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Update section

Sequence

Nucleotide sequence of the rice (*Oryza sativa*) Em protein gene (*Emp1*)

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The Em protein was first identified as a major product of the *in vitro* translation of poly(A) RNA obtained from dry wheat embryos [3, 4]. It was later shown that Em protein accumulation begins late in embryogenesis and that the level of accumulation can be manipulated in culture by abscisic acid (ABA) [20]. The wheat Em protein and its encoding mRNA are rapidly degraded during seed germination under normal conditions [2, 4, 18]. This decline is inhibited by ABA. Em transcripts also accumulate in wheat seedlings when treated with exogenous ABA or when subjected to water stress [1,13]. This correlation between Em accumulation and water stress, along with the interesting physical properties of the Em protein, suggests that Em, along with other factors in the cell, may act as a cytoplasm protectant during desiccation [4, 12]. A cDNA clone (p1015) [8] and a genomic counterpart (λCS41) [9] have been isolated and characterized from wheat. The 5′ flanking region of the wheat gene represented by λCS41 has been shown to contain elements that are responsive to ABA in a rice protoplast transient expression system [10] and in transgenic tobacco [11]. A protein which binds one of these elements has been identified and a cDNA representing this protein has been characterized [5].

A genomic clone λOSg4B containing a rice Em gene was isolated from an EMBL3 library [7] using a fragment of the wheat Em clone λCS41 [9] as a heterologous probe. The nucleotide sequence of the rice Em gene (*Emp1*) is presented in Fig. 1. Southern blot analysis and trisomic mapping experiments have shown that the Em sequence in this clone is single copy in the rice genome and maps to chromosome 5 [16]. The nucleotide sequence includes 733 bp of 5′ flanking region above the putative transcription start site, 114 bp of 5′ untranslated leader, 395 bp of sequence including the coding region and a 107 bp intron, and 683 bp of 3′ flanking region. A comparison of the *Emp1* nucleotide sequence with the sequences of several genes that are similar with respect to their products and/or regulation revealed several interesting similarities. The most interesting of these sequences was found in *Emp1* as a 15 bp perfect, inverted repeat located at 574 and inverted at 654. A similar sequence was found

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X63126.

Fig. 1. Nucleotide sequence of *Emp1* as determined from λOSg4B. The coding region is blocked into codons and translated. The introns and exons are demarcated by vertical bars (|). The various putative control elements are underlined, with the exception of the ABREs which are double-underlined.

at 412. This repeat, ACGTACGTGTCGCGC, contains a sequence (underlined) which is very similar to those identified as ABA-response elements (ABREs) in a wheat Em gene [9,11] and another ABA-responsive rice gene [14,17]. ABREs and their role in gene regulation have been reviewed recently [6, 15].

An analysis of *Emp1* expression in mature rice embryos revealed that it is regulated in a manner similar to that described in detail for the wheat gene [19, 20]. When isolated mature rice embryos were incubated on moistened filter paper there was an 80% reduction in the amount of accumulated mRNA in the first six hours of germination. When similar embryos were incubated for 24 h in the presence of ABA, the level of *Emp1* mRNA was maintained. Like the water control, the levels of mRNA in GA-treated embryos dropped by 80%. Rice *Emp1* mRNA was also found to accumulate in immature seeds.

In summary, based on sequence analysis and preliminary expression studies, the rice *Emp1* gene belongs to a highly conserved family of genes present in both monocot and dicot plants. *Emp1* is the only gene in the rice genome closely related to the Em protein family. *Emp1* contains highly conserved ABREs that are probably responsible for its induction by ABA, as well as other sequences that are conserved in similar genes and which may control its expression in other ways. *Emp1* is regulated very similarly, if not identically, to the wheat Em genes.

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