Schmitt *et al*. 2010). The eIF2 (a G protein) functions in its GTP-bound state to deliver initiator methionyl-tRNA to the small ribosomal subunit.

Delivery of Met-$tRNA_i$ to the AUG start codon in the translation initiation complex requires hydrolysis of GTP associated with eIF2. The eIF2B is a guanine nucleotide exchange factor (GEF) that recharges GDP-charged eIF2 with GTP. It is a heteropentamer composed of α, β, γ, δ, and ε subunits. Its activity is controlled by phosphorylation and dephosphorylation of its eIF2Bε subunit. A new isoform of eIF2B named as eIF2Bβ has been identified in *Nicotiana tabacum* and shows a high homology with β and δ subunits of eIF2B from yeast and animals (Kim *et al*. 2001). The eIF2B is present in a less proportion than eIF2 in cells. The eIF2B is divided into a catalytic domain comprising eIF2Bγ and eIF2Bε, whereas a regulatory domain consisting of eIF2Bα, eIF2Bβ, and eIF2Bδ which are involved in regulation of eIF2B activity (Dever 2002). In the case of plants, various contradictory reports are available regarding the quaternary structure and M_r of eIF2. Initial studies reported the existence of eIF2 as monomeric protein with M_r of ~ 83 kD (Lax *et al*. 1982). Later on it was found to have two polypeptides with M_r of 21 and 19 kD (Osterhout *et al*. 1983). Further, Shaikhin *et al*. (1992) reported that eIF2 consists of four subunits with M_r of 37kD (α), 40 kD (β), 42 kD (γ), and 52 kD (δ). However, Metz and Browning (1997) showed that wheat eIF2 is comprised of three non-identical subunits with an estimated M_r of 38 kD (eIF2a), 42 kD (eIF2b), and 50 kD (eIF2c). The eIF2.GTP binds specifically to Met-$tRNA_i$, whereas GTP hydrolysis or loss of the methionine moiety weakens the interaction of eIF2 with $tRNA_i$. The eIF.GTP formed is not stable unless Met-$tRNA_i$ joins to form the ternary complex. This step is considered as a rate limiting step of translation (Pavitt 2005). The eIF2-GTP-Met-$tRNA_i$ ternary complex binds stably to a 40S ribosomal subunit and the interaction is further stabilized by other factors. Upon start site recognition, eIF2 hydrolyzes GTP and is released as eIF2-GDP from Met-$tRNA_i$.

eIF3: The eIF3 is the most complex of all eIFs and has been found to perform multiple functions including anti-association of ribosomal subunits, stimulation of 43S PIC assembly, and promotion of the binding of ternary complex and other components of 43S PIC to a 40S subunit (Hinnebusch 2006). The eIF3 also plays a key role in recruiting 43S PIC to mRNA, probably interacting with mRNA as it emerges from the exit channel of 40S subunit (Pisarev *et al*. 2008). Recent findings have suggested that this anti-association function of eIF3 is dependent on other factors including a ternary complex, combination of eIF1 and eIF1A, and RNA oligo-nucleotides. These factors stimulate the anti-association activity of eIF3 partly by stabilizing the eIF3.40S subunit interaction and preventing the binding of 60S subunit to 43S PIC. It has also been suggested that eIF3 has a role in dissociating an 80S subunit at the stop codon after termination. The eIF3 of plants and mammals contains 13 nonidentical subunits that are designated eIF3a to eIF3m (Kolupaeva *et al*. 2005). The functions of individual eIF3 subunits remain to be fully characterized. Though eIF3 has been reported to interact with a 40S subunit, mRNA and several factors, some of these interactions appear to vary among species.

eIF4B: The eIF4B is an RNA-binding protein that binds to both mRNA and the 18S rRNA component of 40S subunit simultaneously. The eIF4B stimulates recruitment of mRNA to 43S PIC. The eIF4B has an N terminal domain (NTD), an RNA recognition motif (RRM), an unusual domain composed of seven imperfect repeats of 26 amino acids, and a C terminal domain. The N-terminal domain and seven repeats are most critical domains for the binding of eIF4B to the head of a 40S ribosomal subunit. This interaction induces conformational changes in ribosome mRNA entry channel that facilitates mRNA loading (Walker *et al*. 2013). The eIF4B also promotes a productive interaction of eIF4A with 43S PIC to facilitate an efficient mRNA recruitment. It stimulates eIF4A helicase activity, which unwinds an inhibitory secondary structure in 5'-UTR of mRNA. The eIF4B is one of the least conserved eIFs. Wheat eIF4B exists as heterodimer containing 80 and 28 kD subunits (Lax *et al*. 1985). Some

conflicting reports have suggested that plant eIF4B may exist as monomer or dimer (Cheng *et al*. 2008). The eIF4B has also been found to have ability to discriminate mRNAs during initiation of translation (Mayberry *et al*. 2009).

eIF4F (eIF4A, eIF4E, eIF4G): The eIF4F mediates the binding of ribosomal small subunit to 5' end of capped mRNA. It comprises three proteins: eIF4A, eIF4E, and eIF4G subunits and each eIF4F subunit play a unique and vital role in translation initiation. Formation of the eIF4F complex is dynamic and tightly regulated, and its three subunits exhibit a differential expression in most cell types. Interaction among all the three subunits is critical for eIF4F function, and therefore the trimer formation is regulated under various biological conditions in order to modulate translation. Under conditions in which the rate of translation is low, eIF4E is prevented from binding to eIF4G by an eIF4E binding protein.

The eIF4A is monomer and required for unwinding of mRNA secondary structure both during formation of 48S PIC and scanning process, and the involvement of eIF4A is related to the secondary structure in 5'-UTR of mRNA (Svitkin *et al*. 2001). The eIF4A also exhibits ATPase activity in the absence of RNA, and it enhances RNA-dependent ATP hydrolysis in the presence of either eIF4B or eIF4F (Lax *et al*. 1986). Once 43S PIC is recruited, eIF4A promotes scanning to the start codon and is indispensible if 5'-UTR contains even minor secondary structure elements. The eIF4B, eIF4G, and eIF4H have been shown to stimulate the helicase activity of eIF4A. The most abundant eIF4F subunit, eIF4A, has been demonstrated to have RNA helicase activity. The eIF4A contributes to eIF4F function by unwinding various secondary structures in 5'-UTR of mRNA undergoing translation using energy derived from ATP hydrolysis.

The eIF4E has emerged as target for different types of regulation affecting translation. The eIF4E interacts with a cap structure and, together with eIF4G and eIF4A, forms an eIF4F complex. Due to its scarce availability, eIF4E confers rate limiting properties onto translation initiation. It exists in a dimeric form with one of the cap binding loops of monomer inserted into the cavity of the other. The eIF4E protein also contains an intramolecular disulfide bridge between two cysteines (Cys) that are conserved only in plants. The eIF4E recognizes and binds the m(7) guanosine nucleotide at the 5' end of eukaryotic messenger RNAs; this protein-RNA interaction is an essential step in initiation of protein synthesis. The eIF4E binds to the 5' cap of mRNA and to scaffold protein eIF4G, thus recruiting the rest of the cap-binding complex and 43S PIC to the 5' end.

The eIF4G is a scaffolding protein and contains two eIF4A binding domains. The eIF4G interacts with both the N terminal domain (NTD) and the C terminal domain (CTD) of eIF4A (Nielson *et al*. 2011). The eIF4G functions for organizing the assembly of initiation factors required for recruitment of a 40S ribosomal subunit to mRNA and for interacting with PABP. Many eukaryotes express two highly similar eIF4G isoforms. The eIF(iso)4G, one of the two isoforms in plants, is highly divergent and small in size (Cheng *et al*. 2010). The eIF(iso)4G domain organization differs from other eukaryotes in its N terminal region where an eIF4A binding domain overlaps with eIF4B and PABP binding domains. It was studied that there is functional difference between plant eIF4G and eIF(iso)4G when present as subunits of eIF4F and eIF(iso)4F, respectively. It was suggested that some functions of plant eIF(iso)4F and eIF4F have diverged: eIF(iso)4F promotes translation preferentially from unstructured mRNAs, whereas eIF4F promotes translation from mRNAs that contain a structured 5' leader and are uncapped or contain multiple cistrons. This ability enables eIF4F to promote translation from standard mRNAs under conditions in which cap dependent translation is inhibited (Gallie *et al*. 2001).

eIF4H: The eIF4H (M_r ~25 kD) has similar function in stimulation of helicase activity of eIF4A and eIF4F. The eIF4H is homologous to part of eIF4B, including a conserved RNA recognition motif (RRM) domain near the N-terminus of protein. Both eIF4B and eIF4H bind RNA, and regions outside RRMs are required for a maximum binding.

eIF5A and eIF5B: The eIF5A (also known as eIF5), a small single polypeptide protein having M_r of 16 to 18 kD, is ubiquitous and highly conserved among eukaryotes (Henderson and Hershey 2011). It functions in start site selection for an eIF2-GTP-$tRNA_i$ ternary complex within ribosomal-bound PIC and also stabilizes the binding of GDP to eIF2 (Jennings and Pavitt 2010). A new regulatory function of eIF5 has also been identified in which it mediates recycling eIF2 from its inactive eIF2-GDP state between successive rounds of translation initiation. The eIF5A motif analysis has indicated that it contains a stretch of basic amino acids resembling a nucleic acid-binding domain as well as a leucine-rich region typically capable for both protein-protein interaction and RNA binding (Liu *et al*. 2008). The eIF5A is post-translationally modified to hypusine-eIF5A in a two-step reaction sequence mediated by deoxy-hypusine synthase and deoxyhypusine hydroxylase (Wang *et al*. 2001).

The eIF5B is a ribosome dependent GTPase and mediates displacement of initiation factors from a 40S ribosomal subunit in 48S PIC and joining of 40S and 60S subunits. The eIF5B has variable N-terminal and conserved central GTP binding and C-terminal regions (Pestova *et al*. 2000). The N-terminus of eIF5B has been suggested to interact with the ribosome and to bind across the small subunit extending from the A-site to the E-site. The eIF5B interacts with the extreme C terminal region of eIF1A and stimulates subunits joining, and GTP hydrolysis triggers eIF5B release from the initiation complex (Acker *et al*. 2006). Upon subunits joining, the GTPase activation center (GAC) of the large subunit induces eIF5B-GTP hydrolysis leading to the release of

eIF5B-GDP together with eIF1A (Olsen *et al*. 2003). A highly thermostable eIF5B resisting temperature up to 95 °C has been reported in *Pisum sativum* (Suragani *et al*. 2011). The eIF5B prevents a thermal aggregation of heat labile proteins under heat stress or chemical denaturation and promotes their functional folding. These observations indicate that eIF5B, in addition to its role in protein translation, has chaperone like activity in *Pisum sativum*. So it could be involved in protein folding and protection under stress.

Roles of translation initiation system in plants

From a recent literature, it has been clearly emerging that in plants, apart from synthesis of proteins, translation initiation factors play various other important roles. Also, regulation of protein synthesis is an important determinant of cell proliferation and senescence. The eIFs provide focal points for translational control of gene expression in response to intrinsic and external cues. Thus, eIFs can be exploited for improving growth, development, and adaptation. The established roles of these eIFs are listed in Table 1 and described below.

Growth and development: Apart from protein synthesis, various eIFs, such as eIF5A, eIF4E, eIF4G, eIF3, *etc*., have been reported to influence growth, development, and reproduction of plants. For example, in *Arabidopsis*, a constitutive over-expression of eIF5A from *Arabidopsis*, *Populus deltoides*, and *Solanum lycopersicum* exhibits an enhanced vegetative and reproductive growth (Ma *et al*. 2010). A loss-of-function mutant of eIF5A gene resulted in more severe defects including reduced sizes and numbers of all adult organs, defective development of floral organs, and abnormal sporogenesis. It was proposed that different isoforms of eIF5A function distinctively in regulation of cell division and cell death. Also, eIF5A has been found to play a pivotal role in senescence (Thompson *et al.* 2004, Parkash *et al*. 2014). Thus, the two different types of functions raises the possibility that eIF5A isoforms are elements of a biological switch that is in one position in dividing cells and in another position in dying cells. The eIF5A also plays a role in xylogenesis as increase in both primary and secondary xylem tissue in the stems of plants over-expressing eIF5A is observed (Liu *et al*. 2008). It has also been shown that transgenic plants overexpressing

Table 1. Roles of translation initiation factors in plants.

eIFs	Role	Plant species	Reference
eIF1A	tolerance to NaCl stress	*Arabidopsis*	Rausell *et al*. 2003
eIF3f	pollen germination and embryogenesis	*Arabidopsis*	Xia *et al*. 2010
eIF3h and eIF3e	pollen growth and germination	*Arabidopsis*	Roy *et al*. 2011
eIF4A	growth and development	*Brachypodium*	Vain *et al*. 2011
eIF4A	salinity stress tolerance	tobacco, rice	Sahoo *et al*. 2012
eIF4E	resistance against several potyviruses	*Arabidopsis*, tomato, beans	German-Retana *et al.* 2008, Mazier *et al*. 2011, Piron *et al*. 2010; Naderpour *et al*. 2010
eIF4E	resistance to potato virus Y	potato	Duan *et al*. 2012
eIF4E	resistance to cucumber vein yellowing virus, melon necrotic spot virus, moroccan watermelon mosaic virus, zucchini yellow mosaic virus	melon	Rodriguez-Harnandaz *et al*. 2012
eIF4E and eIF(iso)4E	plant growth	tobacco	Combe *et al*. 2005
eIF4E and eIF(iso)4E	resistance to chilli veinal mottle virus	*Capsicum*	Hwang *et al*. 2009
eIF(iso)4E	root development	*Arabidopsis*	Martinez- Silva *et al*. 2012
eIF4G	resistance to rice tungro spherical virus	rice	Lee *et al*. 2010
eIF(iso)4G	resistance to rice yellow mottle virus	rice	Boisnard *et al*. 2007, Alber *et al*. 2006
eIF(iso)4F and eIF(iso)4G	promotes germination, growth, fertility and seed viability	*Arabidopsis*	Lellis *et al*. 2010
eIF(iso)4F and eIF(iso)4G	tolerance to dehydration, salinity and heat stress	*Arabidopsis*	Lellis *et al*. 2010
eIF5A	senescence	tomato, *Arabidopsis*	Wang *et al*. 2001, Thompson *et al*. 2004
	tolerance to osmotic and nutrient stress	*Arabidopsis*	Ma *et al*. 2010
	phytopathogen mediated programmed cell death	*Arabidopsis*	Hopkins *et al*. 2008
	enhances thermotolerance and oxidative and osmotic stress resistance	rose	Xu *et al*. 2011

eIF5A exhibit an enhanced fitness when exposed to osmotic and nutrient (N, P, and K) stresses (Ma *et al*. 2010). In *Picrorhiza kurrooa*, a plant of a high altitude area of the Himalayan region, expression of eIF5a increases during leaf senescence and in response to abscisic acid thus suggesting its association with leaf senescence (Parkash *et al*. 2014).

The eIF4 has been observed to be involved in a selective translation of mRNAs related to root development during normal growth conditions in *Arabidopsis* (Martínez-Silva *et al.* 2012). Functional roles of eIF4E were studied by antisense downregulation in tobacco by Combe *et al*. (2005). The antisense depletion of both eIF4E and eIFiso4E results in plants with a semi-dwarf phenotype and an overall reduction in polyribosome loading, demonstrating that both eIF4E and eIFiso4E support translation initiation in plants, which suggests their potential role in regulation of plant growth. Recently, Callot and Gallois (2014) have attempted to combine null mutations affecting the two main *Arabidopsis thaliana* 4E factors eIF4E1 and eIFiso4E but have discovered that this combination is lethal. Transmission through the male gametophyte is completely abolished in the eif4e1-eifiso4e double mutant. This shows that eIF4E1 and eIFiso4E are essential for male gametophyte development and act redundantly.

A T-DNA insertion mutant of eIF4A confers a dose-dependent dwarfing phenotype and a slow growth in *Brachypodium distachyon* (Vain *et al*. 2011) due to decrease in both a cell number and a cell size. The findings are consistent with roles of eIF4A in both cell division and cell growth. Linkage between the insertion site and the phenotype has been confirmed, and the content of eIF4A protein is strongly reduced in the mutant. Flowering time is not significantly affected in *Brachypodium* by down-regulation of eIF4A function, unlike in *Arabidopsis*, in which knockdown of several eIF4-related functions lead to a prolonged vegetative growth (Lellis *et al*. 2010). Knockout mutants of plant specific eIFiso4G1 and eIFiso4G2 have been studied in *Arabidopsis thaliana*. Single gene knockouts have a minimal effect on morphology, germination rate, growth rate, flowering time, or fertility. However, double mutant knockout plants show reduced germination rates, slow growth rates, moderate chlorosis, impaired fertility, and reduced long term seed viability (Lellis *et al*. 2010). Chen *et al*. (2014) studied the role of eIFiso4G in plant growth using null mutants for eIF4G isoforms in *Arabidopsis*. The eIFiso4G loss-of-function mutants exhibit smaller cells, leaves, plant size, and biomass accumulation, which correlates with their reduced photosynthetic activity.

The eIF3 plays an important role in embryogenesis in maize (Shen *et al.* 2013) and in a normal cell growth in *Arabidopsis* (Xia *et al*. 2010). Yahalom *et al*. (2008) have suggested that a precise regulation of eIF3e (a subunit of eIF3) content is necessary for a normal development in *Arabidopsis* as an excess of eIF3e inhibits translation. This regulation of eIF3e content is mediated by COP9 signalosome. In *Arabidopsis*, the over-expression of eIF3e results in defects similar to mutations in the COP9 signalosome. Further, eIF3e is degraded in a proteasome-dependent fashion. The eIF3f subunit of eIF3 has been observed to be highly expressed in pollen grains, developing embryos, and root tips, and it interacts with *Arabidopsis* eIF3e and eIF3h proteins. Further, the eIF3f is required for pollen germination and embryogenesis. Thus, eIF3f may play important roles in *Arabidopsis* cell growth and differentiation in combination with eIF3e. Mutations in several subunits of eukaryotic eIF3 cause male transmission defects in *Arabidopsis* (Roy *et al*. 2011). A mutation in eIF3h displays defects in auxin mediated organogenesis and gene expression and thus affects plant development (Zhou *et al*. 2010).

Recently, eIF2a has been found to regulate amino acid homoeostasis (Byrne 2012). Boex-Fontvieille *et al.* (2013) applied phosphoproteomic methods to *Arabidopsis thaliana* rosettes harvested under controlled photosynthetic gas-exchange conditions to characterize the phosphorylation pattern of eIFs. The analyses detected 11 new eIF phosphorylation sites, revealed significant CO_2-dependent and/or irradiance-dependent phosphorylation patterns, and showed concerted changes in 13 eIF phosphorylation sites. The data indicate the involvement of eIF3, eIF4A, eIF4B, eIF4G, and eIF5 phosphorylation in controlling translation initiation when photosynthesis varies. The response of protein bio-synthesis to photosynthesis thus appears to be the result of a complex regulation network involving both stimulating (*e.g*., eIF4B phosphorylation) and inhibiting (*e.g*., eIF4G phosphorylation) molecular events.

Abiotic stresses: Abiotic stresses are major limiting factors affecting plant growth, and desertification covers more and more of the world's terrestrial area (Vinocur and Altman 2005). Thus, unravelling additional stress-associated mechanisms are highly desirable to enable future molecular dissection of abiotic stress tolerant traits in plants.

The eIF1A is an important determinant in response to NaCl stress. Transgenic *Arabidopsis* plants over-expressing eIF1A exhibit an increased tolerance to NaCl (Rausell *et al*. 2003). The eIF2 alpha kinase GCN2 (general control non-derepressible 2) has been reported essential for growth under stress conditions (Lageix *et al*. 2008). The GCN2 kinase is activated during amino acid and purine starvations, UV, cold shock, and wounding which result in phosphorylation of eIF2α and exhibits reduction in global protein synthesis indicating its role in plant growth under stresses. The eIF4A is also an important determinant in tolerance of salinity (Sahoo *et al*. 2012) because the over-expression of gene encoding eIF4A in indica rice cv. IR64 enhances its tolerance against NaCl. It is proposed that eIF(iso)4E mediates a selective mRNA translation during nutrient stress (Martínez-Silva *et al*. 2012). The implication of knocking out plant specific eIFiso4G1 and eIFiso4G2 has also been studied in *Arabidopsis* (Lellis *et al*. 2010) and single gene

knockouts have a minimal effect on morphology, germination rate, growth rate, flowering time, or fertility. However, double mutant knockout plants exhibit altered responses to dehydration, salinity, and heat stresses.

The eIF5A has been shown to have a higher expression under high temperature, oxidative, and osmotic stresses in *Rosa chinensis*. The eIF5A over-expressing transgenic plants exhibit an increased resistance to these stresses, whereas suppressing expression plants show more susceptibility to these stresses. The results reveal a new physiological role for eIF5A in plants and contribute to elucidation of molecular mechanisms involved in the stress response pathway (Xu *et al*. 2011). The eIF5A has been found to impart abiotic stress tolerance and to be regulated by transcription factors WRKY and RAV, both of which can bind to the W-box motif in the promoter region of eIF5A. In addition, the stress tolerance might be mediated by increasing protein synthesis, enhancing ROS scavenging by improving SOD and peroxidase activities, and preventing chlorophyll loss and membrane damage. Also, the over-expression of eIF5A improves salt tolerance in transgenic plants (Wang *et al*. 2012). Thus, eIF5A might be an important target for improving adaptation to changing environmental conditions.

Biotic stresses: In addition to being essential for translation of eukaryotic mRNA, eIFs are also key components of plant-virus interactions. Surprisingly, of 14 natural recessive resistance genes against plant viruses that have been cloned from diverse plant species, 12 encode eIF4E or its isoform eIF(iso)4E. This indicates that eIF4E plays an important role against virus infection and is a potential target gene in development of genetic resistance to viruses for crop improvement (Wang and Krishnaswamy 2012). A novel eIF4E-1 variant, designated as Eva1, provides resistance against potato virus Y (Duan *et al*. 2012). Contreras-Paredes (2013) have observed that the absence of eIF(iso)4E affects the systemic spread of a Tobacco etch virus in *Arabidopsis thaliana*. A knockdown mutant of eif4E illustrates a broad spectrum of virus resistance in *Cucumis melo* (Rodríguez-Hernandez *et al*. 2012**)**. The mutants are resistant against the Cucumber vein yellowing virus, Melon necrotic spot virus, Moroccan watermelon mosaic virus, and Zucchini yellow mosaic virus. Similarly, knockdown mutants of both eIF4E1 and eIF4E2 genes confer a broad-spectrum resistance against potyviruses in tomato (Mazier *et al*. 2011) and suggest a role for eIF4E2 in tomato - potyvirus interactions. Thus, eIF4E has been proven to be an efficient target for identification of new resistance alleles able to confer a broad-spectrum virus resistance in plants. It has been reported that eIF4G is involved in imparting resistance against Tungro spherical virus in rice (Lee *et al*. 2010). Naderpour *et al*. (2010) has reported that potyviral resistance is associated with the homozygotic presence of a mutated eIF4E allele in *Phaseolus vulgaris*. In *Arabidopsis*, there are at least three isoforms of eIF5A, one possibly regulating programmed cell death caused by infection with a virulent bacterial strain (Hopkins *et al*. 2008). The eIF5A is involved in development of disease symptoms induced by a common necrotrophic bacterial pathogen. This is further supported by the finding that transgenic plants with constitutive eIF5A2 over-expression display a phenotype consistent with a precocious cell death. However, recent evidence raises a possibility that eIF5A2 isoform might directly promote apoptosis. Thus, eIF5A2 is a key element of the signal transduction pathway resulting in plant programmed cell death (Hopkins *et al*. 2008).

The 3'-UTR of many plant viral RNAs contain cap-independent translation elements (CITEs) that drive translation initiation at the 5′ end of mRNA. Barley yellow dwarf virus like CITE (BTE) stimulates translation by the binding of the eIF4G subunit of eIF4F with a high affinity (Kraft *et al*. 2013). It has been reported that eIF4E is responsible for providing resistance against Maize rough dwarf disease which cause a significant grain yield loss and is considered as a candidate gene for resistance against it (Shi *et al*. 2013). Recently, eIF4E has been found to provide resistance against Chrysanthemum virus B in *Chrysanthemum* (Song *et al*. 2013). Also, transgenic plants harbouring a silencing construct of eIF4E and eIF(iso)4E exhibit resistance against Plum pox virus (Wang *et al*. 2013), the virus causing the most economically-devastating viral disease in *Prunus* species.

Conclusion and future perspective

In plants, management of biotic and abiotic stresses becomes increasingly important. A strong need, therefore, has been felt for a holistic approach for better performance and adaptation of plants. It further requires identifying and utilizing more and more targets for scientific and technological intervention to achieve these aims. Translation initiation is an important process which can be manipulated for improving plant growth, development, and adaptation. However, for achieving this, systems for deeper and better insights into the exact mechanisms and complex interaction networks will be essential. Among translation initiation factors, the important component of translation process, eIF5, has been found to influence vegetative and reproductive growth and leaf senescence, thus is an important candidate for genetic engineering of important crop plants. The eIF4E and eIF4G are involved in imparting resistance against a range of plant viruses and thus can be utilized for inducing virus resistance. Though there are not many reports regarding roles of eIFs in imparting tolerance to abiotic stresses, eIF1A, eIF2, and eIF4A have been shown to improve stress tolerance and thus can be

utilized through transgenic approaches. The *NCBI GenBank* (http://www.ncbi.nlm.nih.gov/) has more than two hundred entries of full length cDNAs of genes encoding eIFs in plants. Majority of these belong to plants whose genomes have been sequenced and annotated, for example *Arabidopsis*. These genes can be functionally validated and implications of differences in their sequences may be studied. Further, non-canonical translation initiation mechanisms are other areas of a vital importance in terms of utilizing the protein translation initiation mechanisms for improving plants. In some cases the over-expression of desired eIF(s) may be required, whereas in other cases, inhibition or minimizing expression of eIF(s) might be desirable. For example, the over-expression of eIF3e has been shown to inhibit translation in *Arabidopsis* (Yahalom *et al* 2008). Similarly, an attempt of gene pyramiding for pathogen resistance using a combination of eIF4E1 and eIFiso4E has resulted in male gametophyte lethality (Callot and Gallois 2014). Also, in some cases, a combination of different eIFs may be exploited for desired plant performance. Increasing the content of desired eIF(s) in plants using genetic engineering will need availability of convenient vectors harbouring a strong promoter. Although many of these vectors are already available and being used for developing transgenics, further improvements of the vectors are needed for targeted and efficient over-expression of transgenes. Similarly, inhibition of expression or minimizing the expression of eIF(s) can be achieved through gene knockout, antisense RNA, and RNA interference based approaches. These approaches are presently being used in various plants including crop plants. Efforts are on to further improve these technologies for their better efficiencies.

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