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**HORMONAL EFFECT IN *APETALA3*-DEPENDENT PARTHENO-CARPIC FRUIT  
DEVELOPMENT IN *Solanum lycopersicum***

Belo Horizonte – MG

2023

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DEVELOPMENT IN *Solanum lycopersicum***

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"Hormonal effect in APETALA3- dependent parthenocarpic fruit development in *Solanum lycopersicum*"

Às 9 horas do dia 30 de agosto de 2023, reuniu-se a Comissão Examinadora de Dissertação indicada pelo Colegiado do Programa para julgar, em exame final, o trabalho intitulado, "**Hormonal effect in APETALA3- dependent parthenocarpic fruit development in *Solanum lycopersicum***", requisito final para obtenção do grau de Mestre em Biologia Vegetal, área de concentração **Morfologia, Sistemática e Diversidade Vegetal** pelo discente **Paulo Vitor Paiva Ribeiro**. Abriando a sessão, o Presidente da Comissão, professor Dr. Joni Esrom Lima, após dar conhecimento aos presentes do teor das Normas Regulamentares do Trabalho Final, passou a palavra ao candidato para apresentação de seu trabalho. Seguiu-se a arguição pelos examinadores, com a respectiva defesa do candidato. Logo após, a comissão reuniu-se, sem a presença do candidato e do público, para julgamento e expedição do resultado. Foram atribuídas as seguintes indicações:

#### Comissão Examinadora - Resultado

Dr. Joni Esrom Lima (UFMG) - aprovado

Dr. Lázaro Eustáquio Pereira Peres (USP) - aprovado

Dr. Marcel Giovanni Costa França (UFMG) - aprovado

Pelas indicações, o candidato foi considerado **aprovado**.

O resultado foi comunicado publicamente ao candidato pelo Presidente da Comissão. Nada mais havendo a tratar, o Presidente encerrou a reunião e lavrou-se a presente ata, que será assinada por todos os membros participantes da Comissão Examinadora.

Belo Horizonte, 30 de agosto de 2023.

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## RESUMO

Estabelecimento do fruto representa a fase inicial do início do desenvolvimento do ovário após fertilização bem sucedida. Na partenocarpia, estabelecimento e desenvolvimento do fruto ocorrem sem que ocorra a polinização e fertilização, o que faz a formação do fruto ser menos afetada por fatores ambientais, tornando a partenocarpia como uma característica agrônômica vantajosa. Evidência considerável sugere que alguns genes homeóticos *MADS-box* estão envolvidos no crescimento e desenvolvimento do fruto, além da sua função de determinar a identidade de órgãos florais. Nesse trabalho, a função do fator de transcrição do *Tomato APETALA3 (TAP3)* *MADS-box* na regulação do estabelecimento do fruto em tomateiro (*Solanum lycopersicum*) foi avaliada. Usando a mutação de perda de função no gene classe B, *TAP3* no c.v Micro-Tom, nós examinamos o estabelecimento do fruto em níveis bioquímicos e genéticos, usando tratamentos hormonais e mutantes que afetam biossíntese ou sinalização de auxina, giberelinas (GAs), etileno e citocininas (CKs). Ausência de expressão do gene *TAP3* no mutante espontâneo de tomateiro, batizado de *tap3*, produziu flores com desenvolvimento aberrante de estames e estabeleceram frutos partenocárpico alongados, menores do que os frutos polinizados em plantas controle. Nós observamos que aumento dos níveis endógenos de GA no mutante *tap3* resultaram em maior crescimento do fruto partenocárpico. A indução do estabelecimento de frutos partenocárpico foi associada com o aumento dos níveis de auxina em ovários não polinizados em mutantes *tap3*, provavelmente causados pela redução do transporte de auxina do ovário para o pedicelo. Isso é evidenciado pelo bloqueio do transporte polar da auxina usando o mutante *diageotropic (dgt)* no background *tap3*. Além disso, a produção de etileno diminuiu durante o crescimento do ovário não polinizado do mutante *tap3* e a supressão da sensibilidade ao etileno no background mutante *tap3* resultou em frutos partenocárpico maiores. Em conjunto, esses resultados sugerem que *TAP3* tem um papel no estabelecimento do fruto regulando o transporte de auxina originado a partir do ovário e a produção de etileno através da modificação do metabolismo de giberelina. Esses resultados atuais expandem o paradigma para a ação hormonal no desenvolvimento do fruto dependente de *TAP3* ao revelar uma regulação de múltiplos hormônios durante o desenvolvimento do fruto partenocárpico.

Palavras-chave: *Tomato Apetala 3*, *TAP3*, partenocarpia, tomate, *Solanum lycopersicum*, Micro-Tom.

## ABSTRACT

Fruit set is the initial phase of ovary growth. It is stimulated by successful fertilization, leading to the development of fruits with seeds. However, when fruit set occurs without pollination or fertilization, fruits without seeds are developed. These fruits are called parthenocarpic fruit and parthenocarpic fruit set and further development is called parthenocarpy. Since pollination is sensible to environmental factors, parthenocarpy reduces the impact of environmental factors over fruit set, parthenocarpy is useful for agriculture. Considerable evidence suggests that homeotic *MADS-box* genes are involved in the regulation of fruit growth, apart from its function in determining the identity of the floral organs. Here, we examined the *Tomato APETALA3 (TAP3)* *MADS-box* transcription factor function for the regulation of fruit set in tomato (*Solanum lycopersicum*). Using the cv. Micro Tom loss-of-function mutation in class B gene *TAP3*, we examined fruit set at the biochemical and genetic levels, using hormone treatments, and mutants that affect auxin, gibberellins (GAs), ethylene and cytokinins (CKs) biosynthesis or signaling. Lack of *TAP3* expression in a spontaneous mutant tomato plant, called *tap3*, produced flower with aberrant stamens development and development of parthenocarpic and elongated fruit, that is smaller than pollination-dependent fruit in wild-type tomato. We observed increased parthenocarpic fruit growth by increased endogenous GA levels in *tap3* mutant. The induced parthenocarpic fruit-set was associated with an increased auxin level in unpollinated *tap3* ovaries, probably caused by reduced auxin transport from the ovary to the pedicel. This is supported by blocking polar auxin transport using *diageotropic* mutant in *tap3* mutant background. Furthermore, the distribution of ethylene decreased during pollination-independent ovary growth in *tap3* mutant and suppression of ethylene response in mutant background resulted in larger parthenocarpic fruits. Taken together, our results suggest that *TAP3* regulates auxin transport from the ovary and ethylene production by regulating the GA metabolism. Our current findings expand the paradigm for hormone action in *TAP3*-dependent fruit development by showing regulation of multiple hormones during parthenocarpic fruit development

Keywords: *Tomato Apetala 3*, *TAP3*, parthenocarpy, tomato, *Solanum lycopersicum*, Micro-Tom.

## LIST OF FIGURES

Figure 1: <i>tap3</i> mutant homeotic flower development and ovary growth. ....	29
Figure 2: Parthenocarpic and pollinated fruit development in <i>tap3</i> mutant. ....	31
Figure 3: Parthenocarpic fruit of <i>tap3</i> mutant is little and have diferente shape in comparison to MT pollinated fruit. ....	32
Figure 4: Parthenocarpic fruit development in <i>tap3</i> mutant. ....	33
Figure 5: <i>TAP3</i> expression in ovary pericarp during tomato fruit development in ovary. ....	35
Figure 6: <i>TAP3</i> expression in ovary tissues during tomato fruit development. ....	36
Figure 7: Progress of fruit development is positively correlated with exogenous applied gibberellins. ....	38
Figure 8: Progress of fruit development is positively correlated with gibberellins. ....	39
Figure 9: Auxin Transport Reduces in <i>tap3</i> During Parthenocarpic Ovary Development. ....	42
Figure 10: Further blockage of auxin transport does not affect parthenocarpic fruit growth in <i>tap3</i> but is negatively correlated to pollinated MT fruit development. ....	43
Figure 11: Early and late <i>tap3</i> parthenocarpic fruit development is negatively correlated to expression of <i>ARF4</i> . ....	44
Figure 12: Progress of early parthenocarpic fruit development in <i>tap3</i> is negatively correlated to overproduction of ethylene, while progress of pollinated fruit development after anthesis in MT is negatively correlated to ethylene overproduction. ....	47
Figure 13: Progress of early and late parthenocarpic fruit development in <i>tap3</i> is positively correlated to insensibility to ethylene, while only early progress of pollinated fruit development in MT is not affected by insensibility to ethylene. ....	48
Figure 14: <i>TAP3</i> gene expression is positively correlated to the presence of ethylene in the ovary during early fruit development. ....	49
Figure 15: Progress of parthenocarpic unpollinated <i>tap3</i> fruit development is negatively correlated to increased cytokinin degradation, while pollinated MT fruit development is not. ....	51

## LIST OF TABLES

Table 1: Description of the tomato ( <i>Solanum lycopersicum</i> cv. MicroTom) transgenic lines harboring the reporter gene GUS fused with promoter region responsive for hormonal used in this work. ....	23
Table 2: Description of hormonal mutations or transgene insertion introgressed into MT <i>tap3</i> mutant background used in this work.....	23

## LIST OF ACRONYMS

Acronym	-	Meaning
<i>ARF4-AS</i>	-	<i>Auxin Response Factor 4 Antisense transgenic</i>
<i>CKX2-OE</i>	-	<i>Cytokinin Oxidase 2 Overexpressor</i>
<i>dgt</i>	-	<i>diagetropica mutante</i>
<i>DNA</i>	-	<i>Desoxyribonucleic Acid</i>
<i>DR5:GUS</i>	-	Synthetic promoter of auxin response fused to the reporter <i>gus</i> ( $\beta$ - <i>Glucuronidase</i> ) gene
<i>EBS:GUS</i>	-	Synthetic promoter to the ethylene response fused to the reporter <i>gus</i> ( $\beta$ - <i>Glucuronidase</i> ) gene
<i>epi</i>	-	<i>epinastic mutante</i>
<i>GA20ox-OE</i>	-	<i>Gibberellin 20 Oxidase Overexpressor transgenic</i>
<i>GA3</i>	-	<i>Gibberellin 3</i>
<i>MT</i>	-	Micro-Tom cultivar
<i>Nr</i>	-	<i>Never Ripe mutante</i>
<i>RNA</i>	-	<i>Ribonucleic Acid</i>
<i>tap3</i>	-	<i>Tomato Apetala 3 mutant</i>

## SUMMARY

<b>1. INTRODUCTION AND JUSTIFICATION</b> .....	12
<b>2. OBJECTIVES</b> .....	21
<b>2.1 General Objective</b> .....	21
<b>2.2 Specific Objectives</b> .....	21
<b>3. MATERIAL AND METHODS</b> .....	22
<b>3.1 Plant Material and breeding</b> .....	22
<b>3.2 Generating double lines containing <i>tap3</i> mutation</b> .....	24
<b>3.3 Determination of fruit growth</b> .....	25
<b>3.4 Histochemical analysis</b> .....	25
<b>3.5 Statistical Analysis</b> .....	26
<b>3.6 Fruit weight measurement.</b> .....	26
<b>3.7 Fruit area measurement</b> .....	26
<b>3.8 Fruit shape index measurement</b> .....	27
<b>4. RESULTS</b> .....	28
<b>4.1 Regulation of <i>TAP3</i> efficiently results in parthenocarpic fruit set</b> .....	28
<b>4.2 Expression of <i>TAP3</i> in fleshy tomato fruit development</b> .....	34
<b>4.3 Parthenocarpic fruit development of <i>tap3</i> mutant is modulated by GA</b> .....	37
<b>4.4 Interactions between auxin response and <i>TAP3</i> activity in parthenocarpic fruit set</b> .....	40
<b>4.5 Ethylene participates in <i>TAP3</i>-independent parthenocarpic fruit growth</b> .....	45
<b>4.6 <i>TAP3</i>-independent parthenocarpic fruit development in tomato is dependent on cytokinin</b> .....	50
<b>5. DISCUSSION</b> .....	52
<b>5.1 Tomato <i>APETALA3</i> (<i>TAP3</i>) MADS-box acts as a repressor of ovary development</b> .....	52
<b>5.2 Parthenocarpic fruit set in <i>tap3</i> mutant is mediated by auxin affecting GA</b> .....	54
<b>5.3 Temporal regulation by multiple hormones affects <i>TAP3</i>-independent parthenocarpic fruit growth</b> .....	56
<b>6. CONCLUSION</b> .....	57
<b>7. REFERENCES</b> .....	58

## 1. INTRODUCTION AND JUSTIFICATION

Fruit development is an important reproductive process in angiosperms. Fruit can develop from ovaries, upon successful pollination and fertilization (Gillaspy et al., 1993). This process receives the name of fruit initiation and also fruit set. It is the earliest phase of fruit growth, represents the beginning of ovary growth and largely affects fruit yield (Balaguera-Lopez et al.; Molesini et al., 2020). The phase in which occurs the fruit set is more sensitive to hormone signals, endogenous or exogenous, when compared to further stages of growth (Molezini et al., 2020). Nutrient deficiency impacts sucrose importation by phloem and environmental conditions associated with global climatic changes like high, low temperatures and drought (Berti et al., 2005; Li et al., 2012; O’Carrigan et al., 2013; Müller et al., 2016; Carvalheiro et al., 2021) can impair reproduction, leading to abortion of flower, seeds and fruits, and, therefore, decreasing dramatically fruit productivity and crop yield (Boyer et al., 2007; Barnabas et al., 2008; Zinn et al., 2010; Li et al., 2012). For example: fertilization of ovaries by pollination is impaired in the *Arabidopsis thaliana* plants suffering stress caused by extreme temperatures. Without pollination, ovaries of *Arabidopsis thaliana* are inhibited to set fruits (Zinn et al., 2010). Fruit set is also inhibited in tomato plants under temperature stress (Mubarok et al., 2023). However, fruit development may undergo without pollination, or seed formation, producing fruits without seeds through a process called parthenocarpy (Gorguet et al., 2005). Tomato mutants, such as *iaa-9-3*, able to set parthenocarpic fruits under temperature stress, reduces production losses caused by extreme temperatures (Mubarok et al., 2023). Therefore, parthenocarpy is a phenotypic characteristic very important for agriculture because it can stabilize the yield under some environmental stresses (Matsuo et al., 2020). In addition, some fruits bearing no seeds are attractive to consumers and industry because they taste well, are nutritive and easy to process. (Pandolfini et al., 2009). In this regard, if fruit development could be less dependent on efficient fertilization this would be advantageous for fruit crop production in stress prone areas that are now unsuitable for efficient fruit set. This requires an understanding of the regulatory mechanisms involved in fruit set.

Regular consumption of fruits and vegetables reduces mortality by cancer, cardiovascular and respiratory diseases (Wang et al., 2021). Therefore, fruits are an important part of a healthy diet for human diets and, because of that, consumers worldwide are incentivized to consume them. Tomato, a horticultural crop, (*Solanum lycopersicum* L.) belongs to the *Solanaceae* family, is cultivated worldwide, producing millions of tons of tomato fruits, which are a source of health-promoting compounds such as vitamins, carotenoids,

phenolic compounds, and fibers (Quinet et al., 2019; Liu et al., 2022). Long-term domestication and improvement have brought many morphological changes to tomato, such as larger flower and fruit (Frary et al., 2000) and therefore, tomato serves as a model system for fleshy fruit growth, development, and ripening (Liu et al., 2022). Tomato fruit develops from the ovary, that is an expanded basal portion of the pistil. Tomato fruit is classified as a berry composed of a pericarp, that is derived from the ovary wall, a placenta and a pulp. The pulp contains the seeds (Bertin et al., 2005; Molesini et al. 2020). The pistil can originate either from one or more carpels, and the number of carpels in the pistil corresponds to the number of locules in the fruit, which occupies up to 59% of the total fruit area in some tomato cultivar (Musseau et al., 2017). The organogenesis of Tomato fruit is shaped by the cell division and cell expansion that determines the cell number and cell size inside fruit, respectively (Gillaspy et al., 1993; Bohner and Bangerth, 1988, Musseau et al., 2017). Shortly before anthesis, the unpollinated ovary growth is repressed by developmental factors and cell division ceases for a short period. The control on ovary quiescence in tomato can occur partially by negative factors derived from the communication between the anthers and the ovary (De Jong et al., 2009; Molesini et al., 2020). Without pollination, fertilization or another positive stimulus, the pistils senesce and abscise (Serrani et al., 2008). After ovary development, fertilization relieves the repressors of ovary growth. This stimulates the ovary to grow quickly, forming a young fruit (Serrani et al., 2007). This is the fruit set phase (phase I). The phase in that cell divisions enlarge the fruit is called phase II and happens about 7–10 days after the flower anthesis. Cell expansion marks the phase III, which happens 35 days after fertilization in tomato. (Cheniclet et al., 2005). After all stages of growth, fruits start to ripen. (Gillaspy et al., 1993). Tomatoes usually require pollination and fertilization for fruit set, but some cultivars of tomato can develop fruits without pollination, resulting in parthenocarpic fruits (Ho and Hewitt, 1986; Goetz et al., 2007).

The fruit set is regulated by hormones like auxins, gibberellins (GAs), cytokinins (CKs), abscisic acid (ABA), ethylene, and brassinosteroids (BRs). They have roles in different stages of fruit set and growth (Vriezen et al., 2008; Wang et al., 2009; Pascual et al., 2009). Among them, auxins and GAs are very important promoting fruit initiation (Serrani, et al., 2008; Pandolfini, 2009; De Jong et al., 2009;). Auxin and GAs accumulation in the ovaries of tomato is induced by pollination. Response genes for both hormones are upregulated within 48h after pollination, suggesting auxin and gibberellin are positive signals of initial development of the fruits (Mapelli et al., 1978; Sjut e Bangerth, 1982; Vriezen et al., 2008), and do have important roles in phases II and III, respectively (Bohner et al., 1988; Serani et al., 2007a). The exogenous application of these hormones in unfertilized tomato ovaries or the modification in genes

responsible for their signaling can induce parthenocarpic fruit developmental programme (Fos et al. 2000, 2001, Wang et al., 2005; Serrani et al. 2007a, b, de Jong et al. 2009a).

The molecular mechanism by which auxin mediates fruit set remains largely unknown. Auxin levels increases within the ovary right after pollination and fertilization, which binds to the receptor, the TRANSPORT INHIBITOR RESPONSE 1 (TIR1/AFB [TRANSPORT INHIBITOR RESISTANT1/AUXIN SIGNALING F-BOX]), an F-box protein component of the E3 ubiquitin ligase complex, called Skp1/Cullin/F-box complex (SCF<sup>TIR1/AFB</sup>), that promotes ubiquitination and degradation of the Aux/IAA repressor proteins (Dharmasiri et al., 2005; Salehin et al., 2015). Upon degradation of Aux/IAA repressor proteins, AUX/IAA-AUXIN-RESPONSE FACTORS (ARFs) are free to activate transcription of target genes that gives auxin responses. To date, the auxin-dependent mechanism underlying fruit set is understood as coming from mutations or transgenic manipulation of specific *ARF* and *Aux/IAA* genes leading to the development of parthenocarpic fruits in tomato. For instance, parthenocarpic fruit occurs in the tomato *entire* mutant, with loss of function in the gene *INDOLE 3 ACETIC ACID 9 (Aux/IAA9)*, a member of the family of the genes *Aux/IAA* repressors of auxin responses, resulting in seedless fruit, with thickness enhancement of the pericarp with increased number of cell layers (Wang et al., 2005, Wang et al., 2009), which is similar to auxin-induced parthenocarpic fruit development (Serrani et al., 2007a). Comparable phenotypes in parthenocarpic fruit with smaller in size and a thick pericarp due to increased cell expansion were obtained in transgenic plants with RNA-silencing the auxin response factor *SlARF7*, indicating that *SlARF7* act as a negative regulator of fruit set. *SlARF7* is down-regulated within 48h after pollination in the ovary, indicating its repressive effect in fruit set (Pandolfini et al., 2007; De Jong et al., 2008). Because *SlARF7* and *SlIAA9* directly interact, *SlARF7 RNAi*, and *entire (iaa9)* have parthenocarpic fruit development (Hu et al., 2018). In addition, transgenic plants expressing truncated *Arabidopsis ARF8/FRUIT WITHOUT FERTILIZATION (FWF)* protein causes parthenocarpic fruit growth (Goetz et al. 2007). Taken together, these data supports the notion that auxin-signaling components interact to regulate fruit set.

The involvement of GA in fruit initiation is supported by studies showing that exogenous application of GA to unfertilized ovaries causes parthenocarpic fruit development (Groot et al., 1987; Gillaspay et al., 1993). In tomato, naturally obtained parthenocarpic fruit (*pat*) loci, including *pat*, the *pat-2* and *pat-3/pat-4* mutations, have been found to independently, that increases GA content in the unpollinated ovaries (Fos et al., 2000, Fos et al. 2001). Pollination induces an increase of GA content in the ovary (Mapelli et al., 1978; Koshioka et al., 1994) which is associated with increased expression of GA biosynthetic enzymes, such as

the GA 20-oxidase involved with early-13-hydroxylation pathway resulting in endogenous bioactive GA<sub>1</sub> accumulation (Bohner et al., 1988; Serrani et al., 2007), and reduction expression levels of GA catabolic enzymes, such as GA 2-oxidase (Olimpieri et al., 2007; Serrani et al., 2007). Moreover, the growth of pollinated and unpollinated ovaries of wild-type and unpollinated parthenocarpic mutant tomato was reduced applying GA biosynthesis inhibitors. This can be reversed applying a precursor of active gibberellin: GA<sub>3</sub>. This shows that levels of GA are important in fruit set (Olimpieri et al., 2007). Indeed, in the ovary, before fertilization, the signal transduction pathway of GA is inhibited by DELLA proteins acting as transcriptional repressors. Mutations that leads to suppression of DELLA in tomato (*SIDELLA*), a negative regulator of GA signaling, results in parthenocarpic fruits with increased cell elongation (activated phase III), and reduced cell division (phase II) activity in pericarp, resulting in smaller and elongated fruit morphology (Martí et al., 2007; Serrani et al., 2007a). Thus, *SIDELLA*-mediated signaling plays a role in the early phases of tomato fruit development, during anthesis arrest and regulates pericarp cell expansion in subsequent stages (Martí et al., 2007).

The fact that manipulation of GA signaling results in parthenocarpic fruit development with small and elongated mature fruits than seeded fruit, whereas auxin-dependent parthenocarpic fruit is generally similar in size and shape to seeded fruit suggest that these hormones may act in a crosstalking signaling cascade to regulate fruit set. Indeed, in the unpollinated ovary of tomato, changes in GA metabolism were correlated to the fruit set induced by auxin (Serrani et al., 2007; Serrani et al., 2010). Increased auxin levels in the ovary induced transcript levels of genes encoding GA biosynthetic enzymes (*SICPS*, *GA20ox1*, and *GA20ox2*), promoting parthenocarpic fruit-set. Conversely, auxin when transported from the apical shoot hinders GA biosynthesis genes regulation, and fruit-set of unpollinated ovaries are repressed (Serrani et al., 2010). In addition, the fact that pollination triggers transcript accumulation of auxin response genes, but is not modulated in GA-treated tomato fruits (Vriezen et al., 2008), and that auxin-induced parthenocarpic tomato fruits is inhibited by treatment of GA biosynthesis inhibitors (Serrani, et al., 2008) further supports that these hormones may act in a hierarchical cascade to synergistically regulate fruit set, by which GA signaling acts downstream of auxin-response during early fruit development (de Jong et al., 2009; Serrani et al., 2010).

In addition to these hormones, cytokinins (CKs), brassinosteroids (BRs), ethylene, and abscisic acid (ABA) also participates in fruit growth. CKs play important roles in the development of parthenocarpic fruit, promoting more cell division than cell expansion. (Matsuo

et al., 2012). The levels of endogenous bioactive CKs, including tZ, tZR and iPR, decrease after anthesis, but they remained higher at 4 and 8 DPA in pollinated ovaries in tomato (Matsuo et al., 2012, Ding et al., 2013). Parthenocarpic fruit development with higher accumulation of bioactive GAs (GA<sub>1</sub> and GA<sub>3</sub>) and indole-3-acetic (IAA) can be induced by 1-(2-chloro-4-pyridyl)-3-phenylurea (CPPU, an active CK) in tomato, indicating that CKs positively regulates fruit set initiation mediated by auxin and GA signaling pathways (Ding et al., 2013). The effects of brassinosteroids (BRs) hormones on early fruit growth was shown by exogenous application of BR causing parthenocarpy in cucumber (*Cucumis sativus*) also through the synergistic interaction with auxin pathway (Fu et al., 2008). Transcriptional induction of cell cycle-related genes (i.e., cyclins and cyclin-dependent kinases) was found in ovaries after pollination and also treated with BR. suggesting that BRs can promote cell division in the ovary of cucumber (Fu et al., 2008). Interestingly, this BR effect in fruit set and growth seems not to be a conserved mechanism in fleshy fruit development, because BR application to tomato ovaries cannot induce parthenocarpic fruit set or tomato Micro-Tom cultivar defective in *DWARF (D)* locus responsible for BR biosynthesis, cannot display abnormalities in fruit development nor obligatory parthenocarpic fruit development (Martí et al., 2006; Serrani et al., 2007). In contrast to CKs and BRs effect in fruit set, ethylene negatively regulates the ovary growth and the fruit development (Shinozaki et al., 2015). During the progression of ovary expansion, downregulation of ethylene biosynthesis occurs in unpollinated or pollinated WT pistils, suggesting that suppression of ethylene production could contribute to ovary expansion. In addition, increased GA metabolism can be elicited without auxin, by downmodulating the ethylene production or sensibility (Shinozaki et al., 2015). The application of GA biosynthesis inhibitor paclobutrazol (PAC) repressed the pollination-independent ovary growth in *ethylene receptor 1-1* mutant (*Sletr1-1*) which shows ethylene insensitive response. Auxin did not increased its accumulation in *Sletr1-1*, whereas showed cumulative levels of bioactive GAs. Therefore, ethylene can repress the metabolism of GA, and suppression of ethylene repression upregulate GA metabolism, what leads to GA accumulation inside the ovary, without increase in auxin context, leading to parthenocarpy independent from metabolism or signaling of auxin (Shinozaki et al., 2015).

Similar to ethylene, in tomato ovaries the ABA levels also decreases during early fruit development, which is caused by repressed ABA biosynthesis and increased ABA catabolism (Nitsch et al., 2009), however, ABA levels decreased more rapidly in pollination-independent ovary (fruit-growing) than in unpollinated WT ovaries (Shinozaki et al., 2015). High ABA levels are likely play a role to keep the ovary tissues in a dormant state (Vriezen et a., 2008),

and therefore, pollination suppresses ABA repressive action in ovary growth but the simply inhibition of ABA biosynthesis seems not to be sufficient to induce fruit set (Rodrigo and García-Martínez, 1998; Nitsch et al., 2009).

Taken together, complex interactions in between these plant hormones auxin, CKs, ethylene and GA, can function in an hierarchical mechanism and crosstalk with other hormones during fruit set and development in tomato (de Jong et al., 2009; Ding et al., 2013; Shinozaki et al., 2015). In addition, further layer of complexity emerges from functional analysis of floral homeotic genes that discovered that MADS-box transcription factor are key regulators in defining not only, the identity of floral organs (Coen and Meyerowitz, 1991; Weigel and Meyerowitz, 1994; Soltis et al., 2001; Soltis et al., 2009); but also, fruit growth and development (Molesini et al., 2020).

The MADS-box functions was largely investigated using aberrant flower development in *Arabidopsis thaliana* and *Antirrhinum majus* (Schwarz-Sommer et al. 1990; Coen and Meyerowitz, 1991). ABC model of flower development was formulated based on these investigations. In this model, MADS-box gene functions specify floral organ identities. MADS-box gene functions are divided in three classes. A - function specifies sepals, A and B functions together leads to development of petals, B and C together specify development of stamens, and C alone specifies carpels (Bowman et al. 1989; Irish and Sussex 1990; Schwarz-Sommer et al. 1990; Coen and Meyerowitz 1991). The ABC model was modified including D and E homeotic genes. The interaction between the proteins encoded by the genes responsible for the ABCDE functions in the floral quartet model forms quaternary proteins responsible for specify organ identity. (Theissen and Saedler, 2001). In this model, E interacts with A, B, and/or C functions to specify organ identity (Pelaz *et al.* 2000; Honma and Goto 2001; Ma 2005). In *Arabidopsis*, the A-function genes are *APETALA1* (*API*) and *APETALA2* (*AP2*), B function is provided by *APETALA3* (*AP3*) and *PISTILLATA* (*PI*), C function by *AGAMOUS* (*AG*), D genes *SHATTERPROOF1* (*SHP1*), *SHP2* and *SEEDSTICK* (*STK*) regulates ovule development and E function by more than one *SEPALLATA* genes (*SEPI-4*). Excepted for one (*AP2*) gene, all of the ABCDE genes are members of the type II (MIKC<sup>C</sup>-type) MADS-box gene family, that are divided into two groups (type I and type II) (Becker and Theissen 2003) and these genetic regulators to specify floral organs are shared among angiosperms (Theissen and Melzer, 2007).

Many orthologs and/or homologs of known MADS-box transcription factors floral development regulators are also known to have an active role fleshy fruit development (Molesini et al., 2020). For exemple: mutants with altered organ identity during flower

development, also resulted in several phenotypical modifications in fruit growth and development, including parthenocarpy (Pnueli et al., 1994; Ampomah-Dwamena et al., 2002; Geuten et al., 2010; Ribelles et al., 2019). Transgenic tomato plants silencing of *TOMATO AGAMOUS 1 (TAG1)*, an ortholog of the class C function *AGAMOUS (AG)* gene of *A. thaliana*, related to euAG lineage within the core eudicots (Kramer et al., 2004), expressed in the third and fourth whorl, showed stamens (third whorl) replaced by small petaloid organs and pseudocarpel development derived from the pistil whereas, when *TAG1* is ectopically expressed, the identity of sepals is modified and occurs male and female sterility with parthenocarpic fruit development (Pnueli et al., 1994). Another member of the C class of homeotic genes, but from PLENA (PLE) lineage within the core eudicots (Kramer et al., 2004), the ortholog of *SHATTERPROOF MADS-box* genes of *A. thaliana*, *TOMATO AGAMOUS LIKE1 (TAGL1)* was associated with the regulation of flower identity organ formation and fruit development in tomato. During flower development, *TAG1* and *TAGL1* are similarly expressed in stamens and carpels and act redundantly for stamen and carpel development (Pnueli et al. 1994; Gimenez et al. 2010). While the fruit ripening of transgenic *TAGL1* silenced plants showed inhibition, overexpressing *TAGL1* tomato plants presented homeotic conversion of petals into stamenoid structures, and parthenocarpic fruit development (Vrebalov et al., 2009). The involvement of *TAGL1* in flower and fruit development was further confirmed in *Arlequin/TAGL1*, a gain-of-function T-DNA mutant with increased expression of *TAGL1* resulting in conversion of sepals into succulent carpel-like organs and petals into staminoid organs, and the parthenocarpic fruit development is precocious prior to anthesis (Pineda et al., 2010; Ribelles et al., 2019). The analysis of endogenous hormones in *Arlequin/TAGL1* ovaries not only showed a dramatic increase in CK concentration but also reduced level of ABA before anthesis (Ribelles et al., 2019), consistent with the fact that the *TAGL1* gene acts upstream of these hormones during fruit set. Moreover, the roles of *TAG1* and *TAGL1* genes was further revealed in double RNAi silencing lines displaying lack of seed development due to abnormalities in stamen and pollen formation, causing small parthenocarpic fruit development, indicating a cooperative functions of *TAG1* and *TAGL1* genes not only in the specification of carpel identity but also in pollen maturation and fruit development and ripening (Gimenez et al., 2016). This unpollinated fruit phenotype was not associated with changes in the expression of B-class stamen identity genes, e.g *Tomato MADS-box 6 (SITM6)* and *Tomato PISTILLATA (SITPI)* observed in silencing transgenic lines, but rather with *TAG1* and *TAGL1* redundant roles to regulate sepal developmental program during flower by that promoting carpel and fruit development and maintaining proper organ identity (Gimenez et al., 2016).

The identity of petal and stamen in tomato (*S. lycopersicum*) are controlled by the duplicated MADS-box orthologs of *A. majus DEFICIENS* (*euAP3/TAP3* and *TM6*) and *GLOBOSA* (*SIGLO1* and *SIGLO2*) lineage, which play the B class role in ABCDE model (Geuten and Irish, 2010). The loss of function of *TAP3/ DEFICIENS (DEF)/ STAMENLESS (Sl)* caused the conversion of petals into stamens and stamens into carpelloid structures and the formation of partenocarpic fruits (de Martino et al. 2006, Quinet et al. 2014). In transgenic *TAP3* silenced tomato plants, suppression of *TAP3* expression also resulted into male organ sterility with partial conversion of stamens into carpelloid structures, and parthenocarpic fruit development. However, seeded fruit are restored when manually pollinated with wild-type pollen (Okabe et al., 2019). This suggests that the parthenocarpic trait is likely caused due to lack of pollen development in *TAP3*-suppressed plants (Okabe et al., 2019), which is further supported by experiments showing that, in plants with early anther ablation, the ovaries growth occurs without fertilization, producing fruits without seeds (Medina et al., 2013). Interestingly, parthenocarpic fruit in *TAP3*-suppressed ovaries is caused by high GA levels, suggesting that stamen-derived signal negatively regulates fruit set which is likely caused by repression of GA biosynthesis (Okabe et al., 2019). In line with these findings, downregulation of *SIGLO1* and *SIGLO2* in transgenic plants also resulted in homeotic carpelloid structures, fused with the central gynoecium, and parthenocarpic fruit growth (Geuten and Irish, 2010). Moreover, many studies suggests a possible link between defective stamens and parthenocarpy. Downregulating MADS-box protein of the class E, the *Tomato MADS-BOX29 (TM29)*, a homolog of *SEPALATA* in tomato, resulted in flowers with altered morphology with petals and stamens turned green with a partial conversion to a sepalloid identity and the infertile ovaries of transgenic plants are larger than those of wild-type plants (Ampomah-Dwamena et al. 2002). *HYDRA/SES* gene encodes a homologue of Arabidopsis *SPOROCTELESS/NOZZLE (SPL/NZZ)*, *hydra* and *ses* mutants showed parthenocarpic fruit development with a reduced size and weight compared with that of wild-type caused by absence of both male and female sporocyte development (Hao et al. 2017, Rojas-Gracia et al. 2017). Therefore, the roles of floral organ MADS-box genes in regulating fruit initiation and parthenocarpy seem to be a continuation of the floral developmental program (Busi et al., 2003). Indeed, transcript level for *TAG1*, *TAGL1*, *TM29*, *TAP3*, *GLO1*, and *GLO2* sharply declines during the transition from flower to fruit set in tomato, indicating an inhibitory role on ovary growth (Molesini et al., 2020). However, the involvement of MADS-box gene in fruit set is not invariably related homeotic alteration of identity of floral organs. Facultative parthenocarpy fruits was produced in loss-of-function mutant of the transcription factor gene *AGAMOUS-like6 (SILGL6)* in

tomato, while retaining its sexual reproduction capability (Klap et al., 2017). Integument excessive proliferation and undifferentiated endothelium causes enlarged ovules. AGL6 is a MADS-box protein of the E class, and acquired a new function in tomato that is related to fruit initiation acting within the ovule integument to inhibit ovary growth before fertilization (Gupta et al., 2021). Further, transcriptomic analysis showed that auxin hormones signaling components, fruit set repressor *AUXIN RESPONSE FACTOR 5 (SIARF5)*, and the cytochrome P450 cell proliferation regulator *SIKLUH*, are downregulated and upregulated genes, respectively, in the mutant ovules which likely contributes to *slag16* parthenocarpic fruit growth (Gupta et al., 2021). Therefore, these MADS-box genes seem to have maintained the repressive function in the ovary growth (Molesini et al., 2020). Also, evidences point out that that MADS-box transcriptional factor are involved fruit ripening regulation. Like the MADS-box gene *RIPENING INHIBITOR (RIN)*, which is known to crosstalk with ethylene in order to regulate fruit ripening, but also can regulate ripening in ethylene independent manner (Vrebalov et al., 2002). Among the 131 MADS-box genes known in the genome of tomato, many functions still unknown (Wang et al., 2019).

Parthenocarpic fleshy fruit does not always have size and weight of pollinated fleshy fruits (de Jong et al., 2009; Shinozaki et al., 2015; Tanaka et al., 2016; Okabe et al., 2019). Crosstalk in between floral homeotic alterations and plant hormones were demonstrated by function analysis of natural tomato parthenocarpic mutants. (Fos et al., 2000; Mazzucato et al. 2008; Mazzucato et al., 2015; Rojas-Gracia et al. 2017; Okabe et al., 2019). Downstream signaling of MADS-box genes, identifying downstream targets and elucidating the relationship between hormone signaling of MADS-box involved parthenocarpic fruit set and development are a gap in the knowledge to be elucidated. In the current study, we investigated the hormonal regulation in ovaries of parthenocarpic fruits within tomato plants with altered stamen development. We tested the hypothesis that major hormones regulates early tomato fruit development and are associated with molecular mechanisms of B class *tomato APETALA3 (TAP3)* gene function, characterizing its mode of action in coordination parthenocarpic fruit development and growth.

## 2. OBJECTIVES

### 2.1 General Objective

The aim of this work was to understand the contribution of the MADS-box gene *tomato APETALA3 (TAP3)* to the classical hormonal regulation during the formation of the parthenocarpic fruit in *Solanum lycopersicum* cv. Micro-Tom.

### 2.2 Specific Objectives

1. To analyze the relationship in between the development of the stamens and the parthenocarpy in the homeotic *tap3* mutant and in the control cv. Micro-Tom (*Solanum lycopersicum*) plants;
2. To analyze the spatial distribution pattern of the plant hormones auxin and ethylene during the early development of parthenocarpic fruits in *tap3* mutant;
3. To analyze the role and possible crosstalk of the hormones ethylene, auxin, cytokinins, gibberellins, to the development of *TAP3*-independent parthenocarpic fruit by introgressing into *tap3* mutant background mutations or transgene insertion with altered in either synthesis or perception of target hormones.

### 3. MATERIAL AND METHODS

#### 3.1 Plant Material and breeding

Seeds of tomato (*Solanum lycopersicum* ‘MT’) were donated by Dr. Avram Levy (Weizmann Institute of Science) in 1998 and kept through self-pollination as a true-to-type cultivar obtained from the Laboratório de Controle Hormonal do Desenvolvimento Vegetal (ESALQ/Universidade de São Paulo) generated by Prof. Lázaro E. P. Peres (Carvalho et al., 2011).

Micro-Tom (MT) plants were grown under standard greenhouse conditions at Universidade Federal de Minas Gerais. MT, mutant or transgenic seeds were germinated in trays containing a 1:1 mixture of commercial mix Terral Solo® and vermiculite in the proportion 3:1 (v/v), supplemented with 1 g L<sup>-1</sup> 10:10:10 NPK and 4 g L<sup>-1</sup> of dolomitic limestone (CaCO<sub>3</sub> + MgCO<sub>3</sub>). Ten days after germination, plants were transferred to 150 mL pots containing soil mix. After crossing, mature fruits were harvested to obtain seeds. Seeds were further washed and air-dried in preparation for germination for the experimental analysis.

The target mutations or transgene insertions were introgressed into the MT cultivar harbouring *tap3* mutation by a series of crosses and back-crosses (Carvalho et al., 2011; Pino et al., 2022) and selected by mutation-specific morphological phenotype or transgene in antibiotic resistant plants with homeotic flower phenotype alterations related to *tap3* mutation (Tables 1 and 2). The available transgenic plants harboring the GUS reporter genes are described in Table 1. The *DR5::GUS* line in MT was obtained from Dr. Jose Luis Garcia-Martinez (Universidad Politécnica de Valencia, Spain) according to Silva et al., (2018). *EBS::GUS* transgene into MT background was obtained from genetic transformation using a vector generated at Joe Ecker Lab (Salk Institute, USA) and donated by Dr. Anna N. Stepanova and Dr. Jose M. Alonso (North Carolina State University, USA), while MT-expressing *GA2Ox::GUS* was derived from genetic transformation using the vector donated by Dr. Jonathan Dayan and Dr. Tai-Ping Sun (Duke University, USA). Both transgenic homozygous lines in MT background were generated in Lázaro Peres’s laboratory (ESALQ/Universidade de São Paulo- USP). Pollen from transgenic plants with *DR5::GUS*, *EBS::GUS*, transgene were used to pollinate ovaries of MT *tap3* mutant in order to generate a hemizygous plants for the GUS gene reporter. In F2 generation, recombinants showing antibiotic resistance and homeotic flower alterations were used in the analysis for ovary/ fruit growth.

**Table 1: Description of the tomato (*Solanum lycopersicum* cv. MicroTom) transgenic lines harboring the reporter gene GUS fused with promoter region responsive for hormonal used in this work.**

Transgenic lines	Description	Tag used for selecting plants
MT <i>pDR5::GUS</i>	Synthetic promoter of auxin response fused to the reporter <i>gus</i> ( $\beta$ -Glucuronidase) gene (Ulmasov et al., 1997).	Kanamycin 400 mg/L
MT <i>pEBS::GUS</i>	Synthetic promoter to the ethylene response fused to the reporter <i>gus</i> ( $\beta$ -Glucuronidase) gene (Stepanova et al., 2007.).	Hygromycin 75 mg/L

The mutants used in this study are described in Table 2: *diageotropic* (*dgf*), *Never ripe* (*Nr*) and *epinastia* (*epi*) in the MT background were previously described (Carvalho et al., 2011). Transgenic homozygous CKX2-overexpressing plants (CKX2-OE) with lower cytokinin levels (Pino et al., 2022) and GA 20-oxidase-overexpressing plant (GA20-OE) displaying increased GA accumulation, were kindly provided by Dr. Pino and Prof. Peres, respectively. Transgenic plants with transcriptionally silenced line harboring an *ARF4*-antisense (*ARF4*-as) transgene showing reduced auxin response (Sagar et al., 2013) previously described in Moreira et al., (2022).

**Table 2: Description of hormonal mutations or transgene insertion introgressed into MT *tap3* mutant background used in this work.**

Genotype	Hormone	Phenotype	Description
<i>Never ripe</i> ( <i>Nr</i> )	Ethylene	Slow and incomplete fruit ripening due to ethylene insensitive response	Defective in Ethylene Receptor 3 gene in <i>Solanum lycopersicum</i> ( <i>SlETR3</i> ), Never Ripe (NR) (Carvalho R.F. et al., 2011; Wilkinson et al., 1995).
<i>epinastic</i> ( <i>epi</i> )	Ethylene	curled downward leaves and exaggerated stem thickness	Ethylene overproduction. Unknown gene function (Carvalho et al., 2011; Fujino et al., 1988).

<i>tomato</i> <i>APETALA3</i> ( <i>tap3</i> )	Class B-MADS-box <i>tomato</i> <i>APETALA3</i> ( <i>SITAP3</i> )	conversion of petals into stamens and stamens into carpelloid structures and parthenocarpic fruit	spontaneous mutant <i>tap3</i> occur in the sequence of the gene <i>TAP3</i> , with a insertion of approximately 2500 pb in the coding region. This insertion is a retrotransposon M62 Movement transposons of the <i>long terminal repeat (LTR) Copia</i> class generativa a null mutant with loss of function for <i>SITAP3</i> gene (Maniero, 2016).
<i>diageotropic (dgt)</i>	Auxin	reduced auxin sensitivity, with hyponastic leaves mutation in a gene encoding a cyclophilin A protein	mutation in a gene encoding a cyclophilin A protein causing reduced auxin sensitivity and altered auxin polar transport (Carvalho et al., 2011; Oh et al., 2006).
<i>Auxin Response Factor 4 antisense transgenic line (ARF4-as)</i>	Auxin	severe inward leaf curling	antisense down-regulation of <i>SLARF4</i> (Sagar et al., 2013).
<i>GA 20-oxidase (GA20ox)-Overexpressing transgenic line (GA20ox-OE)</i>	gibberellin	higher concentrations of GA <sub>4</sub> causing enhanced stem growth, yield and partial parthenocarpic fruit growth	MT transformed with Citrus gene CcGA20ox1 under the control of a double CaMV 35S promoter inserted in tomato shows CcGA20ox1 overexpression (García-Hurtado et al., 2012).
<i>CYTOKININ OXYDASE 2 (CKX2)-Overexpressing transgenic line (CKX2-OE)</i>	Cytokinin	excessive growth of axillary shoots and shoot branching	<i>AtCKX2</i> under the transcriptional control of the CaMV35S promoter (Pino et al., 2022).

### 3.2 Generating double lines containing *tap3* mutation

Ovaries of the (*Solanum lycopersicum*) of the Micro-Tom cv. with *tap3* mutation in homozygous received pollen from hormonal mutant alleles or transgenic lines (Table 2). As

result of F2 generation, seeds with homozygotic *tap3* mutation were selected according to homeotic flower phenotype and concomitantly abnormal phenotypes in hormonal mutant alleles or transgenic lines (Table 2).

### 3.3 Determination of fruit growth

To determine the fruit growth of tomato plants, one flower was left per truss and only two trusses were left per plant for the experiments. All other flowers were removed before anthesis, except when otherwise stated. The removal of not selected flowers was made to avoid the effects of dominance, flower position and sink balance over ovary growth, as described by Bangerth & Ho (1985), Bohner & Bangerth, (1988) and Bertin et al. (1998). To measure the ovary diameter from eight days before anthesis (-8 DPA) to red ripe stage, flowers with 4mm length of plants of WT and the mutants or transgenic plants were selected. Ovary were measured with digital pachymeter in order to obtain ovary diameter. Ovaries enclosed inside of the flowers during early ovary development were measured indirectly with the pachymeter embracing the flower bud at the height of the ovary. To make measurements more accurate, the ovary size of enclosed ovaries was standardized as a mean of two measurements made at angle of 90° degrees from each other. As ovaries have irregular shapes, measurements of not enclosed ovaries were standardized as pachymeter directly embracing the ovary in the side of the fruit with lesser diameter. This was done to avoid the long and sharp edges of *tap3* parthenocarpic fruits, which could make their measurement imprecise, making the fruit receive a measurement much bigger than its actual size. Emasculation of MT flowers was made when petals and stamens were considered immature (petals and stamens with 6,5 to 7 mm length, having white petals and green stamens), what occurs in between -4 and -2 DPA. Flowers not yet open, but with yellowish stamens were not considered fit to emasculation, since they could already have mature pollen pollinating the carpel stigma. For pollination, the *tap3* mutant flowers were hand-pollinated at 0 DPA.

### 3.4 Histochemical analysis

Histochemical *GUS* (beta-glucuronidase) was performed in the transgenic plants mentioned in Table 1. Flower buds and fruits, from indicated development stages, were cut longitudinally, then vacuum-infiltrated for 5 minutes in paraformaldehyde fixative solution

[0.3% formaldehyde in 10 mM MES, pH 5,6, 0,3 M mannitol], then washed in water for another 5 minutes and then incubated for 4 hours, in the dark, at 37°C, in GUS staining solution [80mM sodium phosphate buffer, pH 7.0; 8mM EDTA; 0.4 mM potassium ferrocyanide; 0.05% Triton X-100; 0.8 mg ml<sup>-1</sup> 5-bromo-4-chloro-3-indolyl-beta-D-glucoronide (X-Gluc); 20% methanol]. Following GUS staining, the flower buds and fruits were washed in water and photographed with a camera in order to record their images. This protocol was adapted from the original protocol described by Jefferson R.A., Kavanagh T.A. and Bevan M.W. (1987).

### **3.5 Statistical Analysis**

Data were analyzed using Student's t-test in the Microsoft® Excel® for Microsoft 365 MSO (Version 2307 Build 16.0.16626.20170) 64-bit software. Settings were adjusted to consider each pair of data sets being compared as having two tails distribution and unequal variance. Two sets of data were considered as having a difference of statistical significance when p value was lesser or equal to 0,05.

### **3.6 Fruit weight measurement.**

The ripen fruits were collected and weighted at a precision weighing scale (SHIMADZU ATY224, maximum weight 220g, minimum weight 10 mg, error of 1mg, deviation from error of 0,1mg). Removal of the stems, sepals and petaloid sepals (when present) from the ripened fruit was done prior to weight measurement in order to weight only the parts of the fruit derived from carpels and carpeloid stamens.

### **3.7 Fruit area measurement**

Collected ripen fruits were transversely cut at equator, leaving two hemispheres. The sliced surface of both hemispheres were placed over a scanner (Epson Perfection V800 Photo), which generated an image of them. The scanned surface of the slices were measured with ImageJ wand tool, which is described by Rasband W.S. (1997 – 2018); Abramoff, Magalhaes, Ram, S.J. (2004) and Schneider, Rasband, Eliceiri, (2012).

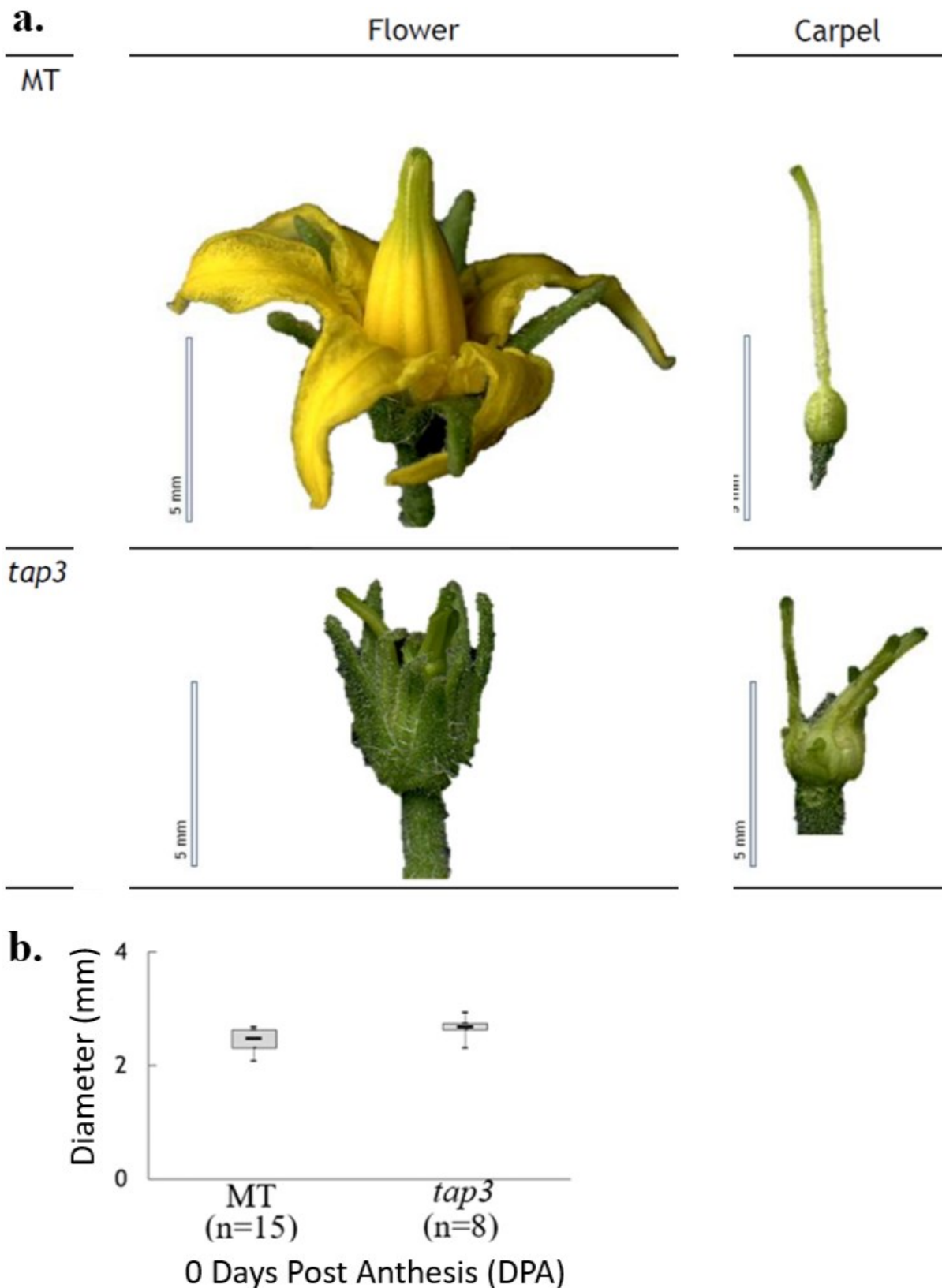
### **3.8 Fruit shape index measurement**

Collected ripen fruits had been scanned at two positions: 1- their profile, 2 – their equatorial transversal slice. Profile was used to measure the height and equatorial transversal slice to measure the width. The height was divided by width in order to obtain the fruit shape index, which can also be called vertical/horizontal ratio.

## 4. RESULTS

### 4.1 Regulation of *TAP3* efficiently results in parthenocarpic fruit set

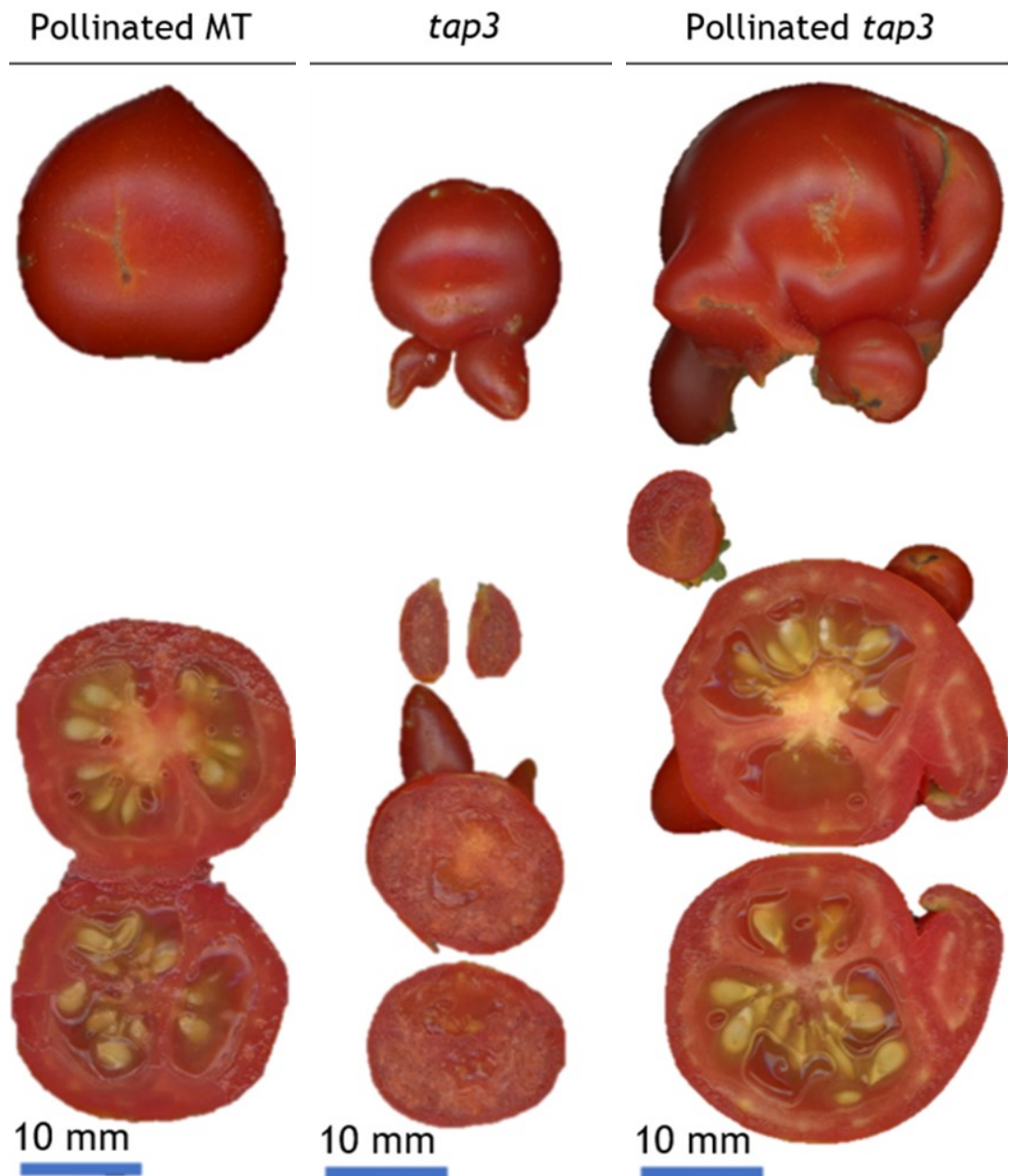
To understand the contribution of the stamen development to the formation of the parthenocarpic fruit, we investigate the spontaneous mutant in Micro-Tom genetic background with loss-of-function for tomato *APETALA3* (*TAP3*) previously described by Maniero (2016). Therefore, here we used Micro-Tom, a dwarf tomato variety, as the wild-type (WT) genotype, as well as the *tap3* mutant in the Micro-Tom background. The analysis of tomato *tap3* mutant presented homeotic conversion of flower in which stamens (third whorl) were replaced into carpelloid organs and of petals (second whorl) into petaloid sepals when compared to WT flower (Figure 1 a). In addition, the ovaries of *tap3* are fused to the carpeloid stamens. However, it shows similar ovary diameter at 0 DPA. (Figure 1 b). Interestingly, the floral homeotic phenotypes of this mutant was similar to those of class B MADS-box homeotic mutants in *Arabidopsis thaliana* with apetalous flower *ap3-1* mutant (Bowman et al. 1989, and loss-of-function of Tomato *APETALA3* (*TAP3*)/ *DEFICIENS* (*DEF*)/*STAMENLESS* (*Sl*) (de Martino et al. 2006, Quinet et al. 2014).



**Figure 1: *tap3* mutant homeotic flower development and ovary growth.**

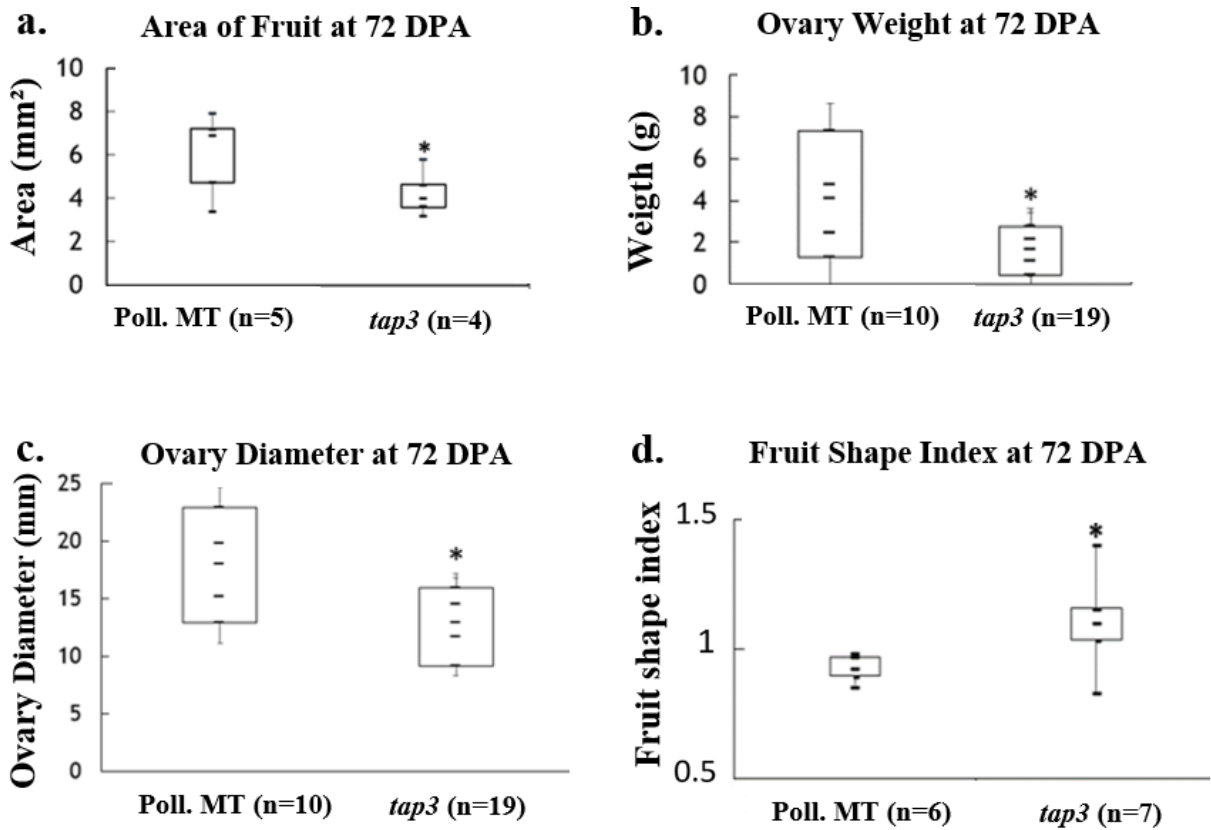
**a** – Morphology of the flower bud and carpels of MT and *tap3* at 0 DPA. **b** – Ovary diameter of MT and *tap3* at 0 DPA with no significant differences between WT and *tap3* mutant according to  $*P < 0.05$  of Student's *t* test. DPA, days post anthesis.

Since *tap3* mutant showed complete male sterility caused by stamens appearing carpelloid, loss-of-function of *TAP3* in ovaries mutant showed parthenocarpic fruit set (Figure 2), whereas *tap3* mutant exhibited reduced fruit area, weight and diameter relative to WT pollinated ovaries growth (Figures 3 a, b and c). Then, the size of the ovaries was compared from WT with or without pollination and *tap3* mutant fruit during early development (Figure 4). The average ovary size of the *tap3* mutant at the time of MT anthesis, referred to here as 0 days post anthesis (0 DPA, i.e. anthesis), was similar to that of the WT (Figures 1 b and 4), although at 6 and 30 DPA the *tap3* mutant ovaries were significantly reduced than those of the WT following pollination (Figure 4). In contrast, the unpollinated WT ovaries, post-anthesis unfertilized ovules did not grow in diameter and changed little between anthesis and 30 DPA when emasculation of flower occurred -2 days before anthesis (-2 DPA). MT ovaries that were emasculated later than -2 DPA and grew up, developed pollination-independent parthenocarpic fruits with significant little size in comparison to pollinated fruits of MT (Figure 4). Although producing carpeloid stamens, *tap3* mutant also developed parthenocarpic fruits from pollination-independent ovaries (Figure 2 and 4). Notably, when *tap3* homeotic mutant flower received WT pollen, fruit set occurs earlier at 4 DPA when compared to pollinated MT, which occurs at 6 DPA, indicating that fertilization-induced ovaries growth occurs greatly in *tap3* mutant (Figure 4). Although, the diameter of *tap3* parthenocarpic mutant ovaries was significantly reduced to that of pollinated WT ovaries in breaker fruit developing stage (Figure 4) and change in fruit shape index (length/width) was observed *tap3* mutant parthenocarpic fruit relative to WT pollinated fruit, showing elongated fruit shape (Figure 3 d). These findings indicate that loss of-function of *TAP3* in ovaries leads to formation of parthenocarpic fruit elongated in shape and smaller in size than WT seeded fruit.



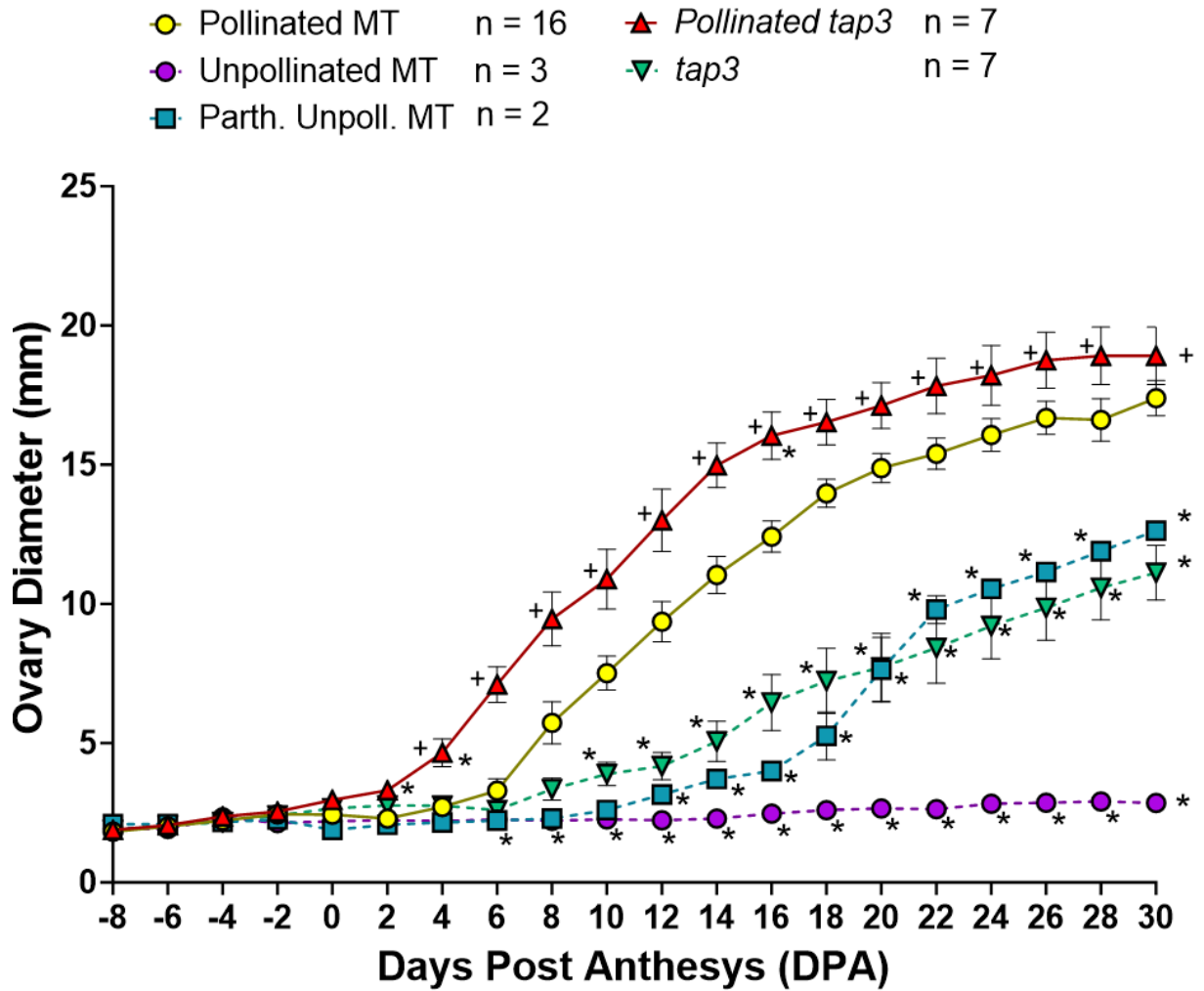
**Figure 2: Parthenocarpic and pollinated fruit development in *tap3* mutant.**

Fruit development at mature breaking phase of MT, *tap3* and pollinated *tap3* with pollen from MT.



**Figure 3: Parthenocarpic fruit of *tap3* mutant is little and have diferente shape in comparison to MT pollinated fruit.**

**a** – Fruit area of MT and *tap3* mutant at 72 DPA. **b** – Ovary weight for MT and *tap3* mutant at 72 DPA. **c** – MT and *tap3* mutant ovary diameter at 72 DPA. **d** – Fruit shape Index (Length divided by height) at 72 DPA for MT and *tap3* mutant. \* Asterisks in **a**, **b**, **c** and **d** indicates significant differences between WT pollinated fruit versus parthenocarpic *tap3* fruit according to  $*P < 0.05$ ; Student's *t* test.

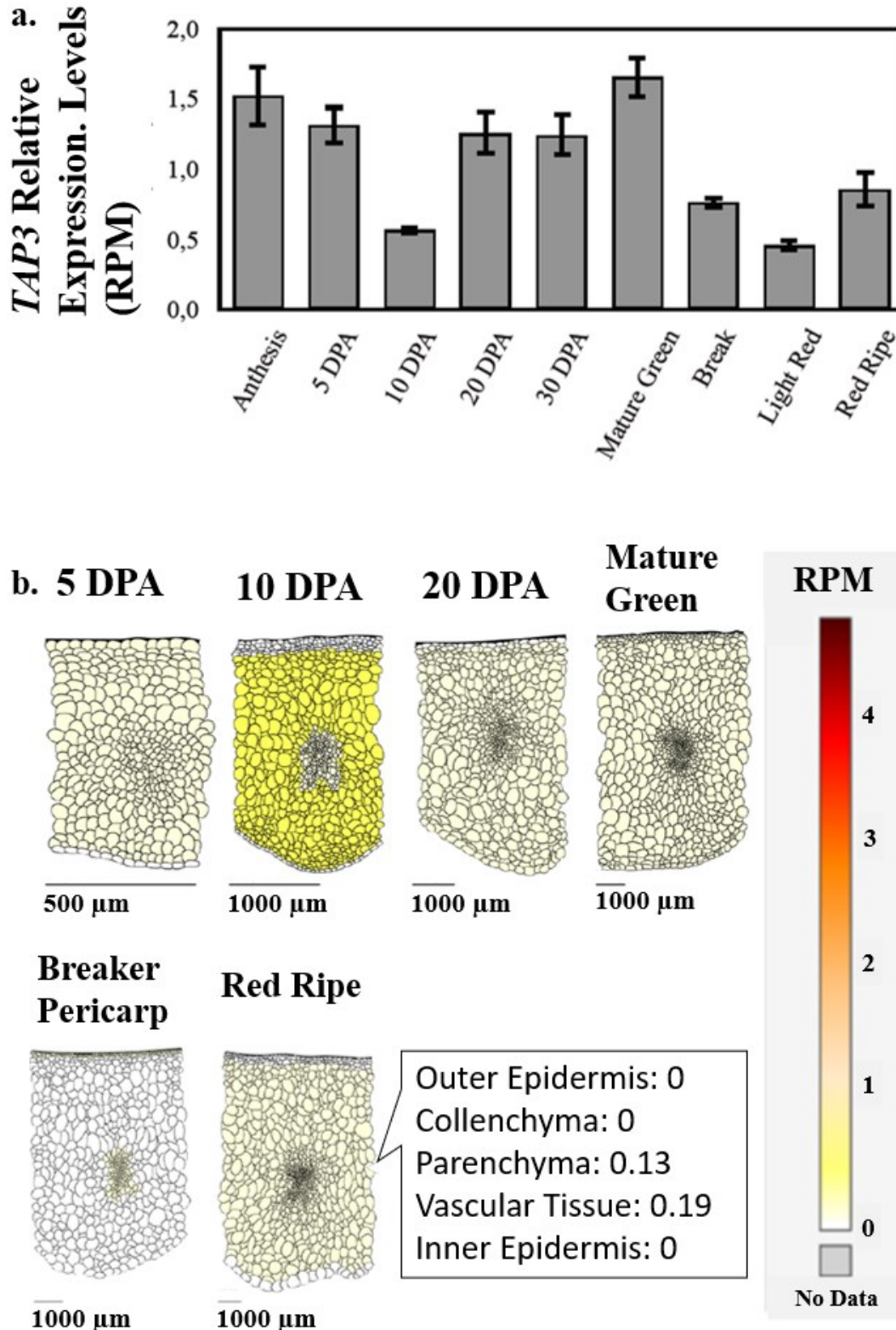


**Figure 4: Parthenocarpic fruit development in *tap3* mutant.**

Ovary diameter during fruit development of pollinated MT, unpollinated MT, parthenocarpic MT, *tap3* and pollinated *tap3* mutant with WT pollen. Values are means with standard error of indicated ovaries number. \* Asterisks indicates significant differences between WT pollinated fruit versus WT unpollinated parthenocarpic fruit, WT unpollinated fruit and parthenocarpic *tap3* fruit according to  $*P < 0.05$ ; Student's *t* test. + Crosses indicates significant differences between unpollinated parthenocarpic *tap3* fruit and pollinated *tap3* fruit according to  $+P < 0.05$ ; Student's *t* test. DPA, days post anthesis.

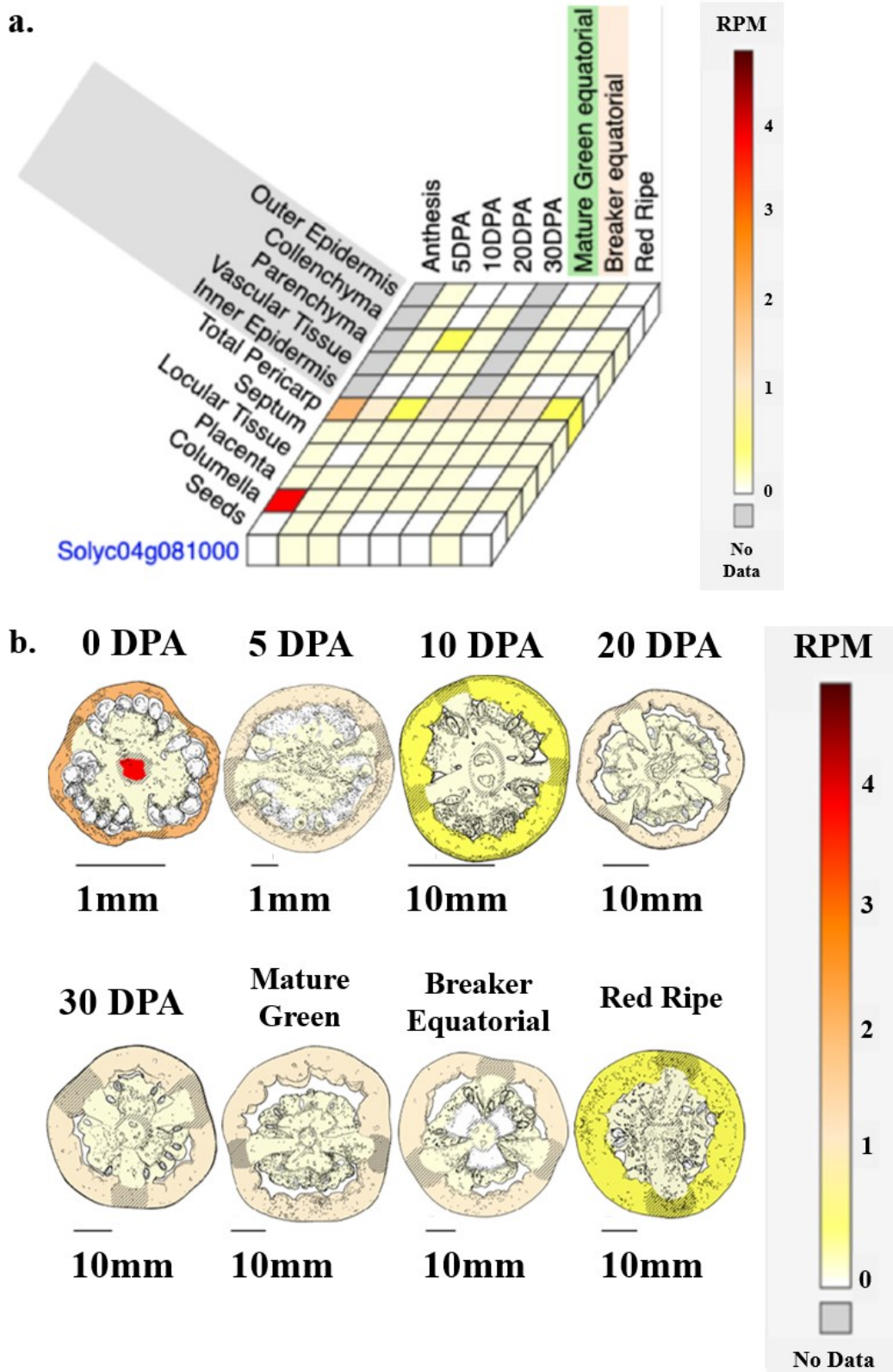
## 4.2 Expression of *TAP3* in fleshy tomato fruit development

To further understand the function of tomato *APETALA3* (*TAP3*) during fruit development, we assessed a spatiotemporally resolved public available transcriptome analysis of fruit ontogeny in manually pollinated tomato ovaries in *S. lycopersicum* cv M82 background (Fernandez-Pozo et al.; 2015, 2017 and 2023; Shinozaki et al., 2018). The *TAP3* (Solyc04g081000.2) expression values in total pericarp (Figure 5 a), indicated by reads per million mapped reads (RPM), peaked twice during fruit development, with higher expression levels at anthesis up to 5 DPA, a steady decreased at 10 DPA, with further increased from 20 DPA to mature green developmental stage (approximately 39 DPA), followed by significantly lower expression levels over-ripe stage (approximately 50 DPA) in fruit development (Figure 5 a). The spatiotemporal *TAP3* expression signatures of individual tissue types accessed during fruit development revealed high transcripts accumulation in columella cells at anthesis, which decreases further in internal tissues during fruit set and development (Figure 6 a and b). We detected the highest expression of *TAP3* in total pericarp, septum and placenta (Figure 6 a and b), with predominant expression in the collenchyma and parenchyma in the pericarp during fruit developmental trajectory, with the exception of breaker stage that presented lower and restricted expression in the vascular tissue and outer epidermal layer (Figure 5 b). Notably, the consistently higher expression in the total pericarp highlighted a specific function of this gene at particular stages at anthesis, 10 DPA and to a lesser extent on ripening stage (Figure 6 a). Collectively, this analysis features the spatial *TAP3* expression levels variation along and throughout fruit development and indicates that this class B *MADS-box* gene might play a role in fruit set and growth during early tomato fruit development.



**Figure 5: TAP3 expression in ovary pericarp during tomato fruit development in ovary.**

**a** – TAP3 expression in the ovary pericarp of M82 tomato cultivar, from flower anthesis to red ripe fruit stage, with three biological replicates in technical triplicate from the transcriptome data integrated into the Tomato Expression Atlas (TEA; <http://tea.solgenomics.net/>) database. **b** - Cell based TAP3 expression of M82 tomato cultivar, from flower anthesis to red ripe fruit stage. Pictograms in **b** were generated through the Tomato Expression Atlas (TEA) database. DPA, days post anthesis.

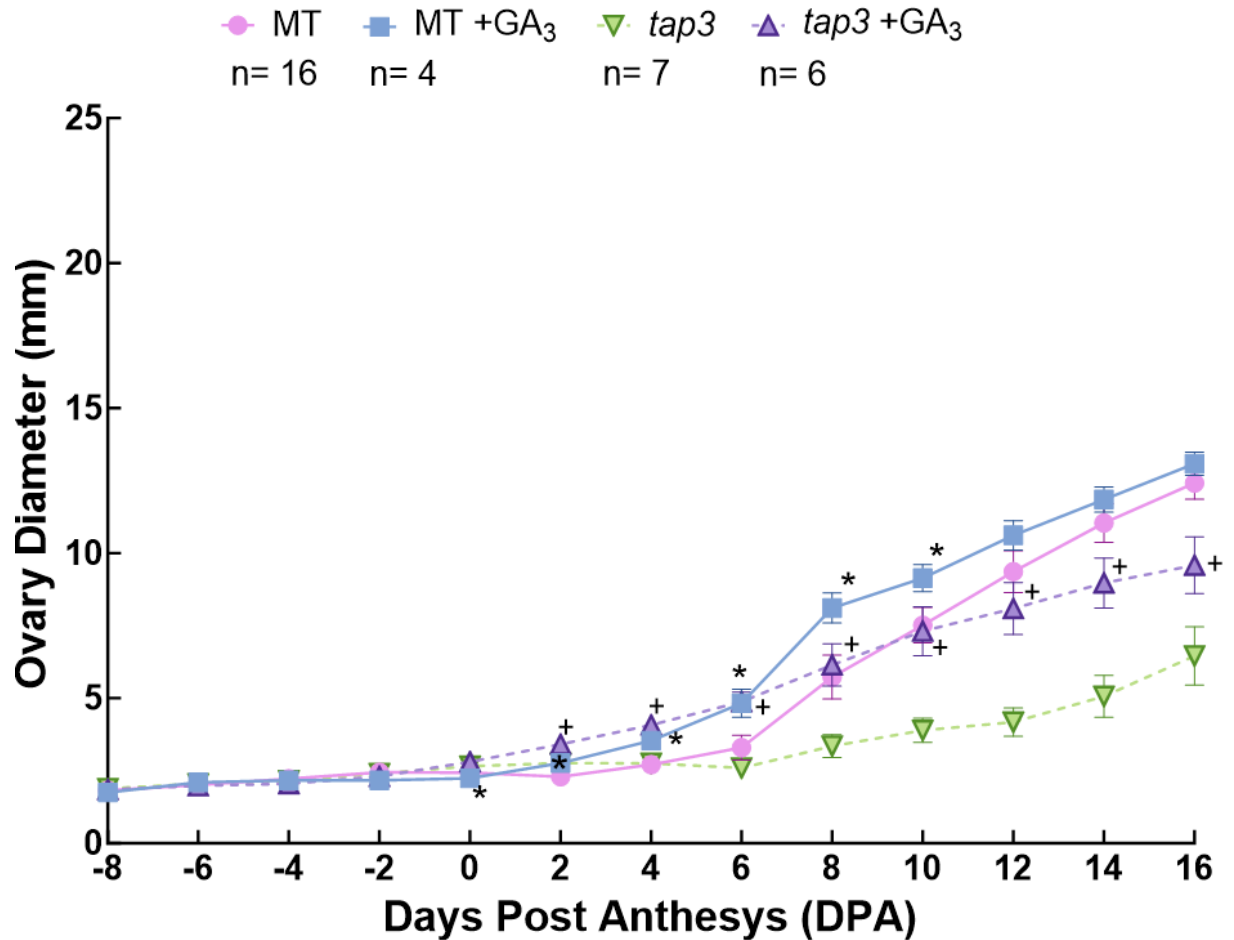


**Figure 6: *TAP3* expression in ovary tissues during tomato fruit development.**

Tissue specific *TAP3* expression of M82 tomato cultivar, from flower anthesis to red ripe fruit stage, with three biological replicates in technical triplicate from the transcriptome data integrated into the Tomato Expression Atlas (TEA; <http://tea.solgenomics.net/>) database. **a** – Expression cube showing *TAP3* expression in different ovary tissues during developmental stages. **b** – Expression map in transversal cuts in equatorial region of ovaries in different developmental stages. Pictograms in **a** and **b** were generated through the Tomato Expression Atlas (TEA) database. DPA, days post anthesis.

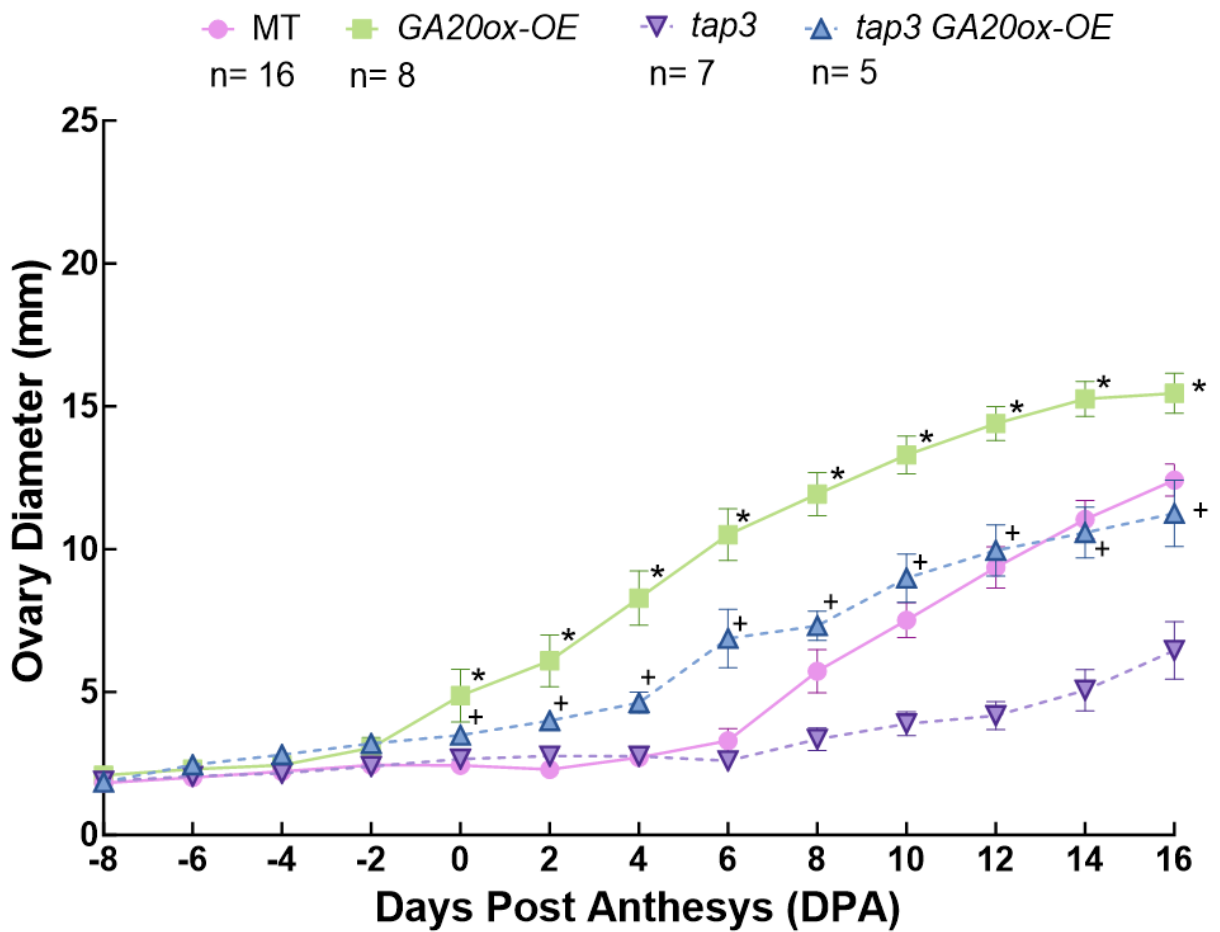
### 4.3 Parthenocarpic fruit development of *tap3* mutant is modulated by GA

Exogenous application of GAs or increases in their biosynthesis or sensitivity can induce pollination-independent fruit set (Serrani et al., 2007; Martí et al., 2007; Shinozaki et al., 2018). To investigate whether the development of *tap3*-induced parthenocarpic fruits were responsive to GAs, pollinated WT and unpollinated *tap3* ovaries were treated with exogenous application of GA3 (Figure 7). Treatment of pollinated WT ovaries with GAs resulted in early fruit growth relative to untreated pollinated WT ovaries plants (Figure 7), whereas no significant differences of ovary growth were found at later stages of fruit development (16 DPA). In contrast, the application of exogenous GA3 led to significantly larger pollination-independent ovary growth in *tap3* mutant than nontreated mutant ovaries (Figure 7). To further evaluate the effect of GA in *tap3* mutant, we generated *tap3* mutant plant with increased endogenous levels of GAs, by crossing with *GA 20-oxidase (GA20ox)-Overexpressing transgenic line (GA20ox-OE)* in the same genetic background. Pollination induced ovary growth in both WT and *GA20ox-OE* fruits, but the *GA20ox-OE* ovaries grew faster than those of the WT following pollination, resulting in greater fruit growth (Figure 8). When compared the size of the ovaries from *tap3* mutant and *tap3 GA20ox-OE* fruit during early development, it was clear that *tap3 GA20ox-OE* presented significantly earlier fruit set and larger ovaries than those of the *tap3* mutant at anthesis (0 DPA), reflecting an increase in the fruit growth during pollination-independent fruit set in the mutant *tap3* (Figure 8). These results suggest that both elevating levels of bioactive GAs or upregulating GA biosynthesis can induce parthenocarpic fruit growth in *tap3* mutant background.



**Figure 7: Progress of fruit development is positively correlated with exogenous applied gibberellins.**

Changes in ovary diameter during fruit development of MT, MT+GA<sub>3</sub>, *tap3* and *tap3*+GA<sub>3</sub>. Values are means with standard error of indicated ovaries number. \* Asterisks indicates significant differences between pollinated WT fruit versus MT+GA<sub>3</sub> pollinated fruit according to \* $P < 0.05$ ; Student's *t* test. + Crosses indicates significant differences between unpollinated parthenocarpic *tap3* fruit and unpollinated parthenocarpic *tap3*+GA<sub>3</sub> fruit according to + $P < 0.05$ ; Student's *t* test. DPA, days post anthesis.



**Figure 8: Progress of fruit development is positively correlated with gibberellins.**

Ovary diameter during fruit development of MT, *GA20ox-OE*, *tap3* and *tap3 GA20ox-OE*. Values are means with standard error of indicated ovaries number. \* Asterisks indicates significant differences between pollinated WT fruit versus pollinated *GA20ox-OE* fruit according to  $P < 0.05$ ; Student's *t* test. + Crosses indicates significant differences between unpollinated parthenocarpic *tap3* fruit and unpollinated parthenocarpic *tap3 GA20ox-OE* fruit according to  $P < 0.05$ ; Student's *t* test. DPA, days post anthesis.

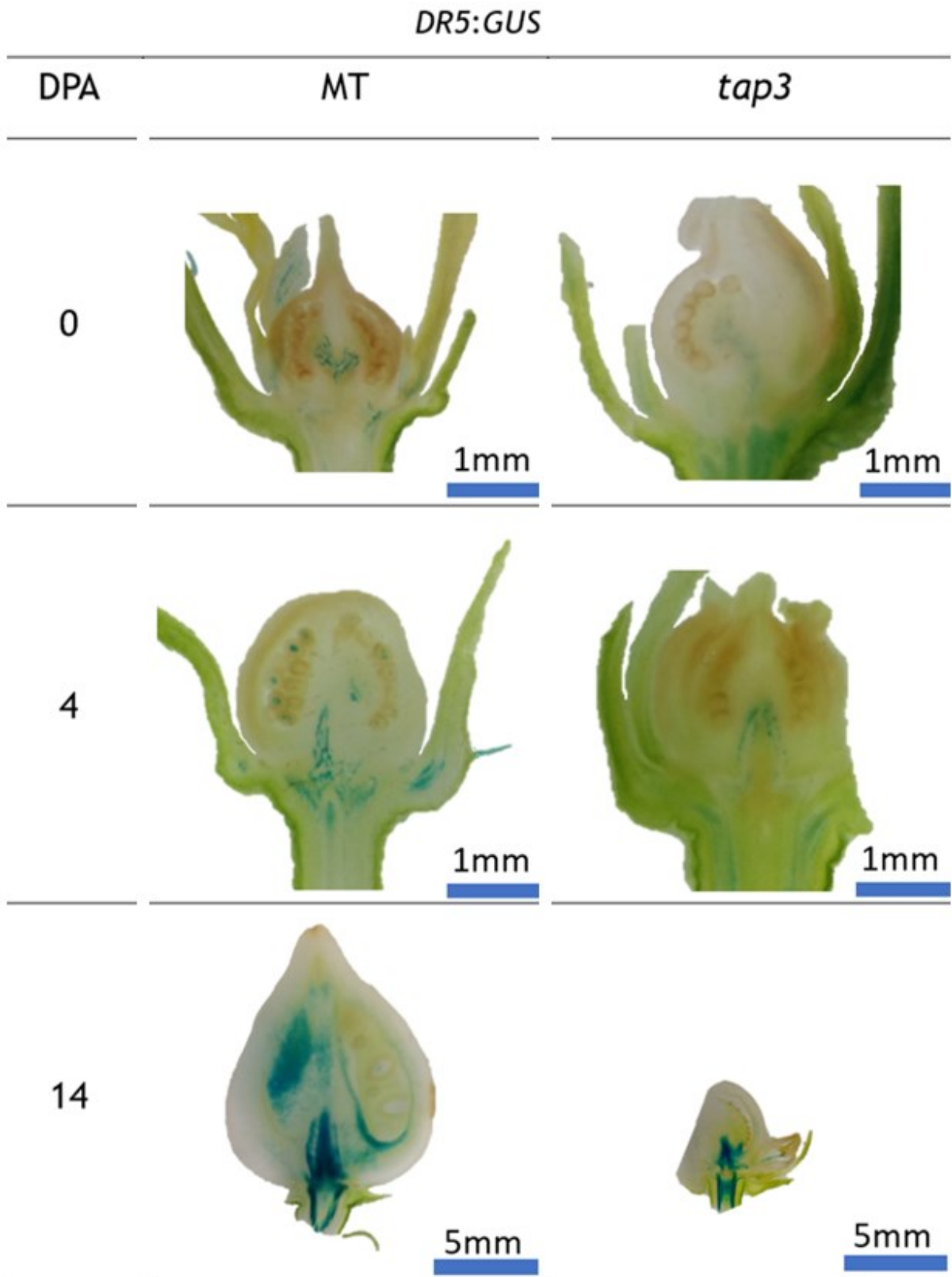
#### 4.4 Interactions between auxin response and *TAP3* activity in parthenocarpic fruit set

Auxins have been shown to interact with GAs by increasing active GA<sub>1</sub> levels in unpollinated ovaries through the upregulation of genes encoding enzymes of GA biosynthesis (Serrani et al., 2010). Unpollinated ovaries are auxin deficient, as this hormone is synthesized in the developing seeds following pollination and ovule fertilization (Varga and Bruinsman, 1976; Martí et al. 2007; Serrani et al., 2008). To investigate the possibility that auxin levels is altered in *tap3*-dependent parthenocarpic fruit, we evaluate the pattern of *DR5* promoter activation in GUS reporter line during fruit development in *tap3* mutant in order to estimate the auxin signaling output (Ulmasov et al., 1995; Liao et al., 2015). Histochemical analysis of *DR5* promoter activity revealed discernible staining difference between WT pollinated and unpollinated *tap3* during fruit development. At anthesis (0 DPA), longitudinal section showed strongly induced GUS expression in the placenta of WT than *tap3* mutant, which was evident at 4 DPA, with GUS expression proceeds auxin flux basipetally up to the vascular tissue in the core towards to the pedicel for WT but not for the unpollinated ovary of *tap3* mutant (Figure 9). Notably, at this stage, pollinated WT ovary showed GUS staining in developing seeds, which was absent in parthenocarpic developing ovary. At later development stage (14 DPA), fainter GUS staining was observed unpollinated ovary of *tap3* mutant whereas GUS activity in WT developing young fruit has expanded expression of the GUS reporter driven by the auxin-inducible *DR5* promoter to locular tissue (Figure 9). These results suggest that pollination-independent induced parthenocarpic fruit-set in *tap3* mutant is associated with perturbed auxin transport and homeostases during ovary growth.

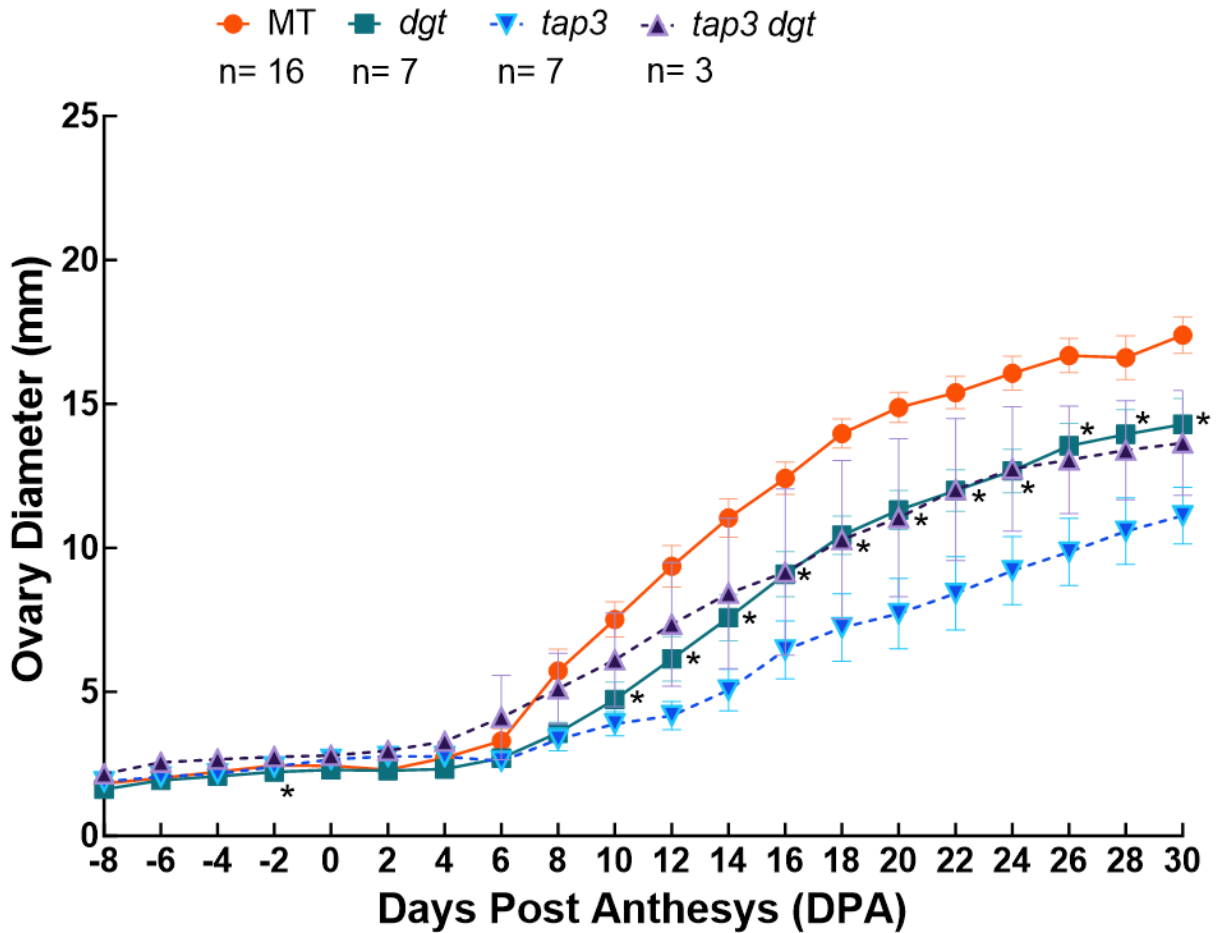
We further assessed the effect of auxin transport in parthenocarpic fruit growth of *tap3* mutant, we used a tomato mutant defective in the gene *DIAGEOTROPICA* (*DGT*). *DGT* is a cyclophilin A protein with peptidyl-prolyl trans/cis-isomerase (PPIase) enzymatic activity (Oh et al., 2006) which is related to rate-limiting steps in the protein-folding pathway (Kumari et al., 2013). The tomato *dgt* mutant has phenotypic defects such as: horizontal shoot growth, thin stems, altered secondary vascular differentiation, and roots lacking lateral branches (Zobel et al., 1993; Coenen et al., 2003) caused by reduced polar auxin transport (Ivanchenko et al., 2015). We produced double mutant *tap3 dgt* to assess the role of auxin transport in both pollination-dependent and -independent early fruit development. In pollinated ovaries, tomato fruit-growth was inhibited in the *dgt* mutant compared to WT (Figure 10). Pollination-independent fruit growth in the double mutant *tap3 dgt* had no with significant differences in

fruit size in comparison to *tap3* single mutant, suggesting that further alteration of auxin transport in *tap3* background is unable to enhance parthenocarpic fruit size (Figure 10).

Auxin modulates plant development, including fruit set and growth, through transcriptional regulation of target genes (Ulmasov et al., 1999) mediated by two gene families, *AUXIN RESPONSE FACTOR (ARF)* and *AUXIN/INDOLE-3-ACETIC ACID (AUX/IAA)*; Ulmasov et al., 1999; Guilfoyle and Hagen, 2007; Audran-Delalande et al., 2012). In particular, ARFs are transcriptional activators and repressors that bind with specificity to TGTCTC AuxREs in promoters of primary/early auxin response genes (reviewed by Guilfoyle and Hagen, 2001). To shed light on the role of auxin response during parthenocarpic fruit development, we used transgenic plants with down-regulation of *Sl-ARF4* gene, *ARF4*-antisense (*ARF4-as*) transgene (Sagar et al., 2013), negative regulator in auxin response in tomato fruits (Jones et al., 2002) and produced *tap3 ARF4-as* line. The higher starch content in developing fruits of *SlARF4* down-regulated lines correlates with increased photosynthetic activity, chlorophyll content in immature tomato fruits, suggesting that *Sl-ARFs* may play a key role in controlling sugar content, an essential feature of fruit quality (Sagar et al., 2013). Pollinated ovaries in *ARF4-as* plants were larger in later developmental stages than those of the WT (Figure 11), whereas the size of unpollinated parthenocarpic ovaries in *tap3 ARF4-as* plants set up earlier, grew up more and became significantly larger than those of the *tap3* mutant (Figure 11). These results suggest that parthenocarpic fruit growth can be additively increased by suppression of ARF4 and TAP3 activity in the ovary.

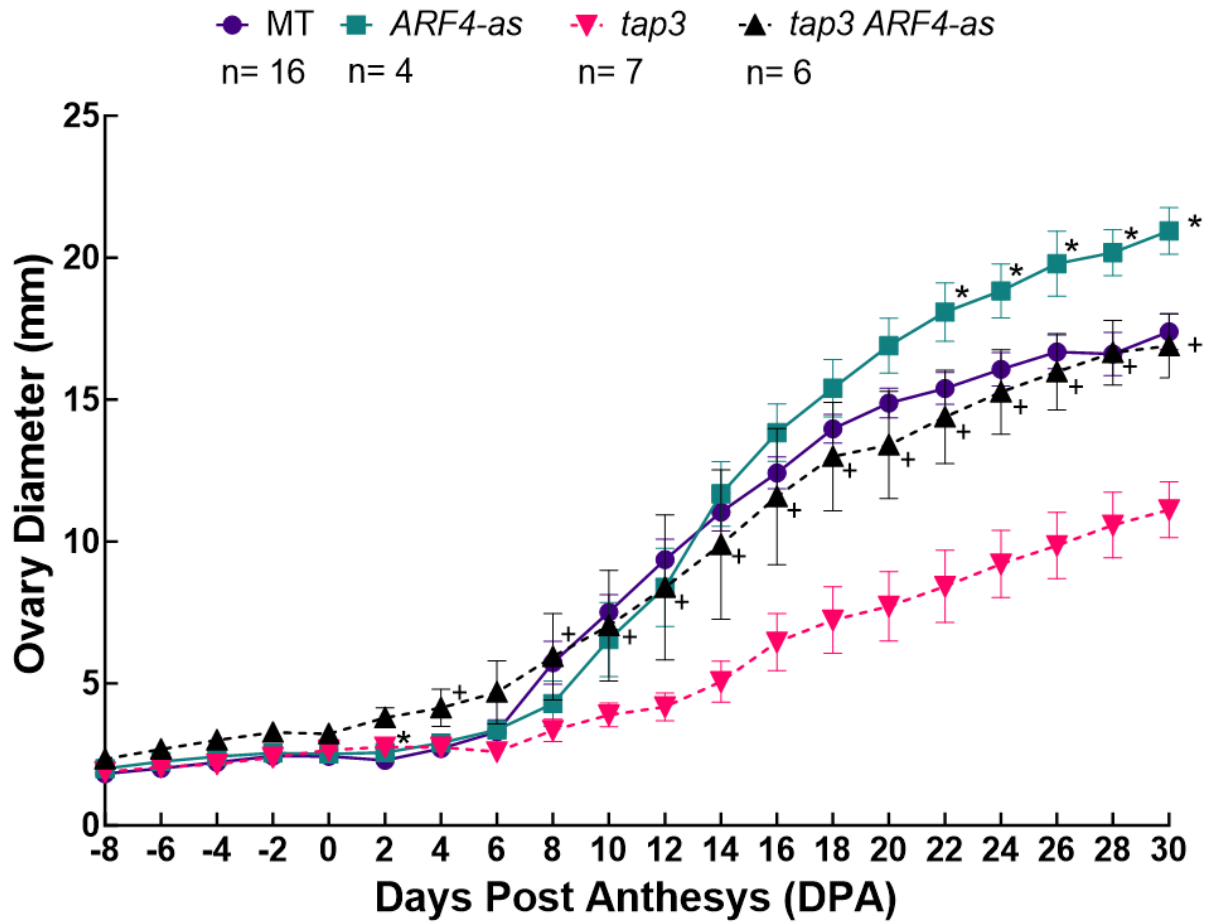


**Figure 9: Auxin Transport Reduces in *tap3* During Parthenocarpic Ovary Development.** Slices of flowers of MT *DR5:GUS* and *tap3 DR5:GUS* at 0, 4 and 14 DPA after staining process. Areas of floral tissue with detectable auxin are stained with blue color. DPA, days post anthesis.



**Figure 10: Further blockage of auxin transport does not affect parthenocarpic fruit growth in *tap3* but is negatively correlated to pollinated MT fruit development.**

Ovary diameter during fruit development of MT, *dgt*, *tap3* and *tap3 dgt*. Values are means with standard error of indicated ovaries number. \* Asterisks indicates significant differences between pollinated WT fruit and pollinated *dgt* fruit according to \* $P < 0.05$ ; Student's t test. + Crosses indicates significant differences between unpollinated parthenocarpic *tap3* fruit and unpollinated parthenocarpic *tap3 dgt* fruit according to + $P < 0.05$ ; DPA, days post anthesis.



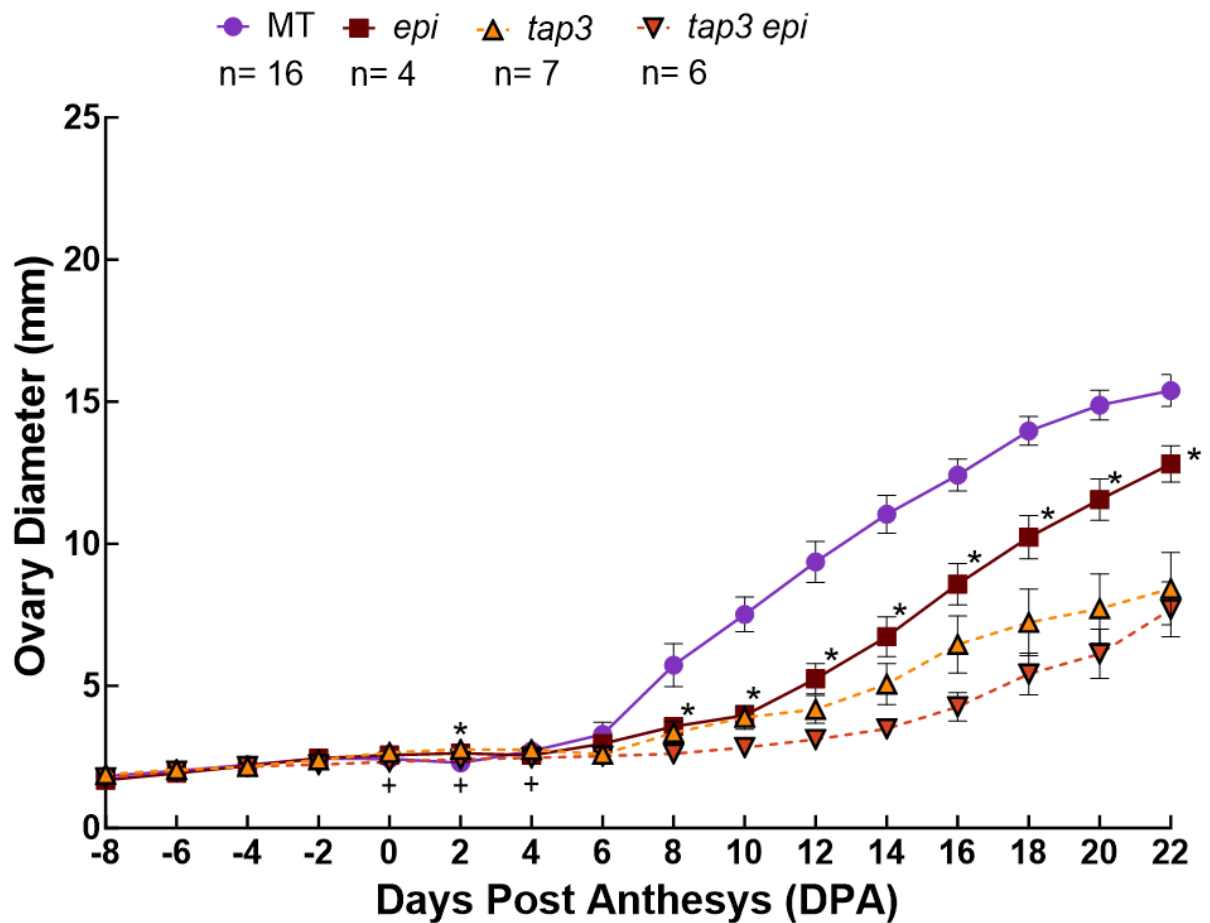
**Figure 11: Early and late *tap3* parthenocarpic fruit development is negatively correlated to expression of *ARF4*.**

Ovary diameter during fruit development of MT, *ARF4-as*, *tap3 ARF4-as*. Values are means with standard error of indicated ovaries number. \* Asterisks indicates significant differences between pollinated WT fruit versus *ARF4-as* pollinated fruit according to \* $P < 0.05$ ; Student's t test. + Crosses indicates significant differences between unpollinated parthenocarpic *tap3* fruit and unpollinated parthenocarpic *tap3 ARF4-as* fruit according to + $P < 0.05$ ; Student's t test. DPA, days post anthesis.

#### 4.5 Ethylene participates in *TAP3*-independent parthenocarpic fruit growth

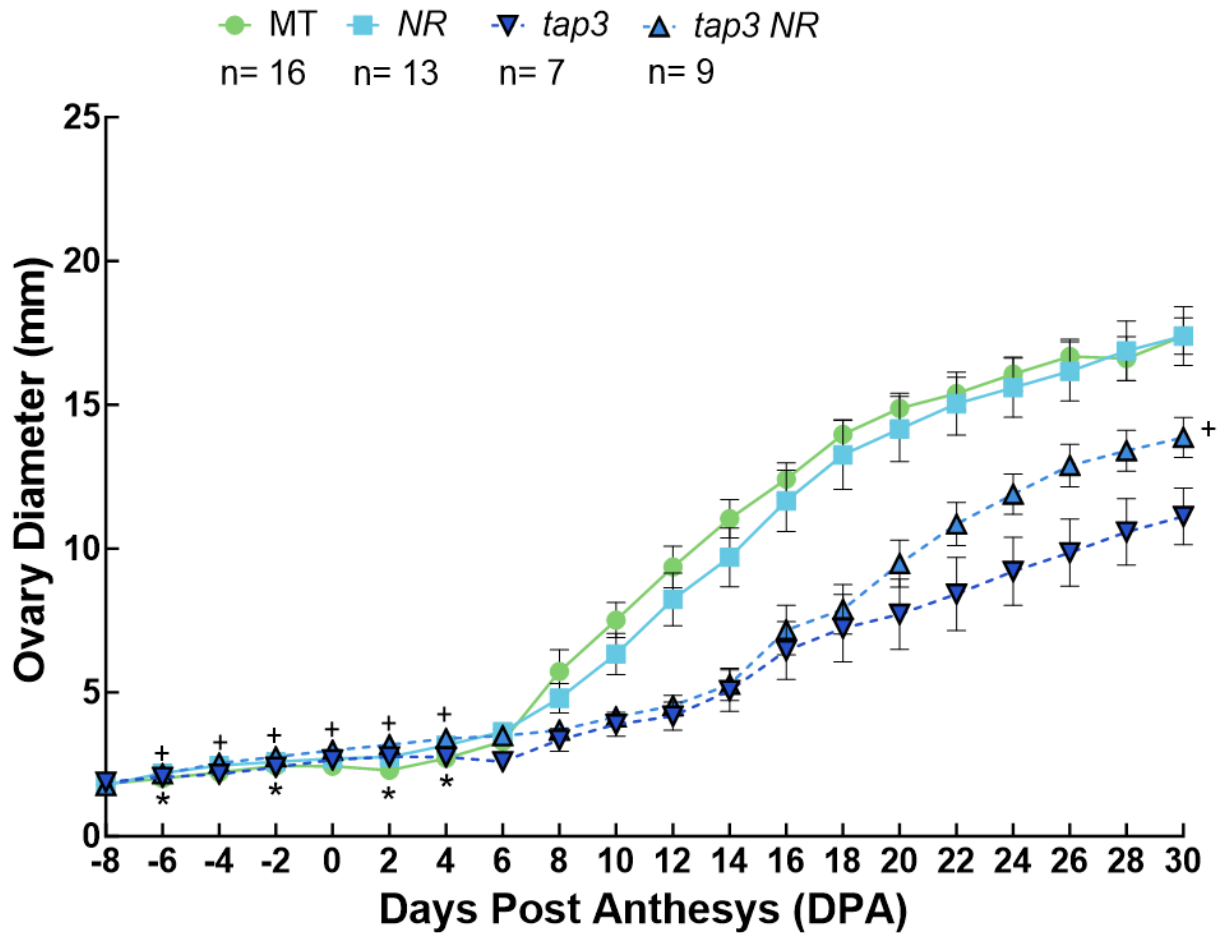
Ethylene negatively regulates fruit set and growth acting antagonistically to downstream auxin-dependent signalling pathway in early fruit development (Shinozaki et al., 2015). To assess the role of ethylene in both pollination-dependent and -independent early fruit development, we first crossed *tap3* mutant with ethylene-overproducing *epinastic* (*epi*) mutant (Fujino et al., 1988; Carvalho et al., 2011) to select double homozygotes mutant. The excess of ethylene production in the *epi* mutant pollinated ovary severely suppressed fruit growth compared to WT (Figure 12) and this ethylene effect was also observed in *tap3 epi* double, but in a lesser extent (Figure 12). Thus, this result might indicate that *TAP3*-independent parthenocarpic fruit formation may have altered sensitivity to ethylene. To test this idea, we blocked the ethylene perception in *tap3* mutant by using the ethylene insensitive *Never ripe* (*Nr*) mutation into the the same genetic background (Wilkinson et al., 1995; Lanahan et al., 1994). A single base substitution in the N-terminal coding region of the *SlETR3* gene (*NR*), homologous to ethylene receptor *ETR1* from *Arabidopsis thaliana* causes typical severe ethylene insensitive phenotypes (Carvalho et al., 2011). In the MT background, *Nr* presented indistinguishable pollinated-ovaries development to WT plants, with similar size of the ovaries (Figure 13), whereas ethylene insensitivity resulted on significantly larger parthenocarpic fruit for the *tap3 Nr* double mutant than those of the *tap3* single mutant (Figure 13), indicating that inhibited pollination-independent ovary growth in *tap3* mutant can be alleviated by suppressing ethylene perception. The fact that ethylene suppresses parthenocarpic fruit growth in *tap3* mutant suggested that ethylene metabolism might be altered during fruit development, so to test this hypothesis, we evaluated the expression pattern of the ethylene reporter *EBS:GUS*, which consists of a tandem of 5 direct repeats of the EIN3-binding site (cctcatgatCAAAGGGGGGATGCACTatttaa) from the promoter of a direct EIN3 target gene and is suitable for detection of ethylene signalling during fruit development (Stepanova et al., 2007). *EBS:GUS* lines were introgressed into the *tap3* mutant background by crossing and temporal fruit growth analysis revealed that the activity of the *EBS:GUS* ethylene reporter in *tap3* genetic background was enhanced in vascular tissue compared to WT ovaries at anthesis (0 DPA) (Figure 14). However, a dramatic decrease in the activity of this ethylene reporter was observed with further fruit development at 4 and 14 DPA in *tap3* unpollinated ovaries than those WT pollinated-ovaries, in which expanded expression of the GUS reporter driven by the

ethylene-inducible *EBS* promoter to vascular tissue and later to placenta and pericarp tissues was also observed (Figure 14). Thus, a temporal regulation of ethylene response was *TAP3*-dependent, in which in early developing *tap3* parthenocarpic fruit, accumulation of ethylene is higher and with the subsequent ethylene production was negatively correlated with the progression of early fruit development compared to pollinated WT ovaries and, therefore, it is likely that *TAP3* functional play a role in parthenocarpic fruit set through ethylene regulation metabolism.



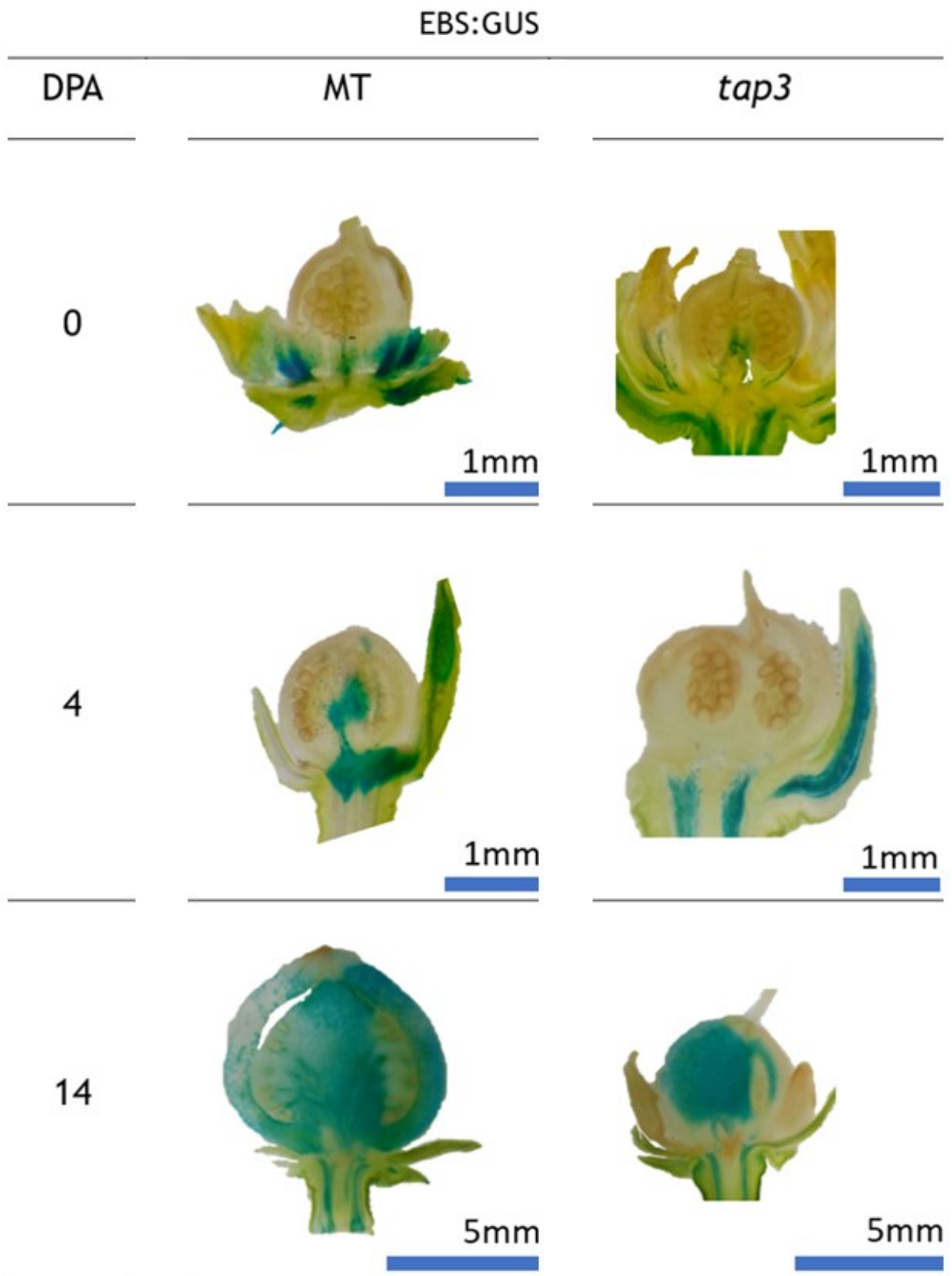
**Figure 12: Progress of early parthenocarpic fruit development in *tap3* is negatively correlated to overproduction of ethylene, while progress of pollinated fruit development after anthesis in MT is negatively correlated to ethylene overproduction.**

Changes in ovary diameter during fruit development of MT, *epi*, *tap3* and *tap3 epi*. Values are means with standard error of indicated ovaries number. \* Asterisks indicates significant differences between pollinated WT fruit versus pollinated *epi* fruit according to \* $P < 0.05$ ; Student's t test. + Crosses indicates significant differences between unpollinated parthenocarpic *tap3* fruit and unpollinated parthenocarpic *tap3 epi* fruit according to + $P < 0.05$ ; Student's t test. DPA, days post anthesis.



**Figure 13: Progress of early and late parthenocarpic fruit development in *tap3* is positively correlated to insensitivity to ethylene, while only early progress of pollinated fruit development in MT is not affected by insensitivity to ethylene.**

Changes in ovary diameter during fruit development of MT, *Nr*, *tap3* and *tap3 Nr*. Values are means with standard error of indicated ovaries number. \* Asterisks indicates significant differences between pollinated WT fruit versus *Nr* pollinated fruit according to \* $P < 0.05$ ; Student's t test. + Crosses indicates significant differences between unpollinated parthenocarpic *tap3* fruit and unpollinated parthenocarpic *tap3 Nr* fruit according to + $P < 0.05$ ; Student's t test. DPA, days post anthesis.

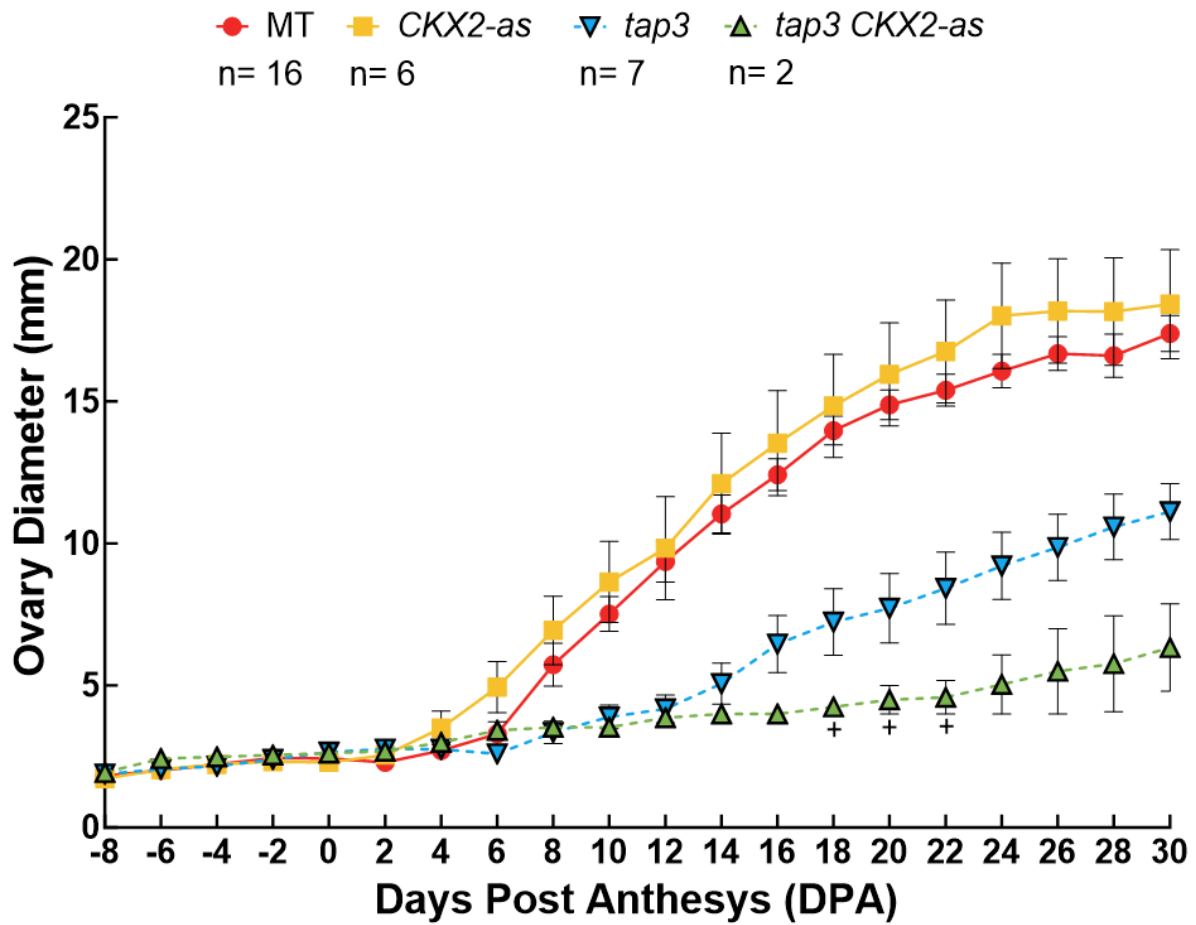


**Figure 14: *TAP3* gene expression is positively correlated to the presence of ethylene in the ovary during early fruit development.**

Slices of flowers of MT *EBS:GUS* and *tap3 EBS:GUS* at 0, 4 and 14 DPA, after staining process. Areas of floral tissue with detectable ethylene are stained with blue color. DPA, days post anthesis.

#### **4.6 *TAP3*-independent parthenocarpic fruit development in tomato is dependent on cytokinin**

Cytokinins (CKs) can induce parthenocarpy in tomato through the stimulation of cell division, rather than cell expansion (Matsuo et al., 2012; Ding et al., 2013). To investigate whether CKs is involved in tomato fruit set and development in *tap3* mutant, tomato plants overexpressing the cytokinin-deactivating gene *CYTOKININ OXYDASE 2 (CKX2-OE)* with reduced cytokinin levels (Pino et al., 2022) were crossed to generate homozygotes *tap3 CKX2-OE* plants. The lower CK levels in *CKX2-OE* plants had no significant effect in pollinated-ovaries growth compared to WT (Figure 15). By contrast, ovary growth was significantly reduced in homozygotes *tap3 CKX2-OE* plants at 18, 20 and 22 DPA compared to *tap3* mutant (Figure 15), suggesting that CKs play an important role in regulating parthenocarpic fruit growth in tomato.



**Figure 15: Progress of parthenocarpic unpollinated *tap3* fruit development is negatively correlated to increased cytokinin degradation, while pollinated MT fruit development is not.** Changes in ovary diameter during fruit development of MT, *CKX2-as*, *tap3* and *tap3 CKX2-as*. Values are means with standard error of indicated ovaries number. \* Asterisks indicates significant differences between pollinated WT fruit versus pollinated *CKX2-as* fruit according to  $*P < 0.05$ ; Student's *t* test. + Crosses indicates significant differences between unpollinated parthenocarpic *tap3* fruit and unpollinated parthenocarpic *tap3 CKX2-as* fruit according to  $+P < 0.05$ ; Student's *t* test. DPA, days post anthesis.

## 5. DISCUSSION

### 5.1 *Tomato APETALA3 (TAP3) MADS-box acts as a repressor of ovary development*

Aberrant stamen formation was associated with the ovary growth that undergo alongside with parthenocarpic fruit formation in tomato and apple (Mazzucato et al. 1998; Yao et al. 2001, Ampomah-Dwamena et al. 2002, de Martino et al. 2006, Medina et al. 2013, Pan et al. 2010, Quinet et al. 2014)). Stamen development acts as a repressor of ovary development prior to fertilization and negatively regulates fruit set and growth during early fruit development (Mazzucato et al. 1998; Vivian-Smith et al. 2001). Indeed, this notion is supported by early stamen ablation experiments resulting in seedless fruit development in tomato plants (Medina et al. 2013) and by the the induction of natural parthenocarpy in the *parthenocarpic fruit (pat)* tomato mutants (*pat; pat-2*) that presented homeotic flower formation with conversion of stamens into carpelloid organs (Mazzucato et al. 1998, Fos et al. 2000).

Duplicated orthologs of *A. majus DEFICIENS (euAP3/TAP3 and TM6)* and *GLOBOSA (SIGLO1 and SIGLO2)* lineage of MADS-box class B genes controls the identity of stamen and petal in *S. lycopersicum* (Geuten and Irish, 2010). The role of the class B of tomato MADS-box genes in regulating parthenocarpic fruit set was previously reported (Okabe et al., 2018; Molesini et al., 2020). Formation of seedless fruit in the absence of pollination and fertilization was observed in transgenic plants with floral organ conversions caused by co-suppression in of *SIGLO1* and *SIGLO2* expression (Geuten and Irish 2010). Similarly, in transgenic downregulation or loss-of-function of *Tomato APETALA3 (TAP3)* tomato plants, exhibits homeotic conversion of stamens into carpel-like organs that accompanies parthenocarpic fruit development (de Martino et al. 2006, Quinet et al. 2014; Okabe et al., 2018). These findings indicate that class B MADS-box transcription factors appeared to be related to the repression of ovary growth before fertilization in tomato. However, the molecular mechanism by which parthenocarpic fruit set and growth is controles by class B MADS-box genes has remained elusive.

In the current study, we used a new allele of the tomato *tap3* mutant as a model system to investigate the function of the stamen in pathernocapic fruit development. Transposon insertion mutation in a MADS-box transcription factor in *Tomato APETALA3 (TAP3)* gene resulted in loss-of-function class B mutant with homeotic changes in first and second whorl, with transformation of petals into sepals and stamens into carpelloid structures causing male

sterility (Maniero et al., 2016; Geuten & Irish, 2010; Figure 1 a). Lack of stamen development in the *tap3* mutant resulted in obligatory parthenocarpic fruit development with significant enhanced fruit size relative to unpollinated WT ovaries that do not develop into fruit but *tap3* ovary growth was smaller than pollinated WT fruits (Figures: 2; 3 a and b; 4). Our findings confirmed that suppression of *TAP3* activity in ovary triggers parthenocarpic fruit development. Furthermore, the fact that upon pollinization with WT pollen, *tap3* mutant sets fruits significantly earlier than pollinated WT plants (Figure 4), might indicate that ovary growth is still responsive to fertilization signal. The current molecular model of fruit set consider that before pollination and fertilization, ovary growth is blocked by a repressor complex, which is inactivated by auxin after fertilization (Pandolfini et al., 2007). Therefore, *TAP3* is a good candidate to take part of the repressor complex that act to maintains in an arrested state the ovary growth until fertilization occurs. This is consistent with *TAP3* expression pattern, with a higher expression in the ovary at anthesis and a sharp decline after fertilization in pollinated WT fruit development (Figures 5 and 6). Consistent with this interpretation, some MADS-box homeotic genes involved in determining the identity of the flowering organs, for instance, *GLOBOSA* (*SIGLO1* and *SIGLO2*) class B genes (Geuten and Irish, 2010) and *Tomato MADS-BOX29* (*TM29*) class E gene (Ampomah-Dwamena et al., 2002), also resulted in seedless fruit growth which is accompanied by male sterility when suppressed in transgenic plants. Furthermore, the silenced MADS-box *TM5/SEPALLATA3* (Pnueli et al., 1994), and the male sterile *parthenocarpic fruit* (*pat*) mutant (Mazzucato et al., 1998) also illustrated that male sporocyte development effect in parthenocarpic trait. These studies and our results support the hypothesis that an anther-derived signal might be involved in the suppression of precocious ovary development before fertilization, which is further supported in tomato, with early but not late anther ablation, resulted in parthenocarpic fruit development (Medina et al., 2013). On the other hand, either tomato mutants or transgenic overexpressing lines exhibiting both parthenocarpy and female sporocyte development, including the mutant *hydra/sporocyteless-like* (Rojas-Gracia et al., 2017; Hao et al., 2017) and mutant defective in *Tomato MADS-BOX29* (*TM29*) *SEPALLATA* family which display absence of both male and female sporocyte development, raises the intriguing relationship between parthenocarpy and male and female gametogenesis. Thus, these last examples of altered expression caused male and female sterility and production of parthenocarpic fruit and therefore cannot rule out a stamen development role affecting negatively fruit set. However, a mutation in *SLAGAMOUS-LIKE 6* (*SLAGL6*) gene that encodes a MADS-box protein of type II lineage MIKCC<sup>C</sup> caused parthenocarpic phenotype in fruit with no homotic alterations in floral organs, indicating a role for *SLAGL6* in fruit set (Takisawa et

al., 2018; Gupta et al., 2021). *SLAGL6* is transcriptionally suppressed by fertilization and play a role within the ovule integument to maintain the quiescent state of ovules and suppress post-fertilization programs until fertilization (Pattinson et al., 2015; Gupta et al., 2021). Interestingly, no direct increased in the levels of GA or auxin was found in the unfertilized mutant ovaries of the *slagl6* compared to that of wild type, but *SLARF5* downregulation in mutant ovules likely contributes to *slagl6* parthenocarpic fruit development (Gupta et al., 2021). Thus, *SLAGL6* function highlight the importance of the ovule integument in the process of fruit set (Klap et al., 2016; Gupta et al., 2021). We found a weak temporal *TAP3* expression in young developing seeds in comparison to columella and pericarpic cells in pollinated-WT developing ovaries (Figure 6) but WT pollen fertilization in *tap3* mutant background led to earlier fruit set relative to pollinated WT ovaries (Figure 4), suggesting that fertilization-triggered fruit initiation in *tap3* mutant is either normal or enhanced upon ovule fertilization, rather than defective. Therefore, we cannot exclude the possibility that *TAP3* plays a role in ovule integument in the process of fruit set and, hence, it would be interesting to investigate whether *TAP3*-independent parthenocarpic fruit set is associated with ovule integument. These observations and our results suggests that, at least, the stamen development acts as a repressor of ovary development (Vivian-Smith et al. 2001, Medina et al. 2013) and, therefore, *TAP3* homeotic *MADS-box* gene is implicated in fruit growth and development, being added to the pool of developmental protein repressors that act in the ovary in the absence of fertilization.

## 5.2 Parthenocarpic fruit set in *tap3* mutant is mediated by auxin affecting GA

It has been well established that the phytohormones GA and auxin play key roles in tomato fruit set and exogenous application of these hormones to unfertilized tomato ovaries or mutations affecting signaling genes can induce parthenocarpy in fleshy fruits (Gillaspy *et al.*, 1993; Wang et al., 2005; Serrani et al., 2008; De Jong et al., 2011). Recently study have shown that seedless fruit development in loss-of-function *tap3* tomato mutant was caused by up-regulation of GA biosynthesis genes, i.e *SIGA20ox1*, *SIGA20ox2*, and *SIGA20ox3*, and concomitant reduced expression of the GA-inactivating gene *SIGA2ox1* resulting in increased GA levels. According to the authors, the parthenocarpic fruit in this *tap3* mutant allele presented increased GA levels to pollinated WT ovaries, but exhibited reduced fruit weight and size (Okabe et al., 2019). Our results show that the small, elongated parthenocarpic fruits of the *tap3* mutant allele presented here are similar to plants treated with GA (Figures 2 and 3), which, in pericarp, shows increased cell elongation and reduced cell division (Martí et al., 2007, Serrani

et al., 2007). This is in agreement with the GA-dependent metabolism involved in the formation of *tap3* tomato parthenocarpic fruits. However, using hormone treatment, and transgenic plants that enhanced GA biosynthesis, we have shown *tap3* mutant ovary growth is responsive to increased GA levels, most likely because of higher levels in the GA content (Figure 7) and biosynthesis (Figure 8) in the *tap3* mutant ovary been additively affected ovary growth (Figures 7 and 8), similar to plants with altered sensibility to GA (Martí et al., 2007). Activation GA metabolism in parthenocarpic tomato fruits was previously shown to be partially an auxin-mediated response (Serrani et al., 2008). This was evidenced by the accumulation of transcripts of auxin signaling genes which are induced by pollination (Serrani et al., 2008). However, these genes were not affected in fruits treated with GA (Vriezen et al., 2008, Serrani et al., 2008). The fact that *tap3* fruit growth is induced by WT pollen fertilization earlier than in WT pollinated ovaries (Figure 4), suggested that auxin-signal derived from fertilization increases within the *tap3* ovary, promoting parthenocarpic fruit set earlier than in pollinated WT ovaries. Thus, alteration of auxin content, sensitivity, perception, transport, or signaling, might be related in seedless fruit production in *tap3* mutant. Indeed, spatial and temporal auxin accumulation was substantially reduced in *tap3* mutant at anthesis, and followed by reduced basipetally auxin transport to the vascular tissue towards to the pedicel (Figure 9), indicating auxins transported from the ovary might be reduced in the absence of function *TAP3*. This conclusion is further strengthened by the observation that *dgt* mutation, which is known to cause blockage of basipetal polar transport of auxin, leading to additional accumulation of endogenous auxin in ovaries, caused no significant effect on parthenocarpic fruit-set and growth in double mutant *tap3 dgt* (Figure 10), indicating that the auxin transport in *tap3* background might be already altered. Together, these results suggest that *TAP3*-independent induced parthenocarpy was the result of auxin accumulation. Supporting this notion, in tomato, application of *N*-1-naphthylphthalamic acid (NPA; an inhibitor of auxin transport) to unpollinated ovaries induced parthenocarpic fruit-set and was associated with increased auxin levels, resulting in higher content of GA (Serrani et al., 2010). This NPA-effect was related to the inhibition of auxin transport from the ovary causing parthenocarpic fruit growth (Serrani et al., 2010). By contrast, pollinated ovaries growth was inhibited by NPA treatment, due to greater than optimal auxin accumulation levels with twice IAA level in unpollinated NPA-treated ovaries than that of untreated pollinated ovaries (Serrani et al., 2010). Taken together, these findings and our results suggest that an optimal auxin level is necessary to induce *tap3* parthenocarpic fruit growth. From this, we infer that auxin signaling pathway could be improved in *tap3* mutant in order to improve parthenocarpic fruit growth. In accordance with

this, unpollinated ovaries in *tap3 ARF4*-as plants enhanced fruit growth than those of the *tap3* mutant (Figure 11), indicating that the relief of SIARF4 repressor effect may provide a significant contribution to regulate the growth of the fruit organ. These results raises the importance of auxin homeostasis in the unpollinated ovary for appropriate control of growth and suggests that reducing the amount of auxin transported basipetally to the pedicel found in the *tap3* mutant induces parthenocarpic fruit growth of unpollinated ovaries.

### 5.3 Temporal regulation by multiple hormones affects *TAP3*-independent parthenocarpic fruit growth

Parthenocarpic growth induced by *TAP3*-independent function was inhibited by excess of ethylene (Figure 12). Moreover, the inhibitory ethylene role on in unpollinated *tap3* ovary growth was alleviated ethylene-insensitive *Nr/Sletr3* mutation, resulted in larger parthenocarpic fruit development than *tap3* single mutant (Figure 13), indicating that ethylene also influences *TAP3*-independent unpollinated fruit development. An ethylene-independent fruit set is required for increased GA metabolism in parthenocarpic ovary growth. Ethylene produced in the pistils was shown to repress GA metabolism, whereas reducing the repression from ethylene, enhances metabolism of GA, inducing parthenocarpy because then, the ovary accumulates GA (Shinozaki et al., 2015). Consistent with this, we found that the ethylene in *tap3* mutant ovary during anthesis decreased according to the progress of early fruit development (Figure 14), indicating that temporal rate of ethylene production in *tap3* differs from the found in WT pistils and, therefore, may contribute to ovary expansion in the mutant background. The *tap3* parthenocarpic fruit growth phenotype resemble the fruit set independent from pollination in the mutant hypersensitive to auxin, which is called *iaa9-3*. In *iaa9-3* mutant, ethylene production is suppressed (Shinozaki et al., 2015). A loss-of-function mutation in, or downregulation of, tomato *INDOLE 3 ACETIC ACID 9 (IAA9)*, negative modulator of auxin responses and member of *AUX/IAA* gene family, results in parthenocarpic fruit (Wang et al., 2009), suggesting that auxin negatively regulates ethylene production during early fruit development (Shinozaki et al., 2015). This is consistent with the idea of auxin-dependent phenotype in ovaries of *tap3* mutant affecting ethylene accumulation, indicating that ethylene suppression participates in the parthenocarpic fruit growth in *tap3* mutant. These results suggests that ethylene is modulated by auxin, meanwhile it does modulates GA which, in turn, induces *tap3* parthecarpic fruit growth.

Additionally, our results show that CKs play important roles in inducing parthenocarpy through the stimulation of fruit growth in *tap3* mutant (Figure 15). As CKs regulate cell division, an increase in bioactive CKs levels associated with the beginning of the ovary of the ovary in unpollinated flowers during ovary maturation. A second increase of CKs is correlated to cell division stimulation in the fruit growth during phase II (Matsuo et al, 2012). By suppressing the CKs levels in *tap3* mutant background by overexpressing the cytokinin-deactivating gene *CYTOKININ OXYDASE 2 (CKX2-OE)* in the ovaries, we found a reduced parthenocarpic fruit growth, indicating that regulation of CK catabolism is required during *TAP3*-independent ovary development for proper growth. This also suggests that CKs can be part of a hierarchical signaling which crosstalks to auxin and gibberellins, as suggested by Ding et. al (2013), or antagonistically with ethylene during parthenocarpic fruit growth in the absence of functional *TAP3* MADS-box gene. Taken together, our results suggest that the effects of multiple phytohormones on the regulation of parthenocarpic fruit set and growth by suppressing *TAP3* function in the tomato ovary.

## 6. CONCLUSION

This study provides insights into the complex hormone regulation of parthenocarpic fruit set and growth in tomato. We report that *TAP3* MADS-box gene is negatively associated with tomato fruit set and growth, a phenomenon that results at least in part through aberrant stamen development and probably through the transcriptional regulation of mediated by *TAP3* affecting hormone homeostases. *TAP3* homeotic *MADS-box* gene is a fruit growth regulator, with an expression pattern similar to ovary growth repressors. Other studies have shown that the mechanisms underlying parthenocarpic fruit set and growth is related with auxin, CK and ethylene affecting GA metabolism (Serrani et al., 2008; Ding et al., 2013; Shinozaki et al., 2015). The inhibitory *TAP3* effect in ovary growth is mediated by auxin, in which downregulation in *TAP3* is required for auxin accumulation in the ovary mediating the activation of GA metabolism and fruit set. An active cell division occurs through increased auxin metabolism and in turn, might suppress the ethylene production, thereby reinforce cell expansion within phase III, via further promotion of GA metabolism. Whether CKs are also an integral part of the regulatory *TAP3* signalling deserves further detailed analysis, but the results of this study confirmed that parthenocarpy is associated with CKs metabolism during fruit set.

## 7. REFERENCES

- ABRAMOFF, M.D., MAGALHAES, P.J., RAM, S.J. 2004.** Image Processing with ImageJ. *Biophotonics International*. 2004, Vol. 11, 7, pp. 36-42.
- AMPOMAH-DWAMENA, C., MORRIS, B.A., SUTHERLAND, P., VEIT, B., YAO, J.-L. 2002.** Down-Regulation of TM29, a tomato SEPALLATA homolog, causes parthenocarpic fruit development and floral reversion. *Plant Physiol*. 2002, Vol. 130, pp. 605–617.
- ARIIZUMI, T., SHINOZAKI, Y., EZURA, H. 2013.** Genes that influence yield in tomato. *Breed. Sci*. 2013, Vol. 63, pp. 3–13.
- AUDRAN-DELALANDE, C., BASSA, C., MILA, I., REGAD, F., ZOUINE, M., BOUZAYEN, M. 2012.** Genome-wide identification, functional analysis and expression profiling of the Aux/IAA gene family in tomato. *Plant Cell Physiol*. 2012, Vol. 53, pp. 659–672.
- BALAGUERA-LÓPEZ, H. E., FISCHER, G., MAGNITSKIY, S. 2020.** Seed-fruit Relationships in Fleshy Fruit Species: Role of Hormones. A Review. *Revista Colombiana de Ciencias Hortícolas*. 2020, Vol. 1, pp. 90-103.
- BANGERTH, F., HO, L.C. 1984.** Fruit Position and Fruit Set Sequence in a Truss as Factors Determining Final Size of Tomato Fruits. *Annals of Botany*. 1984, Vol. 53, 3, pp. 315–320.
- BARNABÁS, B., JÄGER, K., FEHÉR, A. 2008.** The effect of drought and heat stress on reproductive processes in cereals. *Plant Cell Environ*. 2008, Vol. 31, pp. 11-38.
- BECKER, A., THEISSEN, G. 2003.** The major clades of MADS-box genes and their role in the development and evolution of flowering plants. *Mol. Phylogenet. Evol*. 2003, Vol. 29, pp. 464–489.
- BERTIN, N. 2005.** Analysis of the tomato fruit growth response to temperature and plant fruit load in relation to cell division, cell expansion and DNA endoreduplication. *Ann. Bot*. 2005, Vol. 95, pp. 439–447.
- BERTIN, N., GARY, C., TCHAMITCHIAN, M., VAISSIERE, B.E. 1998.** Influence of cultivar, fruit position and seed content on tomato fruit weight during a crop cycle under low and high competition for assimilates. *The Journal of Horticultural Science and Biotechnology*. 1998, Vol. 73, 4, pp. 541-548.
- BOHNER, J., BANGERTH, F. 1988.** Effects of fruit set sequence and defoliation on cell number, cell size and hormone levels of tomato fruits (*Lycopersicon esculentum* Mill.) within a truss. *Plant Growth Regul*. 1988, Vol. 7, pp. 141–155.
- BOHNER, J., HEDDEN, P., BORAHABER, E., BANGERTH, F. 1988.** Identification and quantitation of gibberellins in fruits of *Lycopersicon esculentum*, and their relationship to fruit size in *L. esculentum* and *L. pimpinellifolium*. *Physiol. Plant*. 1988, Vol. 73, pp. 348–353.

- BOWMAN, J. L., SMYTH, D. R., MEYEROWITZ, E. M., 1989.** Genes directing flower development in *Arabidopsis*. *Plant Cell*. 1989, Vol. 1, pp. 37–52.
- BOYER, J.S., MCLAUGHLIN, J.E. 2007.** Functional reversion to identify controlling genes in multigenic responses: analysis of floral abortion. *J. Exp. Bot.* 2007, Vol. 58, pp. 267-277.
- BUSI, M.V., BUSTAMANTE, C., D'ANGELO, C., HIDALGO-CUEVAS, M., BOGGIO, S.B., VALLE, E.M., ZABALETA, E. 2003.** MADS-box genes expressed during tomato seed and fruit development. *Plant Mol. Biol.* 2003, Vol. 52, 4.
- CARRASCO, L., PAPEŞ, M., LOCHNER, E.N., RUIZ, B.C., WILLIAMS, A.G., WIGGINS, G.J. 2021.** Potential regional declines in species richness of tomato pollinators in North America under climate change. *Ecological Applications*. 2021, Vol. 21, 3.
- CARVALHEIRO, L.G., BARTOMEUS, I., ROLLIN, O., TIMÓTEO, S, TINOCO, F.L. 2021.** The role of soils on pollination and seed dispersal. *Philosophical Transactions of Royal Society B*. 2021, Vol. 376, 1834.
- CARVALHO, R.F., CAMPOS, M.L., PINO, L.E., CRESTANA, S.L., ZSÖGÖN, A., LIMA, J.E., BENEDITO, V.A., LÁZARO, E.P. 2011.** Convergence of developmental mutants into a single tomato model system: 'Micro-Tom' as an effective toolkit for plant development research. *Plant Methods*. 2011, Vol. 7, 18.
- CAUSIER, B., SCHWARZ-SOMMER, Z., DAVIES, B. 2010.** Floral organ identity: 20 years of ABCs. *Semin. Cell Dev. Biol.* 2010, Vol. 21, pp. 73–79.
- CHENICLET, C., RONG, W.Y., CAUSSE, M., Frangne, N., Bolling, L., Carde, JP., Renaudin, JP. 2005.** Cell expansion and endoreduplication show a large genetic variability in pericarp and contribute strongly to tomato fruit growth. *Plant Physiology*. 2005, Vol. 139, pp. 1984-1994.
- COEN, E., MEYEROWITZ, E. 1991.** The war of the whorls: genetic interactions controlling flower development. *Nature*. 1991, Vol. 353, pp. 31–37.
- COENEN, C, CHRISTIAN, M, LÜTHEN, H, LOMAX, TL. 2003.** Cytokinin inhibits a subset of diageotropica-dependent primary auxin responses in tomato. *Plant Physiol*. 2003, Vol. 131, pp. 1692–1704.
- D'AGOSTINO, I.B., DERUÈRE, J., KIEBER, J.J. 2000.** Characterization of the response of the *Arabidopsis* response regulator gene family to Cytokinin. *Plant Physiol*. 2000, Vol. 124, 4, pp. 1706–17.
- DAYAN, J., et al. 2012.** Leaf induced gibberellin signaling is essential for internode elongation, cambial activity, and fiber differentiation in tobacco stems. *The Plant Cell*. 2012, Vol. 24, pp. 66-79.
- DE JONG, M., WOLTERS-ARTS, M., GARCIA-MARTINEZ, J.L., MARIANI, C., VRIEZEN, W.H. 2011.** The *Solanum lycopersicum* AUXIN RESPONSE FACTOR 7

(SIARF7) mediates cross-talk between auxin and gibberellin signalling during tomato fruit set and development. *J. Exp. Bot.* 2011, Vol. 62, pp. 617–626.

**DE, JONG, M., MARIANI, C., VRIEZEN, W. 2009.** The role of auxin and gibberellin in tomato fruit set. *Journal of experimental botany.* 2009, Vol. 60, pp. 1523–1532.

**DE, MARTINO, G., PAN, I., EMMANUEL, E., LEVY, A., IRISH, V.F. 2006.** Functional analyses of two tomato APETALA3 genes demonstrate diversification in their roles in regulating floral development. *Plant Cell.* 2006, Vol. 18, pp. 1833–1845.

**DHARMASIRI, N., DHARMASIRI, S., ESTELLE, M. 2005.** The F-box protein TIR1 is an auxin receptor. *Nature.* 2005, Vol. 435, pp. 441–445.

**DING, J., CHEN, B., XIA, X., MAO, W., SHI, K., ZHOU, Y., YU, J. 2013.** Cytokinin-Induced Parthenocarpic Fruit Development in Tomato Is Partly Dependent on Enhanced Gibberellin and Auxin Biosynthesis. *PLoS ONE.* 2013, Vol. 8, 7.

**DORCEY, E., URBEZ, C., BLAZQUEZ, M.A., CARBONELL, J., PEREZ-AMADOR, M.A. 2009.** Fertilization-dependent auxin response in ovules triggers fruit development through the modulation of gibberellin metabolism in Arabidopsis. *Plant J.* 2009, Vol. 58, pp. 318–332.

**FERNANDEZ-POZO, N., MENDA, N., EDWARDS, J.D., SAHA, S., TECLE, I.Y., STRICKLER, S.R., BOMBARELY, A., FISHER-YORK, T., PUJAR, A., FOERSTER, H., YAN, A., MUELLER, L.A. 2015.** The Sol Genomics Network (SGN) âfrom from genotype to phenotype to breeding. *Nucleic Acids Res.* 2015, Vol. 43, D10, pp. 36-41.

**FERNANDEZ-POZO, N., ZHENG, Y., SNYDER, S.I., NICOLAS, P., SHINOZAKI, Y., FEI, Z., CATALA, C., GIOVANNONI, J.J., ROSE, J.K.C., MUELLER, L.A.** “Solyc04g081000.2. 2023. Solyc04g081000.2. *Tea Sol Genomics.* [Online] 2023. [Cited: 06 06 2023.] [https://tea.solgenomics.net/expression\\_viewer/output](https://tea.solgenomics.net/expression_viewer/output).

**FERNANDEZ-POZO, N., ZHENG, Y., SNYDER, S.I., NICOLAS, P., SHINOZAKI, Y., FEI, Z., CATALA, C., GIOVANNONI, J.J., ROSE, J.K.C., MUELLER, L.A. 2017.** The Tomato Expression Atlas. *Bioinformatics.* 2017, Vol. 1, 33, pp. 2397-2398.

**FOS, M., NUEZ, F., GARCIA-MARTINEZ, J.L. 2000.** The gene pat-2, which induces natural parthenocarpy, alters the gibberellin content in unpollinated tomato ovaries. *Plant Physiol.* 2000, Vol. 122, pp. 471–480.

**FOS, M., PROANO, K., NUEZ, F., GARCIA-MARTINEZ, J.L. 2001.** Role of gibberellins in parthenocarpic fruit development induced by the genetic system pat-3/pat-4 in tomato. *Physiol. Plant.* 2001, Vol. 111, pp. 545–550.

**FRANCESCHINELLI, E.V., ELIAS, M.A.S., BERGAMINI, L.L., SILVA NETO, C.M., SUJII, E.R., 2017.** Influence of landscape context on the abundance of native bee pollinators in tomato crops in Central Brazil. *Journal of Insect Conservation.* 2017, Vol. 21, pp. 715–726.

**FRARY, A., NESBITT, T.C., GRANDILLO, S., KNAAP, E., CONG, B., LIU, J., MELLER, J., ELBER, R., ALPERT, K.B., TANKSLEY, S.D. 2000.** fw2.2: a quantitative trait locus key to the evolution of tomato fruit size. *Science*. 2000, Vol. 289, pp. 85–88.

**FU, F.Q., MAO, W.H., SHI, K., ZHOU, Y.H., ASAMI, T., YU, J.Q. 2008.** A role of brassinosteroids in early fruit development in cucumber. *J. Exp. Bot.* 2008, Vol. 59, pp. 2299–2308.

**FUJINO, D.W., NISSEN, S.J., JONES, A.D., BURGER, D.W., BRADFORD, K.J. 1988.** Quantification of indole-3-acetic acid in dark-grown seedlings of the diageotropica, epinastic mutants of tomato (*Lycopersicon esculentum* Mill.). *Plant Physiol.* 1988, Vol. 88, pp. 780–784.

**GARCÍA-HURTADO, N., CARRERA, E., RUIZ-RIVERO, O., LÓPEZ-GRESA, M.P., HEDDEN, P., GONG, F., GARCÍA-MARTÍNEZ, J.L. 2012.** The characterization of transgenic tomato overexpressing gibberellin 20-oxidase reveals induction of parthenocarpic fruit growth, higher yield, and alteration of the gibberellin biosynthetic pathway. *J. Exp. Bot.* 2012, Vol. 63, pp. 5803–5813.

**GEUTEN, K., IRISH, V. 2010.** Hidden variability of floral homeotic B genes in solanaceae provides a molecular basis for the evolution of novel functions. *Plant Cell*. 2010, Vol. 22, pp. 2562–2578.

**GILLASPY, G., BEN-DAVID, H., GRUISSEM, W. 1993.** Fruits: a developmental perspective. *Plant Cell*. 1993, Vol. 5, pp. 1439–1451.

**GIMENEZ, E., CASTAÑEDA, L., PINEDA, B., PAN, I.L., MORENO, V., ANGOSTO, T., LOZANO, R. 2016.** Tomato *Agamous1* and *Arlequin/Tomato Agamous-Like1* MADS-box genes have redundant and divergent functions required for tomato reproductive development. *Plant Mol. Biol.* 2016, Vol. 91, pp. 513–531.

**GIMENEZ, E., PINEDA, B., CAPEL, J., ANTÓN, M.T., ATARÉS, A., PEREZ-MARTIN, F., GARCIA-SOGO, B., ANGOSTO, T., MORENO, V., LOZANO, R. 2010.** Functional analysis of the *Arlequin* mutant corroborates the essential role of the *ARLEQUIN/TAGL1* gene during reproductive development of tomato. *Plos One*. 2010, Vol. 5e, p. 14427.

**GIORNO, F., WOLTERS-ARTS, M., MARIANI, C., RIEU, I., 2013.** Ensuring Reproduction at High Temperatures: The Heat Stress Response during Anther and Pollen Development. *Plants*. 2013, Vol. 2, 3, pp. 489–506.

**GIOVANNONI, J.J. 2004.** Genetic regulation of fruit development and ripening. *Plant Cell*. 2004, Vol. 16, pp. 170–180.

**GOETZ, M., HOOPER, L.C. JOHNSON, S.D. RODRIGUES, J.C.M. VIVIAN-SMITH, A, KOLTUNOW, A.M. 2007.** Expression of aberrant forms of *AUXIN RESPONSE FACTOR8* stimulates parthenocarpy in *Arabidopsis* and tomato. *Plant Physiol.* 2007, Vol. 145, pp. 351–366.

- GÓMEZ, P., JAMILENA, M., CAPEL, J., ZURITA, S., ANGOSTO, T., LOZANO, R. 1999.** Stamenless, a tomato mutant with homeotic conversions in petals and stamens. *Planta*. 1999, Vol. 209, pp. 172–179.
- GORGUET, B., VAN HEUSDEN, A.W., LINDHOUT, P. 2005.** Parthenocarpic fruit development in tomato. *Plant Biol (Stuttg)*. 2005, Vol. 7, pp. 131–139.
- GROEN, S.C., JIANG, S., MURPHY, A.M., NIK, J. 2016.** Virus Infection of Plants Alters Pollinator Preference: A Payback for Susceptible Hosts? 2016, Vol. 12, 8.
- GROOT, S.P.C., BRUINSMA, J., KARSSSEN, C.M. 1987.** The role of endogenous gibberellin in seed and fruit development of tomato: studies with a gibberellin-deficient mutant. *Physiol Plant*. 1987, Vol. 71, pp. 184–190.
- GUILFOYLE, T.J., HAGEN, G. 2007.** Auxin response factors. *Curr. Opin. Plant Biol*. 2007, Vol. 10, pp. 453–460.
- GUPTA, S.K, BARG, R., ARAZI, T. 2017.** Tomato agamous-like 6 parthenocarpy is facilitated by ovule integument reprogramming involving the growth regulator KLUH. *Plant Physiol*. 2017, Vol. 185, pp. 969-984.
- HAO, S., ARIIZUMI, T., EZURA, H.** Sexual sterility is essential for both male and female gametogenesis in tomato. *Plant Cell Physiol*. Vol. 58, pp. 22–34.
- HEDDEN, P., GONG, F., GARCÍA-MARTÍNEZ, J.L. 2012.** The characterization of transgenic tomato overexpressing gibberellin 20-oxidase reveals induction of parthenocarpic fruit growth, higher yield, and alteration of the gibberellin biosynthetic pathway. *J. Exp. Bot*. 2012, Vol. 63, pp. 5803-13.
- HO, L. & HEWITT, J. 1986.** *Fruit development. The Tomato Crop*. New York : Champman and Hall, 1986. pp. 201–239.
- HONMA, T., GOTO, K. 2001.** Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. *Nature*. 2001, Vol. 409, pp. 525–529.
- HU, J., ISRAELI, A., ORI, N., SUN, T.P. 2018.** The interaction between DELLA and ARF/IAA mediates crosstalk between gibberellin and auxin signaling to control fruit initiation in tomato. *Plant Cell*. 2018, Vol. 30, pp. 1710–1728.
- IRISH, V. F., SUSSEX, I. M. 1990.** Function of the *apetala-1* gene during Arabidopsis floral development. *Plant Cell*. 1990, Vol. 2, pp. 741–753.
- IVANCHENKO, M.G., ZHU, J., WANG, B., MEDVECKÁ, E., DU, Y., AZZARELLO, E., MANCUSO, S., MEGRAW, M., FILICHKIN, S., DUBROVSKY, J.G., FRIML, J., GEISLER, M. 2015.** The cyclophilin A *DIAGEOTROPICA* gene affects auxin transport in both root and shoot to control lateral root formation. *Development*. 2015, Vol. 142, pp. 712–721.

- JEFFERSON, R.A., KAVANAGH, T.A., BEVAN, M.W. 1987.** GUS fusions: B-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *The EMBO Journal*. 1987, Vol. 6, 13, pp. 1901-3907.
- JONES, B., FRASSE, P., OLMOS, E., ZEGZOUTI, H., LI, Z.G., LATCHÉ, A., PECH, J.C., BOUZAYEN, M. 2002.** Down-regulation of DR12, an auxin-response-factor homolog, in the tomato results in a pleiotropic phenotype including dark green and blotchy ripening fruit. *Plant J*. 2002, Vol. 32, pp. 603–613.
- JUST, D., JORLY, J., GÉVAUDANT, F., MOING, A., CHEVALIER, C., LEMAIRE-CHAMLEY, M., ROTHAN, C., FERNANDEZ, L. 2017.** Identification of two new mechanisms that regulate fruit growth by cell expansion in tomato. *Front. Plant Sci*. 2017, Vol. 8, pp. 1–15.
- KHAN, U.M., SEVINDIK, M., ZARRABI, A., NAMI, M., OZDEMIR, B., KAPLAN, D.N., SELAMOGLU, Z., HASAN, M., KUMAR, M., ALSHEHRI, M.M., SHARIFI-RAD, J. 2021.** Lycopene: Food Sources, Biological Activities, and Human Health Benefits. *Oxid Med Cell Longev*. 2021, Vol. 19.
- KLAP, C., YESHAYAHOU, E., BOLGER, A.M., ARAZI, T., GUPTA, S.K., SHABTAI, S., USADEL, B., SALTS, Y., BARG, R. 2016.** Tomato facultative parthenocarpy results from SIAGAMOUS-LIKE 6 loss of function. *Plant Biotechnology Journal*. 2016, Vol. 174, 2, pp. 166-178.
- KOSHIOKA, M., NISHIJIMA, T., YAMAZAKI, H., LIU, Y., NONAKA, M., MANDER, L.N. 1994.** Analysis of gibberellins in growing fruits of *Lycopersicon esculentum* after pollination or treatment with 4-chlorophenoxyacetic acid. *J. Hortic. Sci*. 1994, Vol. 69, pp. 171–179.
- KRAMER, E.M., JARAMILLO, M.A., STILIO, D.V.S. 2004.** Patterns of gene duplication and functional evolution during the diversification of the AGAMOUS subfamily of MADS box genes in angiosperms. *Genetics*. 2004, Vol. 166, pp. 1011–1023.
- KUMARI, S., ROY, S., SINGH, P., SINGLA-PAREEK, S.L., PAREEK, A. 2013.** Cyclophilins: proteins in search of function. *Plant Signal Behav*. 2013, Vol. 8e22734.
- LANAHAN, M.B., YEN, H.C., GIOVANNONI, J.J., KLEE H.J. 1994.** The Never ripe mutation blocks ethylene perception in tomato. *Plant Cell*. 1994, Vol. 6, pp. 521-530.
- LI, Z., Palmer, W.M., Martin, A.P., Wang, R., Rainsford, F., Jin, Y., Patrick, J.W., Yang, Y., Ruan, YL. 2012.** High invertase activity in tomato reproductive organs correlates with enhanced sucrose import into, and heat tolerance of, young fruit. *Journal of Experimental Botany*. 2012, Vol. 63, 3, pp. 1155–1166.
- LIAO, CY, SMET, W, BRUNOUD, G, YOSHIDA, S, VERNOUX, T, WEIJERS, D. 2015.** Reporters for sensitive and quantitative measurement of auxin response. *Nat Methods*. 2015, Vol. 12, 3, pp. 207-210.

**LIU, W., LIU, K., CHEN, D., ZHANG, Z., LI, B., EL-MOGY, M.M., TIAN, S., CHEN, T. 2022.** *Solanum lycopersicum*, a Model Plant for the Studies in Developmental Biology, Stress Biology and Food Science. 2022, Vol. 16, 2402.

**MA, H., 2005.** Molecular genetic analyses of microsporogenesis and microgametogenesis in flowering plants. *Annu. Rev. Plant Biol.* 2005. Vol. 56, pp. 393–434.

**MANIERO, R.A. 2016.** Caracterização molecular e regulação hormonal de um mutante macho estéril em tomateiro (*Solanum lycopersicum* L. cv Micro-Tom) envolvendo a transformação de estames em pistilos e pétalas em sépalas. *capstone project presented to obtain the bachelor degree in Agronomy*. University of São Paulo, “Luiz de Queiroz” College of Agriculture : s.n., 2016.

**MAPELLI, S., FROVA, C., TORTI, G. 1979.** Relationship between set, development and activities of growth regulators in tomato fruit. *Plant Cell Physiol.* 1979, Vol. 19, pp. 1281–1288.

**MARTÍ, C., ORZÁEZ, D., ELLUL, P., MORENO, V., CARBONELL, J., GRANELL, A. 2007.** Silencing of DELLA induces facultative parthenocarpy in tomato fruits. *Plant J.* 2007, Vol. 52, pp. 865-876.

**MATSUO, S., KIKUCHI, K., FUKUDA, M., HONDA, I., IMANISHI, S. 2012.** Roles and regulation of cytokinins in tomato fruit development. *J. Exp. Bot.* 2012, Vol. 63, pp. 5569–5579.

**MATSUO, S., MIYATAKE, K., ENDO, M., URASHIMO, S., KAWANISHI, T., NEGORO, S., SHIMAKOSHI, S., FUKUOKA, H. 2020.** Loss of function of the Pad-1 aminotransferase gene, which is involved in auxin homeostasis, induces parthenocarpy in Solanaceae plants. *Proc. Natl. Acad. Sci.* 2020, Vol. 117, pp. 12784-12790.

**MAZZUCATO, A., CELLINI F., BOUZAYEN M., ZOUINE, M., MILA, I., MINOIA, S., PETROZZA, A., PICARELLA, M.E., RUIU, F., CARRIERO, F. 2015.** A TILLING allele of the tomato Aux/IAA9 gene offers new insights into fruit set mechanisms and perspectives for breeding seedless tomatoes. *Mol. Breed.* 2015, Vol. 35, pp. 1–15.

**MAZZUCATO, A., OLIMPIERI, I., SILIGATO, F., PICARELLA, M.E., SORESSI, G.P. 2008.** Characterization of genes controlling stamen identity and development in a parthenocarpic tomato mutant indicates a role for the DEFICIENS ortholog in the control of fruit set. *Physiol.* 2008, Vol. 132, pp. 526–537.

**MAZZUCATO, A., TADDEI, A.R., SORESSI, G.P. 1998.** The parthenocarpic fruit (pat) mutant of tomato (*Lycopersicon esculentum* Mill.) sets seed- less fruits and has aberrant anther and ovule development. *Development.* 1998, Vol. 125, pp. 107–114.

**MCATEE, P., KARIM, S., SCHAFFER, R., DAVID, K. 4.** A dynamic interplay between phytohormones is required for fruit development, maturation, and ripening. *Front. Plant Sci.* 4, Vol. 79.

**MECO, V., EGEE, I., ALBALADEJO, I., CAMPOS, J.F., MORALES, B., ORTÍZ-ATIENZA, A., CAPEL, C., ANGOSTO, T., BOLARÍN, M.C., FLORES, F.B. 2019.**

Identification and characterisation of the tomato parthenocarpic mutant high fruit set under stress (hfs) exhibiting high productivity under heat and salt stress. *Annals of Applied Biology*. 2019, Vol. 15, 5, pp. 634-647.

**MEDINA, M., ROQUE, E., PINEDA, B., CAÑAS, L., RODRIGUEZ-CONCEPCIÓN, M., BELTRÁN, J.P., GÓMEZ-MENA, C.** Early anther ablation triggers parthenocarpic fruit development in tomato. *Plant Biotechnol.* Vol. 11, pp. 770–779.

**MOLESINI, B, DUSI, V, PENNISI, F, PANDOLFINI, T. 2020.** How Hormones and MADS-Box Transcription Factors Are Involved in Controlling Fruit Set and Parthenocarpy in Tomato. *Genes (Basel)*. 2020, Vol. 30, 11(12), p. 1441.

**MOREIRA, J.D.R., ROSA, B.L., LIRA, B.S., LIMA, J.E., CORREIA, L.N.F., OTONI, W.C., FIGUEIRA, A., FRESCHI, L., SAKAMOTO, T., PERES, L.E.P., ROSSI, M., ZSÖGÖN. 2022.** A. Auxin-driven ecophysiological diversification of leaves in domesticated tomato. *Plant Physiol.* 2022, Vol. 190, 1, pp. 113-126.

**MUBAROK S., JADID N., WIDIASTUTI A., MATRA D.D., BUDIARTO R., LESTARI F.W., NURAINI A., SUMINAR E., RAHMAT P.N.B., EZURA H. 2023.** Parthenocarpic tomato mutants, *iaa9-3* and *iaa9-5*, show plant adaptability and fruiting ability under heat-stress conditions. *Front Plant Sci.* 2023, Vol. 1, 14, p. 1090774.

**MÜLLER, F., XU J., KRISTENSEN, L., WOLTERS-ARTS, M., DE GROOT, P.F.M., JANSMA, S.Y., MARIANI, C., PARK, S., RIEU I. 2016.** High-Temperature-Induced Defects in Tomato (*Solanum lycopersicum*) Anther and Pollen Development Are Associated with Reduced Expression of B-Class Floral Patterning. *Genes, PLoS One.* 2016, Vol. 11, 12.

**NITSCH, L.M., OPLAAT, C., FERON, R., MA, Q., WOLTERS-ARTS, M., HEDDEN, P., MARIANI, C. AND VRIEZEN, W.H. 2009.** Abscisic acid levels in tomato ovaries are regulated by *LeNCED1* and *SICYP707A1*. *Planta.* 2009, Vol. 229, pp. 1335–1346.

**O'CARRIGAN, A., HINDE, E., LU, N., XU, X.-Q., DUAN, H., G. HUANG, MAK, M., BELLOTTI, B., CHEN, Z.-H. 2014.** Effects of light irradiance on stomatal regulation and growth of tomato. *Environ. Exp. Bot.* 2014, Vol. 98, pp. 65-73.

**OH, K., IVANCHENKO, M.G., WHITE, T.J., LOMAX, T.L. 2006.** The diageotropica gene of tomato encodes a cyclophilin: a novel player in auxin signaling. *Planta.* 2006, Vol. 224, pp. 133-144.

**OKABE, Y., YAMAOKA, T., ARIIZUMI, T., USHIJIMA, K., KOJIMA, M., TAKEBAYASHI, Y., SAKAKIBARA, H., KUSANO, M., SHINOZAKI, Y., PULUNGAN, S.I., KUBO, Y., NAKANO, R., EZURA, H. 2019.** Aberrant Stamen Development is Associated with Parthenocarpic Fruit Set Through Up-Regulation of Gibberellin Biosynthesis in Tomato. *Plant and Cell Physiology.* 2019, Vol. 60, pp. 38–51.

**OLIMPIERI, I., SILIGATO, F., CACCIA, R., MARIOTTI, L., CECCARELLI, N., SORESSI, G.P., MAZZUCATO, A. 2007.** Tomato fruit set driven by pollination or by the parthenocarpic fruit allele are mediated by transcriptionally regulated gibberellin biosynthesis. *Planta.* 2007, Vol. 226, pp. 877-888.

- PAN, I.L., MCQUINN, R., GIOVANNONI, J.J., IRISH, V.F. 2010.** Functional diversification of AGAMOUS lineage genes in regulating tomato flower and fruit development. *J. Exp Bot.* 2010, Vol. 61, 6, pp. 1795-1806.
- PANDOLFINI, T, and MOLESINI, B., SPENA, A. 2007.** Molecular dissection of the role of auxin in fruit initiation. *Trends Plant Sci.* 2007, Vol. 12, pp. 327–329.
- PANDOLFINI, T. 2009.** Seedless fruit production by hormonal regulation of fruit set. *Nutrients.* 2009, Vol. 2, pp. 168-177.
- PASCUAL, L., BLANCA, J.M., CAÑIZARES, J., NUEZ, F. 2009.** Transcriptomic analysis of tomato carpel development reveals alterations in ethylene and gibberellin synthesis during pat3/pat4 parthenocarpic fruit set. *BMC Plant Biol.* 2009, Vol. 9, pp. 1–18.
- PATTISON, R.J., CSUKASI, F., ZHENG, Y., FEI, Z., KNAAP, V.E., CATALÁ, C. 2015.** Comprehensive tissue-specific transcriptome analysis reveals distinct regulatory programs during early tomato fruit development. *Plant Physiol.* 2015, Vol. 168, pp. 1684–1701.
- PELAZ, S., DITTA, G. S., BAUMANN, E., WISMAN, E., YANOFSKY, M. F. 2000.** B and C floral organ identity functions require SEPALLATA MADS-box genes. *Nature.* 2000, Vol. 405, pp. 200–203.
- PINEDA B., GIMÉNEZ-CAMINERO E., GARCÍA-SOGO B., ANTÓN M.T., ATARÉS A., CAPEL J., LOZANO R., ANGOSTO T., MORENO V. 2010.** Genetic and physiological characterization of the arlequin insertional mutant reveals a key regulator of reproductive development in Tomato. *Plant Cell Physiol.* 2010, Vol. 51, pp. 435–447.
- PINO, L.E., LIMA, J.E., VICENTE, M.H., de SÁ A.F.L., PÉREZ-ALFONCEA, F., ALBACETE A., COSTA, J.L., WERNER, T., SCHMULLING, T., FRESCHI, L., FIGUEIRA, A., ZSOGON, A., PERES, L.E.P. 2022.** Increased branching independent of strigolactone in cytokinin oxidase 2-overexpressing tomato is mediated by reduced auxin transport. *Mol Horticulture.* 2022, Vol. 51, pp. 435–447.
- PNUELI L., HAREVEN D., BRODAY L., HURWITZ C., LIFSCHITZ E. 1994.** The TM5 MADS Box gene mediates organ differentiation in the three inner whorls of tomato flowers. *Plant Cell.* 1994, Vol. 6, pp. 175–186.
- PNUELI, L., HAREVEN, D., ROUNSLEY, S.D., YANOFSKY, M.F., LIFSCHITZ, E. 1994.** Isolation of the tomato AGAMOUS gene TAG1 and analysis of its homeotic role in transgenic plants. *Plant Cell.* 1994, Vol. 6, pp. 163–173.
- QUINET, M., BATAILLE, G., DOBREV, P.I., CAPEL, C., GÓMEZ, P., CAPEL, J., LUTTS, S., MOTYKA, V., ANGOSTO, T., LOZANO, R. 2014.** Transcriptional and hormonal regulation of petal and stamen development by STAMENLESS, the tomato (*Solanum lycopersicum* L.) orthologue to the B-class APETALA3 gene. *J. Exp. Bot.* 2014, Vol. 65, pp. 2243–2256.
- QUINET, M., et al. 2019.** Tomato fruit development and metabolism. *Front. Plant Sci.* 2019, Vol. 10, pp. 1–23.

**RASBAND, W.S., IMAGEJ, U. S. 1997-2018.** ImageJ. [Online] National Institutes of Health, 1997-2018. [Cited: 06 06 2023.] <https://imagej.nih.gov/ij/>.

**RIBELLES, C., GARCÍA-SOGO, B., YUSTE-LISBONA, F.J., ATARÉS, A., CASTAÑEDA, L., CAPEL, C., LOZANO, R., MORENO, V., PINEDA, B., COSTA, F. 2019.** Alq mutation increases fruit set rate and allows the maintenance of fruit yield under moderate saline conditions. *J. Exp. Bot.* 2019, Vol. 70, pp. 5731–5744.

**RODRIGO, M.J. & GARCÍA-MARTÍNEZ, J.L. 1998.** Hormonal control of parthenocarpic ovary growth by the apical shoot in pea. *Plant Physiol.* 1998, Vol. 116, pp. 511–518.

**ROJAS-GRACIA, P., ROQUE, E., MEDINA, M., ROCHINA, M., HAMZA, R., ANGARITA-DIAZ, M.P., MORENO, V., PÉREZ-MARTÍN, F., LOZANO, R., CAÑAS, L., BELTRÁN, J.P., GÓMEZ-MENA, C. 2017.** The parthenocarpic hydra mutant reveals a new function for a SPOROCYTELESS-like gene in the control of fruit set in tomato. *New Phytol.* 2017, Vol. 214, pp. 1198–1212.

**SAGAR, M., CHERVIN, C., MILA, I., HAO, Y., ROUSTAN, J-P., BENICHO, M., GIBON, Y., BIAIS, B., MAURY, P., LATCHÉ, A., PECH, J-C., BOUZAYEN, M., ZOUINE, M. 2013.** SlARF4, an auxin response factor involved in the control of sugar metabolism during tomato fruit development. *Plant Physiol.* 2013, Vol. 161, pp. 1362–1374.

**SAKATA, T., OSHINO, T., MIURA, S., HIGASHITANI, A., 2010.** Auxins reverse plant male sterility caused by high temperatures hao. *Agricultural Sciences.* 2010, Vol. 107, 19.

**SALEHIN, M., BAGCHI, R. and ESTELLE, M. 2015.** ScfTIR1/AFB-based auxin perception: Mechanism and role in plant growth and development. *Plant Cell.* 2015, Vol. 27, pp. 9-19.

**SCHNEIDER, C.A., RASBAND, W.S., ELICEIRI, K.W., 2012.** NIH Image to ImageJ: 25 years of image analysis. *Nature Methods.* 2012, Vol. 9, pp. 671-675.

**SCHWARZ-SOMMER, Z., HUIJSER, P., NACKEN, W., SAEDLER, H., SOMMER, H. 1990.** Genetic control of flower development by homeotic genes in *Antirrhinum majus*. *Science.* 1990, Vol. 250, pp. 931–936.

**SERRANI, J.C., CARRERA, E., RUIZ-RIVERO, O., GALLEGO-GIRALDO, L., PERES, L.E.P., GARCIA-MARTINEZ, J.L. 2010.** Inhibition of auxin transport from the ovary or from the apical shoot induces parthenocarpic fruit-set in tomato mediated by gibberellins. *Plant Physiol.* 2010, Vol. 153, pp. 851–862.

**SERRANI, J.C., FOS, M., ATARES, A., GARCIA-MARTINEZ, J.L. 2007a.** Effect of gibberellin and auxin on parthenocarpic fruit growth induction in the cv Micro-Tom of tomato. *J. Plant Growth Regul.* 2007a, Vol. 26, pp. 211–221.

**SERRANI, J.C., RUIZ-RIVERO, O., FOS, M., GARCIA-MARTINEZ, J.L. 2008.** Auxin-induced fruit-set in tomato is mediated in part by gibberellins. *Plant J.* 2008, Vol. 56, pp. 922–934.

- SERRANI, J.C., SANJUAN, R., RUIZ-RIVERO, O., FOS, M., GARCIA-MARTINEZ, J.L. 2007b.** Gibberellin regulation of fruit set and growth in tomato. *Plant Physiol.* 2007b, Vol. 174, pp. 246–257.
- SHINOZAKI, Y., HAO, S., KOJIMA, M., SAKAKIBARA, H., OZEKI-IIDA, Y., ZHENG, Y., FEI, Z., ZHONG, S., GIOVANNONI, J.J., ROSE, J.K.C., OKABE, Y., HETA, Y., EZURA, H., ARIIZUMI, T., 2015.** Ethylene suppresses tomato (*Solanum lycopersicum*) fruit set through modification of gibberellin metabolism. *The Plant Journal.* 2015, Vol. 83, pp. 237–251.
- SHINOZAKI, Y., NICOLAS, P., FERNANDEZ-POZO, N., MA, Q., EVANICH, D.J., SHI, Y., XU, Y., ZHENG, Y., SNYDER, S.I., MARTIN, L.B.B., RUIZ-MAY, E., THANNHAUSER, T.W., CHEN, K., DOMOZYCH, D.S., CATALÁ, C., FEI, Z., MUELLER, L.A., GIOVANNONI, J.J., ROSE, J.K.C. 2018.** High-resolution spatiotemporal transcriptome mapping of tomato fruit development and ripening. *Nature Communications.* 2018, Vol. 9, p. 364.
- SILVA, W.B., VICENTE, M.H., ROBLEDO, J.M., REARTES, D.S., FERRARI, R.C., BIANCHETTI, R., ARAÚJO, W.L., FRESCHI, L., PERES, L.E.P., ZSÖGÖN, A. 2018.** SELF-PRUNING Acts Synergistically with DIAGEOTROPICA to Guide Auxin Responses and Proper Growth Form. *Plant Physiol.* 2018, Vol. 2916, 4, pp. 2904-2916.
- SJUT, V. & BANGERTH, F. 1989.** Induced parthenocarpy: a way of changing the levels of endogenous hormones in tomato fruits (*Lycopersicon esculentum* Mill.): 1. Extractable hormones. *Plant Growth Regul.* 1989, Vol. 1, pp. 243–251.
- SOLTI, S D. E., SOLTIS, P. S., ALBERT, V. A., OPPENHEIMER, D. G., DEPAMPHILIS, C. W., MA, H., FROHLICH, M.W., THEISSEN, G. 2002.** Missing links: the genetic architecture of flower and floral diversification. *Trends Plant Sci.* 2002, Vol. 7, pp. 22–31.
- STEPANOVA, A.N., YUN, J., LIKHACHEVA A.V., ALONSO J.M. 2007.** Multilevel interactions between ethylene and auxin in *Arabidopsis* roots. *Plant Cell.* 2007, Vol. 19, 7, pp. 2169-85.
- TAKISAWA, R., NAKAZAKI, T., NUNOME, T., FUKUOKA, H., KATAOKA, K., SAITO, H., HABU, T., KITAJIMA, A. 2018.** The parthenocarpic gene Pat-k is generated by a natural mutation of SlAGL6 affecting fruit development in tomato (*Solanum lycopersicum* L.). *BMC Plant Biol.* 2018, Vol. 18, 72.
- TANAKA, N., TANAKA-MORIYA, Y., MIMIDA, N., HONDA, C., IWANAMI, H., KOMORI, S., Wada, M. 2016.** The analysis of transgenic apples with down-regulated expression of MdPISTILLATA. *Plant Biotechnol.* 2016, Vol. 33, pp. 395–401.
- THEIBEN G., SAEDLER H. 2001.** Floral Quartets. *Nature.* 2001, Vol. 409, pp. 469–471.
- THEISSEN, G., MELZER, R. 2007.** Molecular mechanisms underlying origin and diversification of the angiosperm flower. *Ann. Bot.* 2007, Vol. 100, pp. 603–619.

**ULMASOV, T. HAGEN, G. GUILFOYLE, T.J., 1999.** Dimerization and DNA binding of auxin response factors. *Plant J.* 1999, Vol. 19, pp. 309–319.

**ULMASOV, T., et al. 1997.** Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. *The Plant Cell.* 1997, Vol. 9, pp. 1963–1971.

**ULMASOV, T., LIU, Z.B., HAGEN, G., GUILFOYLE, T.J. 1995.** Composite structure of auxin response elements. *Plant Cell.* 1995, Vol. 7, pp. 1611–1623.

**VARGA, A., BRUINSMA, J. 1976.** Roles of seeds and auxins in tomato fruit growth. *Z. Pflanzenphysiol.* 1976, Vol. 80, pp. 95–104.

**VIDAL, A.M., BEN-CHEIKH, W., TALÓN, M., GARCÍA-MARTÍNEZ, J.L. 2003.** Regulation of gibberellin 20-oxidase gene expression and gibberellin content in citrus by temperature and citrus exocortis viroid. *Planta.* 2003, Vol. 217, pp. 442–8.

**VIVIAN-SMITH, A., LUO, M., CHAUDHURY, A., KOLTUNOW, A. 2001.** Fruit development is actively restricted in the absence of fertilization in Arabidopsis. *Development.* 2001, Vol. 128, pp. 2321–2331.

**VREBALOV, J., PAN, I.L., ARROYO, A.J.M., MCQUINN, R., CHUNG, M., POOLE, M., ROSE J., SEYMOUR, G., GRANDILLO, S., GIOVANNONI, J., IRISH, V.F. 2009.** Fleshy fruit expansion and ripening are regulated by the tomato Shatterproof Gene TAGL1. *Plant Cell.* 2009, Vol. 21, pp. 3041–3062.

**VRIEZEN, W.H., FERON, R., MARETTO, F., KEIJMAN, J., MARIANI, C. 2008.** Changes in tomato ovary transcriptome demonstrate complex hormonal regulation of fruit set. *New Phytol.* 2008, Vol. 177, pp. 60–76.

**WANG D.D., LI Y., BHUPATHIRAJU S.N., ROSNER B.A., SUN Q., GIOVANNUCCI E.L., RIMM E.B., MANSON J.E., WILLETT W.C., STAMPFER M.J., HU F.B. 021.** Fruit And Vegetable Intake And Mortality: Results From 2 Prospective Cohort Studies Of Us Men And Women And A Meta-Analysis Of 26 Cohort Studies. *Circulation.* 021, Vol. 143, 17, pp. 1642–1654.

**WANG, H., JONES, B., LI, Z., FRASSE, P., DELALANDE, C., REGAD, F., CHAABOUNI, S., LATCHE, A., PECH, J.C., BOUZAYEN, M. 2005.** The tomato Aux/IAA transcription factor IAA9 is involved in fruit development and leaf morphogenesis. *Plant Cell.* 2005, Vol. 17, pp. 2676–2692.

**WANG, H., SCHAUER, N., USADEL, B., FRASSE, P., ZOUINE, M., HERNOULD, M., LATCHÉ, A., PECH, J.C., FERNIE, A.R., BOUZAYEN, M. 2009.** Regulatory features underlying pollination-dependent and-independent tomato fruit set revealed by transcript and primary metabolite profiling. *Plant Cell.* 2009, Vol. 21, pp. 1428–1452.

**WANG, Y., ZHANG, J., HU, Z., GUO, X., TIAN, S., CHEN, G. 2019.** Genome-Wide Analysis of the MADS-Box Transcription Factor Family in *Solanum lycopersicum*. *International journal of molecular sciences.* 2019, Vol. 20, 12, p. 2961.

**WEIGEL, D., MEYEROWITZ, E.M. 1994.** The ABCs of floral homeotic genes. *Cell*. 1994, Vol. 78, pp. 203–209.

**WILKINSON, J.Q., LANAHAN, M.B., YEN, H.C., GIOVANNONI, J.J., KLEE, H.J. 1995.** An ethylene-inducible component of signal-transduction encoded by Never-ripe. *Science*. 1995, Vol. 270, pp. 1807-1809.

**XIAO, H., RADOVICH, C., WELTY, N., HSU, J., LI, D., MEULIA, T., VAN DER KNAAP, E. 2009.** Integration of tomato reproductive developmental landmarks and expression profiles, and the effect of SUN on fruit shape. *BMC Plant Biol*. 2009, Vol. 9, p. 49.

**YAO, J., DONG, Y., MORRIS, B.A. 2001.** Parthenocarpic apple fruit production conferred by transposon insertion mutations in a MADS-box transcription factor. *Proc. Natl. Acad. Sci*. 2001, Vol. 98, pp. 1306–1311.

**ZINN, K.E., TUNC-OZDEMIR, M., HARPER, J.F. 2010.** Temperature stress and plant sexual reproduction: uncovering the weakest links. *J. Exp. Bot*. 2010, Vol. 61, 7, pp. 1959-1968.

**ZOBEL, R.W. 1973.** Some physiological characteristics of the ethylene-requiring tomato mutant diageotropica. *Plant Physiol*. 1973, Vol. 52, pp. 385–389.