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Short communication

Sequence and functional analyses of the rice gene homologous to the maize *Vp1*

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Abstract

The homologous gene of the maize *Vp1* gene was isolated from rice (*Oryza sativa*). Sequence analysis revealed that the rice *Vp1* gene (*Osvp1*) encodes a protein of 728 amino acids and is interupted by 5 introns at positions identical to those of the maize gene. *Osvp1* transcript was detected in developing embryo as early as 10 days after flowering and decreased toward maturity. *Osvp1* transcript was also detected in dry as well as imbided mature embryos. The ability of *Osvp1* gene product to activate a target gene was shown by transient expression experiments in rice suspension-cultured cell protoplasts using a reporter gene construct carrying the bacterial β-glucuronidase (GUS) gene fused to the promoter of *OsEm* gene, the rice homologue of the wheat *Em* gene.

During the late phase of seed development, the embryo and a part of the endosperm tissues acquire desiccation tolerance and the growth of the embryo is arrested. This process is called maturation. A series of *viviparous* mutants, which have defects in the maturation process of the seeds and germinate precociously on the mother plants, have been reported in maize [11]. Unlike most of the maize viviparous mutants, in which abscisic acid (ABA) synthesis is affected, the *viviparous-1* mutant is characterized as an ABA-insensitive mutant [12]. The *vp1* mutant is also blocked in the synthesis of anthocyanin in the embryo and the aleurone tissues [11]. Transcript analyses of the *vp1* mutant seeds have revealed that *Vp1* controls the steady-state levels of the transcript of the regulatory gene *C1* for the anthocyanin pathway and ABA-regulated genes such as the maize homologue of the wheat *Em* gene [9]. The *Vp1* gene [9] and its cDNA [9, 10] have been cloned. The amino acid sequence of the *Vp1* protein (VP1) shows no detectable homology to proteins with known biochemical function [10]. Recently, the *ABI*(ABA-insensitive)*-3* locus [6] of *Arabidopsis* has been cloned and its gene product has been shown to have significant homology to VP1 at the

The nucleotide sequence data reported will appear in the EmBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number D16640.

amino acid sequence level [3]. Transient expression of C1- and Em-GUS reporter gene is transactivated in protoplasts by the cotransfection with a *Vp1* cDNA expression vector [4, 10]. Furthermore, a chimeric construct composed of the DNA-binding domain of the yeast GAL4 protein and the amino-terminal acidic domain of VP1 activates the CaMV 35S minimal promoter containing GAL4-binding sites [10]. From these observations it has been concluded that *Vp1* encodes a novel type of transcriptional activator. In this communication, to obtain information about evolutionary conserved structures, which are presumed to be important for function, we have cloned the *Vp1* homologue of rice (*Osvp1*). This work provides a basis for future analysis of VP1 function in transgenic plants using the homologous rice system.

A rice (*Oryza sativa* L. cv. Nipponbare) genomic library constructed in λEMBL3 phage vector was screened using the maize *Vp1* cDNA clone cvp23 [9] as a hybridization probe. One of several positive recombinant phage clones obtained in this screening was subjected to sequence analysis after subcloning into plasmid vectors. The nucleotide sequence of *Osvp1* was first compared with the maize *Vp1* cDNA sequence to deduce the initiator methionine and the intron/exon boundaries. *Osvp1* is interrupted by 5 introns at the same positions as those of the maize *Vp1* (Fig. 1). The intron/exon boundaries were further verified by sequencing a cDNA amplified by reverse transcriptase/PCR reaction with poly(A)$^+$ RNA prepared from the immature rice embryo (10–11 days after flowering) using primers shown in Fig. 1. The region of the amplified cDNA sequence is indicated in Fig. 1. *Osvp1* was found to encode a protein with 728 amino acids, which is slightly larger than that of the maize VP1.

In Fig. 2 the amino acid sequence deduced from the exonic sequence of *Osvp1* is aligned with those of the maize VP1 [10] and the *Arabidopsis* ABI3 [3]. When compared with the maize sequence, OSVP1 is 62% identical to VP1 after introduction of gaps. This degree of sequence conservation seems to be fairly low for protein products of homologous genes from maize and rice, both of which belong to Gramineae. For example, the waxy protein [5, 13] and alcohol dehydrogenase II [2, 14] show sequence identities of 88% and 86%, respectively, between the two species. *Arabidopsis* ABI3 is only 35% identical to OSVP1 and VP1. Because of the low degree of sequence identity but with the several localized conserved regions and the slight differences in the mutant phenotype, Giraudat *et al.* [3] have discussed the

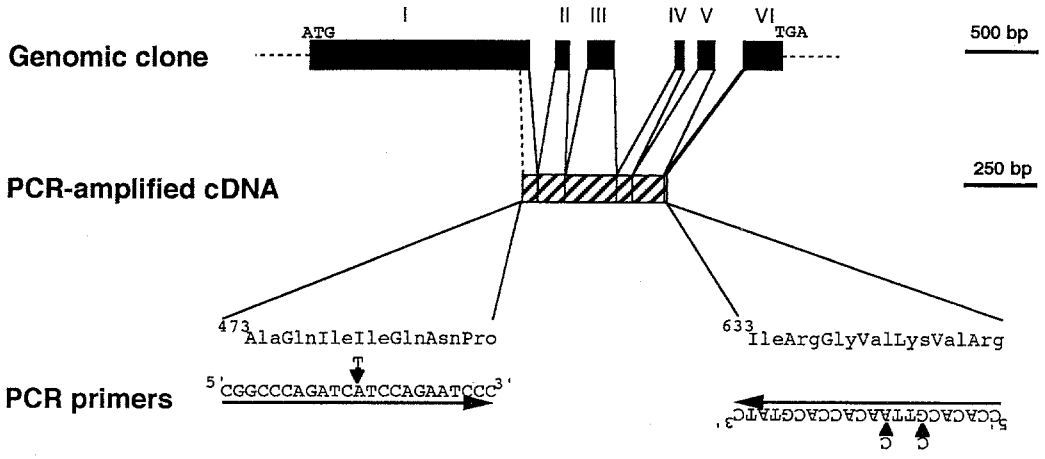

Fig. 1. Schematic illustration of the structures of *Osvp1* and the PCR-amplified cDNA clone. Exons are indicated by filled boxes and numbered with roman numerals. The PCR-amplified cDNA is shown by the hatched box with connecting lines to the corresponding regions of the genomic clone. The nucleotide sequences of the primers used for PCR amplification and the corresponding amino acid sequences are shown at the botom. Base changes were introduced in the primers to facilitate cloning as indicated.

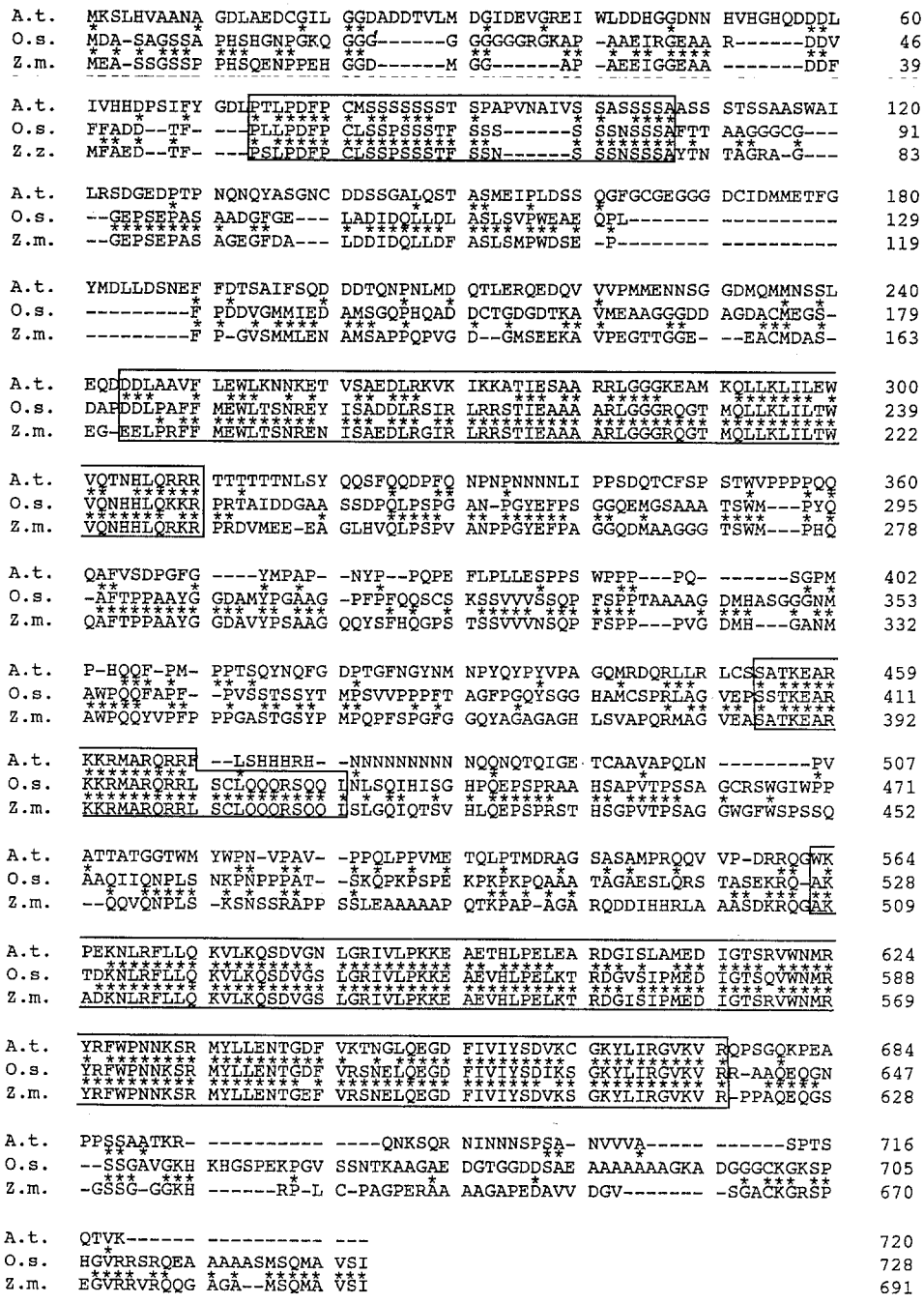

Fig. 2. Alignment of the amino acid sequence of OSVP1 from rice (O. s.), with VP1 from maize (Z. m.) and ABI3 from *Arabidopsis* (A. t.). Identical amino acids are shown by asterisks. Conserved regions among the three species are boxed.

possibility that *ABI3* may not be the functional counterpart of the maize *VP1*. On the other hand, it is hard to doubt that *Osvp1* is the true rice counterpart of the maize *Vp1*, because genomic DNA blot hybridization with the maize probe under stringent conditions shows only the bands that match the restriction map of our *Osvp1* genomic clone (data not shown). Considering the

relatively low degree of sequence identity between *Vp1* and *Osvp1*, the further low level of conservation for the *Arabidopsis* homologue does not seem striking.

Three regions with localized sequence conservation have been pointed out previously in a comparison between the *Arabidopsis* and maize sequences [3]. A similar pattern is obvious even between the two phylogenetically close species rice and maize. The region with the most extensive conservation is that encoded by the exons 2 to 5 (amino acid residues from 527 to 632 of OSVP1). In this region, 95% and 86% of amino acid residues are identical between OSVP1 and VP1, and between OSVP1 and ABI3, respectively. Such high degree of conservation strongly suggests that the region constitutes a domain highly important for its function. The amino acid residues from 183 to 249 constitute the second conserved region. This region contains three stretches of potentially helix-forming sequences [10]. We have found that within this region from 228 to 235, leucine or isoleucine residues are located at every seven residues with an exception for the threonine at the fourth position. In ABI3 at this forth position is methionine. This structure with at least three consecutive hydrophobic amino acids repeating every seven residues could be involved in protein-protein interaction. The sequence from 406 to 432 is the third region of remarkable sequence conservation between OSVP1 and VP1. The sequence conservation extends only to 420 when compared with ABI3, Giraudat *et al.* [5] have pointed out that between 406 and 420, there exists a putative nuclear targeting signal consensus sequence, RKKR. The fourth region of conservation are the serine-rich residues from 54 to 81, which have not previously been discussed but may have functional importance.

The expression of *Osvp1* was examined by RNA gel blot hybridization (Fig. 3). RNAs were fractionated by a 1.2% agarose gel containing formaldehyde and transferred to a nylon membrane (Zetaprobe, Amersham). Hybridization was carried out with the PCR-amplifed *Osvp1* cDNA fragment labeled with [α-^{32}P]-dCTP in a solution containing 50% (v/v) formamide, 7% SDS, 0.25 M sodium phosphate buffer pH 7.2, 0.25 M NaCl at 42 °C. Washes were carried out in 2 × SSC at room temperature and subsequently in 0.1 × SSC at 65 °C. In the developing rice embryos, *Osvp1* transcripts were detected at approximately equal levels 10 to 13 days after flowering and decreased towards the maturity of the embryo. The dry and the 16 h imbided mature embryos, however, contained significant amounts of the transcripts (Fig. 3). These results suggest that *Osvp1* may function not only during embryo development but also at early stages of germination. This is in accordance with the phenotype of *abi3* mutants in *Arabidopsis*, which have been obtained by screening for the ability to germinate in the presence of ABA [6]. *Osvp1* transcripts were not detected in the shoot tissues. Although we have not carried out an analysis with other tissues, this is consistent with the seed specific phenotypes of the *vp1* mutants and the results of transcript analyses performed with maize [9].

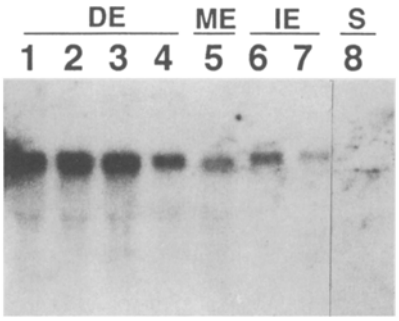

Fig. 3. RNA gel blot hybridization analysis of *Osvp1* transcript. Total RNA (15 μg) from the developing embryo (DE: lane 1, 10 days after flowering (DAF); lane 2, 11 DAF; lane 3, 13 DAF; lane 4, 20 DAF), the dry mature embryo (ME: lane 5), the imbided mature embryo (IE: lane 6, 10 h imbibition; lane 7, 16 h imbibition) and the shoot tissues (S: lane 8) from the 3-week old plants were used.

To establish that *Osvp1* cloned in the present study is functionally homologous to the maize *Vp1*, we tested the ability for OSVP1 to activate transcription of a target promoter in a cotransfection experiment using the protoplasts from the suspension-cultured rice cell line Oc. First we have reconstructed a full-length cDNA from the PCR-amplified partial cDNA and the rest of the exonic sequences taken from the genomic clone.

For convenience, we refer to this construct as the full-length *Osvp1* cDNA. An OSVP1 effector plasmid, 35S-Sh-Osvp1, was constructed by replacing the GUS gene fragment of the 35-Sh-GUS plasmid [10] with the full-length *Osvp1* cDNA sequence with the aid of synthetic oligonucleotide linkers. The resulted plasmid consisted of the CaMV 35S promoter, a short sequence of 5′-untranslated region and the first intron from the maize *Sh1* gene, the full-length *Osvp1* cDNA and the terminator of the nopaline synthase gene. The target promoter tested here was taken from *OsEm*, the rice counterpart of the wheat *Em* gene (T. Hattori *et al.*, in preparation, [8]). The reporter plasmid OsEm-GUS was constructed by replacing the 35S promoter fragment of pBI221

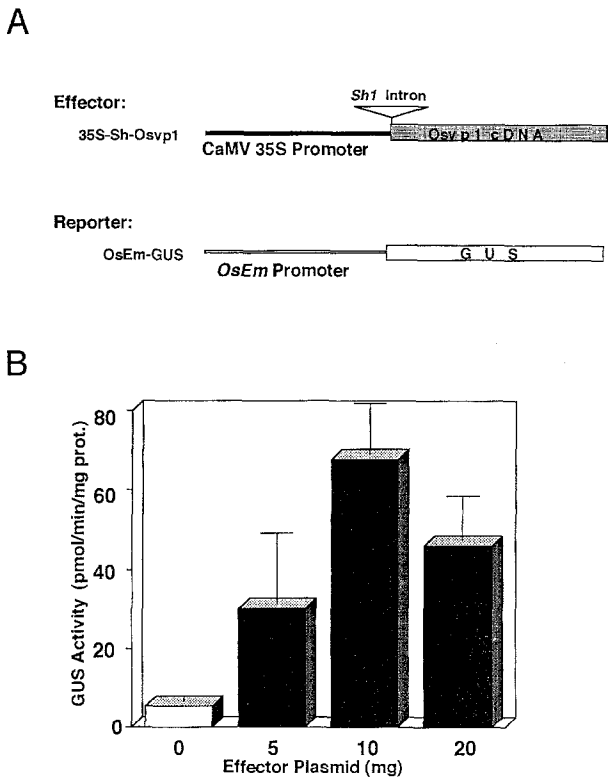

Fig. 4. Transcriptional activation by OSVP1 of the *OsEm* promoter. A. Schematic illustrations of the effector (35S-Sh-Osvp1) and the reporter (OsEm-GUS) constructs used. B. The rice Oc cell line protoplasts were transfected with 10 μg of OsEm-GUS plasmid and the indicated amount of 35S-Sh-Osvp1 plasmid (μg) together by electroporation. After electroporation the cells were cultured for 40 h and GUS activity and protein of the cell were measured.

with an *OsEm* promoter fragment that spans from −502 to + 105 (Hattori *et al.*, in preparation, [8]). The OsEm-GUS plasmid was electroporated into the Oc cell protoplasts [7] together with or without the effector plasmid 35S-Sh-Osvp1. Like in the previously reported transactivation experiments with the maize VP1 effector and the Em-GUS reporter in the maize protoplasts [10], the transcription from *OsEm* promoter, as measured by GUS activity, was activated by OSVP1 expressed from the effector plasmid (Fig. 4). At the optimal dosage (10 μg) of the effector plasmid, around 10-fold activation was observed. This level of transactivation by OSVP1 is lower than that observed in the previous experiments, in which Em-GUS was activated over 100-fold by the maize VP1 effector, 35S-Sh-Vp1 in the maize protoplasts [10]. When the maize VP1 effector plasmid was used in the rice protoplasts, however, a similar level of activation was observed for *OsEm*-GUS or Em-GUS (data not shown). These results indicate that *Osvp1* is functionally as well as structurally, homologous to the maize *Vp1*. The lower degree of activation in the rice protoplasts than that in the maize cells probably resulted from the relatively high basal level of OSVP1 due to the endogenous expression of *Osvp1*, since we have found that the *Osvp1* transcript is present in our rice cell line (T. Hattori *et al.*, unpublished results).

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