Plant Physiol. (1995) 108: 829–830

Plant Gene Register

Nucleotide Sequence of a Sporamin Gene in Sweet Potato[1]

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Sporamin is tissue specific and may account for more than 80% of the soluble protein found in tuberous root of sweet potato (*Ipomoea batatas* Lam.) (Maeshima et al., 1985). SDS-PAGE analysis indicates that sporamin may be resolved into two bands with molecular masses in the range of 25 kD. These two protein bands were characterized and classified into two subfamilies, based on the homology of their nucleotide sequences (Murakami et al., 1986; Hattori et al., 1989). Within the subfamilies, the homology may be greater than 90%, whereas the cDNAs between the two subfamilies share only 80% homology. Recent studies of two sporamin genes, gSPO-A1 and gSPO-B1, which belong to the two distinct subfamilies A and B, respectively, have shown that they are intronless genes (Hattori and Nakamura, 1988). Comparison of the 5′ flanking region also revealed the presence of two conserved sequence blocks, namely Suc box 2 and box 3 (Hattori and Nakamura, 1988; Tsukaya et al., 1991). In this paper, we present a novel sporamin gene, gSPOR5–31, which was identified to be an isogene of the gSPO-A1 within subfamily A.

A λEMBL3 genomic library comprising 6×10^5 plaque-forming units was constructed with partially *Sau*3A-digested genomic DNA of tuberous roots. The library was screened by plaque hybridization using the cDNA probe of SP-B (an antisense strand of sporamin cDNA; K.-W. Yeh, unpublished results). One positive signal was obtained during the primary screening. It was purified to a single plaque, from which the DNA was extracted and characterized by restriction endonuclease and Southern blot analysis (Table I). The results showed that the clone (gSPOR5–31) contained an insert DNA of approximately 7 kb. When the insert DNA was digested by *Bam*HI, resolved by agarose gel electrophoresis, and hybridized with the SP-B cDNA probe, only a 2.2-kb fragment clearly showed a strong signal. Therefore, it was subsequently subcloned into pGEM3Z (Promega) at the *Bam*HI site for further studies.

The nucleotide sequence of the 2.2-kb *Bam*HI fragment was determined by nested deletion and primer walking. It included an open reading frame of 660 nucleotides corresponding to 220 amino acid residues. The genomic clone of gSPOR5–31 was similar to those of the gSPO-A1 and gSPO-B1 containing no intron. These observations suggest that sporamin genes are intronless. Analysis of the coding region demonstrated that gSPOR5–31 shared a high degree of homology with other sporamin genomic clones, such as gSPO-A1 and gSPO-B1 (Hattori and Nakamura, 1988), as well as cDNA clones of pIMO23, pIMO335, etc. (Hattori et al., 1989). Since gSPOR5–31 shows 93.5 and 81% nucleotide homology in the coding region with gSPO-A1 and gSPO-B1, respectively, and 80.8 and 50% in the 5′ flanking region, respectively, gSPOR5–31 may be recognized as a member of the sporamin A gene subfamily. Primer extension data showed that the transcription start site was located at the first nucleotide of the conserved region, 5′-AACACAACACA-3′ and was identical with that of the gSPO-A1. Sequence analysis of the 5′ flanking region revealed several prominent sequences. The TATA box was found at −17 to −24 bp as 5′-TATAAATT-3′. The CAAT box was located at −153 to −158 bp as 5′-GCAATC-3′. In addition, the Suc box 2, Suc box 3, and SP8a box consensus sequences (Ishiguro and Nakamura, 1992) were identified at −79 to −105 bp, −239 to −256 bp, and −145 to −152 bp as 5′-ACTGTGAAAATTTTGAGTAAAAAAAGG-3′, 5′-CAAAATCATTTCTATTTC-3′, and 5′-ACTGTATA-3′, respec-

Table I. *Characteristics of sporamin gene clone gSPOR5–31 from sweet potato*

Organism:
Sweet potato (*Ipomoea batatas* Lam. cv Tainong 57).
Location on Chromosome:
Nuclear genome, chromosome location not known.
Function:
Storage protein.
Clone Type:
Genomic.
Source:
Genomic DNA of Tainong 57 tuberous roots constructed in EMBL3.
Techniques:
Genomic library screening; dideoxy sequencing using nested deletion and primer walking.
Characteristics of the Nucleotide Sequence:
gSPOR5–31 contains an open reading frame of 660 bp, with 44.4% AT content. It is an intronless gene.
Features of the Gene Structure:
Putative TATA box is present at 17–24 bp 5′ to the transcription start site, and the translational start codon is present at 62 bp 3′ to the transcription start site. The SP8a box is present at 145–152 bp 5′ to transcription start site, and Suc box 2 and box 3 are located at 79–105 and 239–256 bp 5′ to the transcription start site, respectively.

[1] This research was supported in part by the National Science Council, Republic of China to K.-W.Y. (NSC 82-0211-B002-218).

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tively. Alignment comparison of the 5′ upstream region among the above-mentioned genomic clones showed that these sequence blocks were highly conserved for sporamin genes in the diverged 5′ flanking region. These observations are consistent with the notion that these sequences are important and might play significant roles in regulating the expression of sporamin genes.

Received October 31, 1994; accepted November 18, 1994.

The GenBank/EMBL accession number for the sequence reported in this paper is U12436.

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