Isolation of cDNAs for Genes Expressed after/during Fertilization and Fruit Set of Melon (*Cucumis melo* L.)

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Summary

Fertilization is an important event leading to fruit set. Tips of pollen tubes reached the ovules at the base of the ovary within 24 hr after pollination. Therefore, cDNAs for genes preferentially expressed after/during fertilization were isolated by suppression subtractive hybridization between cDNAs from pollinated and unpollinated fruits. Using cDNA macroarrays, 384 cDNA clones of the subtracted cDNA library were differentially screened by the probes of the cDNAs from pollinated or unpollinated fruit 1 day after anthesis. By Northern blot analyses, mRNAs, corresponding to 14 cDNA clones, were more concentrated in pollinated fruit than in the unpollinated ones. The deduced amino acid sequence of genes corresponding to these cDNA clones showed a similarity with ascorbate oxidase, hydroxymethyltransferase, phospho-ethanolamine *N*-methyltransferase, short chain alcohol dehydrogenase, histone H2A, auxin binding protein, 14–3–3 protein, extensin-like protein, nucleoid DNA-binding-like protein, type IIIa membrane protein (reversibly glycosylated polypeptide), and hypothetical proteins. In melon, *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea (CPPU) induced parthenocarp without pollination, whereas 4-chlorophenoxyacetic acid (4-CPA) was less effective on producing parthenocarpic fruit. mRNAs corresponding to 14 cDNA clones were expressed in both pollinated and CPPU-treated fruit. Expressions of mRNAs corresponding to all cDNA clones in pollinated and CPPU-treated fruit were higher than those in 4-CPA-treated fruit. These mRNAs were barely detectable in the later stages of fruit development and were expressed preferentially in fruit except for *6E04* and *6H01*. Especially, expression patterns of *8A07* (nucleoid DNA-binding-like protein) and *8D10* (short chain alcohol dehydrogenase) lead us to speculate that they play important roles in fruit set because they are expressed at a higher level in the early stages of both pollinated and CPPU-treated fruit than that of 4-CPA-treated fruit.

Key Words: *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea (CPPU), fertilization, fruit set, pollination, suppression subtractive hybridization.

Introduction

Fertilization is generally a prerequisite to fruit set. It is considered that fruit set depends on the successful completion of pollination and fertilization in normal fruit development. The presence of fertilized ovules generally triggers the continuous development of an ovary into a fruit (Gillaspy et al., 1993). Pollination and fertilization provide growth stimuli or signal(s) for fruit setting by promoting synthesis of plant hormones, such as auxins, gibberellins and cytokinins. For example, indole-3-acetic acid (IAA) content of mesocarp increases in pollinated fruit 1 day after anthesis in melon 'Prince' (Lee et al., 1997).

In some plants, fruit set is induced in the absence of

pollination and fertilization, a phenomenon known as parthenocarp. Exogenously applied CPPU, a synthetic cytokinin, induces parthenocarp in muskmelon (Hayata et al., 2000) and watermelon (Hayata et al., 1995). Several genes, involved in natural parthenocarpic fruit development in tomato (*Lycopersicon esculentum*), were described (Barg et al., 1990; Fos and Nuez, 1996, 1997; Testa et al., 2002). Transgenic eggplants and tomatoes, expressing the coding region of the *iaaM* gene in the ovule, promote parthenocarpic fruit development (Ficcadenti et al., 1999; Rotino et al., 1997). Parthenocarpic fruit was produced by transposon insertion mutations in a MADS-box transcription factor in apple (*Malus domestica*) (Yao et al., 2001). Recently, Carmi et al. (2003) reported that ovary-specific expression of the *rolB* gene induced parthenocarp in tomato.

cDNAs for genes that are preferentially expressed in pollinated fruit 3 days after anthesis in cucumber (*Cucumis sativum*) (Suyama et al., 1999) and those in pollinated fruit 3 days after anthesis (Nagasawa et al., 2001) and 2 days after anthesis (Nagasawa et al., 2004) in eggplant, were isolated by subtractive hybridization.

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cDNAs for genes, preferentially expressed in early fruit development, were isolated by differential display in tomato (Lemaire-Chamley et al., 2000; Tieman and Handa, 1996) and pollinated strawberry (*Fragaria* × *ananassa*) (Hamano et al., 1998), and by differential screening in auxin-treated strawberry (Reddy et al., 1990), peach (*Prunus persica*) (Callahan et al., 1993), kiwifruit (*Actinidia deliciosa*) (Ledger and Gardner, 1994), and apple (Dong et al., 1997, 1998). Interestingly, similar cDNA clones were isolated from several species; e.g. ribosomal protein, histone H2A, polyphenol oxidase, and cytochrome P450. However, the molecular signal(s) for fruit set and early fruit development are not clear.

A number of studies have been reported on the isolation of cDNAs for genes that are preferentially expressed during early fruit development, but they have not targeted the stage of fertilization except for our recent report (Nagasawa et al., 2004). In that report, we isolated cDNAs for genes preferentially expressed after/during fertilization (i.e., in pollinated fruit 2 days after anthesis) in eggplant (Nagasawa et al., 2004). Since fertilization is an important step for fruit set, cDNAs for genes that are preferentially expressed after/during fertilization are assumed to play important roles in the process. In this study, we aimed to isolate cDNAs for genes after/during fertilization in melon. Further, CPPU induces parthenocarp without pollination in melon (Hayata et al., 2000), whereas 4-CPA that induces parthenocarp in eggplant is less effective on parthenocarp in melon. Therefore, we analyzed the expression of genes in pollinated, CPPU-treated-, and 4-CPA-treated melon fruit.

Materials and Methods

Plant material

Anthems on melon (*Cucumis melo* L.) 'Prince' (Sakata Seed Co., Yokohama) seedlings growing in the field of Nagoya University, were removed 1 day before anthesis; the flowers were covered with paper bags to prevent natural pollination. Flowers were treated as follows: 1) hand-pollinated at anthesis (pollinated fruit); 2) sprayed unpollinated ovaries with 100 ppm CPPU containing 0.1% (w/v) Tween 20 (CPPU-treated fruit); 3) sprayed ovaries of unpollinated flowerws with 50 ppm 4-CPA mixed with 0.1% (w/v) Tween 20 (4-CPA-treated fruit); and 4) emasculated unpollinated flowers as a control (unpollinated fruit). After treatments, all flowers were covered with paper bags again. Fruits were sampled periodically, frozen immediately in liquid nitrogen, and stored at -80°C until analyzed.

Observation of pollen tube growth

Pollinated flowers, harvested at 12 and 24 hr after pollination in August 2000, were dissected, fixed, softened, and stained as described in previous paper

(Nagasawa et al., 2004). At least three flowers of each treatment were observed under a fluorescence microscope IX70 (Olympus, Co., Ltd. Tokyo) fitted with a combined filter (Olympus BP330-385, BA420, DM400).

Isolation of poly(A)⁺ RNA

Total RNA was extracted from 2 g of ovaries/fruits from which poly(A)⁺RNA was isolated as described by Suyama et al. (1999).

Construction of the subtracted cDNA library

The PCR-Select cDNA Subtraction Kit (Clontech, Palo Alto, CA) was used to make a cDNA library enriched for transcripts differentially abundant in pollinated fruit 1 day after anthesis. This protocol uses suppression PCR to enrich transcripts that are differentially expressed in the tester material (Diatchenko et al., 1996). The cDNA from pollinated fruit 1 day after anthesis was used as the tester, and the cDNA from unpollinated fruit 1 day after anthesis was used as the driver. Following PCR amplification, subtracted cDNAs were cloned into pT7Blue T-Vector (Novagen, Madison, WI) by using DNA Ligation Kit Ver. 2 (Takara Bio, Shiga).

Sequence of cDNA and analysis

Nucleotide sequences were determined by using a CEQ 2000 DNA sequencer (Beckman Coulter, Fullerton, CA) and CEQ Dye Terminator Cycle Sequencing Quick Start Kit (Beckman Coulter), or cDNA clones were sent to Dragon Genomics (Mie) for sequencing. Sequences were analyzed by using BLASTX against GenBank/EMBL/DDBJ non-redundant sequence databases.

Differential screening using cDNA macroarrays

Three hundred eighty-four cDNA clones were picked up randomly from the subtracted cDNA library; inserts were amplified by PCR (94°C for 30 sec, 55°C for 30 sec, 72°C for 1 min for 35 cycles) using the primers 1 and 2R (Clontech). To prepare membranes for cDNA macroarray, PCR products were denatured with 0.5N NaOH and spotted onto Hybond-N+ membrane (Amersham Biosciences, Piscataway, NJ) by using Multimicro filter DP-96 (Advantec, Tokyo). The first strand cDNA was synthesized from poly(A)⁺RNA isolated from a fruit. ³³P-labeled cDNA probe was synthesized from the first strand cDNA (300 ng) as a template. The membrane was hybridized with the ³³P-labeled cDNA probes (equal specific activities among probes); hybridization was carried out as described by Suyama et al. (1999).

Northern blot analysis

Total RNA (10 µg) was subjected to electrophoresis on 1.0% (w/v) agarose gels and transferred to Hybond-N+ membrane. The membrane was hybridized with ³²P-

labeled cDNA probes as above.

Results

Growth of pollen tubes

Tips of pollen tubes reached near the center of the ovary 12 hr after pollination (Fig. 1A). Almost all tips entered the ovules at the base of the ovary within 24 hr after pollination (Fig. 1B).

Fig. 1. Photomicrographs of pollen tube in melon ovaries. A: The center of an ovary 12 hr after pollination, B: The basal side of ovary 24 hr after pollination. Ovu, ovule; Pt, pollen tubes. Bars = 500 μ m.

Table 1. Fruit set (%) by hand-pollination, CPPU or 4-CPA treatment in melon.

Treatment	Fruit set (%)
Pollinated	100 (25/25) ^z
CPPU	100 (11/11)
4-CPA	8 (1/12)
Unpollinated (Control)	0 (0/15)

Emasculated flowers were treated at anthesis and fruit set was determined 7 days later.

^z Values in parentheses represent fruit set/treated flowers.

Fig. 2. Changes in fruit weight of melon during early fruit development. Values are means of five samples $\pm$ SE.

Fig. 3. Northern blot analyses of positive cDNA clones in pollinated fruit (+) and unpollinated ones (-) 1 day after anthesis. Each lane represents 10 μ g of total RNA extracted from each sample.

Table 2. Characterization of cDNA clones for genes that were preferentially expressed after/during fertilization in melon.

cDNA clone	Accession no.	Best database match	Source	Accession no.	E value
2A10	AB154522	Hypothetical protein	<i>Arabidopsis thaliana</i>	AC007213	2e ⁻⁵⁰
2C05	AB154523	Hypothetical protein	<i>A. thaliana</i>	AB022218	2e ⁻⁰⁸
2C07	AB154524	Ascorbate oxidase	<i>Cucumis melo</i>	AF233593	1e ⁻⁹¹
6A06	AB154525	Histone H2A	<i>Oryza sativa</i>	AP003144	1e ⁻³⁴
6D05	AB154526	Auxin binding protein	<i>Malus domestica</i>	U77952	2e ⁻⁴⁰
6E04	AB154527	14-3-3 protein	<i>Vigna angularis</i>	AB042299	e ⁻¹¹⁴
6F11	AB154528	Hydroxymethyltransferase	<i>A. thaliana</i>	AY086929	e ⁻¹³⁶
6H01	AB154529	Extensin-like protein	<i>C. sativus</i>	AF104392	2e ⁻²⁶
7E10	AB154530	Hypothetical protein	<i>A. thaliana</i>	AB012244	1e ⁻³²
7G02	AB154531	No hit	-	-	-
7G07	AB154532	Phosphoethanolamine N-methyltransferase	<i>Spinacia oleracea</i>	AF237633	5e ⁻⁹³
8A07	AB154533	Nucleoid DNA-binding-like protein	<i>O. sativa</i>	AP003833	6e ⁻⁰⁸
8C01	AB154534	Type IIIa membrane protein	<i>V. unguiculata</i>	AF005279	3e ⁻⁷⁴
		Reversibly glycosylated polypeptide	<i>Gossypium hirsutum</i>	AJ292078	3e ⁻⁷⁴
8D10	AB154535	Short chain alcohol dehydrogenase	<i>V. unguiculata</i>	D88121	7e ⁻⁵³

Fruit set of melon

Pollinated fruit developed rapidly 3 days after anthesis, whereas unpollinated fruit did not develop and aborted within a week. Exogenously applied CPPU, a synthetic cytokinin, induced the growth of ovaries without pollination. An application of 4-CPA, a synthetic auxin, to the ovaries without pollination had little effect on parthenocarpy (Fig. 2, Table 1). A 100% fruit set was obtained with CPPU, which is equivalent to that obtained by hand-pollination (Table 1). Pollinated fruit produced seeds, but CPPU- and 4-CPA-treated fruit were seedless at maturity.

Isolation of cDNA clones for genes expressed after/during fertilization in melon

Using suppression subtractive hybridization, the subtracted cDNA library was constructed and differentially screened by the probes of the cDNAs from pollinated or unpollinated fruit 1 day after anthesis, using cDNA macroarray. Eighty candidates of cDNA clones for genes that were expressed at a higher level in pollinated fruit than unpollinated ones, were selected after screening 384 cDNA clones of the subtracted cDNA library (data not shown). Some of these were used as probes for Northern blot analyses. Fourteen clones showed that hybridization signals with mRNA from pollinated fruit were stronger than those from unpollinated fruit by Northern blot analyses (Fig. 3).

Sequence analyses of isolated cDNAs

Several cDNA clones corresponding to mRNAs, whose expression in pollinated fruit increased compared with the unpollinated one, exhibited a similarity to enzymes: 2C07 showed a similarity to ascorbate oxidase from melon (Accession no. AF233593); 6F11 had a similarity to hydroxymethyltransferase from *Arabidopsis* (AY086929); 7G07 showed a similarity to phosphoethanolamine *N*-methyltransferase from spinach (*Spinacia oleracea*) (AF237633); 8D10 showed a similarity to short chain alcohol dehydrogenase from cowpea (*Vigna unguiculata*) (D88121) and watermelon (*Citrullus lanatus*) (AB018559).

Likewise, the deduced amino acid sequence of 6A06 showed a similarity to histone H2A from rice (*Oryza sativa*) (AP003144). 6D05 had a similarity to auxin binding protein from apple (U77952). 6E04 showed a similarity to 14-3-3 protein from azuki bean (*Vigna angularis*) (AB042299). 6H01 had a similarity to extensin-like protein from cucumber (AF104392). The deduced amino acid sequence of 8A07 had a similarity to nucleoid DNA-binding-like protein from rice (AP003833). 8C01 showed a similarity to both type IIIa membrane protein from cowpea (AF005279) and reversibly glycosylated polypeptide from cotton (*Gossypium hirsutum*) (AJ292078).

2A10, 2C05 and 7E10 showed a similarity to hypo-

thetical proteins from *Arabidopsis*, AC007213, AB022218, and AB012244, respectively. 7G02 had no significant similar sequence in the database. The results of sequence analyses of isolated cDNA clones are in Table 2.

The nucleotide sequence data reported in this paper has been submitted to the DDBJ, EMBL and GenBank nucleotide sequence databases with the accession numbers AB154522 (2A10), AB154523 (2C05), AB154524 (2C07), AB154525 (6A06), AB154526 (6D05), AB154527 (6E04), AB154528 (6F11), AB154529 (6H01), AB154530 (7E10), AB154531 (7G02), AB154532 (7G07), AB154533 (8A07), AB154534

Fig. 4. Northern blot analyses showing temporal expression during fruit development and organ specific expression of mRNAs corresponding to isolated cDNA clones. Each lane represents 10 μ g of total RNA extracted from each sample.

(8C01) and AB154535 (8D10).

Temporal and organ specific expression of mRNAs corresponding to isolated cDNA clones

Expressions of mRNAs corresponding to 14 cDNA clones were detected in the ovary at anthesis. 2C07, 6F11, 7G07 and 8C01 mRNAs were expressed in steady-state levels in the early development of pollinated fruit, whereas expressions of mRNAs corresponding to the other 10 cDNA clones increased during early development of pollinated fruit. mRNAs corresponding to all 14 cDNA clones were also expressed in CPPU-treated fruit between 1 and 3 days after anthesis. Transcripts of these 14 cDNA clones were barely detectable 20 days after anthesis. The expressions of mRNAs corresponding to all cDNA clones in pollinated and CPPU-treated fruit were higher than those in 4-CPA-treated fruit. Expression pattern of only 8D10 in pollinated fruit differed from that in CPPU-treated fruit. 6E04 mRNA was detected at a very low level in leaves, stems, and roots, whereas 6H01 mRNA was expressed at a high level in leaves (Fig. 4).

Discussion

Our photomicrographs (Fig. 1A, B) revealed that to isolate cDNAs for genes that signal fertilization, the ideal time to collect samples was 1 day after anthesis. In the melon 'Prince', pollination promoted the growth of ovaries, and CPPU, a synthetic cytokinin, had a similar effect on unpollinated ones (Fig. 2, Table 1). To understand the mechanism of fruit set, we compared the expression of mRNAs corresponding to isolated cDNAs among pollinated fruit, CPPU-treated fruit, 4-CPA-treated fruit and unpollinated fruit during the early stage of development (Fig. 4). mRNAs corresponding to isolated 14 cDNAs were expressed in both pollinated and CPPU-treated fruit, which suggests that genes corresponding to these cDNAs are related to the pericarp development, but not seed formation. Furthermore, their expression patterns in pollinated fruits were similar to those in CPPU-treated fruits during fruit development except for 8D10, which leads us to speculate that the mechanism of fruit set by CPPU is similar to that by pollination in many aspects.

Previously, we isolated cDNAs for genes preferentially expressed after/during fertilization (i.e. pollinated fruit 2 days after anthesis) in eggplant (Nagasawa et al., 2004). Their mRNAs corresponding to 3 cDNA clones were expressed at a higher level in pollinated and 4-CPA-treated fruit 2 to 4 days after anthesis than before pollination occurred. In this study, cDNAs for genes that were preferentially expressed after/during fertilization (i.e. pollinated fruit 1 day after anthesis) in melon were sought. Expressions of mRNAs corresponding to 10 of 14 cDNA clones increased after/during (i.e. between 1 to 3 days after anthesis) fertilization (Fig. 4) indicating that the mRNAs corre-

sponding to these cDNA clones were expressed increasingly during early fruit development when fruit set was being initiated.

2C07 showed a similarity to ascorbate oxidase. Ascorbate oxidase mRNA was expressed at a high level in ovaries in muskmelon (Diallinas et al., 1997). Ascorbate oxidase is induced by auxin in pumpkin (*Cucurbita* spp.) (Esaka et al., 1992). IAA content in mesocarp of pollinated fruit during early development was higher than that in the later stage in the melon 'Prince' (Lee et al., 1997); that of CPPU-treated fruit increased from anthesis to 2 days later in muskmelon (Hayata et al., 2002). In addition, expression of 2C07 was also induced by exogenous 4-CPA that had little effect on parthenocarp. These results suggest that expression of 2C07 may be induced by endogenous and/or exogenous auxin.

6F11 has a similarity to serine hydroxymethyltransferase, the enzyme that catalyzes the reversible conversion of serine and tetrahydrofolate to glycine and 5,10-methylene tetrahydrofolate (Schirch, 1982), which leads us to speculate that pollination initiates diverse metabolic activities in the early stage of fruit development.

7G07 shows a similarity to phosphoethanolamine *N*-methyltransferase, a key enzyme in the synthesis of choline, which is responsible for membrane synthesis (Rhodes and Hanson, 1993). Phosphoethanolamine *N*-methyltransferase mRNA may be expressed during the early stage of fruit development to synthesize membranes.

8D10 shows a similarity to short chain alcohol dehydrogenase that is expressed in pollinated watermelon seeds but not in the fruit 2 days after pollination (Kim et al., 2003). However, our results showed that 8D10 was expressed in both pollinated fruit and CPPU-treated seedless fruit in melon (Fig. 4). Kim et al. (2003) also reported that short chain alcohol dehydrogenase gene was inducible by gibberellin and sucrose. In melon, expression of 8D10 in CPPU-treated fruit was higher than that in pollinated fruit, which suggests that the treatment induced an increase in gibberellin and/or sucrose during early fruit development. Interestingly, short chain alcohol dehydrogenase cDNA was isolated by differential display from *parthenocarpic fruit (pat)* tomato ovaries (Testa et al., 2002). A possible involvement of short chain alcohol dehydrogenase in fruit set is supported by an increase in transcripts of 8D10 after/during fertilization that subsequently decreased rapidly. The transcripts were readily detected in parthenocarpic fruit induced by CPPU but barely in fruit treated with 4-CPA that had little effect on parthenocarp (Fig. 4).

The deduced amino acid sequence of 6A06 showed a similarity to histone H2A, whose cDNA was also isolated in our previous report (Nagasawa et al., 2001). We showed that histone H2A mRNA which is related to cell

division is expressed during early stage of development in eggplant just as 6A06 is related to cell division in melon.

6D05 has a similarity to an auxin binding protein, a candidate for an auxin receptor (Napier, 2001). In hot pepper (*Capsicum annum*), transcripts of auxin binding protein were found in the early and middle stages of fruit development (Choi, 1996). 6E04 showed a similarity to 14-3-3 proteins that are known to interact with other proteins and regulate their activities, involving in signal transduction (Sehnke et al., 2002). In addition, 14-3-3 protein cDNA was also isolated as a gene that is expressed during the early development of tomato fruit (Lemaire-Chamley et al., 2000). 6D05 and 6E04 may be involved in signal transduction.

The deduced amino acid sequence of 8A07 has a similarity to nucleoid DNA-binding-like protein. Nucleoid DNA-binding-like protein mRNA was activated by cytokinin in tobacco (Schafer et al., 2000). Our results also showed that the expression of 8A07 in CPPU-treated fruit was higher than that of 4-CPA-treated fruit (Fig. 4), suggesting that 8A07 is a cytokinin inducible gene.

8C01 showed a similarity to both type IIIa membrane protein and reversibly glycosylated polypeptide. The latter may play a role in plant cell wall biosynthesis as its mRNA is expressed at a high level during early cotton fiber development (Zhao and Liu, 2001). 6H01 has a similarity to an extensin-like protein whose transcripts were reported by Suyama et al. (1999) during the early stages of cucumber fruit development. Extensins are members of the family of cell wall proteins whose genes are expressed in young fruits of tomato (Salts et al. 1991). Hence, it is possible that 6H01 and 8C01 are related to cell wall synthesis that are required for cell enlargement.

2A10, 2C05 and 7E10 are similar to hypothetical proteins from *Arabidopsis*. To date, their functions are unknown, but 7E10 may play important roles in fruit set because of its specific expression in fruits (Fig. 4).

In melon, CPPU induced parthenocarp without pollination, whereas 4-CPA was less effective on parthenocarp. This strengthens the speculation that 8A07 (Nucleoid DNA-binding-like protein) and 8D10 (Short chain alcohol dehydrogenase) may play important roles in fruit set of melon, because they are expressed at a higher level in the early stages of both pollinated and CPPU-treated fruit than in 4-CPA-treated fruit.

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メロンの結実過程の受精時から受精後初期に発現する遺伝子の cDNA の単離

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摘 要

受精は結実のために特に重要な過程である。メロンでは受粉後 24 時間以内には花粉管の先端は子房の基部の胚珠に到達し、受精が完了していた。そこで、開花後 1 日目の受粉した子房と無受粉の子房に由来する mRNA の間でサブトラクションを行い、Subtracted cDNA ライブラリーを作製した。cDNA マクロアレイを用いたディファレンシャルスクリーニングとノーザン解析により、最終的に開花後 1 日目の受粉果実のほうが無受粉果実より発現の高い 14 個の cDNA クローンを得た。これらは、Ascorbate oxidase, Hydroxymethyltransferase, Phosphoethanolamine *N*-methyltransferase, Short chain alcohol dehydrogenase, Histone H2A, Auxin binding protein, 14-3-3 protein, Extensin-like protein, Nucleoid DNA-binding-like

protein, Type IIIa membrane protein (Reversibly glycosylated polypeptide) および Hypothetical protein と相同性があった。いずれも受粉果実および単為結果が引き起こされる CPPU 処理果実で発現しており、それらの発現は単為結果がほとんど引き起こされない 4-CPA 処理果実より高かった。これらは受粉果実および CPPU 処理果実の開花後 20 日目ではほとんど発現しておらず、また、そのほとんどが果実特異性の高い発現をしていた。これらの遺伝子のうち、CPPU 処理果実のほうが 4-CPA 処理果実より特に高い発現を示した 8A07 (Nucleoid DNA-binding-like protein) および 8D10 (Short chain alcohol dehydrogenase) がメロンの結実に特に重要な役割を果たしているのではないかと考えられた。