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**Mutation at the tomato *EXCESSIVE NUMBER OF FLORAL ORGANS* (*ENO*) locus impairs floral meristem development, thus promoting an increased number of floral organs and fruit size**

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**Abstract**

A novel tomato (*Solanum lycopersicum* L.) mutant affected in reproductive development, *excessive number of floral organs* (*eno*), is described in this study. The *eno* plants yielded flowers with a higher number of floral organs in the three innermost floral whorls and larger fruits than those found in wild-type plants. Scanning-electron microscopy study indicated that the rise in floral organ number and fruit size correlates with an increased size of floral meristem at early developmental stages. It has been reported that mutation at the *FASCIATED* (*FAS*) gene causes the development of flowers with supernumerary organs; however, complementation test and genetic mapping analyses proved that *ENO* is not an allele of the *FAS* locus. Furthermore, expression of *WUSCHEL* (*SlWUS*) and *INHIBITOR OF MERISTEM ACTIVITY* (*IMA*), the two main regulators of floral meristem activity in tomato, is altered in *eno* but not in *fas* flowers indicating that *ENO* could exert its function in the floral meristem independently of *FAS*. Interestingly, the *eno* mutation delayed the expression of *IMA* leading to a prolonged expression of *SlWUS*, which would explain the greater size of floral meristem. Taken together, results showed that *ENO* plays a significant role in the genetic pathway regulating tomato floral meristem development.

**Key words**

*FASCIATED*; flower organogenesis; fruit development; *INHIBITOR OF MERISTEM ACTIVITY*; *Solanum lycopersicum*; *WUSCHEL*

**Abbreviations**

A, flowers at the anthesis stage; FB0, flower buds of 3.0 to 5.9 mm in length; FB1, flower buds of 6.0 to 8.9 mm in length; FB2, flower buds of 9.0 to 12 mm in length; FM, floral meristem; IM, inflorescence meristem; PA, flowers at the pre-anthesis stage; SAM, shoot apical meristem; SEM, scanning-electron microscopy; SNP, single-nucleotide polymorphisms; WT, wild-type.

## 1. Introduction

Plants have the unique ability to produce new organs continuously due to the indeterminate growth of undifferentiated stem cells located in specific regions, the meristems. Reproductive development starts when the shoot apical meristem (SAM) changes its developmental pattern giving rise to the inflorescence meristem (IM), which produces several floral meristems arranged in a species-specific phyllotaxis. In contrast to the SAM, the floral meristem (FM) shows determinate growth leading to the development of a specific number of organs with a particular size and shape before ceasing its meristematic activity [1]. This developmental process, named floral determinacy, is critical for the reproductive success of plants, and requires a precise temporal and spatial control of gene expression to regulate the cessation of stem cell activity in the FM. In *Arabidopsis*, the homeobox gene *WUSCHEL* (*WUS*) is necessary to maintain the stem cell domain in the shoot and floral meristems [2]. The floral identity gene *LEAFY* (*LFY*) and *WUS* are expressed after floral induction and they activate the MADS-box gene *AGAMOUS* (*AG*), which in turn plays an important role for both FM determinacy and floral organ identities [3-7]. In addition, *WUS* expression decreases when *AG* expression is activated. Thus, repression of *WUS* by *AG* is necessary to terminate stem cell activity at the appropriate time during flower development, allowing the cells in the centre of the flower to differentiate into carpels [8,9].

Tomato (*Solanum lycopersicum* L.) is a major crop plant that also serves as a model species for the study of developmental processes [10]. While significant progress has been made on those issues related to fleshy fruit formation and ripening, relatively little is known about floral determinacy in this species. It has been reported that *TOMATO AGAMOUS1* (*TAG1*) silencing lines display defects in FM determinacy resulting in a 'fruit inside fruit' phenotype [11]. However, although floral determinacy in *Arabidopsis* depends on a negative autoregulatory mechanism involving *AG* and *WUS* [8,9], the interaction between *TAG1* and the meristem organizing centre gene *SlWUS* in tomato has not been stated so far. The *INHIBITOR OF MERISTEM ACTIVITY* (*IMA*) gene, which encodes a Mini Zinc Finger (MIF) protein, takes part in the termination of tomato FM by inhibiting the stem cell activity through the repression of *SlWUS* [12]. Therefore, a proper temporal pattern of *SlWUS* and *IMA* expression is necessary to achieve an optimal FM size, which allows for an appropriate production of floral organs. Thus, premature termination of stem cell proliferation in the FM would mean insufficient cell number for floral organ formation, whereas overly extended stem cell activity would result in an excessive number of floral organs [13].

In addition to the *IMA* gene, two loci have been reported – *FASCIATED* (*FAS*) [14] and *LOCULE NUMBER* (*LC*) [15] – to affect FM size and floral organ number in tomato. *FAS* encodes a YABBY-like transcription factor which is expressed during the development of FM. Mutation of this gene is produced by a large insertion in the first intron (estimated to be 6-8 kb) resulting in an increased number of floral organ caused by an alteration of the FM size [14,16]. Regarding *LC* locus, Muños et al. [15] identified two single-nucleotide polymorphisms (SNPs) in a noncoding region located 1,080 bp from the stop codon of *SlWUS*, which have a significant effect on floral organ number. Although it has not yet been possible to identify the function of these two SNPs, they might participate in the regulation of *SlWUS* expression or of other genes that play an important role in the FM development [15]. Furthermore, *FAS* has the strongest effect on FM size and both *FAS* and *LC* interact epistatically to produce flowers with extremely large carpel number [17,18].

During tomato fruit development, both cell division and floral organ number determination control the final size of fruits. The most significant change in cell division is due to a mutation in the cell cycle-control gene *FRUIT WEIGHT 2.2* (*FW2.2*), which encodes a negative regulator of this process [19]. Nonetheless, the development of extreme fruit size is mainly determined by the number of carpels in a flower and hence, by the final number of locules forming the mature fruit. Thus, an increase in the number of locules (carpels) can lead up to a 50% increase in fruit size [17,20]. Fruit size is a major component of tomato yield and a key goal for crop domestication. However, relatively few genes involved in the control of floral organ number have been reported up to now, despite the chance that this trait provides to improve fruit yield potential. This study reports the genetic and phenotypic characterization of a new tomato mutant called *excessive number of floral organs* (*eno*) as its flowers had an increased number of petals, stamens, and carpels compared to wild-type (WT) plants. The detailed examination of the flowers through scanning-electron microscopy (SEM) analysis indicated that *eno* mutation enhances the FM size at an early stage of FM development. This alteration in FM size promotes the development of supernumerary organs in the three inner floral whorls, as well as the formation of larger size fruits. Moreover, genetic and gene expression analyses revealed that *ENO* is a novel gene involved in the control of FM development and which takes part in the pathway regulated by *SlWUS* and *IMA* genes.

## 2. Materials and Methods

### 2.1. Plant material

The *eno* mutant was identified from a phenotypic screening of T-DNA lines obtained from the tomato cultivar P73 (kindly provided by Dr. M.J. Díez, COMAV-UPV, Valencia, Spain). Given that molecular analysis showed that the *eno* mutation was not caused by a T-DNA insertion, a T4 population was obtained from a single selfed T3 *eno* plant that did not contain T-DNA insertion. The T4 mutant population, together with plants of the P73 cv. were used for further characterization. All experiments were carried out under greenhouse growing conditions. Standard management practices were used including regular addition of fertilizers.

The accession 170045 (kindly provided by Dr. R. Fernández-Muñoz, IHSM-CSIC-UMA, Málaga, Spain) homozygous for the mutant allele of *FAS* locus was used for a complementation test between *eno* and *fas* mutations. The PCR-based markers developed by Rodríguez et al. [21] were used to support the homozygous genotypes of the *fas* mutant plants used in this work. The primers EP1070 (5'-ATGGTGGGGTTTTCTGTTCA-3') and EP1071 (5'-CAGAAATCAGAGTCCAATTCCA-3') were employed to amplify the WT allele ; whereas the primers EP1069 (5'-CCAATGATAATTAAGATATTGTGACG-3') and EP1071 were used to amplify the mutant allele. In addition, the primers described by Rodríguez et al. [21] were used to confirm that the WT (P73 cv.), *eno*, and *fas* plants were homozygous for the recessive high-locule-number allele at the *LC* locus. The primers lcn-SNP695-F (5'-GTCTCTTGGATGATGACTATTGCACTTT-3') and lcn-SNP695-R (5'-TCAGCGCCTCATTTTCTATAGTATTTGT-3') were used to amplified the dominant low-locule-number allele; while lcn-SNP695-F-cer (5'-CTTTTCCTAAAAGATTTGGCATGAGGT-3'), and lcn-SNP695-R-lev (5'-AAAGTAGTACGAATTGTCCAATCAGTCAG-3') were employed to amplify the recessive high-locule-number allele. The four primers were used in the same PCR master mix following the method described by Rodríguez et al. [21].

2.2. Phenotypic characterization

Flower buds and flowers were harvested at different developmental stages following description of Mazzucato et al. [22]: FB0, flower buds of 3.0 to 5.9 mm in length; FB1, flower buds of 6.0 to 8.9 mm in length; FB2, flower buds of 9.0 to 12 mm in length; PA, flowers at the pre-anthesis stage; and A, flowers at the anthesis stage. For each developmental stage, fifty measurements (5 samples x 10 plants) of width were taken from the widest diameter by means of a calliper gauge. The number of sepals, petals, stamens, and carpels was evaluated in flowers at anthesis stage. Besides, five mature fruits per plant were used to calculate average fruit weight (g), width (mm), length (mm) and number of locules per genotype. All values were expressed as the mean ± standard deviation. Data were further subjected to analysis of variance, and the least significant difference (LSD) test (SAS Institute, Carry, NC, USA) was used to compare the mean values. A probability of $P<0.01$ was considered statistically significant.

2.3. Scanning-electron microscopy (SEM)

Five stages of tomato FM initiation were defined as previously reported [23,24]. The widest diameter of the meristem (μm) was measured in a minimum of ten samples per stage in WT and *eno* plants. The significance of pairwise comparisons between genotypes was assessed by using LSD test ($P<0.01$). SEM analysis were carried out as described by Lozano et al. [25]. Plant tissue was fixed in FAEG and stored in 70% ethanol. Subsequently, tissues were dehydrated in ethanol and $CO_2$-critical-point dried using a critical point dryer Bal-Tec CPD 030. Lastly, the samples were gold coated in a Sputter Coater (Bal-Tec SCD005) and analysed using a Hitachi S-3500N scanning electron microscope at 10 kV.

2.4. Genetic mapping of *eno* mutation

To determine the chromosomal localization of the *ENO* gene, a total of 503 $F_2$ plants obtained from a cross between the *eno* mutant and the *S. pimpinellifolium* accession LA1589 were individually genotyped. Genomic DNA was extracted by using the DNAzol® Reagent kit (Life Technologies). Eighty single nucleotide polymorphism (SNP) markers distributed at ~10 Mbp intervals along each chromosome were analysed. Marker data was based on the genetic and physical maps available at the Sol Genomic Network database (SGN, http://solgenomics.net/). Genetic linkages and distances were determined using JoinMap® 4 software [26]. The order of markers was determined at logarithm of odds ratio (LOD) threshold of 3.0, and a recombination frequency value of 0.3. The genetic distance between markers was calculated using the Kosambi mapping function.

2.5. RNA Isolation and gene expression analyses

Total RNA was isolated using TRIzol® Reagent (Life Technologies) following the manufacturer's instructions. Contaminating DNA was removed using the DNA-free™ kit (Ambion). M-MuLV reverse

transcriptase (Fermentas Life Sciences) was used for cDNA synthesis from 500 ng of RNA, using a mixture of random hexamer and oligo$(dT)_{18}$ primers. Gene expression analysis by qRT-PCR were conducted on the 7300 Real-Time PCR System (Applied Biosystems) using the SYBR Green PCR Master Mix (Applied Biosystems) kit. Sequence of specific primers used for qRT-PCR are shown in Supplementary Table S1. Amplification data were analysed using 7300 System Sequence Detection Software v1.2 (Applied Biosystems). Data were normalized to the housekeeping gene *Ubiquitin3* and the quantification of gene expression were performed using the ΔΔCt calculation method. A tomato-specific amplicon (intron sequence) was used to confirm the absence of genomic DNA contamination in the qRT-PCR assays. Differences in gene expression levels were statistically analysed by the least significant difference (LSD) test (SAS Institute, Carry, NC, USA). A probability of $P<0.01$ was considered statistically significant.

## 3. Results

### 3.1. *eno* mutation affects floral meristem and fruit development

Phenotypic screening of a T-DNA insertional mutant collection was conducted so as to isolate novel regulators of reproductive development. As a result, the *eno* mutant was initially selected for its larger flowers and fruits as compared with the wild-type, P73 cv. (Fig. 1). Wild-type and mutant plants did not display alterations in any other vegetative or reproductive traits. The segregation observed (42 WT : 16 *eno*) in the T2 progeny was consistent with a monogenic recessive inheritance of the *eno* mutation ($\chi2=0.2$; $P=0.65$). Southern blot analysis showed that the original T1 line carried a single T-DNA insertion. The correlation between T-DNA insertion and the *eno* mutation was studied in the T2 progeny (58 plants). However, it was not associated with the *eno* phenotype.

To characterize flower development of the *eno* mutant, the number of floral organs at anthesis stage was scored. The WT flowers consisted in four whorls of floral organs being composed of 6-7 green sepals in the outer whorl, which alternate to a similar number of yellow petals at the second whorl, about 6-7 yellow stamens in the third whorl forming a staminal cone around the pistil, and 4-5 fused carpels in the innermost whorl (Fig. 1A, D; Table 1). In contrast, the *eno* flowers consisted of 6-7 sepals, 10-13 petals, 12-18 stamens, and 12-18 carpels (Fig. 1B, D; Table 1). The number of petals, stamens, and carpels were significantly higher in *eno* compared to WT, which indicates that *ENO* gene function is required to control organ number during tomato flower development.

In order to elucidate the developmental effects of *eno* mutation at early stages of floral development, several stages of FM development from sepal organ initiation up to carpel differentiation were examined by SEM (Fig. 2A-J). Results showed significant differences in size between WT and *eno* FM from the stage of petal development and stamen initiation to the stage of carpel differentiation (Fig. 2K). Thus, the average size of the wild-type FM at petal development and stamen initiation stage was 564.5±68.9 μm while it increased up to 795.2±175.9 μm in *eno* mutant flower buds, which means a ~ 40% increase in FM size. It is interesting how such size difference becomes greater as the flower development progresses and so, FM size of mutant floral buds was ~ 90% higher than WT ones at carpel development stage (Fig. 2K). With regard to the number of floral organs developed by *eno* mutants, it was found that, with the exception of sepals, the number of organs in the three

inner whorls was significantly higher than in WT at all stages of floral organ initiation. Besides, the width of the flower buds and flowers was measured at different developmental stages (see Material and Methods), finding significant increases in *eno* plants at all stages (Fig. 1C). Therefore, *eno* plants differed significantly from WT with respect to FM size from early stages of organogenesis up to flower anthesis stage.

In tomato the number of carpels in a flower determines the locule number in a fruit. As expected, WT plants produced fruits with 4-5 locules (average 4.3±1.1), while the *eno* plants yielded fruits with 12-18 locules (average 12.9±3.2). Together with this increase in locule number, there was a significant increase in the weight of *eno* fruits (Table 2). With regard to fruit size, *eno* mutation showed effects completely restricted to fruit width (Table 2), making *eno* fruits appear flatter and larger as compared with WT. Nevertheless, WT and mutant tomato fruits showed no differences in ripening patterns (Fig. 1E, F). On the whole, the results indicated that the final size of *eno* fruits is determined by the increase in the number of carpels that occurs during floral development.

### 3.2. *eno* mutation affects a locus different from the *FAS* gene

A similar phenotype to that observed in the *eno* mutant has been previously reported for the *fas* mutant [16]. Although both mutants developed an increased number of floral organs, *fas* flowers showed a weaker phenotype than those of *eno*, while the increase in sepal number observed in *fas* was not apparent in *eno*. Thus, *fas* flowers consisted of 7-8 sepals, 7-9 petals, 7-9 stamens, and 8-13 carpels (Table 1). Likewise, the *fas* mutants produced fruits with an increased number of locules (Table 2). A higher number of locules compared to the number of carpels was found in *eno* and *fas* mutants, most likely due to abortion of some carpels or by failure of septum development. Additionally, in order to check whether *fas* and *eno* were allelic or non-allelic mutations, a genetic complementation test was carried out by crossing *eno* and *fas* homozygous mutant plants. Results showed no significant phenotypic differences between F1 (*eno x fas*) and WT plants (Table 1, 2) proving that *eno* is not an allele of the *FAS* gene.

To determine the chromosome location of the *ENO* gene, floral and fruit phenotypes of a total of 503 $F_2$ plants obtained from the cross between the *eno* mutant and the wild relative species *S. pimpinellifolium* (accession LA1589) were scored. In addition, these plants were genotyped using 80 SNPs markers distributed at ~10 Mbp intervals along the twelve chromosomes of the tomato genome. Genetic mapping placed the *ENO* gene within 6.05 Mbp interval close to the telomere of chromosome 3 (57.76-63.81 Mbp) between solcap_snp_sl_62377 and solcap_snp_sl_33829 markers (Fig. 3), while the *FAS* gene was located on the long arm of chromosome 11 [14]. Overall, genetic complementation and mapping results supports that *eno* phenotype is due to a mutation that affects a different locus of the *FAS* gene.

### 3.3. *eno* mutation affects the temporal expression pattern of *SlWUS* and *IMA* genes

With the aim to analyse the genetic pathway affected by *eno* mutation, the expression patterns of genes involved in the control of floral organ number and fruit size were analysed. Thus, transcript levels of *FAS* (Solyc11g071810), *TAG1* (Solyc02g071730), *SlWUS* (Solyc02g083950), and *IMA* (Solyc02g087970) genes were evaluated in WT, *eno*, and *fas* flowers at five developmental stages (see Material and Methods). Except for

the pre-anthesis stage, similar levels of *FAS* expression were observed in WT and *eno* flowers. As expected, a downregulation of this gene was found in *fas* mutant plants (Fig. 4A). Concerning *TAG1*, expression analysis displayed no differences among WT and *eno*. In *fas* flowers significant differences was only found at the anthesis stage, likely due to the different genetic background of the *fas* and WT flowers (Fig. 4B). In the WT flowers, *SlWUS* transcript accumulation reached a maximum in flower buds of 9.0 to 12 mm in length (FB2 stage). The peak of *SlWUS* expression was delayed in *eno* flowers and occurred at the pre-anthesis stage rather than at FB2 stage (Fig. 4C). Similarly, the increase in *IMA* expression was also delayed in *eno* with transcripts not accumulating until anthesis stage, whereas a large increase in *IMA* expression occurred at the pre-anthesis stage in WT flowers. In contrast, the expression of *SlWUS* and *IMA* was not dramatically impacted by *fas* (Fig. 4C-D). In addition, the expression of *FW2.2* (Solyc02g090730) gene, a negative regulator of cell division associated with carpel cell number [27], did not show differences among WT, *eno*, and *fas* flowers (Fig. 4E).

## 4. Discussion

Developmental analyses here reported showed that the tomato *eno* mutation promotes an increased size of FM, which is in turn associated with an excessive number of floral organs in the three innermost floral whorls (Fig. 1D). Such phenotypic effects were observed at early stages of floral development, since significant differences between WT and *eno* flowers had already been detected when petal and stamen primordia were initiated (Fig. 2K). These results indicated that *ENO* gene function is required to regulate FM development during the first stages of floral organogenesis. Furthermore, as the number of carpels in a tomato flower determines the final number of locules in a mature fruit, the increased number of carpels developed by *eno* flowers is responsible for the large multilocular *eno* fruits suggesting that FM and fruit development are linked developmental processes, which could be connected through *ENO*.

Even though several features of the *eno* mutant phenotypes resemble those reported for the *fas* mutation, genetic complementation test displayed that *ENO* is not an allele of the *FAS* locus. Genetic mapping results showed that the *ENO* gene is located on the long arm of chromosome 3, between marker solcap_snp_sl_62377, at 57.76 Mbp, and marker solcap_snp_sl_33829, at 63.81 Mbp (Fig. 3). Until now, floral organ number in tomato was principally determined by two loci, *FAS* and *LC*. *FAS* gene encodes a YABBY-like transcription factor, located on the long arm of chromosome 11, whose downregulation causes the development of flowers with supernumerary organs [14]. The *LC* locus is defined by two SNPs placed on chromosome 2 at 1,080 bp from the stop codon of *SlWUS,* which have a significant effect on floral organ number [15]. Besides, given that the regulation of floral organ number seems to be closely associated with FM size, Barrero et al. [16] mapped the putative tomato homologs of *Arabidopsis* genes known to be involved in FM development (i.e. *CLAVATA1*, *CLV1*; *CLAVATA2*, *CLV2*; *CLAVATA3*, *CLV3*; *SHEPHERD*, *SHD*; *WIGGUM*, *WIG*; *WUSCHEL*, *WUS*; *POLTERGEIST*, *POL*; *ZWILLE*, *ZLL*; *PINHEAD*, *PNH*). Among all of them, only the Solyc03g043770 gene, which encodes for a CLV1-related receptor-like kinase, is placed on chromosome 3, although it is located at 11.3 Mbp. Therefore, overall results indicate that *ENO* is a novel tomato gene involved in the control of floral organ number.

Little is known about the genetic network underlying FM development in tomato, which leads to the formation of a limited number of organs with a predictable size and shape. The *IMA* gene inhibits meristematic

cell proliferation during floral termination by repressing the meristem organizing centre gene *SlWUS* [12]. Nevertheless, the relationship among *FAS*, *SlWUS*, and *IMA* genes has hitherto not been examined. Gene expression analyses revealed that *eno* mutation delays the expression of *IMA* leading to a prolonged expression of *SlWUS* in *eno* flowers. Thus, the *eno* mutation impairs the temporal expression balance of *SlWUS* and *IMA* transcripts during flower development (Fig. 4C, D). The fact that *eno* is recessive suggests a loss-of-function mutation, and if that occurred, *ENO* would act as a direct or indirect repressor of the meristematic activity in the FM through a pathway involving the *SlWUS* and *IMA* genes. Conversely, although *fas* flowers had an increased number of organs, the expression profiles of *SlWUS* and *IMA* genes were similar between the *fas* and WT flowers (Fig. 4C, D). Thereby, the results suggest that the *SlWUS-IMA* pathway must be only a part of the complex genetic network involved in the formation of a proper number of floral organs. Such hypothesis is also supported by the fact that the *IMA* gene seems to be involved only in the control of the carpel number since flowers of *IMA* overexpression and loss-of-function lines were only affected in the inner floral whorl. Thus, *IMA* loss-of-function lines had supernumerary carpels [12].

The increased size of FM found in *eno* floral buds was correlated to the excessive number of floral organs developed in the three innermost whorls, and particularly, the greater number of carpels leads to the formation of tomato fruits of bigger size. These results suggest a cross-talk between FM activity and tomato fruit development, whose genetic control has been poorly studied so far. It is known that the size of tomato fruits increased during domestication through gene mutations affecting two processes: cell division and floral organ number determination [20]. The *FW2.2* gene is responsible for the most dramatic change in cell division [19], whereas the *FAS* gene is the main regulator of the floral organ number [14]. *FW2.2* is expressed early during floral development and controls the number of carpel cells by inhibiting cell division [27]. These results indicated that the increase in the number of carpels in the *eno* flowers is responsible for the higher size of *eno* fruits, given that a similar expression pattern of the *FW2.2* gene was found in *eno* and WT plants (Fig. 4E). Therefore, *ENO* increases the size of tomato fruit by regulating the number of carpels during the floral development and its function seems not to be involved in the cell division process regulated by *FW2.2*. Consequently, not only *FAS* but also *ENO* are required for the formation of a proper number of floral organs by regulating the FM size. However, given that the first floral whorl (i.e. sepals) is only affected in *fas* mutants and the expression pattern of *FAS* gene is not altered in *eno* flowers, it might be hypothesized that *ENO* function is required downstream of or in parallel to the *FAS* function. In addition, an altered expression profile of *SlWUS* and *IMA* genes was found in *eno* but not in *fas* flowers. These gene expression results suggest one possible scenario where *ENO* participates, independently of the *FAS* gene, in a signal transduction pathway that shares some common components or targets with *SlWUS* and *IMA*. Taken together, results suggest that *ENO* may encode a new regulator of tomato floral meristem and fruit development. Hopefully, positional cloning of the *ENO* gene, which is currently in progress, will allow us to get insight into the functional role of *ENO* and its genetic and molecular interactions with other floral meristem genes.

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**Tables**

**Table 1** Number of floral organs in WT (P73 cv.), *eno*, *fas*, and F1 (*eno* x *fas*) plants

| | Sepals | Petals | Stamens | Carpels |
|---|---|---|---|---|
| WT (P73 cv.) | 6.2±0.4 a | 6.2±0.4 a | 6.6±0.8 a | 4.1±1.2 a |
| *eno* | 6.8±0.5 a | 12.9±1.4 b | 15.2±2.3 b | 15.6±2.4 b |
| *fas* | 7.9±1.5 b | 8.1±1.5 c | 8.7±1.6 c | 10.1±2.4 c |
| F1 (*eno* x *fas*) | 6.4±0.5 a | 6.3±0.5 a | 6.4±0.6 a | 5.4±0.8 a |

Values are expressed as the mean ± standard deviation. Values followed by the same letter (a, b, or c) are not statistically different ($P<0.01$).

**Table 2** Comparison of mature fruits among WT (P73 cv.), *eno*, *fas*, and F1 (*eno* x *fas*) plants

| | Weight (g) | Number of locules | Fruit size | |
|---|---|---|---|---|
| | | | Width (mm) | Length (mm) |
| WT (P73 cv.) | 96.8±22.2 a | 4.3±1.1 a | 57.8±5.3 a | 46.1±3.4 a |
| *eno* | 134.6±42.8 b | 12.9±3.2 b | 74.8±8.8 b | 44.7±6.5 a |
| *fas* | 70.5±12.2 c | 8.9±1.2 c | 55.1±5.9 a | 41.2±2.4 b |
| F1 (*eno* x *fas*) | 87.9±28.2 a,c | 4.9±1.2 a | 55.4±8.6 a | 45.4±4.3 a |

Values are expressed as the mean ± standard deviation. Values followed by the same letter (a, b or c) are not statistically different ($P<0.01$).

**Figure legends**

**Fig. 1.** Flowers and fruits phenotype of WT and *eno* plants. (A) WT (P73 cv.) and (B) *eno* mutant flowers. (C) Mean comparison of flower width at different developmental stages. (D) Mean comparison of organ number in the four floral whorls at the anthesis stage. (E) WT (P73 cv.) and (F) *eno* mutant fruits. Scale bars: 1 cm in A and B; and 5 cm in E and F. FB0, flower buds of 3.0 to 5.9 mm in length; FB1, flower buds of 6.0 to 8.9 mm in length; FB2, flower buds of 9.0 to 12 mm in length; PA, flowers at the pre-anthesis stage; and A, flowers at the anthesis stage. ns, no statistically significant differences, * significant differences at $P<0.01$.

**Fig. 2.** Scanning electron microscopy (SEM) images of floral meristems from WT (P73 cv.) and *eno* plants. (A, F) Sepal initiation. (B, G) Sepal development, petal initiation. (C, H) Petal development, stamen initiation. (D, I) Stamen development, carpel initiation. (E, J) Carpel development. Se, Sepals; Pe, petals; Sta, stamens; and Ca, carpels. Note: sepals were removed in images C, D, E, H, I, and J. Scale bars = 100 μm. (K) Floral meristem size at each stage of organ initiation up to the stage of carpel differentiation. ns, no statistically significant differences, * significant differences at $P<0.01$.

**Fig. 3.** Genetic and physical maps of chromosome 3 of tomato. Markers are displayed in the central column. Genetic distances in centimorgans (cM) are given on the left and physical distances in megabase pairs (Mbp) are shown on the right. The grey boxes along the physical map represent the euchromatic regions.

**Fig. 4.** Analysis of (A) *FASCIATED*, *FAS*; (B) *TOMATO AGAMOUS1*, *TAG1*; (C) *WUSCHEL*, *SlWUS*; (D) *INHIBITOR OF MERISTEM ACTIVITY*, *IMA*; and (E) *FRUIT WEIGHT 2.2*, *FW2.2* expression during flower development. FB0, flower buds of 3.0 to 5.9 mm in length; FB1, flower buds of 6.0 to 8.9 mm in length; FB2, flower buds of 9.0 to 12 mm in length; PA, flowers at the pre-anthesis stage; and A, flowers at the anthesis stage. ns, no statistically significant differences. Values followed by the same letter (a, b, or c) are not statistically different ($P<0.01$).