Response of the Major Allergen Fra a 1.01 in Strawberry to Cold

Shunji Okochi**, Misaki Ishibashi**, Hiroki Yoshikawa and Yuichi Uno*

Department of Plant Resource Science, Graduate School of Agricultural Science, Kobe University, Kobe 657-8501, Japan

Fra a 1 is a strawberry allergen that causes oral allergic syndrome. Fra a 1.01 is a major isoform that accumulates abundantly in fruits during the winter season. Here, we tested the hypothesis that Fra a 1.01 responds to environmental factors, such as cold stress. We analyzed transcriptional and translational levels of Fra a 1.01 in strawberry calli and organs under various cold conditions. First, we incubated strawberry (Fragaria × ananassa ‘Akihime’) calli and post-harvested fruits at low temperatures from several hours to days. Fra a 1.01 did not show significant differences in either gene expression or protein accumulation levels, suggesting that short-term cold treatments did not affect Fra a 1.01 expression. Second, we exposed whole plants to low temperature conditions for ~28 days. Under conditions below 10°C, Fra a 1.01 transcripts were induced gradually throughout the cold treatment (crown and root), or from 2 days to the last day (leaf and fruit). The Fra a 1.01 protein remarkably accumulated in crowns and slightly in fruits after 28 days. Finally, the promoter region of Fra a 1.01e was analyzed to detect tissue-specific expression. The cloned and sequenced promoter included several cis-acting regulatory elements related to cold response. When the Fra a 1.01 promoter region was heterologously expressed in Arabidopsis, the promoter activities, as assessed by GUS staining, were observed mainly around the shoot apices and in roots. Thus, Fra a 1.01 was considered to be expressed in crowns and roots, and was additively induced by cold stress. These organ-specific expressions could be important in elucidating the mechanisms responsible for Fra a 1.01 protein’s accumulation in fruits during the winter season.

Key Words: Fragaria × ananassa, low temperature, oral allergic syndrome, organ, promoter.

Introduction

Strawberry fruits contain various nutrients that promote human health, such as minerals, vitamins, and polyphenols (Afrin et al., 2016; Haugeneder et al., 2019). In addition, strawberries contain an allergen that causes oral allergic syndrome (OAS). Strawberry OAS is an IgE-mediated food allergy that results in oral itching and/or swelling (Ebisawa et al., 2017; Kelava et al., 2014). In severe cases, anaphylactic reactions and asphyxia caused by difficulty breathing have been reported due to strawberry allergy (Bajraktarevic et al., 2011). In the Kanto region of Japan, 13% of OAS patients sensitized with Rosaceae fruits show allergic reactivity to strawberry fruits (Ono et al., 2007). Because these patients tended not to display OAS symptoms when avoiding eating strawberry fruits (Bohle et al., 2006), OAS could lead to a slump in the consumption of strawberry fruit. Recently, an allergen-silenced apple was produced and evaluated through a clinical trial targeting allergic patients sensitized with the major allergen Mal d 1 (Dubois et al., 2015). Thus, the industry desires to produce less allergenic fruit through breeding and cultivation.

In the cultivated species Fragaria × ananassa, the major allergen is Fra a 1 (Hjerno et al., 2006). Fra a 1 is a member of the pathogenesis-related 10 (PR-10) subfamily, and several isoforms have been reported (Amil-Ruiz et al., 2011; Muñoz et al., 2010; Musidlowska-Persson et al., 2007). In the F × ananassa genome, many paralogs were conserved (Hirakawa et al., 2014). RNA sequencing analyses revealed that various paralogs are expressed in each organ (Ishibashi et al.,
2018; Toljamo et al., 2016). Fra a 1.01 and Fra a 1.02 have been reported to be the most important allergens during strawberry development and in OAS (Franz-Oberdorf et al., 2016; Ishibashi et al., 2018). In previous research, we selected Fra a 1.01 as the target Fra a 1 isoform in strawberry plants (Ishibashi et al., 2018).

Fra a 1 shows various expression patterns at both the transcriptional and translational levels. In the fruit, each Fra a 1 paralog is differentially expressed at the ripening stage (Ishibashi et al., 2017; Petriccione et al., 2017). In vegetative organs, the expression levels of several paralogs can be induced by pathogen infection (Besbes et al., 2019; Toljamo et al., 2016). Several external stimuli induce Fra a 1 expression, including UV-C radiation, chitosan treatments, and achene removal (Ishibashi et al., 2017; Petriccione et al., 2017; Severo et al., 2015). Differences in Fra a 1 expression levels in varieties have been reported in some cultivation areas (Alm et al., 2007; Franz-Oberdorf et al., 2016); however, differences, as assessed by Fra a 1 protein accumulation levels, have not been documented in various production areas, including Japan (Franz-Oberdorf et al., 2017; Ishibashi et al., 2019b; Kurze et al., 2018).

We revealed a seasonal variation in Fra a 1.01 accumulation, which was especially enhanced in the winter season (Ishibashi et al., 2019b). We hypothesized that Fra a 1.01 transcription and/or the Fra a 1.01 protein responds to cold stress. Several PR-10 orthologs are induced by cold stress under pre- or post-harvest conditions, including those in Pinus monticola (PmPR10; Liu et al., 2003), Morus bombycis (WAP18; Ukaji et al., 2004), and Prunus persica (Pru p 1; Giraldo et al., 2012). Strawberry plants are basically cultivated as herbaceous annuals, and their shelf lives are shorter than those of other Rosaceae fruits, like apple and peach. Strawberry cultivation and distribution are closely related to low temperature treatments. Low temperatures promote floral differentiation in June-bearing strawberries (Kurokura et al., 2017; Nakano et al., 2015); and cold conditions are required in the transport of fruits (Gross et al., 2016; Itoh et al., 1992; Zhao et al., 2019). Thus, it is important to understand the responsiveness of Fra a 1.01 to cold stress. In this study, we used various low temperature treatments during pre- and post-harvesting, and examined the expression patterns of Fra a 1.01 transcriptional and translational levels in strawberry organs.

**Materials and Methods**

**Plant materials**

Strawberry plants (*F. × ananassa*, Duch. ‘Akihime’) were originally purchased from a nursery company (Takii & Co., Ltd., Kyoto, Japan), and propagated vegetatively by runners. These materials were used for almost all the experiments, except for post-harvest treatments and those involving transgenic *Arabidopsis*. For the post-harvest experiment, ripe fruits (‘Akihime’) were purchased directly from a farmer’s store in Kobe City, Japan in January 2018. *Arabidopsis thaliana* (ecotype Columbia) seeds were obtained from the Arabidopsis Biological Resource Center (https://abrc.osu.edu/; Columbus, OH, USA) and used as a heterologous expression system in a histochemical GUS assay.

**Callus induction, sub-culturing and cold treatments**

Viruses-free plants were generated from the shoot apices of strawberry runners according to Asao et al. (1994). Leaf petioles of the virus-free plants at 2–6 months of age were cut into segments of approximately 1.0 cm, and cultured on MS solid medium (Murashige and Skoog, 1962) supplemented with 0.4 mg·L⁻¹ 3-indolebutyric acid and 1.5 mg·L⁻¹ thidiazuron in a 25-mL culture dish (Yonghua et al., 2005). Each petiole was sub-cultured twice in new medium every two weeks. Induced calli from cut surfaces of the petioles were separated and transferred under the same conditions onto the new MS solid medium in a 50-mL culture vessel. Four pieces of calli per vessel were cultured for approximately 30 days at 25°C with fluorescent lighting (35 μmol·m⁻²·s⁻¹, photoperiod of 16 h) until the cold treatments began. The calli grown in culture vessels were incubated for 1, 2, 5, 10, and 24 h at 25 or 4°C without light in a low-temperature incubator (Nippon Medical & Chemical Instruments Co., Ltd., Osaka, Japan). All the samples were immediately frozen in liquid nitrogen. Frozen samples were ground at 2,000 rpm by a multi-bead shoker (Yasui Kikai Corporation., Osaka, Japan) and stored at −80°C prior to protein and RNA extraction.

**Chilled storage of post-harvested fruits**

Samples of four post-harvested ripe fruits were placed in plastic containers and chilled at 0°C for 1, 5, and 10 days without light in a low-temperature incubator (Nippon Medical & Chemical Instruments). Fruit color was evaluated using a Handy Spectrophotometer NF333 (Nippondenshoku Industries Co., Ltd., Tokyo, Japan). Sampling and cryopreservation were performed as described in the preceding section.

**Growth conditions and the whole-plant cold treatment**

Runner plants were grown in a greenhouse at Kobe University from October 2018 to July 2019. Five plantlets were cultivated per plastic container. The containers were filled with general culture soil according to Ishibashi et al. (2019b). One of the containers, in which some plantlets bore red fruits after artificial pollination, was transferred to a low-temperature room with fluorescent lighting (from 4 to 10°C, 50 μmol·m⁻²·s⁻¹, photoperiod of 16 h; Fig. S1) in June 2019, and the other stayed in a greenhouse to be used as the control. Every strawberry plant was sampled at 2, 14, and 28 days after the low-temperature treatment began. The ripe fruits,
leaves (leaf blades), crowns, and roots of plants were immediately sampled. These samples were frozen, ground and stored as described above.

**RNA expression analysis**

RNA extraction, quantification, reverse transcription, and real-time PCR were performed as described by Ishibashi et al. (2017, 2019a).

For the expression analysis of callus mRNA, RT-PCR was carried out using a T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA). The PCR assay was performed in accordance with the real-time PCR described above, except for the thermal cycling conditions, which were stopped at 22 cycles for Fra a 1.01 and at 18 cycles for the control elongation factor 1α to avoid saturated amplifications.

**Protein extraction and immunoblotting**

For protein extraction and fluorometric determination, we mainly followed the protocol of Ishibashi et al. (2019b), with some modifications. For the detection of Fra a 1.01, 0.05 μg protein from calli, 0.5 μg protein from fruits, and 0.1 μg protein from leaf blades, crowns, and roots were each dissolved in SDS-PAGE sample buffer. To confirm the electrophoretic patterns, each protein was separated by SDS-PAGE using 15% acrylamide gels and stained with CBB Stain One Super solution (Nacalai Tesque, Inc., Kyoto, Japan) (Fig. S2).

**Amplification of the 5' region of Fra a 1.01 using TAIL-PCR**

The gDNA was extracted from young leaves using a Nucleon Phytopure Genomic DNA Extraction Kits (GE Healthcare UK Limited, Buckinghamshire, UK) according to the instruction manual. TAIL-PCR was carried out using a T100 Thermal Cycler as described by Fujita et al. (1998). The first PCR assay solution contained 1 U TaKaRa Ex Taq (TaKaRa Bio Inc., Shiga, Japan), Ex Taq buffer, 0.2 mM dNTPs, 0.2 μM Gene Specific Primer (GSP) 1, 200 ng gDNA, and 5.0 μM degenerated primers. The thermal cycling conditions were as follows: 95°C for 60 s, three-step amplification with 5 cycles of 94°C for 30 s, 65°C for 30 s, and 72°C for 210 s, next 94°C for 30 s, 30°C for 30 s, and 72°C for 210 s, nine-step amplification with 13 cycles of 94°C for 30 s, 68°C for 30 s, 68°C for 210 s, 94°C for 30 s, 68°C for 210 s, 94°C for 30 s, 44°C for 30 s, 68°C for 210 s, and finally, 68°C for 300 s. The second PCR assay solution contained 0.8 U TaKaRa Ex Taq, Ex Taq buffer, 0.2 mM dNTPs, 0.2 μM GSP2, 1/50 diluted first PCR product solution, and 4.0 μM degenerated primers. The thermal cycling conditions were as follows: 95°C for 60 s, nine-step amplification with 13 cycles of 94°C for 30 s, 68°C for 30 s, 68°C for 210 s, 94°C for 30 s, 68°C for 210 s, 94°C for 30 s, 44°C for 30 s, and 68°C for 210 s, and finally, 68°C for 300 s. The third PCR assay solution contained 0.5 U TaKaRa Ex Taq, Ex Taq buffer, 0.2 mM dNTPs, 0.3 μM GSP3, 1/50 diluted second PCR product solution, and 3.0 μM degenerated primers. The thermal cycling conditions were as follows: 94°C for 60 s, 95°C for 120 s, nine-step amplification with 13 cycles of 94°C for 30 s, 68°C for 30 s, 68°C for 210 s, 94°C for 30 s, 68°C for 30 s, 68°C for 210 s, 94°C for 30 s, 44°C for 30 s, 68°C for 210 s, and finally, 68°C for 300 s. Each of the following GSP primers was designed manually: Fra a 1.01e2-TAIL-GSP1, AAACGTCAGCTTAGGTTGGTCA; Fra a 1.01e2-TAIL-GSP2, GAAGTAAATCAGTTATACCTCCA; and Fra a 1.01e2-TAIL-GSP3, GAGGATCTCGGCACCTCA AC. The following degenerated primers were previously published in Fujita et al. (1998): A1, NGTCGASWG ANAWGAA; A2, GTNCGASWCANAWGTT; and A3, WGTGNAWGWACANAGA.

**Cloning and sequencing of the Fra a 1.01 promoter**

The TAIL-PCR-amplified products were gel purified using the Gel/PCR DNA Isolation System (Viogene-Biotek Corp, Taipei, Taiwan), cloned into the pANT vector (Nippon Gene Co., Ltd., Tokyo, Japan), and sequenced. Sequences were aligned using CLC Main Workbench 7.7 (QIAGEN, Aarhus, Denmark). cis-acting elements in the promoter were identified using the PLACE database (Higo et al., 1999).

**Transformation of the promoter region into A. thaliana**

The cloned promoter region was inserted into the SflI site of the pRAFLentr vector (Ogawa et al., 2008) using an In-Fusion HD Cloning Kit (TaKaRa Bio). The sequence was recombined with the upstream region of the GUS open reading frame in the pFAST-G04 destination vector using the LR reaction of the Gateway system (INPLANTA INNOVATIONS Inc., Yokohama, Japan). The vector construct was transformed into Agrobacterium tumefaciens GV3101, and the suspension was used for infecting A. thaliana through the floral dip method (Clough and Bent, 1998). Infected plants were incubated for 24 h in the dark and grown for appropriately 3 weeks at 23°C with a photoperiod of 16 h. T2 lines were used to confirm the transformation by RT-PCR and GUS assays. gDNA was extracted from T2 leaves using Nucleon Phytopure Genomic DNA Extraction Kits. The RT-PCR to confirm the transformation was performed using a T100 Thermal Cycler. The PCR assay and thermal cycling were performed in accordance with the KOD Plus Neo (TOYOBO Co., Ltd., Osaka, Japan) instruction manual. Specific primers were designed for Fra a 1.01-GUS-F, GGAGACCTTCGGAGACCTTC GUS-R, TGATCAATTCCACTGAGCTG; and GUS-R, TGATCAATTCCACTGAGCTG.

**Histological GUS assay**

At 20 days after sowing, T2 seedlings were soaked in GUS reaction solution [1 mM 5-bromo-4-chloro-3-
indolyl β-D-glucuronide cyclohexylammonium salt; 50 mM phosphate buffer (pH 7.5), 5 mM dithiothreitol, and 5% methanol] and evacuated for 30 min. The samples were incubated at 37°C overnight and then decolorized three times with 100% methanol.

**Statistical analyses**

Statistical analyses were performed using JMP 13 (SAS Institute, Cary, NC, USA). We performed Shapiro-Wilk tests to determine the propriety of the normal distribution, and determined significant differences based on non-parametric analyses. A two-way ANOVA was applied to analyze the whole-plant experiments.

**Results and Discussion**

**Expression of Fra a 1.01 in strawberry calli**

The Fra a 1.01 protein is a major allergen encoded by *Fra a 1* paralogs (Ishibashi et al., 2018; Musidlowska-Persson et al., 2007). It accumulates particularly in fruits grown during the winter season (Ishibashi et al., 2019b). Our hypothesis was that the cold might be a key trigger to induce *Fra a 1.01* accumulation in the winter. Therefore, in this study, the responses of *Fra a 1* to low-temperature treatments were investigated in calli and various organs during the early or late stress period. First, calli were used as undifferentiated cells to eliminate the organ-specific functions. The treatment temperature was set at 4°C based on a previous study in which several *A. thaliana* cold-responsive genes under the control of the DREB transcription factor were induced within several hours at 4–10°C (Kidokoro et al., 2017).

The *Fra a 1.01* gene expression level, as assessed by RT-PCR, showed no transcriptional changes at 4°C or 25°C within 24 h (Fig. 1). No translational changes were observed among cold treatments or time courses (Fig. 2). This unchanged expression was maintained for 5 days under cold treatment (data not shown). It was possible that *Fra a 1.01* did not respond to cold during the early stress period. Otherwise the phenomenon may not occur in undifferentiated cells. Next, we focused on longer stress periods and organ-specific responses of *Fra a 1*.

**Effects of post-harvest cold storage on strawberry fruits**

The United States Department of Agriculture reported that strawberry fruits can be stored for up to 7 days at 0°C, depending on disease pressure (Gross et al., 2016). Using this knowledge and some other references (Gross et al., 2016; Itoh et al., 1992), we stored post-harvested ripe fruits at 0°C for 1, 5, and 10 days. Fruits had decreased *b* values, which represent the color differences between yellow (positive values) and blue (negative values), after 5 and 10 days of cold storage (Table S1). In addition, *L* × *b*/*a* values, which indicate ripening degrees (Wang et al., 1988), significantly decreased after 10 days of cold storage (Wicoxon’s test, *P* < 0.05). After 10 days of storage, the fruits’ quality began to decrease. However, fruits in cold storage did not become mildewed like those stored at room temperature (data not shown).

The gene and protein expression levels of *Fra a 1.01* showed no significant differences over the whole storage period (Figs. 3 and 4). This result was not consistent with the report by Li et al. (2015); that *Pru av 1*-like protein (other *Fra a 1* isoform) decreased during the cold storage. In nectarine (*Prunus persica* Batsch, var. *nectarina*) and apple (*Malus × domestica*), cold storage...
increased Pru p 1.05 (Giraldo et al., 2012) and Mal d 1 (Sancho et al., 2006), respectively. The differential expression between strawberry and these Rosaceae fruits might result from the regulatory mechanism of expression or could be based on specific species characteristics, such as after-ripening type (i.e., climacteric or non-climacteric fruits). In this experiment, the strawberry fruits were ripe because they were obtained from a farmer’s store. In the case of the market, unripe fruits are transported because of their long shelf life and transportability. These materials should be examined to decide whether strawberries can be shipped under cold storage without increasing Fra a 1.01.

**Effects of cold treatments on whole plants**

To determine the long-term effects of cold treatments on whole plants, we cultivated runner plants in plastic containers in a controlled low-temperature room at less than 10°C (Fig. S1). The transcript levels of *Fra a 1.01* were gradually up-regulated throughout the cold treatment (crown and root), except for a transient reduction from 0 to 2 days (leaf and fruit) (Fig. 5). In contrast, there was no trend in the control plot. The transcripts increased more than twofold under cold conditions compared with under control conditions in the crown, root, and fruit at 14 days. These inductions were sustained to 28 days with marked levels in both the crown and root. The two-way ANOVA indicated that these two organs were affected significantly by treatment, duration, and their interaction (Table 1). We considered that this effect was due to low temperature, not other environmental factors such as light intensity. This was because shaded strawberry fruits did not show significant changes in the *Fra a 1.01* content (Ishibashi et al., 2019b). In addition, Severo et al. (2015) reported that UV-C treatment caused an accumulation of *Fra a 1* in strawberry fruits. This suggested that *Fra a 1* could not be induced under weak light conditions by a fluorescent lamp including low-level ultraviolet rays in a controlled low-temperature room.

The *Fra a 1.01* protein showed marked (in the crown) and slight (in the fruit) accumulation levels during the cold treatment compared with control conditions after 28 days (Fig. 6).

In previous proteomic research, the amount of *Fra a 1*
The tissue-specific activity of the Fra a 1.01 promoter was observed by ectopic expression in Arabidopsis. In a GUS histochemical assay, transgenic plants harboring the Fra a 1.01 promoter sequence showed strong GUS activity levels specifically around shoot apices and in roots (Fig. 7). The expression pattern was similar to the results of the real-time PCR of Fra a 1.01 in crowns and roots (Fig. 5). PR-10 orthologs, such as PmPR10-1.14 in lateral roots (Pinus monticola; Liu and Ekramoddoullah, 2003), Ypr10 in vascular bundles of leaves (Phaseolus vulgaris; Walter et al., 1996), and PR10g in anthers (Lilium longiflorum;
Hsu et al., 2014), have tissue-specific promoter activities. The activity of Fra a 1.01-pro in this study was similar to that of PmPR10-1.14 (Liu and Ekramoddoullah, 2003). This promoter region contains 19 AAAG boxes, which are cis-regulatory elements of plant-specific transcription factor Dof proteins (Yanagisawa and Schmidt, 1999). Over 30 AAAG elements were conserved in the promoter region of Fra a 1.01e2 (Fig. S3), and these sequences might affect their physiological regulation by some Dof proteins (Yanagisawa, 2002).

In conclusion, short-term low temperature treatments of calli and post-harvest cold treatments of fruits might not affect Fra a 1.01 expression or protein accumulation. On the contrary, long-term cold treatments of whole strawberry plants significantly increased the transcript level of the Fra a 1.01 gene in crown and root. The Fra a 1.01e gene promoter showed tissue-specific expression patterns around shoot apices and in roots. These results suggest that when strawberry plants experience low temperatures for 28 days or longer, they accumulate more Fra a 1.01 protein than under normal conditions. Other organs may be the key to elucidating the accumulation mechanisms in fruit.

Acknowledgements

We thank Dr. Takeshi Nabe (Setsunan University) for providing antibodies. We thank Drs. Ro Osawa (Kobe University), Yoko Nitta (Okayama Prefectural University), Miho Iduhara (Bistir Inc.), Hideyuki Inui (Kobe University), and Michio Kanechi (Kobe University) for their helpful discussions. We thank Mr. Rikuo Furukawa (Kobe University) for his technical assistance.

Literature Cited

Afrin, S., M. Gasparrini, T. Y. Forbes-Hernandez, P. Rehoredo-Rodriguez, B. Mezzetti, A. Varela-López, F. Giampieri and M. Battino. 2016. Promising Health Benefits of the Strawberry: A Focus on Clinical Studies. J. Agric. Food Chem. 64: 4435–4449.

Alm, R., A. Ekefjärd, M. Krogh, J. Häkkinen and C. Emanuelsson. 2007. Proteomic variation is large within as between strawberry varieties. J. Proteome Res. 6: 3011–3020.

Amil-Ruiz, F., R. Blanco-Portales, J. Muñoz-Blanco and J. L. Caballero. 2011. The strawberry plant defense mechanism: A molecular review. Plant Cell Physiol. 52: 1873–1903.

Asao, H., S. Arai, T. Satou, M. Hirai and T. Hibi. 1994. Transformation of strawberry using Agrobacterium tumefaciens. Plant tissue Cult. Lett. 11: 19–25 (In Japanese with English abstract).

Bajraktarevic, A., S. Tominic, S. Penava, A. Mahinic, B. Begovic, A. Selmovic, S. Kurtagic, T. Frankic, J. Gucic, A. Hujic and L. Sporisovic. 2011. Prevalence of strawberry allergen in Bosnian children and management. Clin. Transl. Allergy 1 (Suppl. 1): 45.

Baker, S. S., K. S. Wilhelm and M. F. Thomashow. 1994. The 5’-region of Arabidopsis thaliana cor15a has cis-acting elements that confer cold-, drought- and ABA-regulated gene expression. Plant Mol. Biol. 24: 701–713.

Besbes, F., R. Habegger and W. Schwab. 2019. Induction of PR-10 genes and metabolites in strawberry plants in response to Verticillium dahliae infection. BMC Plant Biol. 19: 128.

Bohle, B., B. Zwo, A. Heratizadeh, B. Jahn-schmid, Y. D. Antonia, M. Alter and W. Keller. 2006. Cooking birch pollen–related food: Divergent consequences for IgE- and T cell–mediated reactivity in vitro and in vivo. J. Allergy Clin. Immunol. 118: 242–249.

Clough, S. J. and A. F. Bent. 1998. Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J. 16: 735–743.

Dan, K., W. Sugeno, S. Nakahara, N. Goto, Y. Iwasaki, I. Takano, M. Okimura, K. Hidaka, T. Takayama and H. Imamura. 2015. Experiment on the crown-temperature control technique in forcing culture of strawberries in Miyagi. Bull. NARO Kyushu Okinawa Agric. Res. Cent. 64: 1–14 (In Japanese with English abstract).

Dubois, A. E. J., G. Pagliarani, R. M. Brouwer, B. J. Kollen, L. O. Dragsted, F. D. Eriksen, O. Callesen, L. J. W. J. Gilissen, F. A. Krens, R. G. F. Visser, M. J. M. Smulders, B. J. Vlieg-Boerstra, B. J. Flookstra-De Blok and W. E. Van De Weg. 2015. First successful reduction of clinical allergenicity of food by genetic modification: Mal d 1-silenced apples cause fewer allergy symptoms than the wild-type cultivar. Allergy Eur. J. Allergy Clin. Immunol. 70: 1406–1412.

Dunn, A. M., A. J. White, S. Vural and M. A. Hughes. 1998. Identification of promoter elements in a low-temperature-responsive gene (hlv4.9) from barley (Hordeum vulgare L.). Plant Mol. Biol. 38: 551–564.

Ebisawa, M., K. Ito and T. Fujisawa. 2017. Japanese guidelines for food allergy 2017. Allergol. Int. 66: 248–264.

Franz-Oberdorf, K., B. Eberlein, K. Edelmann, P. Bleicher, E. Kurze, D. Helm, K. Olbricht, U. Darsow, J. Ring and W. Schwab. 2017. White-fruit strawberry genotypes are not per se hypoallergenic. Food Res. Int. 100: 748–756.

Franz-Oberdorf, K., B. Eberlein, K. Edelmann, S. Hüchterig, F. Besbes, U. Darsow, J. Ring and W. Schwab. 2016. Fra a 1.02 Is the Most Potent Isoform of the Bet v 1-like Allergen in Strawberry Fruit. J. Agric. Food Chem. 64: 3688–3696.

Fujita, T., Y. Hiwatashi and T. Nishiyama. 1999. Isolation of genomic fragment by TAIL-PCR [WWW Document]. PHYSOCmanual ver. 1.4.

Giraldo, E., A. Diaz, J. M. Corral and A. Garcia. 2012. Applicability of 2-DE to assess differences in the protein profile between cold storage and not cold storage in nectarine fruits. J. Proteomics 75: 5774–5782.

Gross, K. C., C. Y. Wang and M. Saltveit. 2016. The commercial storage of fruits, vegetables, and florist and nursery stocks, Agriculture Handbook. United States Department of Agriculture.

Haugeneder, A., J. Trinkl, K. Härtil, T. Hoffmann and J. William. 2019. Answering biological questions by analysis of the strawberry metabolome. Metabolomics 14: 1–10.

Higo, K., Y. Ugawa, M. Iwamoto and T. Korenaga. 1999. Plant cis-acting regulatory DNA elements (PLACE) database: 1999. Nucleic Acids Res. 27: 297–303.

Hirakawa, H., K. Shirasawa, S. Kosugi, K. Tashiro, S. Nakayama, M. Yamada, M. Kohara, A. Watanabe, Y. Kishida, T. Fujisiro, H. Tsuruoka, C. Minami, S. Sasamoto, M. Kato, K. Nanri, A. Komaki, T. Yanagi, Q. Guoxin, F. Maeda, M. Ishikawa, S. Kuhara, S. Sato, S. Tabata and S. N. Isobe. 2014. Dissection of the octoploid strawberry genome by deep sequencing of the genomes of Fragaria species. DNA
Yanagisawa, S. and R. J. Schmidt. 1999. Diversity and similarity among recognition sequences of Dof transcription factors. Plant J. 17: 209–214.

Yonghua, Q., Z. Shanglong, S. Asghar, Z. Lingxiao, Q. Qiaoping, C. Kunsong and X. Changjie. 2005. Regeneration mechanism of Toyonoka strawberry under different color plastic films. Plant Sci. 168: 1425–1431.

Zhao, X., M. Xia, X. Wei, C. Xu, Z. Luo and L. Mao. 2019. Consolidated cold and modified atmosphere package system for fresh strawberry supply chains. LWT – Food Sci. Technol. 109: 207–215.