Isolation and characterization of two cDNA clones for mRNAs that are abundantly expressed in immature anthers of rice (Oryza sativa L.)

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Abstract

The relationship between the length of anthers and the stage of development of microspores was examined in rice (Oryza sativa L. cv. Hayayuki). Anthers of ≤2 mm and 2.1–2.2 mm in length and those ready to dehiscence were determined to be at the uninucleate, binucleate and trinucleate microspore stage, respectively.

Two cDNAs (YY1 and YY2), representing genes that are specifically expressed in anthers at the uninucleate microspore stage, were isolated and characterized. YY1 cDNA encoded an open reading frame of 95 amino acids. Eight cysteine residues with the potential to form disulfide bridges were present in the amino acid sequence. There was a hydrophobic region at the N-terminus of the putative protein, suggesting that the YY1 protein might be secreted. This cysteine motif and the hydrophobic N-terminus are conserved among products of several anther-specific genes or cDNAs isolated from various plant species. These proteins are thought to form a superfamily of proteins that are confined to anthers. The YY1 transcript was localized in the tapetal cells and the peripheral cells of the vascular bundle. YY2 cDNA encoded an open reading frame of 389 amino acids and the deduced amino acid sequence exhibited substantial homology to that of chalcone synthase. Expression of YY2 mRNA was confined to the tapetal cells. The genes correspond to YY1 and YY2 cDNAs were shown to exist as single copies in the rice genome.

Introduction

In higher plants, the formation of pollen is a unique developmental process, involving cytological and biochemical changes in several types of cells [14]. Underlying these processes is a complex program of gene expression in both sporophytic and gametophytic tissues [15]. Genes that are preferentially expressed in anthers during the development of microspores might be expected to

The nucleotide sequence data reported will appear in the GenBank, EMBL, DDBJ and NCBI Nucleotide Sequence Databases under the accession number D50575 (YY1) and D50576 (YY2).
play important roles in anther-specific developmental pathways. To isolate these genes, differential screening of cDNA libraries has been carried out by several groups \[16, 17, 32\]. Mascarenhas \[15\] classified these genes into two classes according to their patterns of expression. The 'early' genes become active soon after meiosis and the levels of their transcripts are reduced or they are undetectable in mature pollen. The 'late' genes become active after microspore mitosis and their transcripts continue to accumulate until the pollen reaches maturity. Based on their nucleotide sequences and the localization of their transcripts, the two classes of genes are likely to have very different roles. The 'early' genes are probably involved in pollen development such as wall synthesis. For example, the Satap35 and Satap44 proteins from Sinapis alba are thought, from their localization, to be involved in sporopollenin formation and/or deposition to the pollen wall \[31\]. The 'late' genes are likely to be required during pollen maturation or growth of pollen tube. LAT56 from tomato \[37\] and P2 from Oenothera organensis \[1\] have amino acid sequences that exhibit homology to those of pectate lyase and polygalacturonase, respectively. They may be required during growth of pollen tubes. Recently, the LAT52 gene, which is expressed in a pollen-specific manner, was shown to play a role in pollen hydration and/or germination from results of antisense inhibition experiment \[18\]. However, with the exception of a few examples, such as LAT52, the functions of these anther-specific genes are still a matter of conjecture. In order to dissect the molecular processes that occur during anther development, we must isolate many more anther-specific genes and determine their functions.

In this report, we describe the isolation and characterization of two cDNAs for mRNAs that are specifically expressed in anthers at the uninucleate microspore stage in rice plants. These two cDNAs can be classified as cDNAs of 'early' genes, and the corresponding genes may participate in microspore development. One of the cDNAs, YY1, encodes an amino acid sequence with eight cysteine residues, as do several other anther-specific genes. It is suggested that the YY1 protein is a member of a superfamily of proteins that are specifically localized in anthers. The protein encoded by another cDNA, YY2, exhibited significant homology to chalcone synthase (CHS) and it might be involved in the synthesis of flavonoids, which play important roles in anthers.

**Materials and methods**

**Plant materials**

Rice plants (Oryza sativa L. cv. Hayayuki) were grown in a greenhouse. For construction of a cDNA library and northern blot analysis, anthers were collected and immediately frozen in liquid nitrogen. Samples of shoots and roots were obtained from 1-week-old frozen tissues were stored at -80 °C until use.

**Determination of anther length and the stage of microspore development**

Lengths of florets and anthers were determined under the light microscope. The stage of microspore development was assessed by light-microscopic examination of microspores (or pollen grains) that had been extruded from anthers squashed on a glass slide. For observations of nuclei, anthers dissected from florets were fixed in a mixture of 99% ethanol and acetic acid (3:1, v/v) for 1 h. They were rinsed first in 70% ethanol and then in water. Microspores in a solution of 4'-6-diamidino-2-phenylindole (DAPI) (1 μg/ml DAPI, 0.05 M Tris-HCl pH 7.0, and 0.5% Triton X-100) were observed under UV light.

**Construction of a cDNA library**

Poly(A)^+ RNA was extracted from anthers at the uninucleate microspore stage with a Fast Track mRNA isolation kit (Invitrogen). cDNAs were synthesized with a cDNA synthesis kit (Pharmacia) and ligated with EcoRI adapters for bidirec-