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Translation initiation factor 5A in Picrorhiza is up-regulated during leaf senescence and in response to abscisic acid

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ABSTRACT

Translation initiation, the first step of protein synthesis process is the principal regulatory step controlling translation and involves a pool of translation initiation factors. In plants, from recent studies it is becoming evident that these translation initiation factors impact various aspects of plant growth and development in addition to their role in protein synthesis. Eukaryotic translation initiation factor eIF5A is one such factor which functions in start site selection for the eIF2-GTP-tRNA$_i$ ternary complex within the ribosomal-bound preinitiation complex and also stabilizes the binding of GDP to eIF2. In the present study we have cloned and analysed a gene (*eIF5a*) encoding eIF5A from Picrorhiza (*Picrorhiza kurrooa* Royle ex Benth.) a medicinal plant of the western Himalayan region. The full length *eIF5a* cDNA consisted of 838 bp with an open reading frame of 480 bp, 88 bp 5′ untranslated region and 270 bp 3′ untranslated region. The deduced eIF5A protein contained 159 amino acids with a molecular weight of 17.359 kDa and an isoelectric point of 5.59. Secondary structure analysis revealed eIF5A having 24.53% α-helices, 8.81% β-turns, 23.27% extended strands and 43.40% random coils. *pk-eIF5a* transcript was found to be expressing during the active growth phase as well as during leaf senescence stage, however, highest expression was observed during leaf senescence stage. Further, its expression was up-regulated in response to exogenous application of abscisic acid. Both high intensity as well as low intensity light decreased the expression of *pk-eIF5a*. The findings suggest *eIF5a* to be an important candidate to develop genetic engineering based strategies for delaying leaf senescence.

1. Introduction

The process of protein synthesis known as translation is universal and essential for all organisms whether eukaryotes, archea or bacteria. Translation 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. 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). Apart from the synthesis of proteins eIFs play various other important roles in plants. eIFs have been found to be influencing plant growth and development. Also, eIFs have been found to play role in imparting biotic and abiotic stress tolerance in plants (Duan et al., 2012; Ma et al., 2010; Wang and Krishnaswamy, 2012; Wang et al., 2012; Xu et al., 2011). Thus, these recent studies provide a strong platform for engineering translation initiation machinery for improving growth, development and adaptation of plants. Our present work pertains to translation initiation factor eIF5A from Picrorhiza (*Picrorhiza kurrooa* Royle ex Benth.). Picrorhiza is a small perennial herb (Family Plantaginaceae) which grows primarily in the north-western Himalayan region, at an altitude of 3000–5000 m above mean sea level. Its underground parts, rhizomes and roots are widely used in traditional system of medicine due to its antioxidative, hepatoprotective, antiproliferative, immunomodulatory, antibacterial and antiviral activities (Banerjee et al., 2008). The plant is self-regenerating but unregulated over-harvesting has caused it to be threatened to near extinction and thus Picrorhiza has been listed in the Red Data Book as an endangered plant species (Kala, 2000). Recently, we reported the presence of picrosides, the main medicinally active compounds in the leaves of Picrorhiza (Dutt et al., 2004). It was observed that, in addition to rhizome and roots, leaves can also be a good source of picrosides. However, the contents of these picrosides

Abbreviations: eIF5A, eukaryotic translation initiation factor 5A; *pk-eIF5a*, *Picrorhiza kurrooa* translation initiation factor 5A; RACE, rapid amplification of cDNA ends; CDD, conserved domain database; RT-PCR, reverse transcription-polymerase chain reaction; SOPMA, self-optimized prediction method with alignment; ABA, abscisic acid.

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Table 1
Oligonucleotide sequences and PCR conditions used in cloning and expression analysis of *pk-eIF5a* gene.

Name	Sequence (5′–3′)	PCR condition
Degenerate primers for amplification of the target gene		
pk-eIF5a-dF1	AAGGGCGATGCCGGAGCTTC	Initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C, 30 s; 55–60 °C, 40 s; 72 °C, 50 s. Final extension at 72 °C for 7 min
pk-eIF5a-dR2	ATCTGCTCCTCTCCCATGGA(C)AGAC	
Primers for RACE PCR		
pk-eIF5a 3′ RACE F1	GCTGACTGATAATGGCAACACCAAGG	Primary PCR
pk-eIF5a 3′ N RACE F2	AAGCTTCCAACTGATGACAGTCTG	5 cycles of 94 °C, 30 s; 72 °C, 3 min, followed by 5 cycles of 94 °C, 30 s; 70 °C 30 s; and 30 cycles of 94 °C, 30 s; 68 °C 30 s 72 °C, 3 min
pk-eIF5a 5′ RACE R1	AGGCAGACATGACACTCACCACAAGA	Secondary PCR
pk-eIF5a 5′ N RACE R2	TCATCCTTGGTGTTGCCATTATCAGT	30 cycles of 94 °C, 30 s; 68 °C, 30 s; 72 °C, 3 min. Final extension at 72 °C for 7 min
Primers for full length cloning of pk-eIF5a		
pk-eIF5a-Fl F1	ATGTCGGATGAGGAGCACCACTTC	Initial denaturation at 94 °C for 3 min., followed by 33 cycles of 94 °C, 30 s; 54 °C, 40 s; 72 °C, 40 s. Final extension at 72 °C for 7 min
pk-eIF5a-Fl R2	TTACTTGGGACCAATATCCTTGAGGG	
Primers for expression studies		
pk-eIF5a-expF1	GCACGGTCATGCTAAATGTCAC	Initial denaturation at 94 °C for 3 min, followed by 31 cycles of 94 °C, 30 s; 54 °C, 45 s; 72 °C, 20 s. Final extension at 72 °C for 7 min
pk-eIF5a-expR1	CAGACTGTCATCAGTTGGAAGC	

Primers name with "*F*" and "*R*" represents forward primers and reverse primers, respectively.

decrease sharply during senescence phase (Singh et al., 2011). Thus, understanding on leaf senescence phenomena in Picrorhiza is of vital importance to devise and utilize the molecular strategies for delaying leaf senescence and increasing biomass production, and thereby improving the picroside contents. In the present study, we cloned the gene encoding eIF5A from Picrorhiza (hereinafter referred to as *pk-eIF5a*) and analysed its expression in relation to leaf senescence, abscisic acid and light.

2. Materials and methods

2.1. Plant material

Picrorhiza (*Picrorhiza kurrooa*) plants used in the present study were collected from its natural habitat at Rohtang Pass (4000 m altitude, 32°23′ N, 77°15′ E, India) during December when the plants were in

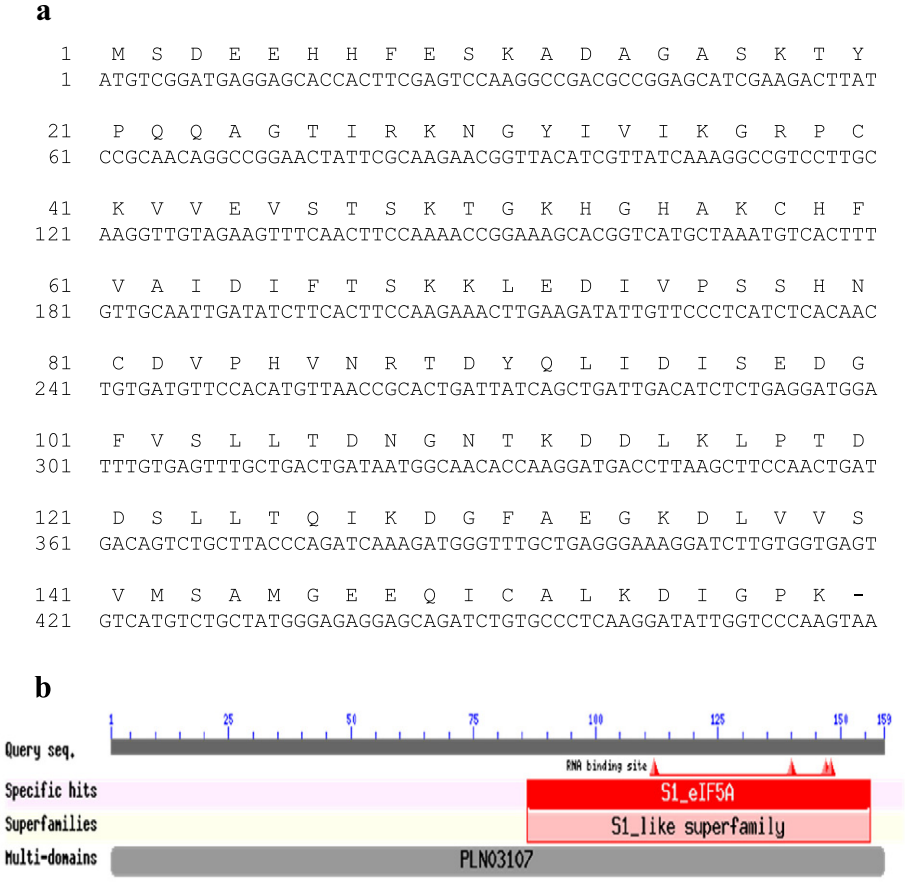

Fig. 1. Nucleotide and deduced amino acid sequences of *pk-eIF5a* cDNA (GenBank accession no. KF019100) (a). Conserved domain of deduced protein pk-eIF5A of Picrorhiza. Deduced amino acid sequences were analysed for the location of conserved domain using conserved domain database available at NCBI website (http://www.ncbi.nih.gov/structure/ccdd/wrpsb.cgi).