Analysis of Differentially Expressed mRNAs during the Early Developmental Stage of Strawberry (Fragaria x ananassa Duch.) Fruit by Differential Display Technique

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Summary

Differential display technique was applied to detect differential gene expression during the early developmental stage of strawberry (Fragaria x ananassa Duch.) fruit. Total RNA was isolated from fruits 0, 2, 4, 7, 10 and 13 days after pollination. Subsequently cDNAs were synthesized and used as templates for differential display analysis. Eleven polymerase chain reaction (PCR) products, related to differential gene expression during fruit development, were observed. Two cDNAs of eleven PCR products, designated as SGR101 and SGR701, were subcloned and sequenced. A homology search revealed that SGR101 shared high homology to S6 kinase homolog of Arabidopsis thaliana and SGR701 shared high homology to the gene which encodes hydroxyproline-rich glycoprotein. Differential display technique can detect changes in gene expression during strawberry fruit growth.

Key Words: strawberry, differential display, fruit development.

Introduction

In Japan about 200,000 tons of strawberries were produced in 1995 (MAFF Statistics and Information Dep., 1997). More information about the influence of the early developmental stage is essential to stabilize strawberry production.

Wilkinson et al. (1995) reported a change in gene expression at the ripening stage of strawberry fruit by the differential display technique which was developed by Liang and Pardee (1992). This technique is based on PCR and it can detect a change in low levels of mRNA.

In this paper, we report a successful application of this technique to analyze differential gene expressions during the early developmental stage of strawberry fruit.

Materials and Methods

Strawberry plant 'Nyoho' were rooted from runners in September 1995 and maintained in a greenhouse. When flower buds appeared, plants were transferred to a growth chamber controlled at 20°C with 16hr photoperiod. Secondary to 6th flowers were hand-pollinated. Fruits from the secondary flower were harvested at 0, 2, 4, 7, 10, and 13 days after pollination. Fruits were frozen in liquid nitrogen and stored at −80°C.

Total RNAs were isolated from strawberry fruit containing receptacle and achene (ca. 100 mg), according to Manning (1991), with a little modification, using 1.5 ml microtube. First-strand cDNA was synthesized, using the First-Strand cDNA Synthesis Kit (Pharmacia Biotech Inc.). The total reaction volume of 33 µl contained 5 µg of total RNA, 2.5 µg anchor primer 5'-TTTTTTTTTTTGT-3', 1 µl of DTT, and 11 µl of reaction mix. The reaction solution was kept at 37°C for one hour. The cDNA was recovered after phenol: chloroform (1:1:v:v) extraction followed by ethanol precipitation, then redissolved in 50 µl sterilized distilled water.

Four decamer oligonucleotides which were used singly as PCR primer were RA01 (5'-GTCTGACGGA-3'), RA03 (5'-CGATCGAGGA-3'), RA05 (5'-AAGCAGCAGGA-3'), and RA07 (5'-AGCAGTCTCGG-3') (Monna et al., 1994). The PCR cocktail of 50 µl was composed of 2 µl of cDNA solution as a template, 1 µM of each primer, 200 µM of dNTP, and 1.25 unit of Taq DNA polymerase (Nippon Gene Co., Ltd.) in the recommended buffer. PCR was performed under the following parameters: 94°C denaturing for 60 sec, 45°C annealing for 60 sec, 74°C extension for 60 sec, repeated for 40 cycles, with preheating 94°C for 60 sec and
followed by a final extension at 74°C for 300 sec. Subsequently, PCR products were separated by electrophoresis on 8% polyacrylamide gel (acylamide:BIS acrylamide = 39:1) and visualized by ethidium bromide stain.

PCR fragments from differentially expressed gene were excised from the gel and eluted into 100 μl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) by heating to 60°C, overnight. One μl of the above solution was used as a template of reamplification by PCR. The PCR condition of reamplification was the same above except denature temperature (60°C).

For subcloning, the reamplified products were ligated with pCR II vector (TA cloning Kit, Invitrogen Co.) and transformed into E. coli according to the manufacturer’s instructions. cDNA-containing clones were selected, the sequence was analyzed by DNA sequencer (Pharmacia Biotech Inc.), a homology search of the DNA Sequence Data Bank was performed.

**Results and Discussion**

Under the growth condition of this experiment, a fruit took about four weeks to ripen, the fruit did not turn red within 13 days after pollination.

The differential display of mRNAs from an early stage of the strawberry fruit revealed significant changes at the molecular level (Fig. 1(A)). Eleven PCR products related to differentially gene expressions were detected by four primers. The numbers of amplified PCR products or electrophoresis patterns were different according to the primer used in PCR.

More differentially expressed genes were detected from strawberry fruits at 7 or 10 days after pollination compared with other developmental stages. Miura et al. (1990) reported that this stage was near the peak of fruit growth rate and achene enlargement. Thus, this stage could be called the cell expansion phase which is physiologically significant for fruit development (Gillaspy et al., 1993). Two polymorphic PCR products which were expressed during this period were designated SGR101 and SGR701 and analyzed.

Then the analysis of nucleotide sequence and homologous research of these PCR products were performed (Fig.1(B)). According to these analysis, we concluded

![Fig. 1. Electrophoretic gel plates illustrating PCR products (A) and partial nucleotide sequences of cDNA SGR101 and SGR701 and comparison of sequence of SGR101 with ATPK19 and SGR 701 with Hyp 2.13 (B).](image-url)
that SGR101 shared high similarity with an Arabidopsis thaliana cDNA clone ATPK19 (Mizoguchi et al., 1995). ATPK19 was a homolog to ribosomal-protein S6 kinase and Mizoguchi et al. (1995) speculated that ATPK19 may play important roles in increasing the capacity of protein synthesis. SGR101 is supposed to be an important gene for protein synthesis or metabolism at this physiologically active stage.

SGR701 which was expressed at the same stage as SGR101 (Fig. 1(A)), shares high similarity with a Phaseolus vulgaris cDNA clone Hyp2.13 which encodes hydroxyproline-rich glycoprotein (HRGP) (Corbin et al., 1987). HRGPs include extensins, (Kieliszewski and Lamport, 1994) and the sequence of Hyp2.13 contains the repetitive peptide motif of extensin. Extensins are found in the cell walls of higher plants and their expression is developmentally regulated (Showalter, 1993). SGR701 may be related to cell wall division or cell wall elongation processes.

We concluded that differential display technique is applicable for gene expression analysis during the early developmental stage of strawberry fruit and estimate genes which relate to fruit development.

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ディファレンシャルディスプレイ法によるイチゴ（Fragaria x ananassa Duch.）果実の生育初期におけるmRNA発現の解析

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摘 要

イチゴ'女峰'の果実生育の初期に発現する遺伝子をディファレンシャルディスプレイ法により解析した。開花後0, 2, 4, 7, 10, 13日の果実から抽出したRNAを基に合成したcDNAをPCR反応の鋤型に用いた。異なる遺伝子発見を示唆するPCR産物101のうちSGR101, SGR701の2本をサブクローニングして塩基配列を決定した。ホモジー検索の結果, SGR 101はリポソームタンパクS6キナーゼのホモロジーアラビドプシスのcDNAと, SGR 701はインゲ

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