Determination of Ethylene Synthetic Genotypes Related to Ripening in Japanese Pear Cultivars

Akihiro Itai*, Takahiro Kotaki, Kenji Tanabe, Mari Fukuda, Yuko Kawata,
Yuri Amano and Naoko Fujita

Laboratory of Horticultural Science, Faculty of Agriculture, Tottori University, Tottori 680–8553

Summary

In Japanese pear (*Pyrus pyrifolia* Nakai), fruit storage potential is closely related to the maximum level of ethylene production. Previously we identified two markers (A and B) that are linked to the high and moderate ethylene production during fruit ripening respectively and developed the gene diagnosis for the process. This gene diagnosis was used to identify the ethylene synthetic genotypes of Japanese and Chinese pear cultivars by using 43 combinations from 1260 seedlings from which genotypes of 32 cultivars were determined. For example, genotypes of 'Kosui', 'Shinsui', 'Chojuro', 'Cili', and 'Yali' are *aaBb*, *aaBB*, *aaBb*, *AaBB*, and *AABB*, respectively. All the progenies of cultivars, such as 'Rokugatsu' (*Aabb*) and 'Shinsui' (*aaBB*), are predicted to produce high or moderate amounts of ethylene during fruit ripening. If the objective of breeding is storage ability, using these cultivars is inadequate. This information is critical to the breeding program for selection of parents and useful for breeding strategies to improve storage ability in Japanese pear.

Key Words: ACC synthase, ethylene synthesis, genotype, MAS (Marker assisted selection), shelf life.

Introduction

In breeding of Japanese pears, improvement of fruit quality is a major objective (Abe et al., 1993a; Machida and Kozaki, 1976), but there are a limited reports on the inheritance of fruit characters in Japanese and European pears (Abe et al., 1993a, 1995; Crane and Lewis, 1949; Machida and Kozaki, 1975, 1976). To increase breeding efficiency, it is important to elucidate the mode of inheritance of the main characters which affect fruit quality. There are wide genetic variations in the harvest season, storage potential, and ripening characters in pear cultivars.

It is generally thought that ethylene production affects the ripening processes and shelf life in many fruits. In Japanese pear, both climacteric and nonclimacteric cultivars exist (Itai et al., 1999; Kitamura et al., 1981). We reported that cultivars, such as 'Shinsui', 'Kosui', and 'Chojuro', exhibit a rapid increase in ethylene production and have a low storage potential, whereas cultivars, such as 'Nijisseiki', 'Shinko', and

'Okusankichi', evolve non-detectable levels of ethylene, while maintaining fruit quality for over a month. Moreover, ethephon, a synthetic ethylene-releasing chemical, is used to accelerate ripening in Japanese pear.

Marker assisted selection (MAS) is a useful tool for increasing selection efficiency in fruit trees as well as other crops. The process facilitates the evaluation of favorable traits at the seedling stage in fruit trees that have a long juvenile phase. To date, three molecular markers are associated with self-incompatibility, black spot disease, and pear scab (Banno et al., 1999; Iketani et al., 2001; Sassa et al., 1997; Takasaki et al., 2004).

Previously, we cloned three ACC (1-aminocyclopropane-1-carboxylate) synthase genes (*PPACS1*, 2, 3) and studied their expression during fruit ripening (Itai et al., 1999, 2003b). *PPACS1* is specifically expressed by cultivars with high ethylene producing potential, whereas *PPACS2* is specifically expressed by those moderate capacity. Moreover, we used RFLP analysis to identify two ACC synthase genes (*PPACS1* and *PPACS2*) (Itai et al., 1999) linked to ethylene evolution rate of ripening fruit. These RFLPs that appeared when the total DNA was digested by *HindIII* were designated as A (2.8 kb of *PPACS1*) linked to high levels of ethylene ($>10 \text{ nL} \cdot \text{g}^{-1} \text{ FW} \cdot \text{h}^{-1}$) and B (0.8 kb of *PPACS2*), linked to moderate levels of ethylene ($0.5\text{--}10 \text{ nL} \cdot \text{g}^{-1} \text{ FW} \cdot \text{h}^{-1}$). Based on this analysis, we classified cultivars into four RFLP types, AB, Ab, aB, and ab in which types AB and Ab showed high levels, while aB showed a moderate level

Received; December 1, 2004. Accepted; February 25, 2005.

This study was partially supported by Grant-in-aid nos. 14760019 and 14360022 from the Ministry of Education, Science, Sports and Culture and by a grant from Venture Business Laboratory (VBL) of Tottori University.

A part of this article was presented at the 2003 Spring Meeting of the Japanese Society for Horticultural Science.

* Corresponding author (E-mail: itai@muses.tottori-u.ac.jp).

of ethylene production during fruit ripening (Itai et al., 1999). Moreover, we transformed two RFLP markers (A, B) into more convenient and easier PCR based CAPS markers (A, 1.57 kb and 0.63 kb of *PPACS1* fragments; B, 0.83 kb and 0.35 kb of *PPACS2* fragments) (Itai et al., 2003b). These markers should be useful for the selection of Japanese pear seedlings with enhanced post-harvest keeping quality.

However, the identified CAPS markers are dominant markers so that our methods cannot distinguish the dominant homozygous genotypes from heterozygous ones. To do so, we hybridized cultivars with an unknown genotype to that which was homozygous recessive to determine their heterozygosity. Here we report that the allele-specific PCR-based method was applied to the test cross lines to determine the ethylene synthetic genotypes of 30 Japanese and 2 Chinese pear cultivars.

Materials and Methods

Plant material

The plant material was obtained from 43 combinations of controlled crosses between Japanese or Chinese pear cultivars. The cultivars used in this study, RFLP types, and ethylene production rate (Itai et al., 1999, 2003a) are listed in Table 1. Young leaf samples taken from 1260 seedlings derived from 43 crosses were stored frozen at -80°C until utilized for DNA analysis.

DNA extraction

DNA extraction protocol was a modified SDS method of Dellaporta et al. (1983) with some additional washing steps. Approximately one hundred mg of leaf material were ground in liquid nitrogen by using a mortar and pestle; the powder transferred to 1.5 mL tube containing 1 mL of washing buffer (0.1 M HEPES pH 8.0, 0.1% polyvinylpyrrolidone (K30), 2% 2-mercaptethanol) and mixed. The mixture was centrifuged at $15000 \times g$ for 10 min; the supernatant was discarded and the pellet was resuspended in 1 mL of the same washing buffer. This washing step was repeated 3 times to remove the soluble polysaccharides. After washing, the pellet was resuspended in 400 μL of extraction buffer (0.5 M NaCl, 100 mM Tris-HCl pH 8.0, 50 mM EDTA-Na, 2% SDS) and incubated at 70°C for 10 min. After incubation, 120 μL of 5 M potassium acetate was added to the mixture and centrifuged at $15000 \times g$ for 15 min. The supernatant was transferred to a new tube and an equal volume of 2-propanol was added to the solution in which DNA precipitated. Upon centrifugation, the supernatant was discarded and the precipitated DNA dissolved in 20 μL TE with 2 μL RNase A ($10\text{g} \cdot \text{L}^{-1}$), incubated at 37°C for 30 min, and stored at 4°C .

PCR analysis

Based on sequences of 5' flanking regions of *PPACS1* of 'Nijisseiki', (AB080678), forward (SYN1PU1: 5'-

GATGAAATAAAGTCCACAATCAAG-3') and reverse (SYN1PD1: 5'-GCGTTTCTGCATAACATGCG-3') primers were designed and used for segregations of marker A linked to high ethylene production (Itai et al., 2003a). The PCR amplification reaction was performed according to the previous report (Itai et al., 2003a). Amplified fragments were digested by *HindIII* for more than 3 h at 37°C ; the products were separated on 1.2% agarose gels in $1 \times \text{TAE}$ buffer and stained by ethidium-bromide. Segregation of marker A in progenies was analysed by the existence or the absence of CAPS marker (1.57 kb and 0.63 kb band; Fig. 1A).

Table 1. Parental cultivars used for the identification of ethylene synthetic genotype.

Cultivar	Ethylene production ^z	RFLP marker type ^y
Akaho	Moderate	aB
Awayuki	High	Ab
Choju	Moderate	aB
Chojuro	Moderate	aB
Cili	High	AB
Doitsu	Moderate	aB
Edoya	High	Ab
Gion	Moderate	aB
Gold Nijisseiki	Low	ab
Gozennashi	High	Ab
Hakuteiryu	Moderate	aB
Hokkaiwase	Moderate	aB
Hosui	Low	ab
Ichiharawase	Moderate	aB
Inugoroshi	Moderate	aB
Kimitsukawase	Moderate	aB
Kinchaku	Moderate	aB
Kosui	Moderate	aB
Kozo	Moderate	aB
Nijisseiki	Low	ab
Ninomiya	High	AB
Ninomiyahakuri	High	AB
Ohiromaru	Moderate	aB
Okuroku	High	Ab
Osanijisseiki	Low	ab
Rikiya	High	Ab
Rokugatsu	High	Ab
Seigyoku	Moderate	aB
Shinchu	Moderate	aB
Shinsui	Moderate	aB
Suisei	Moderate	aB
Suishu	Moderate	aB
Taihaku	Moderate	aB
Tama	Moderate	aB
Wasekoso	Moderate	aB
Yali	High	AB

^z Maximum ethylene production during fruit ripening (High: $>10 \text{ nL} \cdot \text{g}^{-1} \text{ FW} \cdot \text{h}^{-1}$, Moderate: $0.5-10 \text{ nL} \cdot \text{g}^{-1} \text{ FW} \cdot \text{h}^{-1}$, Low: $<0.5 \text{ nL} \cdot \text{g}^{-1} \text{ FW} \cdot \text{h}^{-1}$).

^y A, linked to high level of ethylene; B, linked to moderate level of ethylene (Itai et al., 1999, 2003a).

(A) *PPACS1* (B) *PPACS2*

Fig. 1. CAPS analysis of *PPACS1* (A) and *PPACS2* (B) genes in seedlings derived from test crosses. +, presence of A or B marker; -, absence of A or B marker.

Based on sequences of *PPACS2* cDNA (AB007631), SYN2F: 5'-GTCACAGAATCAACGATTGA-3' and SYN2R: 5'-AGTAGAACGCGAAAACAAAT-3' were designed and used to segregate marker B linked to moderate ethylene production. The PCR procedure was the same as previously reported (Itai et al., 2003a). Amplified fragments were also digested by *Hind*III and separated on 1.2% agarose gels in 1 × TAE buffer. After staining with ethidiumbromide, gels were observed and photographed under UV light. Segregation of marker B in progenies was examined by the existence or the absence of CAPS marker (0.83 kb and 0.35 kb band; Fig. 1B) (Itai et al., 2003a).

Statistical analysis

The fit between observed and expected segregation ratio (1:0 or 1:1) for both markers was tested by Chi-square analysis.

Results

Genotypes in high ethylene producers

The following high ethylene producers: 'Awayuki', 'Cili', 'Edoya', 'Gozenashi', 'Ninomiya', 'Ninomiya-hakuri', 'Okuroku', 'Rikiya', 'Rokugatsu' and 'Yali' were analyzed for genotype determination by using 11 combinations that included 1 reciprocal cross (Table 2). The six cultivars, 'Awayuki', 'Edoya',

'Gozenashi', 'Okuroku', 'Rikiya', and 'Rokugatsu', were RFLP type of Ab (Itai et al., 1999, 2003a). In these test cross combinations (ab × Ab or Ab × ab), all populations segregated 1:1 for high (Ab) and low (ab) except 'Rokugatsu'. The segregation ratio was consistent with the result from the reciprocal cross. The progenies of 'Rokugatsu' fitted the expected 1 Ab:0 ab ratio. These data suggested that 'Awayuki', 'Edoya', 'Gozenashi', 'Okuroku', and 'Rikiya' are heterozygous for A and that 'Rokugatsu' is homozygous for A. So the genotype of 'Awayuki', 'Edoya', 'Gozenashi', 'Okuroku' and 'Rikiya' is *Aabb* and the genotype of 'Rokugatsu' is *AAbb* (Table 3). 'Cili', 'Ninomiya', 'Ninomiya-hakuri', and 'Yali' were revealed to be RFLP type of AB (Itai et al., 1999, 2003a). Progenies of 'Hosui' × 'Ninomiya' did not fit the expected 1 AB:1 Ab:1 aB:1 ab ratio; thus a segregation distortion was observed. However, the genotype of 'Ninomiya' is predicted to be heterozygous for both A and B; hence it is an *AaBb* genotype. If 'Ninomiya' was predicted to be homozygous for both markers (A and B), all seedlings would have segregated as AB. Therefore, we conclude that the genotype of 'Ninomiya' was *AaBb*. Progenies of 'Hosui' × 'Ninomiya-hakuri' populations fit the expected 1 AB:1 Ab:1 aB:1 ab ratio, thus assigning the genotype of 'Ninomiya-hakuri' to be *AaBb*. Two progenies ('Osanijisseiki' × 'Cili' and 'Nijisseiki' × 'Yali') are both products of ab (Japanese pear) × AB (Chinese pear) populations. Consequently, progenies of 'Osanijisseiki' × 'Cili' fit the expected 1 AB:1 aB ratio, whereas those of 'Nijisseiki' × 'Yali' fit the expected 1 AB:1 Ab ratio, indicating that the genotype of 'Cili' is *AbBB* and that of 'Yali' is *AABb* (Table 3).

Genotypes in moderate ethylene producers

Thirty-two crosses including 9 reciprocal ones (Table 2) involving 22 cultivars of moderate ethylene producers were analyzed to determine their genotypes. The RFLP marker type of moderate ethylene producers was aB, so that their genotypes had to be either *aaBB* or *aaBb*. Progenies of 'Nijisseiki' × 'Chojuro' did not fit the expected 1 aB:1 ab ratio as same segregation distortion occurred. However, the genotype of 'Chojuro' was predicted to be *aaBb* because of the existence of ab seedlings. In the other cross combinations (ab × aB or aB × ab), all populations segregated 1:1 for moderate (aB) and low (ab) except 'Shinsui'. Thus, the genotype of 21 cultivars such as 'Akaho', 'Chojuro', 'Doitsu', and the other 17 cultivars except 'Shinsui' was assigned the genotype *aaBb*. The segregation ratio was consistent with results from reciprocal crosses. The progenies of 'Shinsui' fitted the expected 1 aB:0 ab ratio to suggest that 'Shinsui' is homozygous for B and its genotype is *aaBB*.

Table 2. Segregation of both markers in progenies derived from test crosses.

No.	mother	father	No.	AB	Ab	aB	ab	probability
1	Gold Nijisseiki	Akaho	30	0	0	16	14	0.715
2	Gold Nijisseiki	Ichiharawase	30	0	0	17	13	0.465
3	Gold Nijisseiki	Shinchu	30	0	0	15	15	1
4	Gold Nijisseiki	Suishu	32	0	0	15	17	0.724
5	Hosui	Awayuki	40	0	21	0	19	0.752
6	Hosui	Choju	30	0	0	13	17	0.465
7	Hosui	Doitsu	30	0	0	11	19	0.144
8	Hosui	Edoya	40	0	18	0	22	0.527
9	Hosui	Gion	30	0	0	13	17	0.465
10	Hosui	Gozennashi	30	0	18	0	12	0.273
11	Hosui	Hakuteiryu	15	0	0	7	8	0.796
12	Hosui	Hokkaiwase	12	0	0	4	8	0.248
13	Hosui	Inugoroshi	30	0	0	10	20	0.068
14	Hosui	Kimitsukawase	30	0	0	14	16	0.715
15	Hosui	Kinchaku	30	0	0	19	11	0.144
16	Hosui	Kozo	30	0	0	17	13	0.465
17	Hosui	Ninomiya	120	27	12	16	65	$1.20e^{-12}$
18	Hosui	Ninomiyahakuri	48	11	11	16	10	0.607
19	Hosui	Ohiromaru	30	0	0	12	18	0.273
20	Hosui	Okuroku	40	0	20	0	20	1
21	Hosui	Rikiya	40	0	20	0	20	1
22	Hosui	Seigyoku	33	0	0	16	17	0.862
23	Hosui	Suisei	30	0	0	17	13	0.465
24	Hosui	Taihaku	30	0	0	12	18	0.273
25	Hosui	Tama	15	0	0	9	6	0.439
26	Hosui	Wasekozo	30	0	0	15	15	1
27	Nijisseiki	Chojuro	30	0	0	8	22	0.011
28	Nijisseiki	Yali	41	23	18	0	0	0.435
29	Osanijisseiki	Kosui	27	0	0	13	14	0.847
30	Osanijisseiki	Shinsui	17	0	0	17	0	1
31	Osanijisseiki	Cili	24	9	0	15	0	0.221
32	Doitsu	Hosui	23	0	0	12	11	0.835
33	Gion	Hosui	24	0	0	10	14	0.414
34	Ichiharawase	Hosui	19	0	0	11	8	0.491
35	Inugoroshi	Hosui	18	0	0	11	7	0.346
36	Kimitsukawase	Hosui	24	0	0	12	12	1
37	Kinchaku	Hosui	24	0	0	11	13	0.683
38	Kozo	Hosui	14	0	0	9	5	0.285
39	Ohiromaru	Hosui	18	0	0	7	11	0.346
40	Rokugatsu	Hosui	12	0	12	0	0	1
41	Rikiya	Hosui	18	0	5	0	13	0.059
42	Taihaku	Hosui	24	0	0	9	15	0.221
43	Wasekozo	Hosui	18	0	0	10	8	0.637

No. 1–31 are ab × AB, Ab or aB cross combinations.

No. 32–43 are Ab or aB × ab cross combinations.

Table 3. Ethylene synthetic genotypes of Japanese and Chinese pear cultivars.

Genotypes	Cultivars
<i>AABb</i>	Yali
<i>AAbb</i>	Rokugatsu
<i>AaBB</i>	Cili
<i>AaBb</i>	Ninomiya, Ninomiyahakuri
<i>Aabb</i>	Awayuki, Edoya, Gozennashi, Okuroku, Rikiya
<i>aaBB</i>	Shinsui
<i>aaBb</i>	Akaho, Choju, Chojuro, Doitsu, Gion, Hakuteiryu, Hokkaiwase, Ichiharawase, Inugoroshi, Kimitsukawase, Kinchaku, Kosui, Kozo, Ohiromaru, Seigyoku, Shinchu, Suisei, Suishu, Taihaku, Tama, Wasekozo
<i>aabb</i>	Amanogawa, Atago, Gold Nijisseiki, Hosui, Imamuraaki, Konpeito, Niitaka, Nijisseiki, Okusankichi, Osanijisseiki, Ruisannashi, Sekaiichi, Shinko, Shinsetsu, Waseaka, Yokogoshi

Discussion

We previously identified RFLP markers (A and B) as being linked to the ethylene evolution rate of ripening fruit in Japanese pear cultivars (Itai et al., 1999, 2003a). By applying this gene diagnosis of both markers, four types (AB, Ab, aB, ab) were identified, based on RFLPs. RFLP types of AB and Ab show high levels of ethylene synthesis, followed by aB (moderate), and ab (low) during fruit ripening (Itai et al., 1999). The trait for low ethylene production is recessive so that the genotype of low ethylene producers carrying the RFLP type:ab must be *aabb*. A list of 16 *aabb* cultivars in Table 3 was derived from previous data on RFLP types (Itai et al., 1999, 2003a). Cultivars with marker AB cultivars have four possible genotypes (*AABB*, *AABb*, *AaBB*, *AaBb*); those with marker Ab also have two possible genotypes (*AAbb*, *Aabb*); whereas those with marker aB have two possible genotypes (*aaBB*, *aaBb*). Progenies from 'Hosui' × 'Ninomiya' and 'Nijisseiki' × 'Chojuro' in this study did not fit the expected ratio of segregation. Sanada et al. (1994) reported that segregation distortion of resistance to black spot disease was observed in 'Nijisseiki' × 'Chojuro' combination to indicate that lethal genes may exist in this combination. The 'Hosui' × 'Ninomiya' combination, an excess of AB or ab seedlings could segregate because both markers are linked to each other. These two markers are linked to each other at a recombination frequency of 20.8% (Itai et al., 2003a). Genotypes of 32 cultivars, with a high or moderate ethylene production (Table 3) represent a large number of moderate ethylene producers that correspond to the genotype *aaBb*. Of the tested cultivars that show moderate ethylene production, only 'Shinsui' was *aaBB*. Why a large number of *aaBb* cultivars and the scarcity of *aaBB* cultivars segregated is inexplicable. 'Shinsui' (*aaBB*) is one of the earliest maturing cultivar with a short shelf life, which lead us to propose that *aaBB* cultivars may produce more ethylene than *aaBb* cultivars that result in small, early maturing fruits with poor storage potential. This genetic correlation between ripening time and weight of fruits was observed by Abe et al. (1993b). Although *aaBB* seedlings have the economic advantage of ripening early, new cultivars are difficult to develop because they tend to be small and short-lived.

'Shinsui' is one of the earliest maturing cultivars with a short storage life. therefore, it is important as a breeding stock. However, if the objective of breeding is storage ability, using 'Shinsui' is inadequate because all the progenies of 'Shinsui' are predicted to have low storage potential. Our data suggests that seedlings of cultivars such as 'Rokugatsu' (*AAbb*), 'Cili' (*AaBB*), and 'Yali' (*AABb*) possess the potential to evolve high or moderate ethylene production during fruit ripening. Among the Chinese pears, 'Cili' and 'Yali' have good storage potential. Hiwasa et al. (2004) reported that

'Yali' lacked the gene expression of cell wall degrading enzyme, such as polygalacturonase, that may be responsible for their good storage potential. However, hybrids between Chinese and Japanese pears, such as 'Ninomiyahakuri' ('Yali' × 'Shinchu') and 'Kogetsu' ('Yali' × 'Nijisseiki') were reported to have a short shelf life (Kajiura and Sato, 1990). Although these cultivars may exhibit other desirable horticultural traits, they may be eliminated as breeding materials for improving storage quality.

In conclusion, we determined the ethylene synthetic genotypes of 32 cultivars including 2 Chinese cultivars. This information is critical to the breeding program for selection of parents and in breeding strategies to improve storage ability in Japanese pear.

Literature Cited

- Abe, K., Y. Sato, A. Kurihara and K. Kotobuki. 1993a. Inheritance of ripening time of fruit of Japanese pear (*Pyrus pyrifolia* Nakai). Japan. J. Breed. 43: 289–298.
- Abe, K., Y. Sato, T. Saito, A. Kurihara and K. Kotobuki. 1993b. Genetic correlation between ripening time and weight of fruits in Japanese pear (*Pyrus pyrifolia* Nakai). Japan. J. Breed. 43: 439–447.
- Abe, K., Y. Sato, T. Saito, A. Kurihara and K. Kotobuki. 1995. Narrow-sense heritability of fruit characters in Japanese pear (*Pyrus pyrifolia* Nakai). Breed. Sci. 45: 1–5.
- Banno, K., H. Ishikawa, Y. Hamazu and H. Tabira. 1999. Identification of a RAPD marker linked to the susceptible gene of black spot disease in Japanese pear. J. Japan. Soc. Hort. Sci. 68: 476–481.
- Crane M. B. and D. Lewis. 1949. Genetical studies in pears V. Vegetative and fruit characters. Heredity 3: 85–97.
- Dellaporta, S. L., J. Wood and J. B. Hicks. 1983. A plant DNA miniprep: version II. Plant Mol. Biol. Rep. 1: 19–21.
- Hiwasa, K., R. Nakano, A. Hashimoto, M. Matsuzaki, H. Murayama, A. Inaba and Y. Kubo. 2004. European, Chinese and Japanese pear fruits exhibit differential softening characteristics during ripening. J. Exp. Bot. 55: 2281–2290.
- Iketani, H., K. Abe, T. Yamamoto, K. Kotobuki, Y. Sato, T. Saito, O. Terai, N. Matsuta and T. Hayashi. 2001. Mapping of disease-related genes in Japanese pear using a molecular linkage map with RAPD markers. Breed. Sci. 51: 179–184.
- Itai, A., T. Kawata, K. Tanabe, F. Tamura, M. Uchiyama, M. Tomomitsu and N. Shiraiwa. 1999. Identification of 1-aminocyclopropane-1-carboxylic acid synthase genes controlling the ethylene level of ripening fruit in Japanese pear (*Pyrus pyrifolia* Nakai). Mol. Gen. Genet. 262: 42–49.
- Itai, A., T. Kotaki, K. Tanabe, F. Tamura, D. Kawaguchi and M. Fukuda. 2003a. Rapid identification of 1-aminocyclopropane-1-carboxylate (ACC) synthase genotypes in cultivars of Japanese pear (*Pyrus pyrifolia* Nakai) using

- CAPS markers. Theor. Appl. Genet. 106: 1266–1272.
- Itai, A., K. Tanabe, F. Tamura, and M. Tomomitsu. 2003b. Cloning and characterization of a cDNA encoding 1-aminocyclopropane-1-carboxylate (ACC) synthase (PPACS3) from ripening fruit of Japanese pear (*Pyrus pyrifolia* Nakai). J. Japan. Soc. Hort. Sci. 72: 99–106.
- Kajiura, I. and Y. Sato. 1990. Recent progress in Japanese pear (*Pyrus pyrifolia* Nakai) breeding and descriptions of cultivars based on literature review. Bull. Fruit Tree Res. Stn. Extra 1: 97–329.
- Kitamura, T., T. Iwata, T. Fukushima, Y. Furukawa and T. Ishiguro. 1981. Studies on the maturation-physiology and storage of fruits and vegetables II. Respiration and ethylene production in reference to species and cultivars of pear fruit. J. Japan. Soc. Hort. Sci. 49: 608–616.
- Machida, Y. and I. Kozaki. 1975. Quantitative studies on the fruit quality for Japanese pear (*Pyrus serotina* Rehder) breeding. I. Statistical analysis of cultivar population. J. Japan. Soc. Hort. Sci. 44: 235–240.
- Machida, Y. and I. Kozaki. 1976. Quantitative studies on the fruit quality for Japanese pear (*Pyrus serotina* Rehder) breeding. II. Statistical analysis of a hybrid seedling population. J. Japan. Soc. Hort. Sci. 44: 325–329.
- Sanada, T., K. Sagisaka, J. Soejima, T. Moriguchi, S. Teramoto and K. Kotobuki. 1994. Inheritance of intermediate resistance to black spot disease in an induced Japanese pear mutant, 'Gold Nijisseiki'. J. Japan. Soc. Hort. Sci. 62: 689–693.
- Sassa, H., H. Hirano, T. Nishio and T. Koba. 1997. Style-specific self-compatible mutation caused by deletion of the S-RNase gene in Japanese pear (*Pyrus serotina*). Plant J. 12: 223–227.
- Takasaki, T., K. Okada, C. Castillo, Y. Moriya, T. Saito, Y. Sawamura, N. Norioka, S. Norioka and T. Nakanishi. 2004. Sequence of the S-9-RNase cDNA and PCR-RFLP system for discriminating S-1- to S-9-allele in Japanese pear. Euphytica 135: 157–167.

ニホンナシ品種における果実成熟エチレン生成に関する遺伝子型の決定

板井章浩・小瀧貴大・田辺賢二・福田真理・川田裕子・天野由梨・藤田直子

鳥取大学農学部 680-8553 鳥取市湖山町

摘 要

ニホンナシ果実のエチレン生成量は、品種間で著しい差がみられ、その生成能力が貯蔵性と密接に関わっている。著者らはこれまで、ニホンナシ ACC 合成酵素 PPACS1 および PPACS2 遺伝子の RFLP 分析によりエチレン生成量が著しく多い ($>10 \text{ nL} \cdot \text{g}^{-1} \text{ FW} \cdot \text{h}^{-1}$) 品種に特異的なマーカー A および、エチレン生成量が中程度 ($0.5 \sim 10 \text{ nL} \cdot \text{g}^{-1} \text{ FW} \cdot \text{h}^{-1}$) の品種に特異的なマーカー B を同定している。そこで本実験では、検定交雑を行い、様々なエチレン生成を示す品種の遺伝子型の決定を行った。全 32 品種の遺伝子型を決定した結果、例えば、'幸水' は $aaBb$ 、'新水' は $aaBB$ 、'長

十郎' は $aaBb$ 、'慈梨' は $AaBB$ 、'鴨梨' は $AABb$ のように各品種の遺伝子型が決定された。'新水' ($aaBB$) や '六月' ($AAbb$) といった A または B マーカーの遺伝子型がホモの品種は、その後代すべてが多量または中程度のエチレン生成を示すと考えられるため、貯蔵性を目的とした育種を行う場合、これらの品種を育種親と用いるのは不適であると考えられた。このようにエチレン生成に関する遺伝子型を決定することは、今後貯蔵性の良い品種を育種する際に重要な遺伝情報となると考えられた。