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Genetic analysis of reproductive development in tomato

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ABSTRACT Besides being an important commercial crop, tomato (*Solanum lycopersicum* L.) constitutes a model species for the study of plant developmental processes. Current research tends to combine classic disciplines such as physiology and genetics with modern approaches coming from molecular biology and genomics with a view to elucidating the biological mechanisms underlying plant architecture, floral transition and development of flowers and fruits. Comparative and functional analyses of tomato regulatory genes such as *LATERAL SUPPRESSOR (LS), SELF PRUNING (SP), SINGLE FLOWER TRUSS (SFT)* and *FALSIFLORA (FA)* have revealed mechanisms involved in shoot development and flowering time which are conserved among *Arabidopsis*, tomato and other plant species. Furthermore, several regulatory genes encoding transcription factors have been characterized as responsible for singular features of vegetative and reproductive development of tomato. Thus, the sympodial growth habit seems to require a specific control of the developmental fate followed by shoot meristems. In this process, novel genetic and molecular interactions involving *SP, SFT* and *FA* genes would be essential. Also this latter, but mainly *ANANTHA (AN)* and *COMPOUND INFLORESCENCE (S)* have recently been found to regulate the inflorescence architecture of the tomato. Concerning fruit development, genetic and molecular analyses of new genes such as *fw2.2, FASCIATED, OVATE* and *SUN* have proved their contribution to the domestication process and most importantly, their function as key regulators of fruit size and shape variation. Tomato ripening is also being elucidated thanks to the characterization of regulatory genes such as *RIPENING INHIBITOR (RIN), NON-RIPENING (NOR), TDR4* and *COLORLESS NON-RIPENING (CNR)*, which have been found to control early stages of fruit development and maturation. At the same time, much research is dedicated to isolating the targets of the ripening regulators, as well as the key genes promoting the parthenocarpic development of tomato fruits. Hopefully, the ongoing sequencing project and the progress made by integrating several research fields will help to unravel the genetic and molecular pathways controlling tomato development.

KEY WORDS: *tomato, flowering, fruit development, transcription factor*

Introduction

Tomato is a perennial vegetable which is almost universally cultivated as an annual crop. It belongs to the *Solanaceae* family as do its close cousins potato, eggplant, pepper, tobacco and petunia. The origins of cultivated tomato can be traced to the Andean region of South America -from Ecuador to Chile- but its native wild species was less attractive in size, shape and colours than the domesticated cultigens taken to Europe in the mid-sixteenth century. Several data point to Mexico as the probable region of domestication and the word "tomatl" in the Nahuatl language of Mexico is undoubtedly the origin of the modern name (Rick, 1978). While morphological descriptions usually made from herbarium material placed wild tomato as belonging to the genus *Lycopersicon*, a recent study on the tomato variability, including genetic and molecular markers, has shown that it is deeply nested in the *Solanum* genus, forming the sister clade to potato (Peralta *et al*., 2005). Therefore, *Lycopersicon esculentum* Mill. has been renamed *Solanum lycopersicum* L.

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Thought to be poisonous due to its relationship with belladonna and mandrake, tomato is now a major crop for human consumption. Together with its economic importance, it has also become a model plant for research purposes (Emmanuel and Levy, 2002; Van der Hoeven *et al.*, 2002; Giovannoni, 2007). It is easy to cultivate, has a short life cycle and tends itself to horticultural manipulation including grafting or cutting. Various types of explants can be cultured *in vitro* and plant regeneration is feasible, allowing efficient transformation procedures. In addition, tomato has several features that distinguish it from other model plant species: it is phylogenetically distant either from maize, *Arabidopsis*, snapdragon, rice, *Medicago* or poplar, it contains sequences that share no significant similarity to those from other plant species and it grows as an indeterminate plant due to reiterate switches from vegetative to reproductive stages. Together, genetic research has progressed further than in other crop species, and lately, tomato is the most advanced model among species bearing a fleshy berry type of fruit. Other properties of tomato, such as the small genome size (0.9 pg per haploid genome; Arumuganathan and Earle, 1991), the availability of a large set of mutants and the development of genomic and sequencing resources (genetic and physical maps, ESTs and microarrays), have favoured an international sequencing project and are contributing to the current progress in understanding the biological bases of plant development. Extensive details on these topics can be obtained at the following website: http://www.sgn.cornell.edu/.

Noteworthy, tomato mutants constitute not only an essential source of plant material for breeders but also a valuable tool for isolating important genes which regulate developmental patterns of tomato, and whose functional roles are now being elucidated. Many spontaneous mutants are being preserved and characterized by the Tomato Genetic Resource Center (Chetelat, 2005). Also, induced mutations, mainly generated through chemical ethylmethane sulfonate (EMS) and irradiation, are providing available screening populations and the possibility to identify new developmental genes. Exhaustive data about these tomato mutants can be obtained from the following Websites: http://tgrc.ucdavis.edu/ and http://zamir.sgn.cornell.edu/mutants/. Nevertheless, as few insertional mutants have been described to date, more research is required in approach to facilitate the cloning of tomato genes and unveil their functions and molecular interactions.

Shoot architecture

For most tomato cultivars the vegetative phase is short; typically 6 to 12 leaves are produced below the first inflorescence and the floral transition usually starts when the third leaf is expanding. Contrary to *Arabidopsis thaliana* and *Antirrhinum majus*, which have a monopodial growth pattern, tomato shows a sympodial growth habit. While in monopodial species the shoot apical meristem (SAM) is indeterminate and the vegetative or reproductive organs are generated on its flanks, the SAM of tomato is determinate and the primary shoot is completed by the first inflorescence (Figs. 1 and 2). This first stem fragment is called the "initial segment". A new vegetative shoot then arises from the uppermost (proximal) axillary meristem, i.e. the sympodial meristem located at the axil of the youngest leaf just

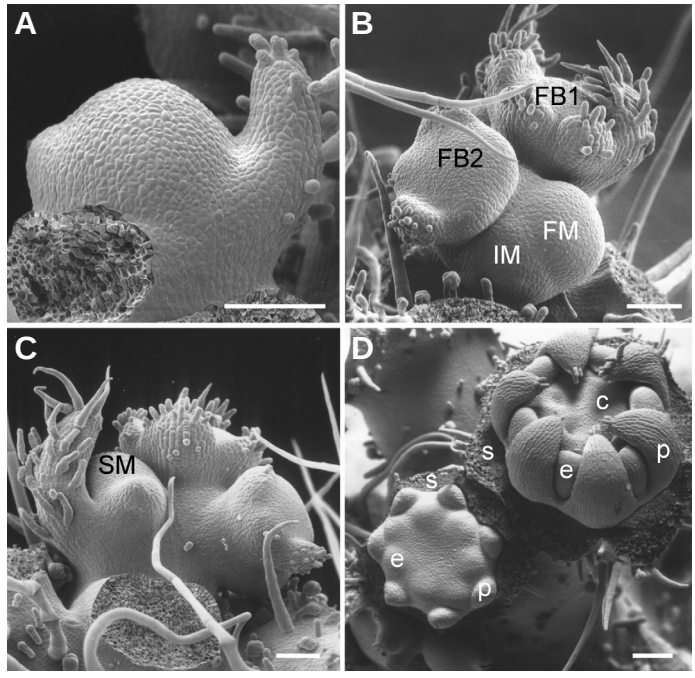

Fig. 1. Development of tomato meristems *(analysed by scanning electron microscopy).* *The shoot apical meristem (SAM) develops leaf primordia* **(A)**. *Upon floral transition, the SAM is converted into a reproductive meristem* **(B)**, *which in turn gives rise to the first floral meristem (FM) and a mound of dividing cells, the latter functioning as an inflorescence meristem (IM). The successive splitting of the IM produces new FMs until the inflorescence development is completed; organ primordia emerging from the floral meristem can be observed in the floral buds (FB). The indeterminate growth of tomato plant occurs by the activity of the sympodial meristem (SM), which is located at the axil of the uppermost leaf* **(C)**. *Two flower buds at different developmental stages can be observed along the inflorescence* **(D)**; *they bear organ primordia (s, sepal; p, petal; e, stamen; c, carpel) placed in four consecutive flower whorls (sepal primordia removed). Scale, 100 μm.*

below the terminating inflorescence (Fig. 1C). The sympodial meristem allows the plant to continue its growth carrying up the leaf which, due to the active growth of this bud and the partial fusion of its petiole with the new vegetative shoot, is finally located above the inflorescence. This sympodial shoot forms three vegetative nodes (leaves) and terminates in a new inflorescence. This growth pattern is repeated by the formation of successive determinate units or sympodial segments, resulting from the newly arisen sympodial meristems (see wt phenotype in Fig. 2). Thus, the architecture of a tomato plant means a regular alternation of vegetative and reproductive phases along the primary and axillary shoots (Atherton and Harris, 1986).

Despite the differences between the monopodial and sympodial systems, genes that maintain the indeterminate state of the shoot apex in *Arabidopsis, TERMINAL FLOWER 1* (*TFL1*), and *Antirrhinum, CENTRORADIALIS* (*CEN*), have an orthologue in the tomato genome, the *SELF PRUNING* (*SP*) gene (Table 1), which controls the regular vegetative-to-reproductive switch of inflorescence meristems (Pnueli *et al.*, 1998). A mutation in *SP* or the suppression of gene activity by antisense RNA has no effect on the architecture of the initial segment or the flowering time (in terms of node number). However, *sp*

mutant allele promotes a gradual reduction in the number of vegetative nodes arising on successive sympodial segments until the vegetative phase is completely by-passed with the development of two consecutive inflorescences (Fig. 2) (Yeager, 1927). Overexpression of *SP* or *CEN* in tomato plants results in an extended vegetative phase of sympodial shoots and in an increased leafiness of the inflorescence itself (Pnueli *et al.*, 1998). *CEN*, *TFL1* and *SP* are members of a novel family of regulatory genes denominated *CETS*, which encode a family of modulator/adapter proteins capable of interacting with a variety of signalling proteins. Among the tomato members of this family, *SINGLE FLOWER TRUSS* (*SFT*) gene is involved both in the transition to flowering (see the following section) and the shoot development (Lifschitz *et al.*, 2006). In fact, *sft* mutation arrests the growth of the sympodial buds and allows the plant to grow from the ectopic vegetative meristems appearing in the inflorescence (Kerr, 1982). Therefore, *SFT* gene is required for the normal development of the sympodial meristem. Reduced expression level of *SP* in the apical buds of the *sft* mutant suggests that the function of *SFT* controlling sympodial development depends on its interaction with *SP*, probably acting as an upstream regulator of *SP* in the sympodial meristem (Molinero-Rosales *et al.*, 2004).

The formation of lateral branches is regulated by *LATERAL SUPPRESSOR* (*LS*) and *BLIND* (*BL*) genes (Fig. 2). While *BL* affects both sympodial and axillary meristems (Rick and Butler, 1956; Schmitz *et al.*, 2002), *LS* is only involved in the development of the latter (Malayer and Guard, 1964; Schumacher *et al.*, 1999). Among the phenotypic abnormalities showed by *ls* mutant, the lack of lateral meristems during vegetative growth of the initial segment is particularly apparent (Fig. 2). Cloning and characterization of *LS* has revealed that it encodes a putative transcription factor of the GRAS family to which some negative regulators of gibberellin response also belong (Schumacher *et al.*, 1999). Furthermore, expression of the *Arabidopsis* orthologous *LAS* gene in the tomato *ls* mutant restores the wild-type phenotype, indicating a conserved mechanism in the control of axillary meristem initiation (Greb *et al.*, 2003). The *BL* gene is a member of the R2R3 class of MYB transcription factors involved in the regulation of various biological processes. The phenotype of the double mutant *ls bl* suggests that *BL* and *LS* participate in different pathways which promote the development of lateral meristems (Schmitz *et al.*, 2002).

Transition to flowering

In tomato, the transition to flowering means that the apical meristem is completely consumed in the development of the first inflorescence. This process is under control of environmental and endogenous factors, the later being of genetic and hormonal nature. This crop species is considered a day-neutral plant since the time to flowering, as measured by the number of leaves developed before floral transition, is not affected by photoperiod (Kinet and Peet, 1997). In fact, some genes have been identified and characterized as members of an autonomous pathway controlling floral transition. In the initial segment, *FALSIFLORA* (*FA*) and *SINGLE FLOWER TRUSS* (*SFT*) promote floral transition (Molinero-Rosales *et al.*, 1999; Molinero-Rosales *et al.*, 2004; Lifschitz *et al.*, 2006), while *SP* regulates this process in the sympodial segments (Pnueli *et al.*, 1998). Mutations at either *FA* or *SFT* loci result in a photoperiod-independent late flowering phenotype and in abnormalities affecting inflorescence development (Fig. 2). *FA* is orthologous to *LEAFY* (*LFY)* and *FLORICAULA* (*FLO*), two floral identity genes of *Arabidopsis* and *Antirrhinum*, respectively, although only *LFY* regulates floral transition as *FA* does (Molinero-Rosales *et al.*, 1999).

SFT is orthologous to the *Arabidopsis FLOWERING LOCUS T* (*FT*) and triggers systemic signals that regulate floral transition and sympodial growth in tomato (Lifschitz *et al.*, 2006). Double mutant *sft fa* is unable to flower suggesting that *FA* and *SFT* regulate floral transition by independent pathways. This agrees with the fact that *FA* expression is not affected by *sft* mutation (Molinero-Rosales *et al.*, 2004). In petunia, floral transition is abolished by a single mutation in the *PETUNIA FLOWERING GENE* (*PFG*), a MADS-box gene highly homologous to *Arabidopsis APETALA1* (*AP1*) (Immink *et al.*, 1999). Interestingly, *AP1* overexpression in tomato caused early flowering with no obvious effects on sympodial development (Ellul

TABLE 1

TOMATO MUTATIONS AFFECTING EITHER INFLORESCENCE OR FLORAL MERISTEM IDENTITY GENES

Mutant	Phenotype	Isolated gene	References	*Arabidopsis* orthologue
self-pruning (*sp*)	Altered sympodial meristem development	*SP*	Yeager, 1927 Pnueli *et al.*, 1998	*TERMINAL FLOWER1*
single flower truss (*sft*)	Late flowering, altered sympodial development	*SPD3* (*SFT* in text)	Kerr, 1982 Lifschitz *et al.*, 2006	*FLOWERING LOCUS T*
lateral suppressor (*ls*)	Lack of axillary lateral meristems	*LS*	Rick and Butler, 1956 Schumacher et al. 1999	*LAS*
blind (*bl*) = *torosa* (*to*)	Absence of sympodial meristem	*BL*	Malayer and Guard, 1964 Schmitz *et al.*, 2002	*RAX* R2R3 Myb
falsiflora (*fa*)	Late flowering, loss of floral meristem identity	*FA*	Stubbe, 1963 Molinero-Rosales *et al.*, 1999	*LEAFY*
uniflora (*uf*)	Late flowering, inflorescence composed by a single flower		Dielen *et al.*, 1998	
compound inflorescence (*s*)	Late flowering, affected inflorescence meristem development	*S*	Quinet *et al.*, 2006 Lippman *et al.*, 2008	*WOX9/STIMPY*
anantha (*an*)	Highly branched inflorescence, altered floral meristem identity	*AN*	Allen and Sussex, 1996 Lippman *et al.*, 2008	*UNUSUAL FLORAL ORGANS*
jointless (*j*)	Altered inflorescence meristem identity	*J*	Rick and Butler, 1956 Mao et al., 2000	*AGL24*

et al., 2004). By contrast, mutations of three flowering genes, each involved in a different regulatory pathway, are required to avoid flowering in *Arabidopsis* (Reeves and Coupland, 2001). The domestication process of petunia (ornamental species) and tomato (crop species) may have favoured a reduction in the number of genes needed to flower by eliminating redundancy in flowering genes. Alternatively, gene interactions controlling floral transition may differ among plant species, yet the functional roles of individual genes may be similar.

Late flowering mutants *uniflora* (*uf*) and *compound inflorescence* (*s*) (Fig. 2) show enhanced phenotypes under winter conditions, namely low irradiance (daily light energy integral) and poor assimilate availability perceived by the apical meristem. Thus, an environment-dependent pathway also seems to regulate floral transition in tomato (Dielen *et al.*, 1998; Quinet *et al.*, 2006b). As the *fa sft* double mutant, introducing *sft* into the *uf* background completely suppresses floral transition, which suggests that *UF* and *SFT* promote flowering but participating in parallel regulatory pathways (Lifschitz and Eshed, 2006). Although the genetic interactions between *UF* and *FA* remain to be clarified, *uf* is epistatic over most of flowering mutations (Quinet *et al.* 2006a), indicating that *UF* is a key regulator of tomato flowering. Moreover, *UF* might function upstream to *SFT* as is indicated by the fact that constitutive expression of *SFT* rescues the flowering time phenotype of the *uniflora* mutant, substituting its high light requirements (Lifschitz *et al.*, 2006).

SP gene does not affect the time to flowering in the initial segment, since its mutation produces a progressive shortening of the floral transition in the sympodial segments (Pnueli *et al.*, 1998). However, the capability of *sp* allele to rescue the flowering phenotype avoided in the non-flowering double mutants *sft fa* and *sft uf* (Lifschitz and Eshed, 2006) agrees with a role of *SP* in floral induction, which deserves greater attention. Additionally, *fa* mutation produces the opposite effect to *sp*, i.e. an increased number of vegetative nodes in the first sympodial segments. This feature, and the expression pattern of *FA*, proves its involvement in sympodial development. Although the function of *SP* seems to be antagonistic to *FA*, the vegetative-to-reproductive switch in the sympodial segment may depend on a balance between *FA* and *SP* transcription levels (Molinero-Rosales *et al.*, 1999). Likewise, a balance in the activities of *SFT* and *SP* could be responsible for the floral transition of both initial and sympodial segments (Lifschitz and Eshed, 2006). Thus, different developmental scenarios may be possible as result of genetic interactions among flowering genes at the apical and sympodial meristems, which should be further investigated.

Additionally, *JOINTLESS* (*J*) and *BLIND* (*BL*) genes seem to promote autonomous flowering in tomato, although the most evident alterations produced by *j* and *bl* mutations affect the inflorescence development (Fig. 2). Molecular characterization of these genes and their interactions with meristem identity genes support this functional role (Mao *et al.*, 2000; Schmitz *et al.*, 2002; Szymkowiak and Irish, 2006).

Although tomato flowers autonomously, environmental cues can modify this developmental pattern. Low temperatures (10-15°C) reduce the number of nodes up to the first inflorescence, and also the rate at which these leaves are produced. Similarly, a scarce but significant reduction in flowering time has been observed in many cultivars grown under short day conditions (see Samach and Lotan, 2007). Most importantly, high irradiance accelerates flowering, an effect associated with a higher rate of leaf initiation and an increased assimilate availability in the meristem (Kinet and Peet, 1997; Dielen *et al.*, 2004). Light is perceived by the plant through photoreceptors, particularly red/far red light PHYTOCHROME (PHY) and blue light CRYPTOCHROME (CRY) receptors. Overexpression of tomato *CRY2* does not alter flowering time but does increase the number of days to the first floral anthesis (Giliberto *et al.*, 2005). Interestingly, a QTL mapping approach has revealed that *PHY2B* gene, as well as *FALSIFLORA*, co-localize with major QTLs responsible for floral transition in this species, making them candidate genes for the domestication process of tomato (Jiménez-Gómez *et al.*, 2007).

Autonomous flowering of tomato is also modulated by hormones, though their roles during floral transition have been poorly studied. Gibberellins (GAs) promote tomato flowering since GA-deficient mutants require exogenous gibberellins to flower (Koornneef *et al.*, 1990). Elevated GA contents increase the number of leaves before flowering and the rate of leaf initiation (Kinet and Peet, 1997). Furthermore, *in vitro* experiments show that cytokinin-mediated stimulating effect on floral initiation could be inhibited by GA. Most probably, plant hormones modulate tomato flowering by gene interactions involving several regulatory pathways although the nature of such interactions is still unknown.

Most flowering mutants already characterized in tomato flower later than the corresponding wild type backgrounds. Of forty one flowering mutants identified by an "*in silico*" screening, only four showed an early flowering phenotype (Menda *et al.*,

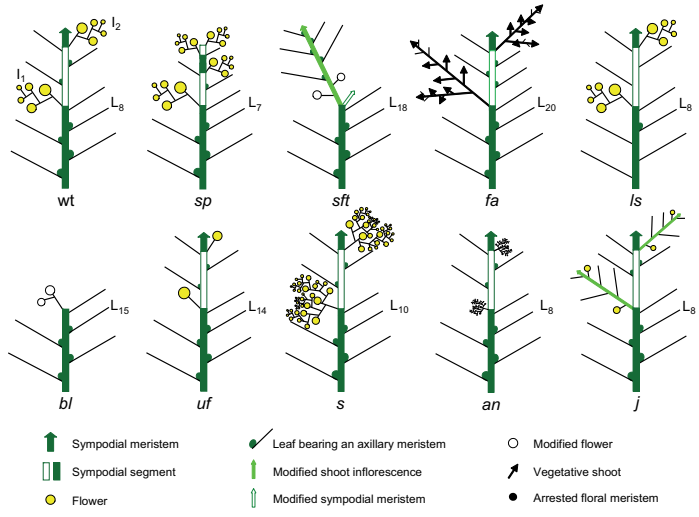

Fig. 2. Schematic representations of shoot architecture and reproductive structures *of wild type (wt) tomato and several mutants altered in floral transition* (sft, fa, bl *and* uf), *sympodial growth* (sp, sft *and* bl) *and inflorescence/floral development* (fa, sft, bl, uf, s, an *and* j). *Note that the wild type genotype used as reference in this paper usually flowers after the formation of seven leaves (L7); however this number may differ in other tomato backgrounds. See text for mutant descriptions. The first vegetative segment (green) is referred as the initial segment in the text; the remaining vegetative segments have a sympodial origin.*

2004). These observations, together with the influence of environmental cues on flowering time, suggest that selection of favourable combinations of flowering genes has played a crucial role throughout the tomato domestication process, enabling early flowering and ensuring fruit yield under several conditions.

Reproductive development

Tomato inflorescence has been classically described as a cyme, although available evidences also permit to interpret it as a raceme (Quinet and Kinet, 2007; Lippman *et al.*, 2008). Irrespectively, initiation of reproductive development entails the conversion of the apical meristem into an inflorescence meristem (IM) from which the floral meristem is produced laterally, giving rise to the first flower. The successive floral meristems developed from the IM are located at the base of each preceding flower bud. This process culminates in the production of a terminal flower, once the determinate inflorescence is composed of about five to ten flowers (Allen and Sussex, 1996).

Inflorescence and flower initiation

In tomato, the activity of the inflorescence meristem is affected by *BL* and *UF* genes, since the inflorescence is prematurely terminated by a reduced number of flowers in the *bl* mutant, or by a single flower in the *uf* mutant (Rick and Butler, 1956; Fehleisen, 1967; Dielen *et al.*, 1998; Schmitz *et al.*, 2002). The unusual reproductive structure of the *uf* mutant (Fig. 2) results from its inability to produce an inflorescence, not from abortion of flower buds (Dielen *et al.*, 2004). Moreover, double-mutants which involve *uf* allele in combinations with *sft*, *bl* or *j* mutations all develop a single normal flower (Quinet *et al.*, 2006a; Quinet and Kinet, 2007), demonstrating the epistatic interaction of *UF* respect to the remaining IM genes.

Phenotypes of *jointless* (*j*) and *macrocalyx* (*mc*) mutants display a reversion of the IM to a vegetative developmental programme (Fig. 2), allowing normal shoot formation and indicating that maintenance of IM identity requires *J* and *MC* genes (Rick and Sawant, 1955; Rick and Butler, 1956; Vrebalov *et al.*, 2002; Szymkowiak and Irish, 2006). Also the identity of IM is lost in the *sft* mutant after one or two flowers are developed (Fig. 2). It then reverts to a vegetative state and the position of the following flower is occupied by a sympodial shoot. Thus, *SFT* prevents the change of identity of the inflorescence meristem once flowering is initiated (Molinero-Rosales *et al.*, 2004).

Before flower development, floral identity is determined in the IM by the *FALSIFLORA* gene (Molinero-Rosales *et al.*, 1999). The *fa* allele promotes a strong inflorescence phenotype due to the replacement of flowers by secondary leafy shoots (Fig. 2 and Fig. 3B), as occurs in *Antirrhinum flo* and *Arabidopsis lfy* mutants. A careful examination of early development of *fa* inflorescence has shown that it is not the inflorescence meristem, but rather derived vegetative meristems which are unable to acquire floral identity (Allen and Sussex 1996). *FA* is the orthologue to the *Arabidopsis LFY* gene, the latter being extensively analysed since it is the major responsible for the transition from inflorescence to floral meristem in the model species (Weigel *et al.*, 1992; Blázquez and Weigel, 2000). The high sequence similarity observed between *FA* and *LFY* affecting DNA-binding domains and other regulatory elements should lead to further analyses about the mechanism determining floral meristem identity in tomato.

Mutations of *ANANTHA* (*AN*) and *S* genes also modify the developmental fate of IM giving rise to highly branched inflorescences composed either by reproductive meristems or normal flowers, respectively (Rick and Butler, 1956; Allen and Sussex, 1996). Such observations indicate the failure of the meristems emerging from the IM to specify floral identity. Recently, Lippmann *et al.* (2008) have demonstrated that *AN* encodes an F-box ortholog of the Arabidopsis floral gene *UNUSUAL FLORAL ORGANS* (*UFO*) while *S* gene codes for a transcription factor homologous to *WUSCHEL-HOMEOBOX9* (*WOX9/STIMPY*). Functional analyses of these two genes indicated that sympodial architecture of tomato inflorescence is made by the sequential expression of *S* and *AN*, which in turn promotes the phase transition of a IM to a floral meristem. Double-mutant analyses have shown that *FA* acts upstream to *AN*, confirming the key function of *FA* gene in the specification of floral identity.

The leafy phenotype of *fa* mutant is also achieved by overexpression of *SP* in wild type tomato and *sp* mutant plants. Similarly, the reproductive nature of *an* proliferating meristems is changed and vegetative shoots are also developed when *SP* is overexpressed. Both results make that the contribution of *SP* to the control of floral meristem identity can not be discarded. Moreover, expression domains of *SP* coincide with those of *FA* being the latter gene, epistatic to *SP* (Pnueli *et al.*, 1998; Molinero-Rosales *et al.*, 1999). Thus, the mutual negative regulation existing in *Arabidopsis* between *LFY* and *TFL1* is unlikely to occur between *FA* and *SP* in tomato. Most probably, *FA* could regulate floral identity by both activating *AN* and decreasing *SP* activity in the reproductive meristems.

In summary, *BL*, *J*, *SFT*, *MC*, *UF*, *AN* and *S* genes play important roles in the maintenance of reproductive meristem identity and together with *FA* and *SP* are required for other flowering-related processes (Fig. 2), i.e. floral transition (*FA*, *SFT, BL* and *UF*), sympodial growth of plant (*SP, SFT* and *BL*), inflorescence architecture (*AN* and *S*) and floral organ development *(MC)*. Therefore, further research should distinguish the main function of these genes from their pleiotropic effects during plant development, as Lifschitz *et al.* (2006) have recently done for *SFT*.

Floral organ development

At maturity, the hermaphrodite and symmetric flower of tomato consists of four whorls each formed from the outermost by 5-6 green sepals, which alternate to a similar number of yellow petals at the second whorl, about 6 stamens displaying anthers forming a cone around the style, and a variable number of fused carpels in the innermost whorl.

Genetic analyses in *Arabidopsis* and *Antirrhinum* have led to propose three main gene functions, A, B and C, each including a few number of genes, which acting alone or in combination determine organ identity in the four floral whorls. The so-called ABC model (Coen and Meyerowitz, 1991; Meyerowitz *et al.*, 1991) has been confirmed in several plant species and assumes that mutations affecting A-, B- and C-class genes promote homeotic changes in the floral organs of two consecutive

whorls. Most of the ABC genes belong to the MADS-box family encoding transcription factors, which are highly conserved among plant species. MADS proteins bind to DNA as multimeric complexes which ultimately control the development of floral organs (see Robles and Pelaz, 2005).

Characterization both of homeotic mutants and transgenic plants where homologous ABC genes have been up- or down-regulated seems to confirm the ABC model in tomato (Table 2). The *macrocalyx* mutation resides in a homologue to the *AP1*, an *Arabidopsis* A-function gene (Vrebalov *et al.*, 2002). Expression of *MC* is detected in sepals, petals and carpels while either mutation or gene silencing of *MC* causes homeotic conversion from sepals to leaf-like structures (Rick and Butler, 1956; Vrebalov *et al.*, 2002). Both the mutant phenotype and the pattern expression of *MC* are however more similar to *SQUAMOSA* (*SQUA*), the *AP1* orthologue of *Antirrhinum*.

Several B-class mutants showing partial or complete transformations in the second and third organ whorls have been identified in tomato (Nash *et al.*, 1985; Sawhney, 1992). Among them, *stamenless* (*sl*) and its allelic mutant *corollaless* (*cs*) show sepals instead of petals in the second whorl and stamens replaced by carpels in third whorl (Fig. 3D; Gómez *et al.*, 1999). Mutation of *SL* affects to a B-class gene homologous to *DEFICIENS* (*DEF*) in *Antirrhinum* and *APETALA3* (*AP3*) in *Arabidopsis*, both involved in the development of petals and stamens. In addition, among the several *TOMATO MADS BOX* genes (abbreviated as *TM* or *TDR*), *TM6* also shares homology to *AP3*, yet mapping data reject it as candidate for *SL* gene (Gómez *et al.*, 1999).

Regarding tomato C-class genes, tomato mutants with homeotic changes in both reproductive organs (whorls 3 and 4) have not been described until now. However, *TOMATO AGAMOUS1* (*TAG1*), a tomato orthologue to the *Arabidopsis AGAMOUS* (*AG*) gene has been isolated. Tomato plants expressing sense and antisense *TAG1* transcripts corroborate the role of *TAG1* in the specification of stamen and carpel identities (Pnueli *et al.*, 1994a).

Recently, the ABC model has been extended with two new classes of genes. D-class genes control ovule identity and were initially described in *Petunia* after the functional and molecular analyses of *FLORAL BINDING PROTEIN7* (*FBP7*) and *FBP11* (Angenent *et al.*, 1995). The Arabidopsis D-class gene is *SEEDSTICK* (*STK*) which, like *FBP7* and *FBP11*, is specifically expressed in ovules (Pinyopich *et al.*, 2003). Furthermore, it has been proven that A, B and C genes require an additional function which cooperates with them in the development of the four floral organs. Such function is carried out by the E-class *SEPALLATA* (*SEP*) genes. Strong evidence was found to support the formation of multimeric complex involving A, B, C and SEP proteins, as mechanism which triggers flower development (see Robles and Pelaz, 2005 for references). In tomato, *TM5* (Pnueli *et al.*, 1994b) and *TM29* (Ampomah-Dwamena *et al.*, 2002) have been described as two *SEP*-like genes on the basis of their expression pattern and down-regulated phenotypes. These and other tomato genes involved in carpel and ovule development are further described in the next section.

Activity of floral organ identity genes seems to depend on *FA* gene, as suggested by the inhibition of *TM5*, *TM6* and *TAG1* expression in *fa* inflorescences, as well as by the phenotype of weak mutant alleles of *FA* which allow floral organ development (Kato *et al.*, 2005; R. Lozano, unpublished). Therefore, *FA* plays in important role in the control of floral meristem identity but it may also induce floral organ identity genes.

More about carpel and ovule development

Bearing in mind the importance of the carpels as sexual floral organs which protect ovules and allow seed formation, much progress has been made in the identification of genes and protein interactions regulating carpel development. As previously mentioned, *AG* gene determines the identity of carpel primordia located at the fourth whorl of a developing flower, which also requires the activity of *SEPALLATA* genes in *Arabidopsis* (Pelaz *et al.*, 2000). However, carpelloid organs instead of sepals can be developed in the absence of *AG*, as happens in the first whorl of *ag ap2* flowers, indicating that an additional *AG*-independent pathway can also specify carpel features (Pinyopich *et al.*, 2003). *SHATTERPROOF1* (*SHP1*), *SHP2* and *SEEDSTICK* (*STK*), members of the same *AG* clade, have been identified as key genes of this pathway. *AG* acts redundantly to *SHP* genes to promote carpel development, while *AG, SHP* and *STK* play redundant roles in the specification of ovule identity (Liljegren *et al.*, 2000; Pinyopich *et al.*, 2003; Favaro *et al.*, 2003). While no protein-protein interactions were observed among *AG, STK* and *SHP* genes, all three interact with *SEP3*, suggesting that SEP proteins mediate the formation of a protein complex directing ovule and carpel identity (Favaro *et al.*, 2003). Likewise, overexpression and down-regulation of *FBP7* and *FBP11*, two *STK* orthologous genes of *Petunia*, confirm that an ovule-specific function is carried out by these MADS-box transcription factors, which in

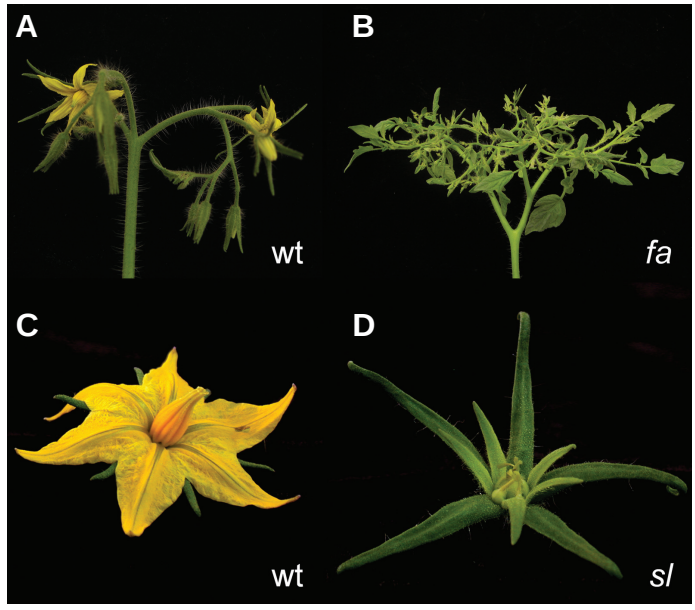

Fig. 3. Tomato mutants affected in floral meristem identity or flower development. *Respect to the wild type inflorescence* **(A)**, *flowers of the* falsiflora *(*fa*) mutant are replaced by secondary vegetative shoots* **(B)**, *demonstrating that* FA *gene controls floral meristem identity. The development of a normal tomato flower* **(C)** *is altered in the* stamenless *(*sl*) mutant, which shows homeotic conversion of petals into sepals in whorl 2, and carpels instead of stamens in whorl 3* **(D)**.

TABLE 2

TOMATO MUTANTS AND GENES IMPLICATED IN FLOWER DEVELOPMENT

Mutant or gene		Phenotype	Gene	Reference	Arabidopsis orthologue
A-class	*macrocalyx* (*mc*)	Large sepals, indeterminate inflorescence	*LeMADS-MC* (*MC* in text)	Rick and Sawant, 1955 Verbalov *et al.*, 2002	A-class
B-class	*stamenless* (*sl*)	Homeotic conversion of petals and stamens into sepals and carpels, respectively	*SL*	Nash *et al.*, 1985 Gomez *et al.*, 1999	B-class
	TM6	Silencing of *TM6* alters stamen development	*TM6*	de Martino *et al.*, 2006	
C-class	*TAG1*	Down-regulation of *TAG1* causes homeotic transformations of stamens and carpels	*TAG1*	Pnueli *et al.*, 1994a	C-class
E-class	*TM5*	Down-regulation alters organ differentiation in the inner three floral whorls	*TM5*	Pnueli *et al.*, 1994b	E-class
	TM29	Down-regulation of *TM29* affects the maintenance of floral meristem identity (flowers altered in the inner three whorls)	*TM29*	Ampomah-Dwamena *et al.*, 2002	

turn are able to interact with SEP-like proteins such as *FBP2, FBP5* and *FBP9* (Angenent *et al.*, 1995; Ferrario *et al.*, 2003).

Genes homologous to some of those mentioned above as being involved in the control of carpel and ovule development have been also described in tomato (Table 2). *SEPALLATA* homologues *TM5* and *TM29* (*TAGL2*) regulate floral organ identity and fruit development (Pnueli *et al.*, 1994b, Ampomah-Dwamena *et al.*, 2002). Co-suppression or antisense expression of these tomato *SEP* genes result in homeotic alterations in the inner three whorls resembling those observed in petunia transgenic plants lacking activity of *FBP2* or *FBP5* genes. After fertilization *TM29* expression is confined to the ovary, in particular to developing seeds and vascular bundles, which would link the post-fertilization process to fruit formation in tomato.

As previously mentioned, *TAG1,* the tomato *AG* orthologue, is required for the appropriate development of carpels at the fourth whorl of tomato flower (Pnueli *et al.*, 1994a). Other *AG*-like MADS-box genes expressed during tomato reproductive development have been isolated (Busi *et al.*, 2003). Nucleotide sequences of *TAGL1* and *TAGL11* genes show a high similarity to *SHP1 (AGL1),* and *STK (AGL11*), respectively. Moreover, expression patterns of *TAGL1'*and *TAGL11* are quite similar, being their transcripts detected at the inner integument of the ovules and the carpel walls. These results suggest overlapping functions of the two genes in the specification of ovule identity and in the control of fruit development, as occurs with their homologues *SHP1* and *STK* in *Arabidopsis*. Accordingly, yeast two-hybrid experiments have revealed dimer formation between *TM29* and each of *TAG1*, *TAGL1* and *TAGL11.* Taken together, these data seem to indicate that the proteins encoded by *TM29*, *TAG1*, *TAGL11* and *TAGL1* play an important role in the regulation of gene expression during early fruit development (ovule, seed and carpel development), functioning together as transcriptional complexes (Busi *et al.*, 2003). Despite the formation of different fruit types (siliques in *Arabidopsis*, berries in tomato), the molecular pathway involved in fruit development is most likely conserved, as suggested by the presence of genes sharing similarities in their structure and function, as well as comparable expression patterns and interactions.

The development of a flower as a whorled and determinate reproductive structure implies that determinacy of the floral meristem should be achieved once the carpel identity has been acquired by the fourth whorl organ primordia. In *Arabidopsis*, this process is regulated by the homeodomain encoding genes *WUSCHEL* (*WUS*) and *SHOOTMERISTEMLESS* (*STM*) which operate at different meristematic domains. The ceasing of activity in the floral meristem depends on a negative feedback loop involving *WUS*, *LFY* and *AG*, in such a way that the first two genes would activate *AG*, which in turn would repress *WUS* once the flower has completed floral organ development (Lohmann *et al.*, 2001). In petunia, the formation of multimeric complex involving C-, D- and E-class MADS-box transcription factors could be responsible for the repression of *TERMINATOR*, the *WUS* homologue in this species (Ferrario *et al.*, 2006). Concerning tomato, down-regulation of *TAG1* promotes flower indeterminacy featured by the replacement of carpels at the fourth whorl by ectopic floral-like structures bearing indeterminate floral meristems. Such homeotic changes support a role of *TAG1* in the determinacy of floral meristem (Pnueli *et al.*, 1994a).

Molecular isolation of new mutants and orthologous genes affected in the floral meristem size are required in tomato, as well as new insights into the genetic interactions they keep with floral meristem identity genes such as *FA* and *TAG1*. They could provide evidence about the conservation of the genetic pathway which regulates floral meristem determinacy in different plant species, as well as genetic and molecular singularities that distinguish the sympodial meristem from the SAM.

Tomato as model system for fruit development

Upon fertilization of the ovules, the carpels become a complex organ, forming the mature fruit, this ensuring seed dispersal and therefore survival of the plants. Mature fruits can be classified generally as either fleshy or dry, which mainly differ in the mechanism achieved to permit seed dispersal. A senescence program leading to fruit dehiscence is needed before some external agent (e.g. wind, rain, and physical contact) can force seeds to be released from dry fruits. However, fleshy fruits have evolved edible components making them attractive for animals, which facilitate dispersion of the seeds without any other requirements. Tomato plants produce fleshy red fruits as result of a developmental process which includes three phases (Gillaspy *et al.*, 1993). The first phase starts just at anthesis and involves the development of the ovary and the decision to abort or to proceed with fruit development (i.e. fruit set). In the second phase, fruit growth is due primarily to cell division and the embryos begin their development. Finally, cell division ceases at the third phase and fruit growth continues by cell expansion

until the fruit reaches its final size. Once a fully developed fruit has been formed and seeds are mature, respiration and ethylene synthesis are significantly increased allowing ripening and maturation. As result, biochemical and physiological changes affecting colour, texture, flavour, aroma, nutritional content and susceptibility to opportunistic pathogens are made visible from the onset of ripening. Later, a softening process occurs as part of ethylene-induced gene activities which promote degradation of cell walls in different fruit compartments (Giovannoni, 2004). On the other hand, the sharp increase in respiration rate, which usually occurs in combination with elevated ethylene production at the onset of fruit ripening, are considered specific features of climacteric fruits like tomato. Indeed, such features are absent during ripening of non-climateric fruits such as strawberry, grapes, legumes or citrus.

Taking into account the economic and nutritional importance of the fruits as essential components of human and animal diets, considerable scientific work is required to improve fruit yield and quality. *Arabidopsis thaliana* has proven to be an exceptional model for gaining insight into the genetic, molecular and hormonal factors which regulate development and dehiscence of fruits. However, significant contributions in the fields of hormonal regulation of ovary growth, physiology of ripening, and genetic control of fruit size and shape have taken tomato as a model system given the developmental features of fleshy fruits (see reviews by Giovannoni, 2004; Tanksley, 2004; Gorguet *et al.*, 2005). Recent discoveries on the regulatory mechanisms of fruit development and ripening have revealed the key role played by certain transcriptional factors, suggesting that some developmental regulators are conserved among plant species. Furthermore, ethylene perception and signalling pathways which control fruit development seem to share a common molecular basis in different species, although alterations in gene expression patterns and gene family composition may account for differences in fruit developmental patterns among species. This review focuses on the early stages of fruit development and ripening. Subsequent stages of fruit maturation is not considered since exhaustive reviews have recently been published on these topics (Giovannoni, 2004, 2007).

Hormonal control of fruit development and parthenocarpy

Fertilization of the ovules usually triggers development of the ovary into a fruit as pollen germination generates growth stimuli (Gillaspy *et al.*, 1993). Some of the growth factors controlling fruit set by pollen include auxins and gibberellins. Furthermore, auxins and ethylene control early stages of fruit development by inducing the expression of several gene families (Balbi and Lomax, 2003). However, successful development of tomato fruits can also occur in the absence of fertilization, a physiological event called parthenocarpy, which leads to the formation of seedless fruits (Lukyanenko, 1991).

Classic research into plant physiology has suggested that tomato parthenocarpy is related to an imbalance in hormonal control, as revealed by the fruit phenotypes observed after hormone applications or when plants grow under adverse conditions, mainly extreme temperatures (see Gorguet *et al.*, 2005). Parthenocarpic development has also been observed in tomato backgrounds displaying deficiencies in pollen formation and/or anther dehiscence (male sterility) which result in failure of fertilization. The low level of both cytokinins and gibberellins detected in the male-sterile *stamenless-2* of tomato suggests that these hormones may alter fertility (Sawhney and Shukla, 1994). Accordingly, anther- and pollen-specific expression of negative regulatory genes of cytokinin- or GA-signalling produce abortion of anther and pollen in transgenic plants of maize, tobacco and Arabidopsis (Huang *et al.*, 2003). These results agree with the functional implications of cytokinins and GAs in the reproductive development of plants. Additionally, jasmonic acid and ethylene have been found to synchronize pollen maturation, anther dehiscence and flower anthesis (see references in Gorguet *et al.*, 2005). Parthenocarpic fruits can be developed in absence of this normal synchronous development of male and female gametophytes, particularly in self-pollinating crops.

Besides the importance of plant hormones during pollination, gibberellins constitute a key factor for setting and development of tomato fruit. GA produced by developing seeds promotes a normal fruit development (García-Martínez *et al.*, 1991). Accordingly, exogenous GA can replace the promoter growth effects of GA-producing seeds, which result in a successful fruit set and parthenocarpy. Together with gibberellins, auxins are also involved in the parthenocarpic development of fruits. Enzyme activities involved in GA biosynthesis are regulated by auxins, which in turn are needed to maintain the level of active GAs (García-Martínez *et al.*, 1997; Ross *et al.*, 2000). Recent evidences have shown that early stages of fruit development in tomato are also dependent on auxin- and ethylene-mediated gene expression and that both auxin and ethylene responses are regulated specifically by the *Diageotropica* (*DGT*) gene (Balbi and Lomax, 2003). Therefore, it is likely that GA is involved in later stages of fruit and seed development, whereas the remaining hormones regulate early stages of these processes.

The physiological mechanisms underlying parthenocarpic development of the fruits remain largely unknown. Nevertheless, characterization of parthenocarpic tomato mutants has favoured a better understanding of the genetic and molecular basis of this process (Table 3). The recessive *parthenocarpic* (*pat*) mutant exhibits abnormal flower development, mainly premature ovary growth, reduced number of viable ovules and increased number of pericarp cell layers (Soressi and Salamini, 1975; Mazzucato *et al.*, 1998), the former two being responsible for the impaired fertilization and development of seedless fruits. Parthenocarpy is also induced by an independent recessive mutation *pat-2* (Philouze and Maisonneuve, 1978; Nuez *et al.*, 1986), which also produces unfavourable pleiotropic features (i.e. less plant vigour and reduced fruit set and yield) depending on the genetic background. The third genetic source of parthenocarpy is polygenic, with two genes *pat-3* and *pat-4* as the most probable determinants of this trait (Nuez *et al.*, 1986).

It has been suggested that parthenocarpy of *pat* mutant could be related to a gene with homeotic functions in accordance with the phenotypic similarities observed when *TM5* or *TM29* MADS-box genes are down-regulated (Mazzucato *et al.*, 1998). Nevertheless, it remains unclear whether the parthenocarpy is directly regulated by TM proteins or is caused by

hormonal changes related to abnormal development of reproductive organs which finally results in unsuccessful fertilization. Interestingly, steady-state levels of expression of *TM4* (*TDR4*), a tomato MADS-box gene homologous to *FUL*, are highly increased in parthenocarpic ovaries grown under low temperatures (Lozano *et al.*, 1998). Similarly, transcripts of *Le-DEF*, a tomato B-class MADS-box required for petal and stamen development, are significantly accumulated in GA-treated flowers, which give rise to parthenocarpic fruits, while they are completely abolished by paclobutrazol, an inhibitor of GA biosynthesis (authors' unpublished data). These results suggest that GA-mediated expression changes of MADS-box genes could be at the origin of tomato parthenocarpy, whether through regulation of reproductive organ development (stamens and carpels) or changes in GA-signalling or biosynthesis. Additionally, *PAT* interactions may regulate the ovary-pollen developmental synchrony and hence, fruit formation. Beraldi *et al.* (2004) has recently mapped the *PAT* gene, which will allow not only the cloning of the gene but also a more detailed analysis of genetic interactions regulating parthenocarpic development of fruits.

Differential expression analyses have found that *pat2* and *pat3/pat4* alleles enhance GA biosynthesis pathways, the high level of active GAs being responsible for parthenocarpic development of tomato fruits observed in both mutant backgrounds (Fos *et al.*, 2000; Fos *et al.*, 2001). Moreover, seedless fruit development of *pat2* requires *LATERAL SUPPRESSOR* activity since parthenocarpy is avoided by the presence of *ls* mutant allele (Philouze, 1983). These results agree with the function proposed for *LS* as a gene regulator of GA sensitivity (Schumacher *et al.*, 1999). Therefore, mechanisms which explain parthenocarpic development in *pat2* and *pat3/pat4* tomato mutants should include changes in the GA regulation.

Taking as a whole, these results indicate that parthenocarpy is a complex developmental process regulated by genetic, hormonal and environmental factors. Its scientific and agronomic interest should promote additional efforts to elucidate the molecular and genetic basis of the mechanisms underlying parthenocarpic fruit development. To this end, tomato is an excellent model system, even more so when current progress in functional genomic studies and the ongoing sequencing project can be exploited.

Tomato genes controlling fruit size and shape

Among the evolutionary features affected by the domestication are those related to external appearance of fruits. In this context, tomato is an excellent model to study natural variation and gene function since a wide diversity of phenotypes affected in fruit size and shape can nowadays be observed in the cultivated and wild-related tomato germplasm. Genetic mapping approaches have identified a small number of quantitative genes as responsible for the great variation affecting tomato fruit size (Grandillo *et al.*, 1999). Among them, only *fw2.2* has been isolated to date and its function is involved in the evolution of small fruited wild tomatoes in to large fruited cultivated tomatoes (see review of Tanksley, 2004). Detailed studies on the expression pattern and the gene dosage effect of this gene have proved that the FW2.2 protein is a negative regulator of cell proliferation in tomato that causes changes in overall fruit size but not in fruit shape (Frary *et al.*, 2000; Liu *et al.* 2003). In fact, remarkable results on the physical interaction between FW2.2 and *Le*CKIIβ1, the regulatory subunit of casein kinase II, have been published (Cong and Tanksley, 2006). Nevertheless, essential questions remain to be determined, mainly how this novel gene function has arisen in the plant kingdom and how it has evolved to interact with the well-known component of the cell cycle signaling pathway. Together with the control exercised by the cell cycle, the fruit size also depends on the number of carpels which are determined during early development of tomato fruit and hence, the number of locules forming the mature fruit. Two independent mutations, *locule-number*

TABLE 3

TOMATO MUTANTS AND GENES ISOLATED TO DATE AS INVOLVED IN FRUIT DEVELOPMENT AND RIPENING

Mutant or gene	Phenotype	Isolated Gene	Reference	Arabidopsis orthologue or encoded protein
pat	Parthenocarpic fruit, homeotic alteration of reproductive floral organs		Soressi and Salamini, 1975 Mazzucato *et al.*, 1998	
pat-2	Parthenocarpic fruit (altered GA metabolism)		Phylouze and Maisonneuve, 1978 Fos *et al.*, 2000	
pat-3/*pat-4*	Parthenocarpic fruit (altered GA metabolism)		Nuez *et al.*, 1986 Fos *et al.*, 2001	
TM29	Parthenocarpic fruit	*TM29*	Ampomah-Dwamena *et al.*, 2002	*SEPALLATA*
TDR4	Gene silencing makes a fruit unable to complete ripening and alters cell wall structure	*TDR4*	Seymour *et al.*, 2002 Eriksson *et al.*, 2004 Angosto *et al.* (unpublished)	*FRUITFULL*
fw2.2	Quantitative variation of fruit size	*Fw2.2*	Frary *et al.*, 2000 Cong *et al.*, 2002	Negative regulator of cell division
fasciated	Higher number of carpels/locules in the fruit	*FAS*	Cong *et al.*, 2008	YABBY-like transcription factor
ovate	Pear-shape or elongated fruit	*OVATE*	Liu *et al.*, 2002	New class of nuclear-localized proteins
sun	Quantitative fruit size variation	*SUN*	Xiao *et al.*, 2008 Jiang *et al.*, 2009	IQ67-domain family
nor	Non-ripening fruits	*NAC*	Tigchelaar *et al.*, 1978 Giovannoni *et al.*, 2004	NAC domain transcription factor
Rin	Non-ripening fruits	*LeMADS-RIN* (*RIN* in text)	Dostal *et al.*, 1974 Vrebalov *et al.*, 2002	*SEPALLATA*-like
Cnr	Unripe fruit, loss of cell-to-cell adhesion	*LeSPB-CNR* (*CNR* in text)	Thompson *et al.*, 1999 Manning *et al.*, 2006	*SBL3*

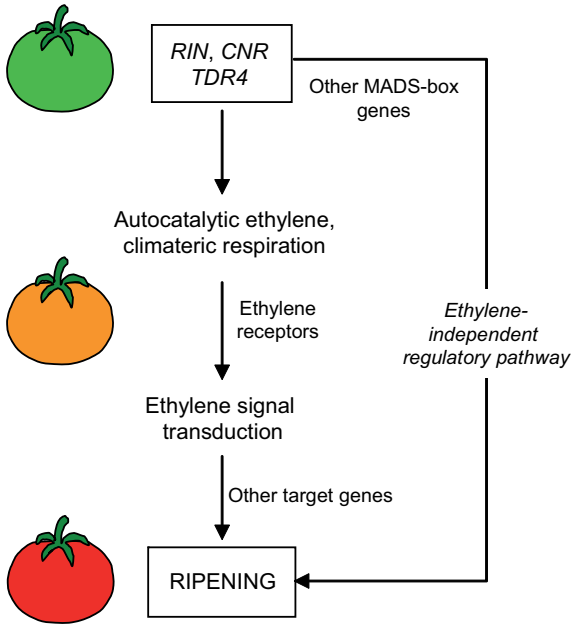

Fig. 4. Genetic regulation of tomato ripening. *Transcription factors encoded by* RIN *and* CNR *genes are involved in an ethylene-mediated control of fruit ripening and also participate in an ethylene-independent pathway. Presumably,* TDR4 *gene is also involved in this mechanism of ripening control, while other regulatory and target genes remain to be characterized. Ethylene receptor proteins involved in the ethylene signal transduction promote a cascade of gene activation allowing the ripening of tomato fruits (adapted from Giovannoni, 2007).*

and *fasciated (fas)*, identified the quantitative loci involved in this feature, the latter having a greater effect (Barrero and Tanksley, 2004). Positional cloning of the *FASCIATED* gene has revealed that it encodes a YABBY-like transcription factor and that the high locule-number-phenotype of modern tomato cultivars is caused by downregulation of this gene during floral development. An insertion in the first intron is likely to be responsible for this regulatory change (Cong *et al.*, 2008). Comparative sequencing of either *fw2.2* or *fas* alleles, has led to two important conclusions: i) fruit size variation is modulated not by changes in the coding sequence of either the 22-kD FW2.2 or the FAS protein but due to differences in the transcriptional activity during flower development associated to changes in the 5' regulatory sequence of the corresponding genes (Cong *et al.*, 2002; Cong *et al.,* 2008), and ii) *fw2.2* and *fas* represent early and late steps, respectively, in the fruit size variation accounted for domestication (Tanksley, 2004; Cong *et al.,* 2008).

Genetic pathways which regulate fruit size and shape of tomato seem to share some components as the organ-determining genes *fasciated* and *locule-number,* which affect both external features (Tanksley, 2004). Nevertheless, two major genes involved in fruit shape variation (described as the ratio of fruit height over width) with little or no effects on fruit size, have recently been isolated. *OVATE* was cloned and found to code a new class of hydrophyllic proteins with a putative nuclear localization signal (Liu *et al.*, 2002). Recessive effects of the single *ovate* mutation promote an elongated or pear-shaped fruit as a result of a premature stop codon in the second exon which generates a truncated protein. This locus is early expressed during flower development although its transcripts can be detected two weeks after anthesis coinciding with the first step of fruit formation. However, the second major locus, the *SUN* gene, affects fruit shape after anthesis (Xiao *et al.,* 2008). Besides, whereas *ovate* mutation usually results in an asymmetric elongation of fruits, *sun* mutant plants yield elongated and oval shaped tomatoes that maintain the bilateral symmetry. The *sun* phenotype was caused by an unusual 24.7-kilobase interchromosomal gene duplication event mediated by retrotransposon *Rider*. When situated in a new genomic context, *SUN* is expressed at increased levels regarding the ancestral copy, most likely driven by regulatory control of the *defensin* gene (*DEFL1*), which culminates in an elongated fruit shape (Xiao *et al.,* 2008; Jiang *et al.,* 2009). The *SUN* locus encodes an IQD12 protein belonging to the IQ67 domain-containing family and its transcription levels are responsible for the variation of tomato fruit shape (Xiao *et al.,* 2008). Together with these two loci, quantitative mapping studies (Grandillo *et al.,* 1999; Ku *et al.,* 2000; Brewer *et al.,* 2007; Gonzalo and Van der Knaap, 2008) have shown that several QTLs, emphasizing the well studied *fruit shape 8.1* QTL (*fs8.1),* are also associated to fruit shape variation. Like *fasciated* and *ovate*, *fs8.1* functions mostly during early floral development and has little if any activity after anthesis (Ku *et al.*, 2000). Presumably, gene interactions controlling shape and size of tomato fruit will soon be elucidated and with this, the molecular mechanisms underlaying morphological variation of tomato fruit.

Genetic regulation of fruit ripening

The onset of ripening in tomato occurs after the cell expansion stage in the developing ovary has finished and seeds are mature. Physiological studies have concluded that ripening process is characterized by a higher respiration and the autocatalytic synthesis of ethylene (Lelievre *et al.*, 1997), the latter being a major determinant of the phenotypic changes affecting colour, texture, aroma and pathogen susceptibility of fruits. Given the importance of ethylene, most of the research into fruit development has focused on the ethylene-dependent regulatory genes (Cara and Giovannoni, 2008), mainly those involved in the perception and biosynthesis of this hormone (Fig. 4). However, here we review the genes acting upstream or in parallel to the ethylene-regulated pathway. Their functional roles should help us to understand the genetic framework, hormonal interactions and molecular pathways which together regulate fruit development in tomato.

Tomato *TDR4* gene was one of the first reported to be involved in tomato fruit development (Seymour *et al.*, 2002; Busi *et al.*, 2003). *TDR4* encodes a SQUAMOSA MADS-box transcription factor and is expressed early in the floral meristem, while later at anthesis, *TDR4* transcripts are accumulated in ovules and carpel walls. Upon fruit set and during cell division stage, expression of *TDR4* is observed in several tissues of the growing ovary. Although the expression level of this gene seems to be low during fruit development, a significant increase is detected at the onset of ripening. Taken into account the expression pattern and the high sequence homol-

ogy, Seymour *et al.* (2002) proposed *TDR4* as an orthologue of *FRUITFULL* (*FUL*) gene of *Arabidopsis*, the latter being a negative regulator of *SHP* genes during fruit dehiscence (Ferrándiz *et al.*, 2000). Moreover, the putative formation of TDR4-TM29 and TDR4-TAG1 dimers described by Busi *et al.* (2003) would support that *TDR4* may participate as a linking factor between flower and fruit development. In fact, tomato MADS-box genes involved in fruit development are also expressed in different stages of floral development, suggesting that fruit and seed development may be considered as a continuation of the floral development program. *TM29*, *TAGL1* and *TAGL11* are induced immediately after anthesis in the ovary (Busi *et al.*, 2003), making them candidates to participate in the signalling pathways which trigger fruit development once successful fertilization has taken place (Fig. 4). Nevertheless, other functional similarities between *FUL* and *TDR4*, in particular the existence of genetic interactions of *TDR4* with MADS-box genes involved in fruit ripening, similar to those described between *FUL* and *SHP* genes, remain to be investigated.

Some of the first evidences supporting an upstream transcriptional control of fruit development came from the characterization of tomato mutants altered in the ripening process (reviewed by Giovannoni, 2004, 2007). Among them, *ripening-inhibitor* (*rin*), *non-ripening* (*nor*) and *Colorless non-ripening* (*Cnr*) mutants all produce fruits which are unable to ripen (Table 3) even after being treated with exogenous ethylene. Also, the lack of ethylene production and increased climacteric respiration are features shared by these mutants (Vrebalov *et al.*, 2002; Giovannoni *et al.*, 2004; Manning *et al.*, 2006). Such results indicate that the affected genes *RIN, NOR* and *CNR* promote fruit ripening by a regulatory pathway acting upstream to the ethylene biosynthesis and signalling. Additionally, these genes should also participate in an ethylene-independent pathway (Fig. 4) as indicated by the expression changes of ethylene-regulated genes induced by this hormone in *rin, nor* and *Cnr* fruits (see Giovannoni, 2007). *RIN* is a MADS-box gene of the *SEP*-clade whose mutant recessive allele is caused by a partial deletion which results in a chimeric transcript (Vrebalov *et al.*, 2002). *Cnr* mutation promotes a dominant epigenetic alteration in the promoter of a SBP-box (SQUAMOSA promoter binding protein) gene (Manning *et al.*, 2006), while the less analysed *NOR* gene encodes a NAC domain transcription factor (Giovannoni *et al.*, 2004).

Genetic interactions among ripening regulators and other fruit developmental genes are being elucidated, but hierarchic relationships among the encoded proteins remains unknown. In agreement with the increased level of *TDR4* transcripts detected when tomato fruits start to ripen (Seymour *et al.*, 2002; Eriksson *et al.*, 2004), *TDR4* expression is reduced in the non-ripening mutants *rin*, *nor* and *Cnr*. Furthermore, *TDR4* loss-of-function produces a slight increase in cell wall stiffness of fruits consistent with a *TDR4*'s role in regulating cell wall structure (Eriksson *et al.*, 2004). In agreement, RNAi-mediated inactivation of *TDR4* makes tomato fruits unable to complete ripening (Angosto *et al.*, unpublished data). Therefore, *TDR4* gene has been proposed as a suitable candidate to regulate the ripening process, together *RIN, NOR* and *CNR* (Eriksson *et al.*, 2004). Even more, the formation of putative dimers between TDR4 and other MADS-box proteins (TM29 and TAG1) detected by yeast two-hybrid analysis could be extended to RIN, as a hypothetical mechanism of ripening control similar to that operating for the specification of floral organ identity (Giovannoni, 2004). In the same way, putative interactions between CNR and TDR4 proteins have been proposed by Manning *et al.* (2006) based on the ability of SBP-box gene products to interact with a sequence motif of the *SQUAMOSA* promoter.

The expression of *RIN* and *NOR* appear unaffected in *Cnr* mutant fruits, suggesting that they either participate in a separate pathway from *CNR* or act upstream in the ripening cascade (Eriksson *et al.*, 2004; Giovannoni, 2007). Additional work is needed to determine genetic and molecular relationships among ripening genes and to identify their targets.

Future prospects

Significant contributions to the developmental genetics of tomato have been made during the last two decades. Genetic analysis of tomato mutants and candidate gene approaches are providing excellent tools to isolate key genes involved in vegetative and reproductive development. The cloning and functional analysis of tomato homologous genes have revealed that many developmental processes are controlled by genes and proteins which are highly conserved among plant species (e.g. *Arabidopsis*, *Anthirrinum*, petunia). However, specific features of tomato fruit seem to require novel gene functions, which have begun to be identified with the isolation of transcription factors involved in fruit development and ripening. Recent genomic research indicates that new tomato regulatory genes should be characterized as regulators of the reproductive development in this crop species. In fact, new mutations affecting floral transition and fruit development have been isolated from an *in silico* screening of a saturated tomato library (Menda *et al.*, 2004) and by insertional mutagenesis (R. Lozano, unpublished data). In addition, EST and microarray analyses have allowed the identification of thirteen additional MADS-box genes and more than fifteen putative transcription factors which are expressed in ripening fruits (Giovannoni, 2007). In coming years, major challenges will focus on isolating new developmental genes and bridging the gap between these genes and their functions. Genomic and sequencing initiatives currently in progress can be expected to unveil the genetic pathways and molecular interactions among transcription factors which regulate when and how flowers and fruits are formed. Taken as a whole, these results would favour novel strategies to improve the productivity and fruit quality of tomato.

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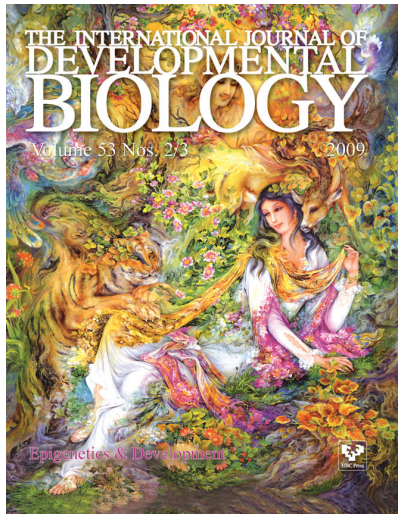

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