Effect of Parthenocarpic Genes pat-2 and pat-k on Vegetative and Fruit Traits in Tomato (Solanum lycopersicum ‘Micro-Tom’)

Rihito Takisawa*, Eri Maai, Ryohei Nakano and Tetsuya Nakazaki

Graduate School of Agriculture, Kyoto University, Kizugawa 619-0218, Japan

Parthenocarpy is a phenomenon that induces fruit set and development without pollination and fertilization. In tomato (Solanum lycopersicum L.) cultivation, parthenocarpy is considered to be an attractive trait, as it reduces the financial and labor costs involved in fruit set. Parthenocarpic genes pat-2 and pat-k are used as the source of parthenocarpic tomato cultivars in Japan. However, the effect of pat-2, pat-k, and their combined effect on vegetative and fruit traits are poorly understood, even though they are important for breeding parthenocarpic tomato cultivars. In this study, we introduced pat-2 and/or pat-k into a model tomato cultivar, ‘Micro-Tom’, to compare their effects on one genetic background, and then examined their vegetative and fruit traits. Lines carrying pat-2 showed greater leaf and stem size and stomatal conductance compared with the wild-type (WT), whereas there was no significant difference in vegetative traits between a line carrying only pat-k (pat-k) and WT. In addition, the content of total soluble solids (TSS) in pollinated fruits of pat-k and the lines carrying pat-2 was higher and lower than that of WT, respectively. Although it is possible that genes linked to pat-2 or pat-k caused these effects, these results suggest that pat-2 and pat-k have pleiotropic effects on these vegetative and/or fruit traits. Moreover, the rate of fruit set of unpollinated fruits in a line carrying pat-2 and pat-k (pat-2/pat-k) was significantly higher than that in pat-k, and unpollinated fruits of pat-2/pat-k were significantly heavier than those of pat-k and a line carrying only pat-2, suggesting that the effects of pat-2 and pat-k on parthenocarpic are additive. The results of this study can be used as fundamental information for the breeding of parthenocarpic tomato cultivars using pat-2 and pat-k.

Key Words: pleiotropic effect, stomatal conductance, total soluble solids, vegetative size.

Introduction

Parthenocarpy is a trait in which fruit set and development occur without pollination and fertilization. It is a desirable trait in tomato (Solanum lycopersicum L.) cultivation, as it reduces the financial and labor costs involved in fruit set and increases yield under unfavorable conditions, such as low or high temperature and humidity, which disturb pollination and fertilization (George et al., 1984; Picken, 1984; Takisawa et al., 2017). In tomato, nine genes have been identified as parthenocarpic genes in different tomato lines: pat in ‘Soressi’ and ‘Montfavet 191’, pat-2 in ‘Severianin’, pat3/pat4 in ‘RP75/59’, pat4.1/pat5.1 in ‘IL5-1’, pat4.2/pat9.1 in ‘IVT-line1’ and pat-k in ‘MPK-1’ (Gorguet et al., 2005, 2008; Takisawa et al., 2017). Among these parthenocarpic genes, the pat gene causes partial aberrations of stamens and ovules, which inhibits seed set and reduces fruit size; the fruit size of the pat mutant is approximately two-thirds that of WT fruits (Mazzucato et al., 1998; Philouze and Pecaut, 1986). In the pat-3/pat-4 mutant, seeded fruits grow larger than seedless fruits on the same plant (Philouze, 1989). Therefore, pat and pat-3/pat-4 mutations are not considered suitable as sources of genetic variation in breeding programs for parthenocarpic tomato cultivars. Additionally, pat4.1/pat5.1 and pat4.2/pat9.1 exhibit polygenic inheritance, which makes their use less attractive for breeding. In this context, pat-2 and pat-k genes have been used to develop parthenocarpic tomato cultivars in Japan. There are some commercial pat-2 parthenocarpic tomato cultivars, such as ‘Renaissance’ (Sugahara et al., 2002), ‘Paruto’ and ‘House Paruto’ (SAKATA SEED CORPORATION, Yokohama, Japan), and a commercial pat-k parthenocarpic tomato cultivar.
The parthenocarpic genes pat-2 and pat-k have been cloned; pat-2 is located on chromosome 4 and encodes a zinc-finger homeodomain (ZHD) protein (Nunome, 2016), whereas pat-k is located on chromosome 1 and encodes an E-class MADS-box gene, SLAG MOUSE-LIKE6 (SLAGL6) (Takaiwa et al., 2018). Klap et al. (2017) have shown that the Slag6 mutation does not affect vegetative development, vegetative to reproductive transition, pollen viability, or fruit size and shape. On the other hand, the effect of pat-2 on vegetative and fruit traits has not been reported, although it is important to identify the effects of this gene to evaluate its use in breeding parthenocarpic tomato cultivars. Additionally, the effect of pat-2, pat-k, and their combined effect on vegetative and fruit traits are not well understood because there is no experimental material which carries wild type and mutant alleles with the same background.

In this study, we first developed lines carrying pat-2 and/or pat-k singly or in combination in a ‘Micro-Tom’ background by repeated backcrossing in order to carry out comparative studies of the parthenocarpic genes in one background. ‘Micro-Tom’ is a model tomato cultivar because of its small size and rapid life cycle (Meissner et al., 1997); it is suitable for developing backcrossed lines. After developing the lines, we investigated the effect of pat-2, pat-k and their combined effects on vegetative and fruit traits.

Materials and Methods

Plant materials and plant growth conditions

We used a non-parthenocarpic tomato cultivar, ‘Micro-Tom’ (TOMJPF00001; Tsukuba University, Japan) and two parthenocarpic tomato cultivars: ‘Renaissance’ carrying pat-2 and ‘MPK-1’ carrying pat-k. To introduce pat-2 into ‘Micro-Tom’, ‘Micro-Tom’ was crossed with ‘Renaissance’. After the resulting F1 plants were backcrossed with ‘Micro-Tom’ as a recurrent parent, BC1 plants heterozygous at the pat-2 locus were selected using a DNA marker, Pat-2 (Nunome et al., 2013; Takaiwa et al., 2017) (Table S1). After the process was repeated four times, the resulting BC2 plants were selfed to obtain BC2F2 plants homozygous for the pat-2 allele, and these were selected using the DNA marker, Pat-2. To introduce pat-k into ‘Micro-Tom’, ‘Micro-Tom’ was crossed with ‘MPK-1’. The resulting F1 plants were selfed to obtain the F2 population. After screening parthenocarpic F2 plants, which were considered to be homozygous for the pat-k allele by examining the expansion of unpollinated ovaries, the selected plants were backcrossed with ‘Micro-Tom’ as the recurrent parent. After the process was repeated four times, the resulting BC3 plants were selfed to obtain BC3F2 plants homozygous for the pat-k allele, which were selected using two DNA markers, an insertion/deletion marker, Del22-1 (SL2.50: 85354768–85355860), and a cleaved amplified polymorphic sequence marker, SNP19 (Takaiwa et al., 2018), flanking the pat-k locus (Table S1).

To generate Micro-Tom lines homozygous for pat-k and/or pat-2 alleles, BC2F2 plants homozygous for the pat-2 allele were crossed with BC2F2 plants homozygous for the pat-k allele, the parthenocarpy of which was confirmed by examining the expansion of unpollinated ovaries. After the resulting F1 plants were selfed to obtain an F2 population, four kinds of F2 plants homozygous for pat-2 and/or pat-k alleles were selected using the following DNA markers: Pat-2, Del22-1, and SNP19; WT: wild-type alleles for both loci; pat-2: pat-2 allele and wild-type allele for the pat-k locus, pat-k: pat-k allele and wild-type allele for the pat-2 locus, and pat-2/pat-k: pat-2 and pat-k alleles for each locus.

Plants were grown in plastic pots containing a commercial substrate (Yasai no tuti; Takii & Co., Ltd., Tokyo, Japan) at 20–26°C under a 16-h light/8-h dark photoperiod and a photosynthetic photon flux density (PPFD) of 100–200 μmol photons·m⁻²·s⁻¹ using fluorescent lights. We supplied plants 1000-fold diluted Hypoxen liquid fertilizer (N-P-K, 6-10-5) (HYPONEX JAPAN CORP., LTD, Osaka, Japan) after the flowering stage about twice a week. A total of 11–14 plants of each genotype were grown to examine the vegetative traits, the rate of fruit set of unpollinated ovaries, fresh and dry weight of pollinated and unpollinated fruits, and transcript levels of Pat-k/SLAGL6. Additionally, 10–12 plants of each genotype were used to investigate locule number, TSS (Brix), acidity (% citric acid), chlorophyll content, stomatal conductance, and photosynthetic rate.

Evaluation of vegetative and fruit traits

To determine the rate of fruit set of unpollinated fruits and the fruit weight of pollinated and unpollinated fruits, two flowers in different clusters of the same plant were emasculated one day before anthesis and pollinated at anthesis, respectively. We removed all flowers which were not examined. After harvesting all fruits (116 days after seeding (DAS)), vegetative traits were investigated in the plants. First, the fresh weights of stems and leaves were measured, including lateral buds. After they were dried at 80°C for three days, the dry weights were measured. The fresh weights of pollinated and unpollinated fruits were measured after maturation, and their dry weights were determined after drying them using a freeze dryer (FDU-1200; TOKYO RIKAKIKAI CO., LTD., Tokyo, Japan) for three days. To determine the locule number, TSS (Brix) and acidity (% citric acid), two pollinated fruits were sampled in 10–12 plants for each genotype at maturity. After the number of locules was counted, TSS (Brix) and acidity (% citric acid) were measured using a digital refractometer (PAL-BX|ACID3; Atago Co., Ltd., Tokyo, Japan).
Measurements of chlorophyll content, photosynthetic rate, and stomatal conductance

Measurements of chlorophyll content, photosynthetic rate, and stomatal conductance were performed on two out of the three uppermost mature leaves of each plant. The chlorophyll content in the leaf was measured as the SPAD value using a Minolta chlorophyll meter (SPAD-502 Plus; Konica Minolta Sensing Inc., Tokyo, Japan) at 75 DAS. Individual leaf photosynthetic rate and stomatal conductance were measured in three plants per genotype at 51 DAS using a portable photosynthesis system (LI-6400; Li-Cor, Inc., Lincoln, NE, USA) at a PPFD of 1500 μmol·m⁻²·s⁻¹ and CO₂ concentration of 1000 μmol·mol⁻¹.

Evaluation of parthenocarpy in pat-k under low and high light conditions

Plants were grown under a 16-h light (25°C)/8-h dark (20°C) photoperiod with CO₂ concentration of 400 μmol·mol⁻¹ in a closed cultivation system, Naeterasu (Mitsubishi Plastics Agri Dream Co., Ltd., Tokyo, Japan). We supplied plants with a standard nutrient solution (Otsuka A; Otsuka Chemical Co., Ltd., Osaka, Japan). Six plants each of pat-k were under low light conditions (a PPFD of 60–150 μmol photons·m⁻²·s⁻¹) and high light conditions (a PPFD of 300–350 μmol photons·m⁻²·s⁻¹), respectively. To examine the rate of fruit set and the fruit weight of pollinated and unpollinated fruits, four flowers in different clusters of the same plants were emasculated one day before anthesis and pollinated at anthesis, respectively. The fresh weights of pollinated and unpollinated fruits were measured after maturation.

Quantitative RT-PCR analysis of SlAGL6

Three flower buds were collected from four plants per genotype one day before anthesis, frozen in liquid N₂, and stored at −80°C until used for RNA extraction. Total RNA was extracted using Sepasol®-RNA I Super G (NACALAI TESQUE, INC., Kyoto, Japan), according to the manufacturer’s instructions, and was used for cDNA synthesis using a ReverTra Ace qPCR RT Kit (TOYOBO CO., LTD., Osaka, Japan). Quantitative RT-PCR was performed as described previously (Takisawa et al., 2018). Table S2 lists primers used for quantitative RT-PCR.

Statistical analysis

Statistical analysis of data was performed using Tukey’s test and Steel’s test to determine differences among genotypes at a 5% level of significance. On the other hand, data under low and high light conditions were analyzed for statistical significance by Student’s t-test.

Results

Development of lines carrying pat-2 and/or pat-k

We developed lines carrying pat-2 and/or pat-k in the ‘Micro-Tom’ background (Fig. 1). The F₁ progeny of ‘Micro-Tom’ and ‘Renaissance’ carrying pat-2 or ‘Micro-Tom’ and ‘MPK-1’ carrying pat-k were backcrossed with ‘Micro-Tom’ until the fifth generation (BC₅). In the introgression process for pat-k, the plants were selfed after each backcross to screen for plants homozygous for the pat-k allele. After the backcrossing process, we crossed the BC₅F₂ plants carrying pat-2 with a BC₄F₂ plant carrying pat-k to obtain F₂ plants with all combinations of pat-2 and pat-k genes. We selected four genotypes of F₂ plants homozygous for pat-2 and/or pat-k alleles: WT, pat-2 homozygous plants (pat-2), pat-k homozygous plants (pat-k), and pat-2/pat-k homozygous plants (pat-2/pat-k) using DNA markers.

Vegetative traits of lines carrying pat-2 and/or pat-k

Using the developed lines, we examined the effects of pat-2 and/or pat-k alleles on vegetative traits. The fresh and dry weights of leaves and stems were significantly higher in pat-2 and pat-2/pat-k compared with WT, whereas there was no significant difference between pat-k and WT (Table 1). In addition, the fresh and dry weights of leaves and stems were significantly lower in pat-2/pat-k than in pat-2. No differences were observed in the leaf architecture between pat-k and WT. On the other hand, pat-2 and pat-2/pat-k had larger leaves compared with WT (Fig. 2).

Parthenocarpy and fruit traits of lines carrying pat-2 and/or pat-k

Next, we examined the effects of pat-2 and/or pat-k alleles on fruit traits. The rate of fruit set of unpollinated fruits was 0% in WT, 92% in pat-2, 72% in pat-k, and 100% in pat-2/pat-k (Table 2). There were significant differences among all genotypes except for the pat-2 and pat-2/pat-k pair. The fresh weight of unpollinated fruits was greatest in pat-2/pat-k, followed by pat-2 and lastly pat-k. Significant differences were observed in the fresh weights of unpollinated fruits among the different genotypes. Additionally, the dry weight and water content percentage of unpollinated fruits were significantly lower in pat-k than pat-2/pat-k. Although no significant differences were observed in the fresh weights of pollinated fruits among all four genotypes, those of pat-k and pat-2/pat-k seemed to be smaller than those of WT and pat-2. On the other hand, the dry weights of pollinated fruits were significantly lower in pat-2 and pat-2/pat-k than WT and pat-k. Consequently, the water content percentage in pollinated fruits was significantly higher in pat-2 and pat-2/pat-k than in WT and pat-k. The locule number did not show any significant differences among the genotypes (Table...
Fig. 1. Scheme of introgression of pat-2 and pat-k genes into ‘Micro-Tom’ (MT) and development of wild-type plants (WT), pat-2 homozygous plants (pat-2), pat-k homozygous plants (pat-k), and pat-2/pat-k homozygous plants (pat-2/pat-k). In pat-2 and pat-k introgressions, MT was crossed with ‘Renaissance’ homozygous for the pat-2 allele and ‘MPK-1’ homozygous for the pat-k allele, respectively. After backcrossing five times, the resulting BC1 plants were selfed to obtain BC1F2 plants homozygous for the pat-2 allele and pat-k allele, respectively. To generate homozygous pat-k and/ or pat-2 lines, BC1F2 plants carrying the pat-2 gene were crossed with BC1F2 plants carrying the pat-k gene. After the resulting F1 plants were selfed, we selected four kinds of F2 plants homozygous for pat-2 and/or pat-k allele by DNA markers: P2, Pat-2; p2, pat-2; Pk, Pat-k; pk, pat-k.

| Genotypes | Leaf fresh weight (g/plant) | Stem fresh weight (g/plant) | Leaf dry weight (g/plant) | Stem dry weight (g/plant) |
|-----------|-----------------------------|-----------------------------|---------------------------|--------------------------|
| WT        | 21.3 ± 0.7 ^c ^z            | 13.7 ± 0.7 e                | 2.9 ± 0.1  c              | 2.1 ± 0.2  c             |
| pat-2     | 40.7 ± 2.7 a ^b             | 29.7 ± 1.4 a ^d             | 5.2 ± 0.3 a ^e            | 5.0 ± 0.3 a ^f            |
| pat-k     | 26.2 ± 0.9 c ^g             | 16.7 ± 1.1 c ^h             | 3.4 ± 0.1 c ^i            | 2.5 ± 0.2 c ^j           |
| pat-2/pat-k | 33.3 ± 2.0 b ^k             | 24.2 ± 1.3 b ^l             | 4.2 ± 0.2 b ^m            | 3.8 ± 0.3 b ^n           |

^ Values are means ± SE (standard error).
^ Different letters indicate significant differences among genotypes (Tukey’s test; P < 0.05).

3). Compared with WT fruits, the TSS of pat-2 and pat-2/pat-k fruits were significantly lower under low light conditions than high light conditions; however, the fresh weights of pollinated fruits decreased by 33% under low light condition, whereas those of unpollinated fruits decreased by 79%.

Evaluation of parthenocarpy in pat-k under low and high light conditions

We examined the effects of the light condition on the degree of parthenocarpy in pat-k. The rate of fruit set of unpollinated fruits was 37.5% and 100% under low and high light conditions, respectively (Table 4). Those of pollinated fruits were 100% under both conditions. The fresh weights of pollinated and unpollinated fruits were significantly lower under low light conditions than high light conditions; however, the fresh weights of pollinated fruits decreased by 33% under low light condition, whereas those of unpollinated fruits decreased by 79%.
Table 3. Locule number, total soluble solids (TSS) and acidity of pollinated fruits of wild-type (WT), pat-2, pat-k, and pat-2/pat-k genotypes.

| Genotype     | Number of locules | TSS (Brix) | Acidity (% citric acid) |
|--------------|-------------------|------------|------------------------|
| WT           | 2.8 ± 0.1± a      | 4.7 ± 0.2 b| 1.92 ± 0.12 a          |
| pat-2        | 2.8 ± 0.1 a       | 3.7 ± 0.2 b| 1.37 ± 0.14 b          |
| pat-k        | 2.9 ± 0.1 a       | 5.6 ± 0.2 a| 1.64 ± 0.06 b          |
| pat-2/pat-k  | 2.7 ± 0.2 a       | 3.5 ± 0.2 c| 0.97 ± 0.03 c          |

Values are means ± SE (standard error).

Different letters indicate significant differences among genotypes (Tukey’s test; P<0.05).

Expression of Pat-k/SlAGL6 in flower buds of all four genotypes

To clarify the transcript level of Pat-k/SlAGL6 in flower buds of all four genotypes, we conducted expression analysis. Transcript levels of Pat-k/SlAGL6 were mostly undetected in of pat-k and pat-2/pat-k flower buds, and the difference between pat-2 and WT was non-significant (Fig. 3).

Table 4. Fruit set (%) and fresh weight of pollinated and unpollinated fruits of pat-k under low (60–150 PPFD) and high (300–350 PPFD) light conditions.

| Conditions  | Pollinated | Unpollinated | Pollinated | Unpollinated |
|-------------|------------|--------------|------------|--------------|
| Weak light  | 100.0      | 37.5         | 5.6 ± 0.3 a| 0.9 ± 0.2    |
| Strong light| 100.0      | 100.0        | 8.4 ± 0.4  | 4.3 ± 0.5    |

Values are means ± SE (standard error).

** and NS indicate significant difference at P<0.01 and no significant difference by t-test, respectively.
Table 5. Chlorophyll content (SPAD value), photosynthesis rate, and stomatal conductance in wild-type (WT), pat-2, pat-k, and pat-2/pat-k genotypes.

| Genotype       | SPAD value     | Photosynthesis rate (μmol m⁻² s⁻¹) | Stomatal Conductance (mol m⁻² s⁻¹) |
|----------------|----------------|------------------------------------|-----------------------------------|
| WT             | 66.0 ± 1.7 a   | 23.2 ± 1.5 a                       | 0.09 ± 0.01 bc                    |
| pat-2          | 63.5 ± 2.2 a   | 22.3 ± 1.7 a                       | 0.18 ± 0.01 a                     |
| pat-k          | 65.4 ± 1.5 a   | 20.7 ± 0.9 a                       | 0.05 ± 0.01 c                     |
| pat-2/pat-k    | 63.3 ± 2.3 a   | 24.4 ± 1.7 a                       | 0.16 ± 0.04 ab                    |

* Values are means ± SE (standard error).

Different letters indicate significant differences among genotypes (Tukey’s test; *P* < 0.05).

Discussion

Our results showed that lines carrying pat-2 had larger leaves and stems compared with WT, whereas there was no significant difference in vegetative traits between pat-k and WT, suggesting that Pat-2 is involved in leaf and stem development, but pat-k is not. Nunome (2016) showed that Pat-2 encodes a ZHD protein. ZHD proteins function as transcription factors and contain a homeodomain and a zinc-finger motif with cysteine and histidine residues (Windhovel et al., 2001). In Arabidopsis thaliana, ZHD proteins are involved in regulating floral development and stress responses (Tan and Irish, 2006; Tran et al., 2007). Khatun et al. (2017) identified and characterized 22 ZHD genes in tomato. Most of the ZHD genes were preferentially expressed in flower buds, but some of them, including Pat-2 (SIZHD17), were highly expressed in leaves and stems. These results support the view that Pat-2 is related to leaf and stem development in tomato. In addition, stomatal conductance is determined predominantly by the stomatal aperture and density (Franks and Beerling, 2009). In this study, the stomatal conductance of lines carrying pat-2 was higher than that of WT, suggesting that Pat-2 plays a role in regulating the stomatal aperture and/or the stomata density. However, the lines used in this study were backcrossed only five times. Therefore, it is possible that these phenotypes were caused by one or more genes linked to the pat-2 locus. Further research is needed to demonstrate the role of Pat-2 in leaf and stem development and in leaf function.

The rate of fruit set of unpollinated ovaries and unpollinated fruit weight were significantly lower in pat-k than pat-2, showing that the effect of pat-k on parthenocarpy is weaker than that of pat-2 in the ‘Micro-Tom’ background. Takisawa et al. (2018) reported that down-regulation of Pat-k/SlAGL6 caused parthenocarpy in ‘MPK-1’ grown under natural light conditions. However, in this study expression of parthenocarpy was weak in pat-k regardless of the fact that the expression level of Pat-k/SlAGL6 was largely undetectable. It is assumed that there are some factors (e.g., environmental conditions and genetic background) that are necessary for expression of parthenocarpy by pat-k in the ‘Micro-Tom’ background. Tomato plants need ample amounts of light during development, and the expression of parthenocarpy is high under high light intensity (George et al., 1984). Therefore, we examined the effects of light conditions on the degree of parthenocarpy in pat-k. The results showed that the rate of fruit set and fresh weight of unpollinated fruits in pat-k were significantly higher under the high light condition than the low light condition, suggesting that light intensity is a major factor in determining the degree of parthenocarpy in pat-k.

Compared with the parthenocarpic lines, pat-2, pat-k, and pat-2/pat-k, the rate of fruit set of unpollinated ovaries was much higher in pat-2/pat-k than pat-k. In addition, unpollinated fruit weights were significantly higher in pat-2/pat-k than pat-2 and pat-k. These results suggest that the effect of pat-2 and pat-k on parthenocarpy is additive. It was shown that the stage when the transcript levels are highest in tomato ovaries was different between Pat-2 and Pat-k/SlAGL6, with expression levels of Pat-2 and Pat-k/SlAGL6 being highest at flower bud (2 mm) and one day before anthesis, respectively (Nunome, 2016; Takisawa et al., 2018). Additionally, we showed that the expression levels of Pat-k/SlAGL6 in pat-2 and WT were similar in this study. These results suggest that pat-2 does not induce parthenocarpy through the down-regulation of Pat-k/SlAGL6.
**SLAGL6**, and that *pat-2* and *pat-k* play independent roles in the induction process of parthenocarpy.

The TSS of pollinated fruits was higher in *pat-k* than WT. Klap et al. (2017) showed significantly higher TSS content in seedless fruits in a homozygous *Slagl6* line compared with that of seeded fruits in WT plants. These results suggest that mutated *SLAGL6* increases TSS content, regardless of whether fruits are pollinated or not. In addition, we showed that the TSS of pollinated fruits was significantly lower in *pat-2* and *pat-2/pat-k* compared with WT, whereas water content was significantly higher than that of WT. These results indicated the possibility that the reduction in TSS in unpollinated fruit weights were higher in *pat-2/pat-k* compared with WT, suggesting that the effect of *pat-2* and *pat-k* on parthenocarpy is additive. Our results provide useful information on the effect of *pat-2* and *pat-k* that could contribute to the development of *pat-2* and *pat-k* parthenocarpic tomato cultivars.

**Acknowledgements**

Tomato seeds (TOMJPF00001) were provided by the University of Tsukuba Gene Research Center, through the National Bio-Resource Project of the Japan Agency for Research and Development (AMED), Japan.

**Literature Cited**

Franks, P. J. and D. J. Beeching. 2009. Maximum leaf conductance driven by CO2 effects on stomatal size and density over geologic time. Proc. Natl. Acad. Sci. USA 106: 10343–10347.

George, W. L., J. W. Scott and W. E. Splittstoesser. 1984. Parthenocarpy in tomato. Hort. Rev. 6: 65–84.

Gorguet, B., P. M. Eggink, J. Ocaná, A. Tiwari, D. Schipper, R. Finkers, R. G. F. Visser and A. W. van Heusden. 2008. Mapping and characterization of novel parthenocarpy QTLs in tomato. Theor. Appl. Genet. 116: 755–767.

Gorguet, B., A. W. van Heusden and P. Lindhout. 2005. Parthenocarpic fruit development in tomato. Plant Biol. 7: 131–139.

Khatun, K., U. K. Nath, A. H. K. Robin, J. I. Park, D. J. Lee, M. B. Kim, C. K. Kim, K. B. Lim, I. S. Nou and M. Y. Chung. 2017. Genome-wide analysis and expression profiling of zinc finger homeodomain (ZHD) family genes reveal likely roles in organ development and stress responses in tomato. BMC Genomics 18: 695. DOI: 10.1186/s12864-017-4082-y.

Klap, C., E. Yeshayahu, A. M. Bolger, T. Arazi, S. K. Gupta, S. Shahbazi, B. Usadel, Y. Salts and R. Barg. 2017. Tomato facultative parthenocarpy results from SIAGAMOUS-LIKE 6 loss of function. Plant Biotechnol. J. 15: 634–647.

Mazzuccato, A., A. R. Taddei and G. P. Soressi. 1998. The parthenocarpic fruit (pat) mutant of tomato (*Lycopersicon esculentum* Mill.) sets seedless fruits and has aberrant anther and ovule development. Development 125: 107–114.

Meissner, R., Y. Jacobson, S. Melamed, S. Levyatuv and G. Shalev. 1997. A new model system for tomato genetics. Plant J. 12: 1465–1472.

Nunome, T. 2016. Map-based cloning of tomato parthenocarpic *pat-2* gene. Regulation of Plant Growth & Development 51: 37–40 (In Japanese with English abstract).

Nunome, T., I. Honda, A. Ohyama, H. Fukuoka, H. Yamaguchi and K. Miyatake. 2013. Parthenocarpic regulation gene and use thereof. Patent WO 2014021398 A1.

Philouze, J. 1989. Natural parthenocarpy in tomato. IV. A study of the polygenic control of parthenocarpy in line 75/59. Agronomie 9: 63–75.

Philouze, J. and P. Pecaut. 1986. Natural parthenocarpy in tomato. III. Study of the parthenocarpy due to the gene *pat* (parthenocarpic fruit) in the line Montfavet 191. Agronomie 6: 243–248.

Picken, A. J. F. 1984. A review of pollination and fruit set in the tomato (*Lycopersicon esculentum* Mill.). J. Hort. Sci. 59: 1–13.

Sugahara, S., S. Enomoto, T. Oyabu, Y. Yabe and H. Noguchi. 2002. Breeding of parthenocarpic tomato cultivar ‘Renascence’. Res. Bull. Aichi. Agric. Res. Ctr. 34: 37–42 (In Japanese with English abstract).

Takisawa, R., T. Maruyama, T. Nakazaki, K. Kataoka, H. Saito, S. Koeda, T. Nunome, H. Fukuoka and A. Kitajima. 2017. Parthenocarpic fruit in the tomato (*Solanum lycopersicum* L.) cultivar ‘MPK-1’ is controlled by a novel parthenocarpic gene. Hort. J. 86: 487–492.

Takisawa, R., T. Nakazaki, T. Nunome, H. Fukuoka, K. Kataoka, H. Saito, T. Habu and A. Kitajima. 2018. The parthenocarpic gene *Pat-k* is generated by a natural mutation of *SLAGL6* affecting fruit development in tomato (*Solanum lycopersicum* L.). BMC Plant Biol. 18: 72. DOI: 10.1186/s12870-018-1285-6.

Tan, Q. K. and V. F. Irish. 2006. The Arabidopsis zinc finger-homeodomain genes encode proteins with unique biochemical properties that are coordinately expressed during floral development. Plant Physiol. 140: 1095–1108.

Tran, L. S., K. Nakashima, Y. Sakuma, Y. Osakabe, F. Qin, S. D. Simpson, K. Maruyama, Y. Fujita, K. Shinozaki and K. Yamaguchi-Shinozaki. 2007. Co-expression of the stress-inducible zinc finger homeodomain ZFHD1 and NAC transcription factors enhances expression of the *ERD1* gene in Arabidopsis. Plant J. 49: 46–63.

Windhuvel, A., I. Hein, R. Dabrowa and J. Stockhaus. 2001. Characterization of a novel class of plant homeodomain proteins that bind to the C4 phosphoenolpyruvate carboxylase gene of *Flaviera trinervia*. Plant Mol. Biol. 45: 201–214.