Nucleotide Sequence of the α-Amylase Gene from Vigna mungo

Hajime Takeuchi, Daisuke Yamauchi*, Sachiko Wada, and Takao Minamikawa

Department of Biology, Tokyo Metropolitan University, Minami-ohsawa 1-1, Hachioji-shi, Tokyo 192-03, Japan

The amount of α-amylase increased in cotyledons of germinating seeds of Vigna mungo (Minamikawa et al., 1992). To examine the genomic organization of the gene, DNA from V. mungo was analyzed by Southern hybridization with 32P-labeled α-amylase cDNA (Yamauchi and Minamikawa, 1990). Approximate estimates of the fragment sizes in kb were: EcoRI, 8 and 2.2; HindIII, 15; and XbaI, 4. Since HindIII and XbaI do not cut the cDNA, the results indicate that there is a single copy of the α-amylase gene. After the DNA from V. mungo digested by EcoRI was separated by 1% agarose gel electrophoresis, about 2.2-kb fragments cut out from the gel were ligated to λgt10. From this library we isolated a genomic clone (λVMAMY1A) containing a 1499-bp transcribed region and a 639-bp 5′-flanking region. The rest of the transcribed region was isolated by polymerase chain reaction. A 1.4-kb fragment was amplified and subcloned. This clone, designated pVMAMY1B, included a part of the third exon, the third intron, and the fourth exon, indicating that the α-amylase gene from V. mungo has three introns of 235, 421, and 637 bp separating four exons of 113, 130, 806, and 492 bp. The sites of the introns are the same as those of the monocot α-amylase gene subfamily 2 (Huang et al., 1992).

Table I. Characteristics of the α-amylase gene from Vigna mungo

| Organism: | Vigna mungo |
| Location of Gene: | Nuclear genome |
| Function: | Encodes α-amylase |
| Cloning Technique: | A genomic DNA concentrated library in λgt10 was constructed from fragments of V. mungo nuclear DNA digested by EcoRI. A part of the transcribed region was amplified by polymerase chain reaction using oligonucleotides 5′-CTGCAGGTGG-AGCTATTACTGCA-3′, position +767 to +789 of the cDNA (Yamauchi et al., 1990), and 5′-ATAATAACTCTTGAGGA-CAAATC-3′, corresponding to the 3′ terminus of the cDNA. |
| Features of Gene Structure: | Transcription initiation site is 28 bp downstream from the initiation codon determined by S1 nuclelease mapping and primer extension analysis. Three introns are present in the gene. |

LITERATURE CITED


Copyright Clearance Center: 0032-0889/93/103/1459/01.

The EMBL accession number for the sequence reported in this article is X73301.

* Corresponding author; fax 81–426–77–2559.

Received June 21, 1993; accepted July 19, 1993.