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Plant Gene Register

Cloning of an α-Amylase cDNA from Aleurone Tissue of Germinating Maize Seed[1]

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A number of hydrolases are required for the conversion of stored carbohydrate reserves in cereal grains into metabolizable products during germination. α-Amylase (EC 3.2.1.1) plays a key role in this process by catalyzing the endoglycolytic cleavage of amylose and amylopectin, the principal components of starch granules in the endosperm (Bewley and Black, 1985).

Studies of α-amylase characterization and expression in cereals have been conducted mainly in barley, wheat, and rice, with very limited information available for maize (*Zea mays* L.). Multiple isozymes of this enzyme are synthesized in two tissues, the aleurone and scutellum of the embryo, and can be separated on the basis of their pI. In barley and wheat, the isoforms fall into two distinct groups (low pI [≤5.5] and high pI [≥5.5]); however, in rice and maize, there is only one group of isozymes, between pI 3 and 6 (McGregor et al., 1988; Sanwo and DeMason, 1993). To facilitate a more detailed understanding of gene structure and regulation in barley, wheat, and rice, a number of α-amylase genes have been cloned and characterized, and these studies have revealed that the cereal α-amylases are encoded by multigene families (Baulcombe et al., 1987; Knox et al., 1987; Khursheed and Rogers, 1988; Huang et al., 1990; O'Neill et al., 1990). Furthermore, it has been shown that although some of the isozymes are derived from different genes, others differ as a result of posttranslational modifications (Fincher, 1989; Jones and Jacobsen, 1991; Sticher and Jones, 1992). The function and significance of the multiple isozymes are not known.

To further advance our understanding of α-amylase expression in maize, we have isolated cDNAs from maize libraries and report here the cloning of a full-length α-amylase cDNA (Table I). Three λgt11 cDNA libraries were constructed from aleurone and scutellar tissues of germinating seeds of the maize inbred OH43. To accomplish this, total RNA was extracted approximately 7 d after inhibition from isolated aleurones of both a dent (wild type) and a *shrunken-2* isogenic line and from scutella of the dent isoline. Poly(A)$^+$ RNA was isolated from each using the PolyATtract mRNA isolation system (Promega) and was subsequently used to synthesize cDNAs according to the specifications of a cDNA synthesis kit (Boehringer Mannheim).

Table I. *Characteristics of α-amylase cDNA (pMAs5) from germinating maize aleurone*

Organism:	
	Zea mays L. (OH43).
Gene Products; Function:	
	1,4-D-Glucan glucanohydrolase, α-amylase (EC 3.2.1.1); α(1-4)endoglycolytic cleavage of amylose and amylopectin.
Clone Type; Designation:	
	cDNA, full-length; pMAs5 (pGEM-11Zf+).
Source:	
	cDNA libraries in λgt11 constructed from poly(A)$^+$ mRNA isolated from aleurone and scutellar tissue of germinating maize seed.
Techniques:	
	Libraries screened with rice (*Oryza sativa* L.) α-amylase clone pOS103 (O'Neill et al., 1990); partial clone subcloned into pGEM-11Zf(+); single-stranded dideoxy-nucleotide sequencing of partial clone; second screening with partial maize clone to obtain full-length clone; both strands of full-length clone (pMAs5) sequenced in their entirety using single-stranded dideoxy-nucleotide sequencing of exonuclease-generated deletions.
Method of Identification:	
	Sequence homology to other α-amylase clones.

A heterologous rice α-amylase clone (pOS103) obtained from R.L. Rodriguez was used as a probe to screen the *shrunken-2* aleurone library. Initial screening resulted in the isolation of a partial maize α-amylase cDNA, the identity of which was confirmed on the basis of sequence homology with other known α-amylases. This clone was subsequently used to rescreen for additional clones in all three libraries. Following this second round of screening, a full-length cDNA (designated pMAs5) of 1627 bp was isolated and subcloned into the plasmid vectors pGEM-11Zf (+ and −) for sequencing. Several additional putative clones were isolated and saved for future work. A single, 1.6-kb band was identified on northern blots of total maize RNA isolated from aleurone and scutellar tissue (7 d after germination) that were probed with either the rice or maize α-amylase cDNA. Sequence comparisons of pMAs5 with other cereal α-amylases showed that the maize cDNA shared the greatest homology within the open reading frame to that of a rice α-amylase (λOSg1;

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Abbreviation: pI, isoelectric point.

GenBank accession No. M24941) with 85% similarity and identity at the nucleic acid level and 94% similarity and 88% identity at the amino acid level.

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