A cDNA for Nuclear-encoded Chloroplast Translational Initiation Factor 2 from a Higher Plant Is Able to Complement an infB Escherichia coli Null Mutant*

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The synthesis of prokaryotic proteins requires complex interactions between the different translation factors, ribosomal subunits, nucleotides, and tRNAs. The initial step consists of the formation of a 30 S subunit ternary complex with the mRNA. This complex includes three initiation factors, IF1, IF2, and IF3, tRNA^Met^, and GTP, which is required for its formation. Furthermore, the 30 S subunit is guided to the initiation region by RNA-RNA base pairing between 16 S rRNA and the Shine–Dalgarno sequence, where this complex is converted into a stable initiation complex (1). During initiation, IF3 binds to the small subunit of the ribosome (30 S), preventing the joining of 50 S subunits, whereas IF1 and IF2 bind to the 30 S-IF3 complex. IF2 promotes the binding of the initiator tRNA to this complex in a GTP-dependent manner (1). This factor belongs to the family of GTPases that are molecular switches capable of alternating between an active (IF2-GTP) and an inactive (IF2-GDP) conformation (2–4). Under steady state conditions, initiation is the predominant rate-limiting step for translation of most mRNAs; thus, protein synthesis is often regulated at this early stage.

The process of translation in chloroplasts resembles prokaryotic protein synthesis more closely than eukaryotic cytoplasmic translation (1, 5). Chloroplast initiation factors are postulated to be functionally analogous to their Escherichia coli counterparts (6, 7). Organellar IF2 has recently been characterized from human, bovine, and yeast mitochondria and from Euglena gracilis chloroplast (5, 8–10). Purified mitochondria IF2 (IF2mt) is a monomeric protein of 85 kDa (8), whereas E. gracilis chloroplast IF2 (IF2cp) is present in multiple high molecular mass forms ranging from 200 to >700 kDa. Subunits ranging in size from 97 to >200 kDa have been detected in the purified factor (5, 11).

Plastids and mitochondria are semiautonomous organelles that possess their own genome but rely on the nucleus for most of their structural proteins and the regulatory factors that control the expression of their genes. It has been proposed that the chloroplasts from green algae and land plants arose from an ancestor related to actual free-living cyanobacteria by endosymbiosis with a nonphotosynthetic host (12–14). Many common features between chloroplasts and prokaryotes support this theory (15). For example, many components of the chloroplast transcriptional and translational machinery are homologous to the eubacterial counterpart and have been identified by their similarity with the corresponding cyanobacteria or E. coli genes (7, 16–18).

The chloroplast genomes from many land plants have been completely sequenced (15). Sequence analysis from these genomes indicates that only IF1 is encoded in the chloroplast, suggesting that IF2 and IF3 are nuclear-encoded in land plants (16). Although some chloroplast translational initiation factors have been identified and purified from algae, none of these factors have been characterized from plants. In this work, we report the molecular characterization of a nuclear-encoded chloroplastic IF2 gene from common bean (PvIF2cp). The PvIF2cp gene encodes a putative
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108.7-kDa polypeptide that presents a chloroplast translocation signal peptide. Our data indicate that the PvIF2cp transcript accumulation is modulated by light. An increase in mRNA levels was detected when plants were transferred from dark to light growth conditions. Also, high accumulation of PvIF2cp transcript was found in photosynthetic tissues contrasting with the low mRNA levels detected in etiolated plants as well as in nonphotosynthetic organs. We also show that the PvIF2cp gene encodes a functional factor, since the PvIF2cp conserved region containing the G-domain and the C-terminal end is able to complement an E. coli inB null mutation in vivo. Finally, phylogenetic analysis using the PvIF2cp conserved region, which seems to be a useful marker for organism phylogeny reconstruction, suggests that PvIF2cp gene originated via endosymbiotic gene transfer to the nucleus and favors the monophyletic origin of primary plastids. To our knowledge, this is the first nuclear-encoded chloroplastic IF2 gene characterized in higher plants.

EXPERIMENTAL PROCEDURES

Plant Material and Growth Conditions—Phaseolus vulgaris L. cv. ng or cv. AC455550 was used for PCR amplification of lipid transfer protein gene. National de Investigaciones Forestales y Agropecuarias, Texcoco, Mexico). Seeds germinated after 3 days were transferred to pots and grown under greenhouse conditions with natural light as indicated (19, 20). Mature plants were harvested 25 days after sowing, and leaves, stems, and roots were collected. For the light induction experiments, 3-day-germinated seedlings were transplanted to vermiculite and grown in the dark at 27 °C for 2 days; seedlings were then transferred to a greenhouse for 24 h. Control seedlings were kept in the dark. To obtain etiolated plants, 25-day-old plants grown under greenhouse conditions were transferred to dark conditions for 72 h. Abscisic acid and water-deficit was performed as described (21). From this screening, proteins are as follows:

anti-taxus were used for detection of lipid transfer protein mRNA (21). Hybridization conditions and membrane washing were carried out at high stringency (26). To detect the PvIF2cp transcript, a SmaI-PstI fragment from pJAS1 was used as a probe (Fig. 1). As controls, additional transcripts were detected. Rubisco mRNA was identified using as a probe a Ph. vulgaris rbcS cDNA (27). The Ph. vulgaris cDNA insert in PLACT-6 was used to probe actin transcripts (19). rps24, a Ph. vulgaris cDNA was obtained from a cdna library (21). Antibody Production and Western Blot Analysis—Antibodies were raised against the PvIF2cp fused to glutathione S-transferase. To obtain the fusion protein, the Smal-BglII fragment, encoding the PvIF2cp-conserved region, was inserted into the pGEX2T expression vector (28). After IPTG induction, the glutathione S-transferase-PvIF2cp protein was purified using glutathione-agarose beads (29). Antibodies were raised in rabbits using purified glutathione S-transferase-PvIF2cp fusion protein. SDS-boiling lysates were obtained from C118 and SL059R cultures grown at an Ao_0 of 1.0. To induce the PvIF2cp overexpression in C112 strain, IPTG (1 mm) was added to cultures grown to an Ao_0 of 0.5, and lysates were obtained when cultures reach an Ao_0 of 1.0. Strains were grown at 57 °C, except in the case of SL059R, which was grown at the permissive temperature (30 °C). Total protein extracts were separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membrane (30). A 1:1000 dilution of the anti-PvIF2cp antibody and a 1:5000 dilution of goat anti-rabbit IgG conjugated to alkaline phosphatase were used (Roche Molecular Biochemicals).

Construction of Green Fluorescent Protein (GFP)-PvIF2cp Fusion—To distinguish encoding the first 92 amino acids of PvIF2cp, which corresponds to the putative transit peptide, was amplified by polymerase chain reaction using the following primers: 5-GAGGGATCCGTTTAT-3 and 5-TATCCATGTACTTACATCTTATACACC-3'. The amplified fragment was digested with NcoI and BamHI and inserted in-frame into the Neo-BamHI site of the GFP expression vector (HB7-SGF-TYG-nos plasmid) (31, 32). The regions surrounding the insertion sites in the resulting plasmid, pJAs1, were verified by sequencing.

Isolation and Transformation of Tobacco Mesophyll Protoplasts—Protoplasts were isolated from 5–6-week-old tobacco leaves (ecotype W38) grown in a controlled growth chamber with a 16:8-h light-dark photoperiod at 24 °C. The protoplast preparation and the transformation procedure were carried out according to Abel and Theologis (33). To allow for expression, protoplasts were incubated in the dark for 24 h.

Fluorescence Microscopy—GFP was visualized using a Zeiss Axioskop universal microscope equipped with epi-fluorescence condenser III RS and a fluorescein isothiocyanate filter set comprising exciter filter (BP 450-490), chromatic beam splitter (FT 510), and barrier filter (LP 520). The light source was provided by a HBO 50 W high pressure mercury bulb. The microscope is equipped with an interference filter to block the chlorophyll autofluorescence. Photographs were taken using Kodak Gold400 color film.

In Vivo Complementation of an E. coli inB Null Mutation—An N terminus deletion of the PvIF2cp gene was constructed by polymerase chain reaction using the PvIF2cp cdna as template and specific primers (F56796). The reaction condition was as follows: 10 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min. This amplification did not generate any change in the amino acid sequence and left the methionine at position 411 as the initial amino acid. The NcoI-BglII

The abbreviations used are: IPTG, isopropyl-1-thio-β-D-galactopyranoside; GFP, green fluorescent protein; kbp, kilobase pair(s); Rubisco, ribulose-bisphosphate carboxylase/oxygenase.

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2.0-kilobase fragment containing a PvIF2cp cDNA-truncated version was inserted into the E. coli expression vector pTrc99A (Amersham Pharmacia Biotech) to produce the pJAS3 plasmid (see Fig. 1). In vivo complementation experiments were carried out as described (34). This protocol is based on the presence of a thermosensitive λ lysogen containing the infB gene in the SL598R strain, which lacks a functional chromosomal IF2. Under permissive temperatures, the wild type infB gene, present in the λ lysogen, will provide the IF2, and cells will survive. Under nonpermissive temperatures (42 °C), bacterial IF2 is not produced, and cells die unless a homologous or heterologous IF2 is able to complement the infB function (35). Strain SL598R was transformed with plasmids pJAS3 or pB18-1. Plasmid pB18-1, a pBR322 derivative containing the E. coli nusA-infB genes, was used as positive control (35). Complementation experiments were carried out as follows. Transformed cells were grown overnight in Luria broth with ampicillin (100 μg/ml) and chloramphenicol (10 μg/ml), diluted in fresh medium, and grown at 30 °C to an A600 of 1.0. Cultures were diluted to A600 0.3, and 0.5-ml aliquots were transferred to 5 ml of 42 °C prewarmed Luria broth, shaken vigorously for 5 min, and immediately chilled on ice. Aliquots of 1.5 ml were concentrated by centrifugation at 4 °C, plated on Luria broth plates containing ampicillin and chloramphenicol, and incubated at 42 °C. In the case of strain SL598R containing plasmid pJAS3, PvIF2cp expression was induced with 1 mM IPTG for 1 h. Survivors were purified several times at 42 °C, and the loss of λ lysogen phage was verified by their sensitivity to λ cI-Complementation was recorded as frequency of cured cells.

RESULTS

Molecular Cloning of a Gene Encoding a Eubacterial-like Translational Initiation Factor-2 from Ph. vulgaris—By screening a bean water-stressed leaf cDNA library, several cDNA clones down-regulated by water deficit were isolated (27). Nucleotide sequence analysis showed that the deduced amino acid sequence of one of these clones, pJAS1 (2.4 kbp), has significant similarity to prokaryotic and organellar translation initiation factors (IF2). Since these factors have not been described in higher plants, we decided to further characterize this gene. To isolate a full-length IF2 cDNA clone, 1 × 10⁶ plaque-forming units from a stressed leaf λ library were probed with the 5'-end of the insert in pJAS1, obtained as a 0.75-kbp Smal-EcoRI fragment (Fig. 1A). A positive clone containing a 2.5-kbp insert (pJAS2) was isolated and sequenced. Even though the insert in pJAS2 contains most of the PvIF2 cDNA, the corresponding initiation codon was not present. Thus, a bean genomic library was screened using as probe the insert in pJAS2. From this screening, we isolated an IF2 λ clone containing a 10-kbp NotI genomic fragment (Fig. 1A). Restriction mapping and sequence analysis of both genomic and cDNA clones indicated that they correspond to the same IF2 gene. Fig. 1A shows the physical map of the PvIF2 genomic fragment, in which two introns were detected. Genomic hybridizations suggest that the PvIF2 gene is present as a single copy in the Ph. vulgaris genome (data not shown). The putative PvIF2 transcript contains a 3.027-kilobase open reading frame and a 336-bp 3'-untranslated region, which includes the poly(A) tail. The deduced protein sequence corresponds to a polypeptide of 1069 amino acid residues with a predicted molecular mass of 108.7 kDa (Fig. 1B).

The PvIF2 Gene Encodes a Putative Chloroplast Translation Initiation Factor-2—Analysis of the deduced amino acid sequence of the PvIF2 gene using the BLASTP program indicated that this protein is similar to the eubacterial and organellar translational initiation factor 2. A low similarity was detected to the archaeal-type IF2 (Fig. 2). A region with extensive similarity among different IF2s compared was found between amino acids 488 and 604 of the PvIF2 polypeptide (Figs. 1B and 2). This domain corresponds to a putative GTP-binding site shown to be functional in several translation factors (2–4). The PvIF2 G-domain showed all characteristic motifs identified in this region (2) (Figs. 1B and 2). Whereas at the C-terminal region the similarity to different IF2s is also appreciable (31–99%), the N terminus of PvIF2 exhibits nonsignificant similarity to other known IF2s (28–35%) (Fig. 2).

Analysis of the PvIF2 N terminus using the program PSORT
revealed that the first 90 amino acids from this gene present the features of chloroplast transit peptides (Fig. 1B). This region also contains a putative cleavage site at position 48–49, predicted by the program ChloroP (37). Although no consensus amino acid sequences for chloroplast transit peptides have been defined (38), the PvIF2 putative 48-amino acid chloroplast-targeting peptide shows an overall amino acid composition similar to that present in bona fide chloroplast transit peptides (39). This analysis suggested that this PvIF2 corresponds to a nuclear-encoded chloroplast translation initiation factor 2 (PvIF2cp).

To determine the functionality of the PvIF2 N terminus as a chloroplast transit peptide, a recombinant plasmid (pJAS11) encoding a chimeric protein in which the first 90 amino acids from PvIF2 are fused to the GFP was introduced into tobacco protoplasts via electroporation (see “Experimental Procedures”). The fluorescence analysis showed that the GFP fusion containing the putative PvIF2 chloroplast transit peptide (pJAS11) was localized into tobacco chloroplast (Fig. 3, a–d). A similar localization pattern was observed for the GFP fusion containing the transit peptide of the nuclear Rubisco gene, RBCS1A, from A. thaliana (Fig. 3, e and f) (32). In contrast, GFP without the targeting sequence showed a diffuse accumulation pattern in tobacco protoplasts, with no particular localization (Fig. 3, g and h). These results lead us to conclude that indeed this sequence corresponds to a true transit peptide and targets PvIF2 to chloroplasts.

The Accumulation of PvIF2cp Transcript Is Modulated by Light and by Stress Conditions—To determine the relative abundance of PvIF2cp transcript in different organs from bean plants, Northern blots were carried out using total RNA from roots, stems, and leaves from mature photosynthetic plants. As shown in Fig. 4, a transcript of the expected size (3.5 kbp) was detected in leaf tissues but not in root or stem tissues. Since these results suggested that the PvIF2cp transcript abundance might be modulated by light, the PvIF2cp transcript accumulation levels were examined in leaves from etiolated and greening seedlings. The results in Fig. 4B showed that PvIF2cp...
transcript is highly accumulated in leaf tissues from greening seedlings, but it was undetectable in etiolated seedlings as well as in mature 3-day dark-grown plants (Fig. 4B, lanes 4 and 2, respectively). Additionally, the PvIF2cp light response was confirmed in experiments where dark-grown seedlings were transferred to light conditions. An increase in the accumulation levels of the PvIF2cp transcript was detected after 24 h in the presence of light (Fig. 4B, lane 3). In contrast, when plants were transferred from light to dark growth conditions, the PvIF2cp transcript accumulation levels declined after 72 h (Fig. 4B, lane 2). The Rubisco transcript, known to be up-regulated by light (40), presents an expression pattern similar to that observed for the PvIF2cp mRNA in response to the different light treatments (Fig. 4, A and B).

Since the original PvIF2cp cDNA was isolated from a group of water-deficit down-regulated clones, a transcript accumulation pattern analysis was carried out using total RNA obtained from leaves of bean plants subjected to water deficit. Northern blots in Fig. 4C show that PvIF2cp transcript levels notably decreased under these stress conditions. A similar behavior was observed when plants were treated with the phytohormone abscisic acid (Fig. 4C), one of the mediators of the plant response to water deficit (41).

The PvIF2cp Complements an E. coli infB Null Mutation—Given the high similarity between PvIF2cp and the bacterial IF2, we asked whether the PvIF2cp gene was able to complement a null mutation in the IF2 gene (infB) of E. coli. To address this question, the PvIF2cp was provided in trans to the infB deleted E. coli SL598R mutant strain. Since the lack of a functional IF2 causes lethality, the SL598R strain is maintained by providing a functional IF2 in trans, which is produced by a wild type infB gene, contained in a thermosensitive λ lysogen. Hence, when λinfB is heat-cured at 42 °C, the SL598R strain will survive only if the PvIF2cp present in a plasmid is able to complement the infB function. Since the complete PvIF2 gene showed a toxic effect in E. coli (data not shown), to carry out this experiment, a truncated version of the PvIF2cp gene lacking the nonconserved N-terminal region was sub-cloned into the pTrc99A vector (see “Experimental Procedures”). The resulting plasmid, pJAS3, as well as plasmid pB18-1 was able to complement the infB null mutation. The frequencies of cured cells in both cases were similar, 1 × 10−4. No survivors were detected when the empty pTrc99A vector was used. As shown in Fig. 5A, the infB heat-cured strains carrying either pB18-1 (C118) or pJAS3 (C112) were able to grow at two different nonpermissive temperatures (37 and 42 °C). Both presented the same growth kinetics at 37 °C and, for the infB heat-cured strains carrying pJAS3 (C112), a slower growth rate was observed at 42 °C (Fig. 5A). No growth was detected for the parental SL598R strain (Fig. 5A). Expression of the PvIF2cp protein was verified by Western blot analysis using a polyclonal anti-PvIF2cp antibody (see “Experimental Procedures” for details). The results in Fig. 5B show that PvIF2cp protein accumulates in the heat-cured SL598R strain carrying plasmid pJAS3 (C112) upon IPTG induction. PvIF2cp synthesis was detected even without IPTG treatment, in agreement with the fact that complementation can be attained under this condition (data not shown). These data indicate that the PvIF2cp conserved region is able to carry out the bacterial IF2 essential functions.

Phylogenetic Relationships between Ph. vulgaris IF2cp, Organellar, and Bacterial IF2—From the comparison of available IF2 sequences and from the results obtained in this work it can be concluded that the region containing the GTP binding domain and the C terminus are the most conserved segments in this protein. To address whether this sequence can be used as a species phylogenetic marker and to determine its relationship to other IF2 sequences, we performed phylogenetic analyses using the amino acid sequences of this conserved region. We used all available eubacterial and organellar IF2 sequences (48 sequences), 6 mitochondrial, 5 chloroplastic (3 from algae and 2 from higher plants), and 36 from eubacteria. The eubacterial sequences include 2 from cyanobacteria, 11 from Gram-positive, 15 from proteobacteria, 2 from chlamydia, 2 from spirochetes, and 2 from ambiguous eubacterial species (Aquifex and Thermotoga) (42) (Fig. 6). This analysis did not include the archaean-type IF2 sequences since the low sequence similarity between them and the eubacterial ones hinder the possibility of resolving the phylogenetic relationship of the latter. However, all the archaean-type IF2s fall in a distinct and sister clade to the eubacterial ones, which are resolved in more detailed in this paper.

The strict consensus maximum parsimony tree suggests that
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PvIF2cp is most closely related to a putative chloroplastic IF2 sequence from A. thaliana. This relationship is supported by a high Bootstrap value. It also groups in a clade with the conserved amino acid sequences of selected organellar and eubacterial IF2. The heuristic maximum parsimony procedure of PAUP4b* was used for this analysis (Ref. 24; see “Experimental Procedures” for details). The tree was rooted with the mitochondrial sequences used as out-groups, and Bootstrap support was based on 100 pseudo-replicates and shown as percentage values on each node. Nodes without values had Bootstrap support lower than 50%. Accession numbers of the analyzed IF2 proteins are indicated under “Experimental Procedures.” A. mellifera, Apis mellifera; A. aeolicus, Aquifex aeolicus; T. pallidum, Treponema pallidum.

FIG. 6. Phylogenetic analysis of organellar and eubacterial IF2. A maximum parsimony strict consensus tree of eight shortest trees (6569 steps; consistency index = 0.461; retention index = 0.478; rescaled retention index = 0.221; homoplasy index = 0.539) was constructed with the conserved amino acid sequences of selected organellar and eubacterial IF2. The heuristic maximum parsimony procedure of PAUP4b* was used for this analysis (Ref. 24; see “Experimental Procedures” for details). The tree was rooted with the mitochondrial sequences used as out-groups, and Bootstrap support was based on 100 pseudo-replicates and shown as percentage values on each node. Nodes without values had Bootstrap support lower than 50%. Accession numbers of the analyzed IF2 proteins are indicated under “Experimental Procedures.” A. mellifera, Apis mellifera; A. aeolicus, Aquifex aeolicus; T. pallidum, Treponema pallidum.

According to the endosymbiosis theory, the chloroplast lost most of its self-encoded functions to the nuclear genome during its evolution. One of the consequences of the transfer of a large fraction of the chloroplast genome to the nucleus is the requirement for coordination of nuclear and chloroplast gene expression. To reach such coordination, one strategy has been the establishment of a protein-synthesizing system, shared with the nuclear genome that is responsible for the translation of mRNAs encoded in chloroplast DNA. Among translation factors, only IF1 is encoded in the chloroplast genome (7, 43), whereas other factors are thought to be encoded in the nuclear genome in most land plants (6, 44). However, only in the case of the green algae E. gracilis, it has been demonstrated that the two chloroplast translational initiation factors, IF2 and IF3, are encoded in the nuclear genome (45, 46). The work presented here represents the first identification of a nuclear-encoded chloroplast translational factor IF2 from higher plants. As expected for a nuclear gene, the PvIF2cp genomic clone showed the presence of two introns, and the isolated cDNA clone contains a typical poly(A) tail at its 3′ end. In agreement with the characteristics of chloroplast proteins encoded by nuclear genes, here we show that the PvIF2cp gene contains a 90-amino acid peptide at the PvIF2cp N terminus that carries the information required to target the precursor polypeptide into higher plant chloroplasts.

The alignment of the PvIF2cp protein sequence with other IF2 sequences revealed a conserved GTP binding domain and a conserved region of the PvIF2cp is able to complement the IF2 of S. cerevisiae (35, 47). The alignment by light assures a good coordination of the translation of chloroplast mRNAs:chloroplast proteins will be synthesized under guest on July 26, 2018http://www.jbc.org/Downloaded from

DISCUSSION

The coordination of the nucleus and chloroplast gene expression may require a communication capacity that would mirror the demand for alteration in gene expression in one compartment to the other. In particular, in the case of post-transcriptional steps of chloroplast gene expression such as initiation of translation, nuclear-encoded factors may be good candidates for participating in the nuclear control over the chloroplast genome (7, 48). Modulation by light is a process for which such coordination is essential. The results in this work suggest that PvIF2cp gene expression is regulated by light. Since Northern blot experiments allow us to determine only the accumulation of transcripts, at this point we cannot discard this factor as also regulated at the post-transcriptional level. Modulation by light has also been observed in the case of the E. gracilis IF2cp, where light induction of its activity involves the synthesis of new polypeptide (11). For the E. gracilis IF3cp, it has been shown that the increased activity after light induction is post-transcriptionally regulated (46). The fact that chloroplast translational initiation factors encoded in the nucleus are regulated by light assures a good coordination of the translation of chloroplast mRNAs:chloroplast proteins will be synthesized only when they are needed. An opposite effect can be observed when plants are exposed to a stress condition, where we have observed that PvIF2cp transcript levels decrease in response to

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The alignment of the PvIF2cp protein sequence with other IF2 sequences revealed a conserved GTP binding domain and a conserved region of the PvIF2cp is able to complement the IF2 of S. cerevisiae (35, 47). Consistent with this observation, we show that the conserved region of the PvIF2cp is able to complement the absence of the IF2 factor in E. coli. These data support the idea that this region of the protein contains the essential functional domains, and therefore, it is capable of supporting protein biosynthesis. The fact that the wild type phenotype was not fully recovered when bacteria were grown at 42 °C suggests that the truncated PvIF2cp may have a deficient interaction with bacterial ribosomes. Evidence suggesting that the C-terminal part of IF2 is needed for interaction with ribosomes (35) supports this hypothesis. Further experimentation is needed to define the different functional domains in PvIF2cp.

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water limitation, consistent with the fact that photosynthesis is one of the processes more sensitive to low water availability (49).

Our phylogenetic analysis shows that IF2 from higher plants groups in the same clade as cyanobacteria support the endosymbiotic theory and suggest that this gene was transferred from a pre chloroplast endosymbiont to the nucleus. The fact that IF2 from algae classifies in the same clade as IF2 from higher plants favors a single origin of primary plastids (50). This relationship is well resolved in the strict consensus tree, but it is supported by a Bootstrap value <50%. This relationship needs to be confirmed with additional sequences when available. The phylogenetic analysis using IF2 sequences is also in agreement with the hypothesis that obligate intracellular bacteria were the ancestors of the mitochondria (51). Finally, the IF2 maximum parsimony tree seems to rescue the relationships.

...combining this molecule with others used by previous authors sequence can be useful in inferring phylogenetic relationships of...
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