Study of parthenocarpy and inhibition of seed formation in 'MPK-1', a parthenocarpic tomato (*Solanum lycopersicum* L.) cultivar

-Abstract version-

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Abstract

Tomato (*Solanum lycopersicum* L.) is a popular vegetable fruit that is used in many cuisines and is consumed by people worldwide. Although fruit development is triggered by pollination and fertilization in tomato, lack of wind and pollinating insects in greenhouses result in reduced fruit set. Under such conditions, the use of growth regulators or the introduction of pollinating insects is required to stabilize fruit production. However, they incur additional labor and costs on farmers.

Parthenocarpy is a trait which fruit set and growth are triggered without fertilization. In tomato, parthenocarpy is a desirable trait for farmers because it can reduce the cost of fruit setting in a greenhouse. 'Severianin', a parthenocarpic tomato cultivar with the *pat-2* gene, has been used for parthenocarpic tomato breeding in Japan. However, tomato cultivars which were developed using the *pat-2* gene, produce only few seeds, and it limits their availability on a commercial scale. 'MPK-1' is a parthenocarpic tomato cultivar which was derived from a cross between a non-parthenocarpic cultivar and a variant from a self-fertilization posterity of 'Severianin', which exhibited strong parthenocarpy. 'MPK-1' also exhibits stable parthenocarpy and low seed set. However, the genetic mechanisms underlying parthenocarpy and the inhibition of seed formation in 'MPK-1' remain elusive. In this study, I investigated the mechanisms of these processes to obtain useful information for the parthenocarpic tomato breeding using 'MPK-1'.

In Chapter 1, I investigated pollen tube elongation in the style and ovary and ovule morphology to understand the factors that inhibit seed formation in 'MPK-1'. I observed pollen tube elongation in two parthenocarpic cultivars with low seed set, 'Renaissance' and 'MPK-1' which were derived from 'Severianin'. In 'Renaissance', pollen tube elongation was inhibited at the style base; however, in 'MPK-1', the pollen tubes elongated in the style normally but did not enter the ovules.

From observing transverse sections of ‘MPK-1’ ovules, there were many ovules with an abnormal micropyle. Because these anomalous ovules were not observed in ‘Renaissance’, in which the *pat-2* gene is known to be responsible for the parthenocarpy, genetic factors other than the *pat-2* gene should relate to the anomalous ovule formation and seed formation restriction in ‘MPK-1’.

In Chapter 2, to determine any association between the *Pat-2* genotype and seed formation restriction, a DNA marker to distinguish between the mutant (*pat-2*) and wild-type (*Pat-2*) alleles of *Pat-2* was developed. Using the developed marker, I firstly performed PCR-based marker analysis to confirm *Pat-2* alleles of ‘MPK-1’. As a result, surprisingly ‘MPK-1’ genotyping revealed that it did not harbor the mutant *pat-2* allele. Therefore, I conducted genetic analyses to identify the genes responsible for parthenocarpy in ‘MPK-1’. I found that parthenocarpy in ‘MPK-1’ is controlled by a novel parthenocarpic gene. This novel gene, which was designated as *Pat-k*, is semi-dominant and located on chromosome 1. I also showed that the size of the parthenocarpic fruit of ‘MPK-1’ is similar to that of the pollinated fruit at maturity, which showd that ‘MPK-1’ should be used as a new parthenocarpic resource for breeding.

In Chapter 3, I performed QTL analysis and fine mapping using the cross population of ‘Micro-Tom’ and ‘MPK-1’ and map-based cloning to isolate the *Pat-k* gene and to elucidate the association between parthenocarpy and low seed set in ‘MPK-1’. In addition, I observed the structure of ovules in plants that are homozygous for the ‘Micro-Tom’ and ‘MPK-1’ allele at the *Pat-k* locus to clarify the effect of the *Pat-k* gene on ovule formation. By QTL analysis for parthenocarpy and seed production, I detected a major QTL for each trait on the nearly same region of the *Pat-k* locus in chromosome 1, which suggests that the *Pat-k* gene causes not only parthenocarpy but also low seed set. To isolate the *Pat-k* gene, I performed fine mapping using an

F_4 population following the cross between a non-parthenocarpic cultivar ‘Micro-Tom’ and ‘MPK-1’. The results showed that the *Pat-k* gene was located in the 529 kb interval between two markers, where 60 genes exist. By using data from a whole genome re-sequencing and genome sequence analysis of ‘MPK-1’, I found that the *SlAGAMOUS-LIKE 6* (*SlAGL6*) gene of ‘MPK-1’ was mutated by a retrotransposon insertion, and that there was no polymorphism in the exon of all genes in the region delimited by fine mapping. In addition, the transcript level of *SlAGL6* was significantly lower in ovaries of ‘MPK-1’ than in a non-parthenocarpic cultivar. From these results, I could conclude that the *Pat-k* gene is *SlAGL6*, and its down-regulation in ‘MPK-1’ causes parthenocarpy and low seed set. In addition, I observed abnormal micropyles only in plants homozygous for the ‘MPK-1’ allele at the *Pat-k*/*SlAGL6* locus. This result suggests that *Pat-k*/*SlAGL6* is also related to ovule formation and its down-regulation is likely caused the low seed set in ‘MPK-1’ by abnormal ovule formation.

In Chapter 4, to understand the mechanism by which *Pat-k*/*SlAGL6* down-regulation induces parthenocarpy, I investigated the metabolism of IAA and GA during fruit development by monitoring the levels of IAA and its metabolites and the transcript levels of genes involved in IAA and GA metabolism. I observed an increase in the level of IAA accompanied by elevated expression of genes encoding IAA synthesis enzymes in the parthenocarpic ovaries of ‘MPK-1’ after anthesis. In addition, the level of IAA-Glu, one of the IAA conjugates comprising a potential IAA inactivation pathway was noted to be more than 10 times higher than that of IAA after anthesis. These results suggest that substantial amount of IAA was synthesized in the parthenocarpic ovaries of ‘MPK-1’. The transcript level of *SlGA20ox1*, the gene encoding one of the GA biosynthetic enzymes greatly increased after anthesis, whereas that of genes encoding the GA inactivating enzymes, *SlGA2ox1*, *SlGA2ox2*, *SlGA2ox4*, and *SlGA2ox5* remained low in the parthenocarpic

ovaries of ‘MPK-1’. These results suggest that *Pat-k*/*SlAGL6* stops the ovary development by repressing the genes related to IAA and GA biosynthesis and promoting GA catabolism genes directly or indirectly, and down-regulation of *SlAGL6* initiates the ovary development.

In conclusion, it was demonstrated that the parthenocarpy of ‘MPK-1’ is controlled by not *pat-2* but *Pat-k*/*SlAGL6*, which is the MADS-box gene with E function on chromosome 1. Retrotransposon insertion in the first intron of *Pat-k*/*SlAGL6* down-regulates its expression, causing parthenocarpy in ‘MPK-1’. In addition, the down-regulation of *Pat-k*/*SlAGL6* is also involved with low seed set through abnormal ovule formation. These results showed that *Pat-k*/*SlAGL6* is related to parthenocarpy and ovule formation. Furthermore, the down-regulation of *Pat-k*/*SlAGL6* up-regulates the expression of IAA and GA biosynthesis genes and down-regulates that of GA catabolic genes, thus promoting the growth of parthenocarpic fruits in ‘MPK-1’. Similar transcript profiles were observed in pollinated ovaries when the expression of *Pat-k*/*SlAGL6* was down-regulated by pollination. These results suggested that *Pat-k*/*SlAGL6* acts to repress ovary development by regulating the expression of genes involved in IAA and GA metabolism.