Chapter 7
Tomato Fruit Set and Its Modification Using Molecular Breeding Techniques

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7.1 Introduction

Breeding objectives have become more multifaceted over the centuries, but yield performance remains the most important trait for numerous crops. In fruit crops, yield is determined by the number and weight of fruits, and so efficient fruit set is essential for achieving good yield. Fruit set is a developmental process in which ovaries differentiate into fruits, and it is generally stimulated by successful pollination and fertilization, leading to embryo and seed development. Fruit set in tomato (*Solanum lycopersicum*) is very sensitive to environmental conditions and in particular to temperature because pollination is inhibited under excessively low or high temperatures (Iwahori and Takahashi 1963; Charles and Harris 1972). To avoid unfavorable conditions and achieve multiple cropping, greenhouse production is adapted to optimal conditions, but the maintenance of suitable temperatures is costly and consumes large amounts of energy. Greenhouse production also poses another problem for pollination in tomato, since closed greenhouses are barriers for wind- or pollinator-dependent flower vibration, which stimulates pollen diffusion from the anthers and hence self-pollination. Greenhouse growers often use insect pollinators, such as bumblebees, or hormone treatments to facilitate fruit set, but these methods are costly or time-consuming.

Parthenocarpy, or fruit set without pollination and fertilization, is a valuable trait for efficient tomato fruit production, in particular under unfavorable conditions for pollination and fertilization, such as during hot summers and cold winter weathers. Furthermore, parthenocarpic fruits are seedless, which is also generally desirable for both processing and fresh market production. In the processing industry, tomato seeds are usually removed from the paste, and seedless fruits often have longer shelf

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life than seeded fruits because seeds may produce ethylene, a ripening hormone (Varoquaux et al. 2000; Fei et al. 2004; Martinelli et al. 2009).

There is considerable evidence that fruit set is tightly controlled by several plant hormones, such as auxin, gibberellin (GA), and cytokinin (CK) (Gillaspy et al. 1993; Ruan et al. 2012; Ariizumi et al. 2013; McAtee et al. 2013), and application of these phytohormones to unpollinated tomato ovaries can induce parthenocarpic fruit set (Serrani et al. 2007a; Matsuo et al. 2012). In addition, several parthenocarpic tomato varieties have been developed, but most of the genes responsible for the traits remain unknown. The ovaries of major parthenocarpic varieties contain high amounts of GAs, even before pollination, and it has therefore been suggested that their parthenocarpy may be caused by altered hormonal activities (de Jong et al. 2009a). Tomato mutants and transgenic plants exhibiting parthenocarpy have also been developed, based on altered expression of hormone-related genes. Studies with *Arabidopsis thaliana* and tomato have led to model of fruit set mediated by auxin and GA (McAtee et al. 2013), wherein successful pollination and fertilization triggers the synthesis of auxin in the ovule. Auxin is then transported to the pericarp, where it induces de novo GA synthesis and the newly synthesized GA promotes pericarp expansion. In the early stages of tomato fruit development, CKs are also synthesized and they function to stimulate cell division (Matsuo et al. 2012). Application of *N*-(2-chloro-4-pyridyl)-*N*-phenylurea (CPPU), a synthetic CK, to unpollinated tomato ovaries can induce parthenocarpic fruit development, and auxin and GA concentrations in the ovaries will transiently increase after CPPU treatment (Ding et al. 2013). Thus, modification of genes associated with CK biosynthesis and/or signaling pathway may lead to further understanding of the role of CK and the cross talk between CK and other plant hormones in fruit set.

Even though parthenocarpy represents a potentially valuable trait for many fruit crops, parthenocarpic varieties have been widely cultivated for only a few species (e.g., cucumber and banana) because high-quality fruits and a high yield are seldom combined with the parthenocarpic trait (Varoquaux et al. 2000; Pandolfini et al. 2002). This limitation is also true for tomato. In addition, the environment in which the fruits are cultivated has a major influence on parthenocarpy, and so the consistent control of the phenotypes in a commercial setting has proven difficult. These barriers have made it difficult to develop parthenocarpic varieties that stably produce high-quality fruits. Molecular techniques, such as genetic modification and marker-assisted selection (MAS), represent powerful tools for the development of new practical varieties showing parthenocarpy in the form of efficient introduction, selection, and pyramiding of useful genes. Here, we review studies of tomato fruit set and parthenocarpy, focusing particularly on parthenocarpic genes, loci, and variations associated with plant hormones and other components (Table 9.1). We also provide suggestions for genetically improving tomato fruit set using molecular breeding strategies. The induction of fruit set by modification of plant hormone-related genes is described, and we review other genetic modifications leading to fruit formation, such as MADS-box transcription factors, anther ablation, and the

Table 7.1 Genetic modifications, mutations, and variations conferring parthenocarpic fruit set in tomato

Gene/locus symbol	Defined function	Genetic modification/ mutation/variation	Parthenocarpic fruit phenotypes (compared to seeded control fruits)	Vegetative and reproductive phenotypes	References
DefH9-iaaM	Auxin biosynthesis	Expression of *iaaM* under an ovary-specific promoter	Normal size and weight, normal or increased soluble solid, increased beta-carotene content	Normal vegetative organs	Ficcadenti et al. (1999), Rotino et al. (2005)
DefH9-RI-iaaM	Auxin biosynthesis	Moderate expression of *DefH9-iaaM*	Decreased weight, increased fruit number, normal yield, increased beta-carotene content	Normal vegetative organs	Pandolfini et al. (2002)
INO-iaaM	Auxin biosynthesis	Expression of *iaaM* under an ovary-specific promoter	Normal size and weight, increased linoleic acid and succinic acid	–	Martinelli et al. (2009)
TPRP-F1::rolB	Auxin response	Expression of *rolB* under an ovary- and young-fruit-specific promoter	Normal size, shape, and jelly, increased weight and soluble solids, lower yield, decreased ripening time	–	Carmi et al. (2003)
DefH9-rolB	Auxin response	Expression of *rolB* under an ovary- and young-fruit-specific promoter	Increased size, increased soluble solids and several nutrient metabolites	–	Martinelli et al. (2009)
INO-rolB	Auxin response	Expression of *rolB* under an ovary- and young-fruit-specific promoter	Increased size and several nutrient metabolites	–	Martinelli et al. (2009)
AUCSIA	Auxin biosynthesis or transport	RNAi silencing of *AUCSIA1* and *AUCSIA2*	Decreased size and weight	Fused and curled leaves	Molesini et al. (2009)
SlPIN4	Auxin transport	Constitutive silencing (RNAi)	Facultative and obligate parthenocarpy, depending on the lines	Normal vegetative organs	Mounet et al. (2012)

(continued)

Table 7.1 (continued)

Gene/locus symbol	Defined function	Genetic modification/ mutation/variation	Parthenocarpic fruit phenotypes (compared to seeded control fruits)	Vegetative and reproductive phenotypes	References
SlIAA9	Auxin signaling	Constitutive silencing (antisense)	Normal	Reduced leaf complexity	Wang et al. (2005)
		Three loss-of-function mutations induced by EMS treatment or γ-ray irradiation	Different parthenocarpic rates, fruit sizes, and ripening times among lines	Reduced leaf complexity	Saito et al. (2011)
SlARF7	Auxin signaling	Constitutive silencing (RNAi)	Heart shaped and a thicker pericarp, seedlike structure formation	Curled leaves, randomized growth orientation	de Jong et al. (2009b, 2011)
AtARF8	Auxin signaling	Constitutive overexpression of a mutated *A. thaliana ARF8*	Normal size, weight, and shape	–	Goetz et al. (2007)
SlTIR1	Auxin signaling	Constitutive overexpression of the *S. lycopersicum TIR1*	–	Dwarf, reduced leaf complexity, increased leaf size, increased petiole diameter	Ren et al. (2011)
CcGA20ox1	GA synthesis	Constitutive overexpression of the citrus hybrid Carrizo citrange *GA20ox1*	Decreased size, normally filled locular tissue	Taller height, non-serrated leaf, longer style, delayed flowering	García-Hurtado et al. (2012)
SlDELLA	GA response	Constitutive silencing (antisense)	Decreased size, elongated shape, elongated cells and decreased number of cells in pericarp, lower growth of locular tissue	Taller height; elongated flower trusses, style, and stylar hair primordia; blunt growth of stigma	Martí et al. (2007)
		Loss-of-function mutation	Decreased weight, increased fruit number, lower yield, increased soluble solids	Altered branching pattern, longer hypocotyls and internodes, thinner stem, delayed flowering, shorter roots	Bassel et al. (2008), Carrera et al. (2012)

SlTPR1	Ethylene signaling	Constitutive overexpression of the *S. lycopersicum TPR1*	Normal ripening, often fused parthenocarpic fruits	Dwarf, epinasty, pleiotropically altered leaf morphology	Lin et al. (2008)
TM29	Flower development	Constitutive silencing (cosuppression or antisense)	Increased size, delayed ripening, misshapen due to growth of internal tissues	Normal vegetative organs, floral homeotic conversion	Ampomah-Dwamena et al. (2002)
TM5	Flower development	Constitutive silencing (antisense)	Frequent parthenocarpy	Normal vegetative organs, floral homeotic conversion	Pnueli et al. (1994)
TM6	Flower development	Constitutive silencing (RNAi)	Occasional parthenocarpy	Normal vegetative organs, floral homeotic conversion	de Martino et al. (2006)
TAP3	Flower development	AC/DS transposon-inserted mutation	Occasional parthenocarpy	Normal vegetative organs, floral homeotic conversion	de Martino et al. (2006)
		Frameshift mutation (*sl-Pr*) or mutation in promoter region (*sl-LA0269*)	Modified floral organs remaining attached to fruits	Normal vegetative organs, floral homeotic conversion with more (*sl-Pr*) or less (*sl-LA0269*) severity	Quinet et al. (2014)
		Constitutive silencing (antisense)	Carpel-like stamens remaining attached to fruits with different severity correlated with the silencing level	Normal vegetative organs, floral homeotic conversion with different severity correlated to the silencing level	Quinet et al. (2014)
PsEND1::barnase	Cytotoxic ribonuclease	Expression of *barnase* under an anther-specific promoter	Decreased size, increased fruit number, normal yield, increased soluble solids and several nutrient metabolites, lower acidity	Normal vegetative organs, early anther ablation	Medina et al. (2013)
StSy	Resveratrol biosynthesis	Constitutive overexpression of the grape *StSy*	Slightly decreased size, increased soluble antioxidants (ascorbate and glutathione)	Normal vegetative organs	Giovinazzo et al. (2005), Ingrosso et al. (2011)
CHS	Flavonoid biosynthesis	Constitutive silencing (RNAi against *CHS* family genes)	Decreased size, misshapen in several lines, normal sugar and organic acid	Normal vegetative organs	Schijlen et al. (2007)

(continued)

Table 7.1 (continued)

Gene/locus symbol	Defined function	Genetic modification/ mutation/variation	Parthenocarpic fruit phenotypes (compared to seeded control fruits)	Vegetative and reproductive phenotypes	References
pat	Parthenocarpy	Natural variation ("*Soressi*" and "*Montfavet 191*")	Decreased size due to decreased cell enlargement	Aberrant anther and ovule development	Mazzucato et al. (1998)
pat-2	Parthenocarpy	Natural variation ("*Severianin*")	Almost normal size	Different yields and plant vigor depending on genetic background	Philouze et al. (1988), Philouze and Maisonneuve (1978)
pat-3/*pat-4*	Parthenocarpy	Natural variation ("*RP75/ 59*")	Considerably decreased size	–	Philouze (1989)
pat4.1/ *pat5.1*	Parthenocarpy	Introgression line (IL5-1)	Normal size, lower parthenocarpic rate than *pat4.2*/*pat9.1*	–	Gorguet et al. (2008)
pat4.2/ *pat9.1*	Parthenocarpy	Natural variation (IVT-line 1)	Decreased size, higher parthenocarpic rate than *pat4.1*/*pat5.1*	–	Gorguet et al. (2008)

–, not described; *ARF*, *auxin response factor*; *AUCSIA*, *AUXIN CUM SILENCING ACTION*; *CHS*, *chalcone synthase*; *DefH9*, *Deficiens homolog 9*; EMS, ethyl methyl sulfonate; *END1*, *ENDOTHECIUM 1*; GA, gibberellin; *INO*, *INNER NO OUTER*; *PIN*, *PIN-FORMED*; RNAi, RNA interference; *TAP3*, *Tomato APETALLA3*; *TM*, *TOMATO MADS-box*; *StSy*, *stilbene synthase*

flavonoid synthesis pathways. Parthenocarpic variation developed through inter-variety or interspecies crossing is also discussed.

7.2 Genetic Modifications and Mutations Influencing Fruit Set

7.2.1 Modifying the Expression of Genes Related to Auxin Synthesis, Responses, and Transport

The effect of auxin-related compounds on fruit set was first described in the early twentieth century (Gustafson 1936, 1937, 1939), and several natural and synthetic auxins were subsequently shown to induce parthenocarpic fruit set (Ho and Hewitt 1986). For example, application of the synthetic auxin, 4-chlorophenoxyacetic acid (4-CPA), can induce parthenocarpic fruits with comparable size to pollination-induced fruits (Bünger-Kibler and Bangerth 1982). More recently, several reports have described the induction of tomato parthenocarpy by altering the expression of several genes related to auxin metabolism and signaling (de Jong et al. 2009a; Ariizumi et al. 2013). For example, a chimeric gene, *DefH9-iaaM*, was used in early trials to produce transgenic parthenocarpic fruits in Solanaceae. This chimeric gene comprises the following two components: (1) the promoter of *Deficiens homolog 9* (*DefH9*), which is an *Antirrhinum majus* MADS-box gene that is expressed specifically in the ovule, and (2) *iaaM*, derived from *Pseudomonas syringae*, which can induce auxin biosynthesis via synthesis of the indoleacetic acid (IAA) precursor indolacetamide. The expression of *DefH9-iaaM* was detected in the developing buds of the transgenic plants (Ficcadenti et al. 1999), which showed normal vegetative growth and developed fruits from emasculated flowers at a rate similar to that of pollinated flowers from non-transformed control plants. The fresh weights and soluble solid concentrations of the parthenocarpic fruits were unchanged or higher than the seeded fruits of control plants; however, the fruits produced by several transgenic lines were misshapen, likely because of excessive auxin levels (Pandolfini et al. 2002). To solve this problem, Pandolfini et al. (2002) altered the 5′ untranslated leader region (5′ ULR) of *iaaM* (*DefH9-RI-iaaM*), leading to a reduction in the translation efficiency of *iaaM* mRNA and, consequently, reducing the IAA content in flower buds compared with that in buds of the *DefH9-iaaM* transgenic plants. This resulted in optimal parthenocarpy, and when fruit productivity and quality of the *DefH9-RI-iaaM* transgenic plants were then evaluated under open-field conditions (Rotino et al. 2005), no obvious differences in fruit quality were detected, with the exception of a higher β-carotene level. The fresh weight of individual transgenic fruits was lower, but was compensated for by an increased fruit number, resulting in a yield comparable with that of parental non-transgenic lines. Expression of *iaaM* under the promoter of the ovule-specific *INNER NO OUTER* (*INO*) gene, which is expressed in only one cell layer in the

ovule outer integument, has also been shown to induce parthenocarpy (Martinelli et al. 2009), but with fruits showing no significant morphological abnormalities.

Another chimeric gene, comprising *Agrobacterium rhizogenes rolB* fused to the ovary- and young-fruit-specific promoter, *TPRP-F1*, has also been used to generate parthenocarpic fruits (Carmi et al. 2003). Tomato plants transformed with *rolB* showed auxin-responsive phenotypes, although the underlying molecular mechanism is not yet known. The highest expression of *rolB* under the control of the *TPRP-F1* promoter was detected in early developing fruits. The transgenic plants developed seedless fruits with a size and morphology comparable to those of seeded fruits from the parental lines, but fruit yield and several other qualities were different in greenhouse-cultivated plants. Expression of *rolB* under the control of the *DefH9* and *INO* promoters also conferred parthenocarpy, but altered fruit qualities were observed (Martinelli et al. 2009).

AUXIN CUM SILENCING ACTION (*AUCSIA*) was identified as a gene that was repressed in parthenocarpic flower buds of *DefH9-iaaM* and *DefH9-RI-iaaM* transgenic plants (Molesini et al. 2009). The tomato genome has two *AUCSIA* genes (*AUCSIA1* and *AUCSIA2*) that encode small polypeptides. RNA interference (RNAi)-mediated simultaneous suppression of *AUCSIA1* and *AUCSIA2* has been shown to result in parthenocarpy and an approximately 100-fold increase in total IAA content in the buds. The parthenocarpic fruit size and weight were smaller than those of wild-type fertilized fruits. *AUCSIA1* and *AUCSIA2* are highly expressed in flower buds, although these expression levels were substantially reduced after pollination (Molesini et al. 2009). The role of *AUCSIA* during fruit set is still unclear, but it might be involved in either auxin synthesis or transport.

PIN-FORMED (PIN) auxin efflux transporters play important roles in fruit set by controlling polar auxin transport between ovules and nearby tissues. Of the ten *PIN* genes (*SlPIN1–SlPIN10*) identified in tomato (Pattison and Catalá 2012), *SlPIN4* has been shown to participate in fruit set (Mounet et al. 2012). *SlPIN4* is predominantly expressed in flower buds and young developing fruits, where the expression level is higher in the placenta than in the locular tissue and pericarp. Specific silencing of *SlPIN4* using an RNAi strategy resulted in parthenocarpy, suggesting a negative role in fruit set (Mounet et al. 2012).

7.2.2 *Modifications and Mutations of Genes Associated with Auxin Signal Transduction*

Auxin signaling is mediated by both transcription-dependent and -independent pathways, but only a few molecular components underlying the latter pathway have been identified (Mockaitis and Estelle 2008; Hayashi 2012). In the transcription-dependent pathway, protein–protein interactions among several key components lead to auxin responses. At low auxin levels, auxin/IAA (Aux/IAA) transcriptional repressors interact with auxin response factor (ARF) transcription

factors, which repress the transcriptional expression of auxin-responsive genes (Tiwari et al. 2004; Guilfoyle and Hagen 2007). At high auxin levels, auxin promotes the degradation of the Aux/IAA proteins via a ubiquitin–proteasome system (Gray et al. 1999; Dharmasiri and Estelle 2002). Auxin is perceived by the TIR1/AFB family of F-box proteins acting as auxin receptors, which form SCF E3 ubiquitin ligase complexes, leading to the ubiquitination of Aux/IAA (Gray et al. 2001; Kepinski and Leyser 2005; Dharmasiri et al. 2005; Maraschin et al. 2009). Auxin-dependent proteolysis of Aux/IAA leads to the induction of auxin-responsive gene expression via activation of ARF transcription factors.

A total of 26 Aux/IAA repressor family genes (*SlIAA1–SlIAA26*) have been found in tomato (Wu et al. 2012). *SlIAA9*, which is highly expressed throughout the plant, has been show to play a regulatory role in fruit development, and antisense lines of *SlIAA9* show a wide range of auxin-related growth alterations, including reduced leaf complexity and the production of parthenocarpic fruit with size, color, and flesh consistency that are similar to those of wild-type fruits (Wang et al. 2005). The accumulation of *SlIAA9* transcripts at anthesis forms a gradient, where the transcript levels are higher in the ovule, sporogenous tissue, placenta, and funiculus but lower in the ovary wall and columella (Wang et al. 2009). Rapid dissipation of the signal gradient occurs approximately one day after pollination, suggesting an important role for *SlIAA9* in the early stages of fertilization-induced fruit set. Three independent mutants of *SlIAA9* (*iaa9-3*, *iaa9-4*, and *iaa9-5*) exhibiting altered vegetative phenotypes and parthenocarpy have been identified in ethyl methyl sulfonate (EMS)-mutagenized or γ-ray-irradiated populations (Saito et al. 2011). The rates of parthenocarpy and seedless fruit expansion vary among the mutants, suggesting that the functional activity and extent to which parthenocarpy is conferred vary for the different *SlIAA9* alleles.

The tomato ARF family comprises at least 17 members (*SlARF1–SlARF17*; Kumar et al. 2011). *SlARF7* is predominantly expressed in unpollinated tomato ovaries and its expression rapidly decreases after pollination (de Jong et al. 2009b). RNAi transgenic tomato lines with reduced *SlARF7* mRNA levels produce parthenocarpic fruits, suggesting that this ARF gene acts as a negative regulator of fruit set. Furthermore, the parthenocarpic fruits showed GA-related phenotypes, such as a thick pericarp due to extensive cell expansion, in addition to auxin-related phenotypes, specifically a heart-shaped fruit and the formation of seedlike structures resembling pseudoembryos. These findings suggest that SlARF7 could be involved in the cross talk between auxin and GA during fruit set, and one model suggests that SlARF7 activates auxin response-attenuating genes (such as *Aux*/*IAA*s) in unpollinated ovaries, while downregulation of *SlARF7* after pollination results in an activation of both auxin and GA signaling that is required for fruit set (de Jong et al. 2011).

In *A. thaliana*, *atarf8* mutants produce parthenocarpic fruits (siliques) without fertilization, suggesting that *AtARF8* is a negative regulator of fruit set (Goetz et al. 2006). Goetz et al. (2007) further showed that the introduction of aberrant forms of *AtARF8* led to parthenocarpy in *A. thaliana* and tomato. Since the expression of *AtARF8* was not reduced in the transgenic plants, the mutated form

of the AtARF8 protein may have functionally competed with endogenous AtARF8 protein and its tomato homolog. A model was proposed by the authors in which ARF8 forms a regulatory complex with Aux/IAA, and this complex directly or indirectly represses transcription of fruit set-regulating genes (Goetz et al. 2006, 2007).

A putative tomato auxin receptor, SlTIR1, plays an important role in the early stage of fruit set (Ren et al. 2011). *SlTIR1* is highly expressed in the ovary and sepal at anthesis, but its expression decreases after pollination. Overexpression of *SlTIR1* results in an auxin-responsive phenotype, including altered vegetative morphology, sterility, and parthenocarpy, and SlTIR1 has been suggested to positively regulate the auxin response via the 26S proteasome-mediated signaling pathway (Ren et al. 2011).

7.2.3 Altered Expression and Mutations of GA-Related Genes

The regulatory effect of GAs on fruit development has been well documented (Wittwer et al. 1957; Sastry and Muir 1963; Serrani et al. 2007a). Endogenous GAs in plants are synthesized in two parallel pathways, the non-13-hydroxylation and early 13-hydroxylation pathways, and in tomato fruit set, the early 13-hydroxylation pathway appears to predominate (Bohner et al. 1988; Fos et al. 2000). Expression of genes in the tomato GA20ox family (*SlGA20ox1*–*SlGA20ox3*) that mediate bioactive GA synthesis increases in the ovaries after pollination, suggesting a central role for *GA20ox* genes in GA synthesis during fruit set (Serrani et al. 2007b). Additionally, overexpression of a citrus *GA20ox* gene (*CcGA20ox1*) in tomato resulted in pleiotropic phenotypes similar to those of GA-treated plants, including parthenocarpy (García-Hurtado et al. 2012).

DELLA family proteins act as key repressors of GA signaling. GA induces the degradation of DELLA proteins via the ubiquitin–proteasome system, leading to GA responses (Dill et al. 2001; Mcginnis et al. 2003; Sun 2010). Tomato has a single DELLA gene (*SlDELLA*), and antisense-mediated silencing of this gene results in constitutive GA-responsive phenotypes, such as elongated plant shape and parthenocarpy (Martí et al. 2007). In addition to being smaller and elongated, the parthenocarpic fruit had a reduced number of cells, which were elongated in the pericarp. These features are similar to those seen in GA-induced fruits (Serrani et al. 2007a), suggesting that parthenocarpic fruit development in *SlDELLA* antisense bypasses auxin-regulated cell division (Martí et al. 2007). A loss-of-function mutant of *SlDELLA*, *procera* (*pro*), also exhibits a constitutive GA-responsive phenotype, including parthenocarpy (Bassel et al. 2008). The *pro* mutation influences auxin signaling with a reduction of *SlARF7* expression during fruit set (Carrera et al. 2012), suggesting a role of SlARF7 in the cross talk between GA and auxin signaling during fruit set.

7.2.4 Modification of the Expression of Ethylene-Related Genes

Ethylene plays a critical role in many developmental processes, such as senescence and abscission of leaves (Lim et al. 2007) and flowers (van Doorn and Woltering 2008), and fruit ripening (Barry and Giovannoni 2007). Llop-Tous et al. (2000) showed that pollination induces transient increases in the production of ethylene in tomato pistils for several hours, although this apparently does not induce ovary senescence (Vriezen et al. 2008) and ethylene production decreases after 12 h of pollination (Llop-Tous et al. 2000). The expression of various genes related to the biosynthesis and signaling of ethylene has been observed to change during the early development of both pollinated and parthenocarpic tomato fruits (Vriezen et al. 2008; Pascual et al. 2009; Wang et al. 2009), all suggesting that ethylene also plays a regulatory role in tomato fruit set; however, further studies are required to elucidate the exact mechanisms.

Overexpression of a tomato signaling component of ethylene (*S. lycopersicum TETRATRICOPEPTIDE REPEAT PROTEIN 1, SlTPR1*) that interacts with the ethylene receptors NEVER RIPE (NR) and *S. lycopersicum* ETHYLENE RECEPTOR 1 (SlETR1/LeETR1) results in ethylene-related pleiotropic effects and parthenocarpic fruit set (Lin et al. 2008). The upregulation of an auxin-responsive gene in the buds of SlTPR1-overexpressing plants suggests that SlTPR1 is directly or indirectly involved in auxin signaling, while downregulation of *SlIAA9* in the ovaries of *SlTPR1* transgenic plants may contribute to parthenocarpic fruit set. Further studies are required to clarify the role of SlTPR in ethylene signaling and fruit set.

The application of ethylene biosynthesis or action inhibitors has been found to induce parthenocarpy in zucchini (*Cucurbita pepo*), indicating that ethylene negatively regulates its fruit set (Martínez et al. 2013). In *A. thaliana*, ethylene is involved in ovule senescence and negatively regulates parthenocarpic fruit set induced by GA (Carbonell-Bejerano et al. 2011). Recently, either the treatment of 1-methylcyclopropene (1-MCP), an ethylene action inhibitor, or ethylene-insensitive *Sletr1* mutation was found to induce parthenocarpic fruit set from emasculated flowers, most likely due to the accumulation of bioactive GAs (Shinozaki et al. 2015). The efficiency of the pollination-independent fruit set induced by *Sletr1* mutation was different depending on the genetic background, and unidentified locus/loci in a dwarf cultivar Micro-Tom, other than *dwarf*, can enhance the parthenocarpic efficiency. Ethylene may play a role in tomato fruit set by suppressing GA metabolism before pollination, while the molecular mechanism of the suppression has not yet been uncovered.

7.2.5 *Altered Expression and Mutations of MADS-Box Transcription Factor Genes*

MADS-box proteins are multifunctional transcription factors found in a wide range of eukaryotic organisms, and plant MADS-box proteins function in the regulation of organ and cell differentiation in flower development (Theissen and Saedler 2001). Tomato has at least 36 MADS-box genes (Hileman et al. 2006), and several studies have indicated a relationship between tomato parthenocarpic fruit development and MADS-box proteins. In *A. thaliana*, three MADS-box genes *SEPALLATA1–SEPALLATA3* (*SEP1–SEP3*) are required for normal floral organ development (Pelaz et al. 2000). Studies with tomato have shown that *Tomato MADS-box 29* (*TM29*), a homolog of *A. thaliana SEP1*, is continuously expressed in developing flowers and preferentially in the peripheral region of well-differentiated ovaries and fruits (Ampomah-Dwamena et al. 2002). Transgenic tomato plants constitutively expressing an antisense construct of *TM29* produce morphologically altered flowers and parthenocarpic fruits, suggesting that TM29 not only is required for normal flower development but may also function as a negative regulator of fruit set. In apple (*Malus pumila*), antisense suppression of *MADS8* and *MADS9* (homologs of *SEP1* and *SEP2*, respectively) resulted in an increased expression of auxin biosynthetic genes, a reduced expression of the GH3 auxin-conjugating enzyme genes, and a high accumulation of free auxin in the fruits during the early ripening stage (Schaffer et al. 2013). Although auxin concentration in the *TM29*-suppressed ovaries had not been measured, production of parthenocarpic fruits by silencing of these *SEP* family genes might be a consequence of the elevated auxin concentration.

TM5, a tomato ortholog of *A. thaliana SEP3*, is known to function in both flower and fruit development (Pnueli et al. 1994). *TM5* is continuously expressed in the central apical zone of the floral meristem throughout differentiation in the tissues of petals, stamens, and pistils. Antisense suppression of *TM5* expression resulted in parthenocarpy and altered identities of floral organs, manifested by sepaloid green petals and abnormal sterile anthers, but with no obvious change in vegetative organs (Pnueli et al. 1994). In addition, parthenocarpic fruit development resulted from a mutation in, or silencing of, the duplicated MADS-box genes, *Tomato APETALLA3* (*TAP3*)/*SlDEF* and *TM6* (de Martino et al. 2006). These genes belong to the *AP3* group, a subfamily of class B MADS-box genes that are required for specification of petals and stamens. *TAP3* is expressed predominantly in developing petals and stamen primordia until the late stage of flower differentiation, when the expression is restricted to several floral tissues. A *tap3* null mutant was shown to have sepaloid petals and carpel-like anthers, and occasionally exhibited parthenocarpy. Recently, two *stamenless* (*sl*) mutants (*slPr* and *sl-LA0269*), which exhibit floral homeotic conversion to different degrees, were found to develop parthenocarpic fruits, and their phenotypes most likely resulted from mutation(s) in the coding sequence and promoter region, respectively, of *TAP3* (Quinet et al. 2014). *TAP3*-antisense plants exhibited similar floral homeotic conversion to *tap3*

mutants, developing parthenocarpic fruits to which carpel-like stamens remained attached, and the severity was found to correlate with the extent of gene silencing. Similarly, *TM6* is weakly but constitutively expressed in the primordia of petals, stamens, and carpels (de Martino et al. 2006), and *TM6*-RNAi transgenic plants are defective in stamen development and exhibit occasional parthenocarpy.

Even with efficient parthenocarpy and normal vegetative development, the floral reversion-like phenotype in the expression of downregulated or loss-of-function mutants of MADS-box genes described above leads to sterility, which limits their use for breeding. More spatially and temporally specific and/or more finely tuned regulation may be needed to develop practical parthenocarpic varieties using these genes. Both parthenocarpy and anther ablation were observed in the mutants and MADS-box gene transgenic plants described above. In the following section, we provide another example of a transgenic tomato line that supports the idea of a relationship between parthenocarpy and anther ablation.

7.2.6 Early Anther Ablation

It has been suggested that the stamens of *A. thaliana* play a regulatory role in preventing initiation of fruit in the absence of fertilization (Vivian-Smith et al. 2001). Parthenocarpy with male sterility has been reported in tomato *parthenocarpic fruit* (*pat*), a mutant derived from natural parthenocarpic variation (Mazzucato et al. 1998), implying that dysfunction of male organ, stamen, is involved in the parthenocarpic fruit development. Roque et al. (2007) showed that the induction of a *Bacillus amyloliquefaciens* ribonuclease gene (*barnase*) under the control of an anther-specific promoter from the *Pisum sativum ENDOTHECIUM 1* (*PsEND1*) gene of pea resulted in specific ablation of the anther at early stages of the development and male sterility in *A. thaliana*, tobacco, oilseed rape, and tomato. Interestingly, tomato plants transformed with the *PsEND1::barnase* construct showed not only early anther ablation but also highly efficient parthenocarpy, while the other species completely impaired fruit development (Roque et al. 2007; Medina et al. 2013). The fruit productivity of the transgenic tomato plants was not significantly changed compared to wild-type plants, since although several transgenic lines produced smaller fruits, a significant increase in the number of fruits per plants was observed in all of the transgenic lines. Furthermore, *PsEND1::barnase* tomato plants produced high-quality fruits with respect to nutritional components such as γ-aminobutyric acid (GABA), glutamic acid, neoxanthin, and tocopherols. This approach in tomato crop production is attractive because it confers effective parthenocarpy without adverse effects on vegetative tissues. Further studies are required to understand the mechanisms underlying the link between early anther ablation accompanied by male sterility and parthenocarpic fruit development.

7.2.7 *Modifications of Flavonoid Synthesis-Related Pathways*

Genetic engineering of the flavonoid biosynthesis pathway represents yet another method to obtain parthenocarpic fruit. RNAi-mediated suppression of *chalcone synthase* (*CHS*), which encodes an important enzyme in flavonoid biosynthesis, has been reported to reduce total flavonoid levels and induce parthenocarpy (Schijlen et al. 2007). Transgenic plants showed normal vegetative growth, but their fruits were seedless and smaller than those of the seeded control fruits. Flavonoids play an essential role in reproductive processes such as pollen development and pollen tube growth. The parthenocarpic fruit development in the *CHS* RNAi plants appeared to be pollination associated, as pollen tube growth was impaired, and so fertilization was prevented. These findings suggest that pollination is required and sufficient to trigger fruit set and that fertilization leads to subsequent normal fruit development and expansion.

Seedless fruits with reduced flavonoid levels were also generated by introduction of the grape (*Vitis vinifera*) *stilbene synthase* (*StSy*), a key enzyme in the synthesis of the antioxidant resveratrol (Giovinazzo et al. 2005; Ingrosso et al. 2011). It was suggested that the altered flavonoid metabolism in *StSy*-transformed plants was caused by the competition between the biosynthetic pathways of resveratrol and chalcone and the parthenocarpic fruits contained high concentrations of soluble antioxidants, ascorbate and glutathione (Giovinazzo et al. 2005). Flowers of *StSy*-transformed plants displayed an open anther structure and were disturbed in pollen development, resulting in reduced seed set. The possibility that male sterility in the transgenic plants may be associated with its parthenocarpy was also suggested (Ingrosso et al. 2011; Medina et al. 2013).

7.3 Natural Variants and Introgression Lines Associated with Parthenocarpy

Several parthenocarpic variants have been developed for efficient fruit production, even under unfavorable conditions (Ho and Hewitt 1986), and three major natural variants for facultative parthenocarpy, *pat*, *pat-2*, and *pat-3*/*pat-4,* have been reported. In addition, two different lines exhibiting parthenocarpy have been described and used for quantitative trait locus (QTL) analysis (Gorguet et al. 2008). Identification of the associated genes will provide not only completely linked (so-called perfect) markers for parthenocarpy but also the identification of important pathways that confer parthenocarpy.

The *pat* mutant, harboring a recessive mutation in a single gene associated with parthenocarpy, was independently found in the tomato cultivars "*Soressi*" and "*Montfavet 191*." The *pat* mutation results in several growth defects, including abnormal anthers, female gametes with lower viability and fewer seeds, and

reduced fruit size and weight (Mazzucato et al. 1998). Altered development of stamens and parthenocarpy in the *pat* mutants suggests homeotic functions of *PAT*; however, while the *pat* locus has been mapped to the long arm of chromosome 3 (Beraldi et al. 2004), the candidate gene has not yet been identified.

The tomato cultivar "*Severianin*" exhibits strong parthenocarpy and its fruits reach almost normal size even under unfavorable conditions (Philouze and Maisonneuve 1978). "*Severianin*" was developed from crosses between several cultivars of tomato (*S. lycopersicum*) and the wild tomato species *S. habrochaites*. A genetic analysis suggested that the resulting parthenocarpy is controlled by two recessive loci, with major (*pat-2*) and minor (*mp*) effects (Vardy et al. 1989). Difference in yield and vigor has been observed in plants in which *pat-2* was introduced, depending on the genetic background (Gorguet et al. 2008). Another strong parthenocarpic cultivar, "*PR75/59*," was developed from a cross between the two weaker parthenocarpic cultivars, "*Atom*" and "*Bubjekosoko*," and so two loci are thought to be responsible for the parthenocarpic phenotype, which have been designated *pat-3* and *pat-4* (Philouze 1989). However, the considerable variation in fruit size in *pat-3*/*pat-4* makes this a less attractive cultivar for commercial production and hence for breeding.

The high accumulation of GAs in *pat*, *pat-2*, and *pat-3*/*pat-4* ovaries may be associated with their parthenocarpic fruit set (Fos et al. 2000, 2001; Olimpieri et al. 2007). Olimpieri et al. (2007) showed that *GA20ox1* is constitutively expressed in the ovaries of the *pat* mutant, while *GA20ox3* has been shown by others to be highly expressed in *pat-3*/*pat-4* ovaries (Pascual et al. 2009). In addition, the early growth of parthenocarpic *pat-2* fruits is associated with polyamine metabolism. Polyamines are required for the parthenocarpy in *pat-2*, and that treatment of wild-type unpollinated tomato ovaries with exogenous polyamines induces partial parthenocarpy, proposing a model that the elevated GA content in the *pat-2* ovaries induces polyamine synthesis (Fos et al. 2003).

In a QTL analysis using two tomato introgression lines (IL5-1 and IVT-line 1) carrying *S. habrochaites* chromosome segments and exhibiting highly stable parthenocarpy (Gorguet et al. 2008), four QTLs associated with parthenocarpy on chromosomes 4 (*pat4.1*, *pat4.2*), 5 (*pat5.1*), and 9 (*pat9.1*) were found. The two QTLs, *pat4.1* from IL5-1 and *pat4.2* from IVT-line 1, are allelic and closely linked to *SlARF8*, indicating that this gene is potentially responsible for parthenocarpy in these ILs, providing new target loci for breeding.

7.4 Perspectives

Parthenocarpic tomato fruits can be artificially produced using chemicals or via genetic modification. Although parthenocarpic varieties may contribute to efficient fruit production, as well as reductions in labor and cost for many crops, they have not yet been widely introduced in tomato cultivation, mainly because of a lack of genetic resources. The parthenocarpic plants described above provide possible resources for the development of commercially viable tomato varieties; however,

several parthenocarpic genotypes exhibit undesirable traits, such as small-sized fruit and morphological defects. Techniques targeting spatial and temporal regulation of gene expression at adequate levels, for instance, using promoters that function specifically in ovarian tissues such as ovule, pericarp, and placenta during early stages of fruit development, may be helpful in eliminating such undesirable traits. In addition, the examples of varied phenotypes among mutant alleles of *SlIAA9* (Saito et al. 2011) and *TAP3* (Quinet et al. 2014) indicate that screening or generating various mutant alleles of genes associated with fruit set, via techniques such as targeting-induced local lesions in genomes (TILLING) (Gady et al. 2009; Okabe et al. 2011) and genome editing (Osakabe and Osakabe 2015), may contribute to increasing genetic variation and obtaining better breeding resources for parthenocarpic cultivars. Until now, only a few parthenocarpic genotypes have been evaluated for productivity performance, such as fruit quality, vegetative phenotypes, and yield, in commercial field environments that include exposure to low and high temperatures. To identify appropriate breeding resources, further practical trait evaluation is necessary. Parthenocarpic *pro* plants often show severe fruit malformation under greenhouse conditions (Carrera et al. 2012), but we have found that a different mutated allele of *DELLA* results in fewer defects in morphology of the parthenocarpic fruits under both moderate- and high-temperature condition compared with the *pro* mutant (unpublished data). This illustrates the necessity and benefit of trait evaluation in practice. Genes and loci with potential for improving tomato fruit set are now emerging (Table 9.1), but genetic resources and understanding of the fruit set mechanisms are still incomplete. Although molecular pathways and genes that are required for fruit set, including those downstream of hormone signaling, are poorly understood, continuing advances in genetic, genomic, and molecular tools and associated bioinformatic platforms are likely to accelerate their discovery.

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