University of São Paulo
"Luiz de Queiroz" College of Agriculture

The impact of tomato domestication-related alleles on plant development

Mayara Caroline Carvalho Pinto

Thesis presented to obtain the degree of Doctor in Science.
Area: Plant Physiology and Biochemistry

Piracicaba
2021

Mayara Caroline Carvalho Pinto
Bachelor and Licensed in Biological Sciences

The impact of tomato domestication-related alleles on plant development

versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:
Prof. Dr. **LÁZARO EUSTÁQUIO PEREIRA PERES**

Thesis presented to obtain the degree of Doctor in Science.
Area: Plant Physiology and Biochemistry

Piracicaba
2021

Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA – DIBD/ESALQ/USP

Pintol, Mayara Caroline Carvalho

The impact f tomato domestication-related alleles on plant development / Mayara Caroline Carvalho Pinto. - - versão revisada de acordo com a resolução CoPGr 6018 de 2011. - - Piracicaba, 2021.

68 p.

Tese (Doutorado) - USP / Escola Superior de Agricultura “Luiz de Queiroz”.

1. Tomate 2. Domesticação 3. Tamanho de fruto 4. Formato de fruto I.Título

To my parents and sisters

For all unconditional love, help, and support,

I dedicate.

ACKNOWLEDGEMENTS

I would like to thank everyone who contributed, from the beginning, to the work described in this thesis since.

To the advisor Dr. Lázaro Peres, thank you very much for the opportunity given me and for the support during my research. Thank you so much for everything you taught me, for your patience, encouragement and friendship. It was a pleasure to learn from you, I will always have deep gratitude to you.

To all professors of the Plant Physiology and Biochemistry program, thank you for the scientific teachings, in particular to the professors Dr. Fabio Tebaldi Silveira Nogueira, Dr. Daniel Scherer de Moura, Dra. Beatriz Appezzato da Glória and Helaine Carrer for their shared knowledge.

I am thankful to Francisco Vitti and, especially, Cássia Figueiredo for all the help, during the development of the work.

To my laboratory companions, Rodrigo Therezan, Lilian Pino, Maísa Siqueira, Ariadne Felício, Ignácio Achon, Marcela Notini, James Gattward and Eloísa Vendemiati, thank you for all your shared knowledge. Special thanks to Karla Gasparini, Matheus Vicente and Diego Sevastian for fundamental help during the development of the experiments. To Karla, Diego and Cássia I especially thank you for the friendship and support you gave me when I need it most, your friendship inside and outside the laboratory was very important for me to complete my doctor degree.

I am grateful to the Escola superior de Agricultura "Luiz de Queiroz" for the opportunity to pursue a doctor degree, for all the opportunities granted over the years.

To Maria Solizete Granziol Silva I thank you for all your help, for always being helpful and very kind, showing support and availability at any time.

I am grateful to CAPES (Agência de Apoio e Avaliação da Pós-Graduação, Financial Code 001) for the financial support throughout the doctor degree.

Special thanks to the friends who have always supported me, helping in the most difficult moments and sharing the most fun with me. Patrícia Iwagaki, Vívian Brancaglioni, Pórtya Piscitelli, Camila Casagrande, Fernanda Lamede, Gleice Rodrigues, Solange Holshuch and Pollyane Vieira, you were a family that ESALQ gave me.

To my parents, Euza and Marcos, my sisters, Gabriela and Mariana, to my stepmother, Grazielle, my love for you is immense. Thank you so much for all the support and encouragement you have given me. And to you, father, I thank you, mainly, for always having been my greatest supporter throughout all these years of study, taught me the importance of education, and for having done everything I could to reach where my dreams could reach.

Finally, I thank God for daily blessings and constant protection.

“I never lose.

I either win or learn”

Nelson Mandela

“Even when everything seems to collapse, it is up to me to decide between laughing or crying, going or staying, giving up or fighting; because I descovered, in the uncertain path of life, that the most important thing is to decide.”

Cora Coralina

CONTENTS

RESUMO

Impacto de alelos relacionados à domesticação do tomateiro no desenvolvimento vegetal

A domesticação de plantas e animais a partir de progenitores selvagens iniciou-se independentemente em vários locais do mundo a milhares de anos. Durante esse processo, os humanos selecionaram características importantes para eles. Potanto, muitas espécies possuem características domesticadas em comum. Esse tipo de característica, que pode ser usada para diferenciar uma espécie selvagem de uma espécie domesticada, é denominado síndrome de domesticação. Acredita-se que o ancestral mais próximo do tomateiro (*Solanum lycopersicum*) seja S. *pimpinellifolium*, uma espécie com frutos do tamanho de uma ervilha. Para criar as variedades atuais disponíveis para consumo, foi necessário selecionar características que modificassem tanto o tamanho quanto a forma dos frutos. Muitos genes estão envolvidos nessas características e seus alelos domesticados são agora conhecidos. Entre os genes mais importantes para aumento de peso dos frutos encontram-se *FRUIT WEIGHT 11.3*, um regulador do tamanho da célula, e *FRUIT WEIGHT 2.2*, que codifica para um regulador do número de células. Para o formato de frutos, temos *SUN*, um regulador positivo do crescimento, e *OVATE,* um regulador negativo do crescimento. Frutos alongados podem ser produzidos por um alelo de ganho de função de *SUN* ou por um alelo de perda de função de *OVATE*. Muito se sabe sobre o impacto desses genes nos frutos, mas sua influência no desenvolvimento da planta como um todo nem sempre é clara. Sabe-se também que os hormônios vegetais são importantes para o controle fisiológico do desenvolvimento das plantas. Portanto, neste trabalho buscamos entender o impacto dos genes de tamanho do fruto nas características do caule e da raiz, bem como a influência dos genes do formato do fruto na expressão de vias hormonais durante o desenvolvimento do ovário/fruto.

Palavras-chave: Tomate, Domesticação, Formato de frutos, Tamanho de frutos, Fitohormonios, Micro-Tom

ABSTRACT

The impact of tomato domestication-related alleles on plant development

The domestication of plants and animals from wild parents occurred independently in various parts of the world thousands of years ago. During this process, humans selected the characteristics of most interested to them. Therefore, many species have domesticated traits in common. This sort of traits, which can be used to differentiate a wild species from domesticate ones, is termed domestication syndrome. The closest ancestor of tomato (*Solanum lycopersicum*) is believed to be *S. pimpinellifolium*, a species with pea-sized fruits. To create the current varieties available for consumption, it was necessary to select traits that would modify both the size and the shape of the fruits. Many genes are involved in these traits and their domesticated alleles are now known. Among the most important genes for weight gain are *FRUIT WEIGHT 11.3*, a cell size regulator, and *FRUIT WEIGHT 2.2*, which codes for a cell number regulator. As for genes controlling fruit shape, the *SUN*, is positive growth regulator, and *OVATE*, a negative growth regulator. Elongated fruits can be produced by either a gain-of-function allele of *SUN* or a loss-of-function allele of *OVATE*. Much is known about the impact of these genes on fruits, but their influence on the whole plant development is not always clear. It is also known that plant hormones are important for the physiological control of plant development. Therefore, in this work we sough to understand the impact of fruit weight genes on shoot and root traits, as well as the influence of fruit shape genes on the expression of hormonal pathways during ovary/fruit development.

Keywords: Tomato, Domestication, Fruit shape, Fruit size, Phytohormones, Micro-Tom

1. INTRODUCTION TO TOMATO DOMESTICATION

Abstract

The domestication of wild species dates back to the dawn of civilization. However, the long process of selection of spontaneous mutations and alleles recombination make it difficult for this process to be restarted for new wild species. Such a limitation is one of the causes of the genetic erosion, in which humankind may have its food security compromised by depending on few species. Recently, CRISPR/Cas9 gene editing technology, which makes it possible to induce specific mutations, coupled with the growing identification of genes that were mutated during domestication, opened the prospect for *de novo domestication* of wild species. Wild species are sources of resistance to biotic and abiotic stresses but these traits have a polygenic inheritance, which hampers the effort to obtain such characteristics in cultivated species. On the other hand, the number of genes needed to confer domestication traits is surprisingly small. In tomato, one of the most consumed vegetables in Brazil and worldwide, some of the genes involved in domestication and improvement are *FRUIT WEIGTH 2.2* (*FW2.2*), *FRUIT WEIGHT 11.3* (*FW11.3*), *FRUIT WEIGHT 3.2* (*FW3.2*), *FASCLATED* (*FAS*), *OVATE* (*O*) and *SUN*. The alleles present in the cultivated forms promote fruit weight increases (*fw2.2*, *fw11.3*, *fw3.2* and *fas*) and modifications in fruit shape (*fas*, *o* and *sun*). Determining the impact of domestication genes on plant development can provide for new biotechnological tools. This fundamental knowledge can also guide *de novo domestication* of wild plants, predicting positive and negative interactions in plant architecture and yield, when harnessing their characteristics of interest, such as nutritional value and resistance to pathogens and abiotic stresses.

Keywords: Tomato; Domestication; Fruit weight; Fruit size

The United Nations released a report on June 17, 2019 with projections for population growth worldwide. It is estimated that the current population of 7.7 billion people will reach 9.7 billion in 2050, with an increase of 2 billion over the next 30 years (https://nacoesunidas.org/). In addition to population growth, the increased concentration of people in urban centers and the increase in the *per capita* revenue in the coming years will culminate in greater demand for food (Saath and Fachinello, 2018).

In the past, an alternative to this problem would be the expansion of areas destined to agriculture, however, currently they are already close to their capacity limits (Saath and Fachinello 2018). In addition, other factors interfere in agricultural production, such as climatic aspects and associated environmental deterioration (Zhang et al., 2017; Zhu et al., 2014). With this, it is emphasized the importance of studying other methods that can help in solving this problem, such as increased plant productivity, food nutritional value and resistance to biotic and abiotic stresses.

Among the crops of economic importance in the national and worldwide scenario, the tomato (*Solanum lycopersicum*) (Piotto and Peres, 2012) represents the largest harvest of fruits, being the greatest value vegetable in the world, contributing substantially to the human diet (Zhu et al., 2018). However, to reach the current cultivars found on the market shelves, tomatoes have undergone a process of domestication over thousands of years. During this process, the characteristics that were of greatest interest of man during cultivation practices were selected, such as vigorous growth, determined growth habit and large fruits, among others (Anne Frary and Doganlar, 2003; Meyer and Purugganan, 2013).

Plant and animal domestication occurred from wild parents and happened independently in several areas of the world (Fuller 2007; Meyer and Purugganan, 2013; Purugganan and Fuller, 2009). The human being started the process of domesticating plants approximately 12,000 years ago, in the Middle East and the Fertile Crescent; 10,000

years ago in Mesoamerica, Andes, China and near Oceania; 8,000 years in Sub-Saharan Africa and 6,000 years ago in eastern North America (Meyer, Duval, and Jensen, 2012). There are about 160 taxonomic families, 2500 species subjected to domestication, and 250 fully domesticated species (Dirzo and Raven, 2003; Duarte, Marbá, and Holmer, 2007; Meyer and Purugganan, 2013).

With the development and establishment of cultivation, the selection of interesting phenotypes in new crops were allowed, starting the domestication and crop diversification. Thereafter, it was possible to amplify the desirable alleles that made it possible to increase yields, and thus to select favorable crop phenotypes. As domesticated crops evolved and spread from their initial locations (centers of origin), plant populations were adapted to new environments and local preferences. In this way, it was possible to deliberately create varieties to maximize yield and to allow easy cultivation, uniformity and quality of different crops (Fernie and Yan, 2019; Meyer and Purugganan, 2013).

Many different crops species share common domestication-related traits (G. Zhu et al, 2018). This set of traits that distinguish crops from their wild ancestors is termed domestication syndrome (Evans, 1996). The domestication of fruits and vegetables have resulted in an enormous diversity of shapes and sizes of plant organs. The selection of alleles that took place over thousands years, which increased the fruit weight and changed the fruit shape for specific culinary uses, provide a wealth of resources to study the molecular bases of crop diversity (Van der Knaap et al, 2014). In this context, the tomato is considered a model plant for genetic studies, as it has a relatively small genome (950Mb) distributed in 12 chromosomes, it is a diploid species and it has a relatively fast life cycle (http: //solgenomics.net/).

Tomatoes originated in South America, in the todays Peru territory. There are, however, indications that they were domesticate in Mexico (Jenkins, 1948). In the XVI century, during the colonization of the Americas, the Spaniards took the tomato from Mexico to Europe. There are reports of the fruit insertion in Italian cuisine in 1554, describing it as flat, segmented and yellow in color, which gave rise to the name Italian Pomo d'Oro (golden apple) (Mattiollus, 1544). In North America, tomatoes were introduced from Europe in the XIX century (Paran and Van Der Knaap, 2007). In Brazil, the better-documented history of tomato production started in 1930, when the first cultivars were created, probably from genotypes introduced by European and Japanese immigrants in the beginning of the XX century. The first Brazilian cultivar documented was the Santa Cruz, which gave rise to the cultivars Angela, in 1969, and Santa Clara, in 1985 (Nagai, 1993).

Due to its widespread occurrence in Central America, and its close genetic relationship with cultivated tomatoes, the cerasiforme varieties of *S. lycopersicum* are believed to be direct ancestors of cultivated tomatoes. *S. lycopersicum* var. *cerasiforme* originated from the wild species *S. pimpinellifolium*, which had a habit of indeterminate growth and reduced apical dominance, resulting in a large shrub with many inflorescences, and small, round and red fruits (Rick, 1995). A recent work involving genomic analyses of 49 *S. pimpinellifolium*, 153 *S. lycopersicum* var. *cerasiforme* and 92 cultivated *S. lycopersicum* accessions, from South America, Mesoamerica and elsewhere, evidence that *S. lycopersicum* var *cerasiforme* originated in S. America as pre-domesticated form 78,000 years ago and spread northwards given origin to cultivated *S. lycopersicum* in Mexico, 7,000 years ago (Razifard et al., 2020).

One of the main changes observed during tomato domestication was the increase in fruit size (Frary et al., 2000). Currently, it is possible to find varieties of tomato with an increase of up to 100 times compared to wild ancestors (Zhu et al., 2018). The increase in the size of the organs can be explained by the increase in the number of cells and/or cell expansion (Krizek 2009). This is a polygenic characteristic, being therefore controlled by a series of genes, but with some genes with great effect. The effect of positive alleles in a single major gene can be responsible for 20-30% increase in fruit size (Cong et al., 2002, 2008; Frary et al., 2000). In addition to the increase in fruit size, variations in the shape

of these organs were also observed during domestication. The wild and semi-wild tomato species produce basically round fruits, while the current cultivars present varied shapes (round, pear-shaped, oval and flattened) (Tanksley, 2004; Rodriguez et al., 2011).

Some of the major genes related to fruit weight are *FRUIT WEIGHT 2,2- FW2.2* (Solyc02g090730), which codes for a negative regulator of cell number; *FRUIT WEIGHT 3,2- FW3.2* (Solyc03g114940), which is a P450 monoozygenase from the CYP78A subfamily, homolog to the Arabidopsis *KLUH* and rice *PLASTOCHRON1*; and *FRUIT WEIGHT 11.3- FW11.3* (Solyc11g071940), which is a previously uncharacterized protein that was named *CELL SIZE REGULATOR* (*CSR*). As for genes affecting fruit shape, some prominent ones are *FASCIATED-FAS* (Solyc11g071380), which is a mutation in the *CLAVATA3* gene that controls the size of shoot meristem and, consequently, the number of carpels (locule) in fruits; *OVATE* (Solyc02g085500), which is a hydrophilic protein with a protein–protein interaction domain probably involved in the negative regulation of organ growth; and *SUN* (Solyc10g079240), which encodes a member of the IQD family of calmodulin-binding proteins, promoting fruit elongation (Tanksley, 2004; Esther van der Knaap et al., 2014; Xu et al., 2015).

The *FW 2.2*, located on chromosome 2, is involved in the control of the cell cycle, being related to the initial events that increase fruit size. The alleles of this gene, responsible for the smaller or larger size of the fruit, cause differences in the time of gene expression (heterochronic allelic expression) and in the level of total transcript, resulting in a great phenotypic difference in the fruits weight (Cong, Liu, and Tanksley, 2002). When cultivated tomato were transformed with constructions containing the *fw2.2* allele from the wild species *S. pennellii*, there was a drastic reduction in the fruit size, proving that this gene can modify the fruit weight by up to 30% (Frary et al., 2000). Therefore, a key transition in the domestication process may have occurred with the selection of the large-fruit *fw2.2* allele (Frary et al., 2000). The small-fruit *fw2.2* alleles from *S. pennellii* and from other wild species are semi dominant to the *S. lycopersicun* large-fruit allele (Alpert, Grandillo, and Tanksley, 1995).

The *FW 3.2*, located on chromosome 3, acts post anthesis and probably controls cell division in the pericarp. The mutated allele, selected during domestication, has a SNP in the putative promoter region (512 bp upstream of the start of transcription) that was linked to its increased expression (Chakrabarti et al., 2013). However, a further study (Alonge et al., 2020) proved that a 50-kbp tandem duplication at the *fw3.2* locus containing three genes, including two identical copies of *SlKLUH*, leads to an increased expression of *SlKLUH*. Hence, the mutated large-fruit *fw3.2* allele is dominant over the small-fruit wild type allele. Besides the effect in cell division, the mutated *fw3.2* allele causes pleiotropic phenotypes, most contributing to increased fruit size. Among the phenotypes, there is an increase in seed masses and a delay in the time from anthesis to ripe fruit. This delayed ripening was associated to prolonged cell division phase in the developing pericarp. Plants with the mutated allele also reduce the number and the length of side shoots. Therefore, the increase in fruit mass also coincides with a reduction of the number of fruits per plant caused by the reduction in side shoot number and length, yielding fewer inflorescences (Chakrabarti et al, 2013).

The *FW 11.3*, located on chromosome 11, codes for a cell size-regulating group, resulting in a protein that has not been well characterized yet and that is involved in cell expansion. The mutated allele, selected during domestication, shows a deletion of about 1406 kb at the 3' end and an addition of 22 bp, which caused the exclusion of 194 amino acids, generating a shorter protein. The domesticated allele is dominant and it is likely a gain of function mutation increasing the size of the fruit. This increase does not affect the number of cells directly, but the cell size (Mu et al, 2017).

Still on chromosome 11, there is the *fasciated* (*fas*), a mutation affecting fruit shape that also contributes to the increase in fruit size. The *fas* mutation promotes the formation of fruits with a greater number of locules, since it

increases the number of carpels during flower development, consequently increasing the fruit weight. Previously, this mutation was attributed to a loss of function of the *YABBY* transcription factor (Cong, Barrero, and Tanksley, 2008). However, when trying to complement the *fas* mutant with a functional *YABBY* gene, there was only a weak recovery of the phenotype. Further studies found that the *fas* locus is a 294-kb inversion with breakpoints in the first intron of the *YABBY* gene and 1 kb upstream of the *CLAVATA 3* (*CLV3*) start codon. This causes a partial loss of *CLV3* function (Xu et al. 2015). In *Arabidopsis*, the CLV3 peptide is a negative regulator of the *WUSCHEL* (*WUS*) gene, which, in its turn, is a positive regulator of stem cell proliferation (Schoof et al, 2000). In tomato, the loss of function of *CLV3* presumably causes an augmented expression of *WUS*, increasing the size of shoot meristem and the consequent increase in the number of ovary carpels that will form the fruit.

Both *OVATE* and *SUN* control fruit elongation. Whereas *OVATE* is a negative growth regulator, leading to shorter fruits, *SUN* is a positive growth regulator, leading to more elongated fruits (Azzi et al, 2015). The mutated *ovate* is recessive and the locus is located on chromosome 2 (Liu et al., 2002a). The occurrence of mutations in *OVATE*, as well as in *FAS*, in some accessions of *S. lycopersicum* var cersiforme suggests that these mutations appeared very early in the selection of the cultivated tomato (Rodriguez et al, 2011). The *OVATE* gene codes for a hydrophilic protein with a protein–protein interaction domain probably involved in the negative regulation of organ growth, culminating in reducing the length of the fruit. The allele responsible for the elongated fruit phenotype has a premature stop codon and is presumably a null allele (Liu et al., 2002a).

It is not known whether the *OVATE* gene is related to disturbances in the hormonal status in tomatoes (Azzi et al., 2015), but there are reports showing that polyamines have a function in the shape of fruits (Anwar et al 2019) and that there is a interaction between these compounds and plant hormones that influence various developmental processes (Anwar, Mattoo and Honda, 2015). It is also known that in *Arabidopsis*, proteins similar to OVATE act as transcriptional repressors affecting the expression of *AtGA20ox1*, necessary for gibberellin biosynthesis, causing cell elongation (Wang et al., 2007, 2011).

The *SUN* gene has a much more pronounced effect in fruit elongation compared to *OVATE* (Esther van der Knaap et al., 2014). The gene is located on chromosome 7, having arisen from a highly unusual duplication of 24.7 kb from chromosome 10 to chromosome 7, and codes for a member of the IQD family, calmodulin-binding proteins (Jiang et al., 2009; Xiao et al., 2008). There is no other allele known for the *SUN* gene (Rodriguez et al., 2011), which can be explained by the fact that this mutation arose from an unusual and rare gene duplication from one chromosome to another. Actually, this kind of event creates a new locus with no correspondent wild type allele in the genome of either wild species or non-mutated tomato cultivars. Since the cv Heinz does not have the *sun* mutation, there is no a gene ID correspondent to *SUN* in the tomato reference genome. The closest gene to *SUN* in the tomato reference genome is the *IQD* gene there was duplicated from the chromosome 10 (Solyc10g079240).

The extremely elongated fruit shape of the *sun* and the lack of proper seed development, resembling parthenocarpic fruits, in addition to their potential biochemical function, suggest that either the *SUN* can affect the levels of auxin or its distribution in the fruit (Xiao et al., 2008). *SUN* can be related not only to the development of terminal organs, such as fruits, but also to the plant lateral development. The gene probably defines the pattern of elongated fruits before anthesis, during the gynoecium development, lasting after fertilization, in addition of controlling sepal shape and of producing atrophied leaves and twisted stems (Esther van der Knaap et al., 2014).

The change in the fruit shape caused by *SUN* is due to the cellular redistribution that occurs towards the proximal-distal region, accompanied by a cellular decrease in the columella region and in the septum in the mediolateral

region in the whole fruit (Wu et al., 2011). Therefore, *SUN* is related to changes in the cell division pattern so that there is a change in the fruit shape (Esther van der Knaap et al., 2014).

Over thousands of years of domestication, the number of genes involved in the domestication process, including those no yet identified at molecular level, but already detected in different types of genetic analyses, is surprisingly low. The crop improvement process was summarized by Meyer & Purugganan (2013) as divided in four steps or generations. The authors explain that the first generation started with selections made independently by local farmers, based on the phenotypes of interest and the subsequent domestication of wild species, making it possible to feed about 50 million people. With population growth during the 20th century, it was necessary to develop other strategies in order to increase food production. This includes the emergence of hybrid breeding and the use of semi-dwarf cultivars, as well as techniques such as mechanization, herbicides, pesticides and fertilizers. The sum of these techniques met the needs of approximately 1.65 billion people, which characterized the second generation. After 1980, the third generation emerged with the sequencing of genomes, the use of transgenic genotypes, and molecular markers for assisted selection, which provided the supply of the current population of more than seven billion people. Currently, the improvement that corresponds to the fourth generation is emerging, through precision agriculture with a deeper exploration of functional genomics and with gene editing technologies incorporated into breeding strategies.

CRISPR/Cas9 is one of the technologies developed that made gene editing possible. This system makes it possible to cleave the DNA at specific sites, using two components, a small molecule of guide RNA (gRNA) and an endonuclase, Cas9 (Jinek et al., 2012). The complexation of the gRNA in the gene of interest signals for cleavage by means of the Cas9 endonuclease. The applications of this technique are not restricted to mutagenesis only, so that after cleavage of the target site it is possible to insert or replace the sequence by recombination. In transgenic plants containing CRISPR/Cas9 constructions, the mutation can occur following a single allele (monoallelic) or each allele can be mutated independently (biallelic) to produce a phenotype (Luo, Gilbert, and Ayliffe, 2016). The technology has already proved efficient in the transformation of cultivated plants such as rice, wheat and tomatoes (Shan et al., 2013; Zsögön et al., 2018).

In view of this, using CRISPR/Cas9 it is possible to manipulate the genome of wild species with characteristics of interest to improve them, facilitating the "*de novo domestication*" process (Zsögön et al. 2018). In this way, it is possible to modificate the genome of wild species, containing desirable characteristics, such as nutritional value, stress tolerance and resistance to pathogens, by editing key genes already known to be involved in crop domestication (Falke et al., 2013; Gasparini et al., 2021).

Taking into account the expansion of the human population, environmental degradation, as well as the growing competition for land, it is important to invest efforts in studies that help to solve these problems. The advanced status of genotyping tools and the creation of new technologies for the manipulation of genes, such as CRISPR/Cas9, paved the way to create ideal crops for human needs in a short time (Gasparini et al., 2021). In addition, the study of crop domestication process has enabled the identification of key genes involved in this process. Despite this, our knowledge of the effect of domestication genes on different aspects of plant development and their potential impact on genes that confer resistance to biotic and abiotic stresses remains to be improved and is the objective of the present study.

REFERENCES

Alonge M, Wang X, Benoit M, Soyk S, Pereira L, Zhang L, Suresh H, Ramakrishnan S, Maumus F, Ciren D, Levy Y, Harel TH, Shalev-Schlosser G, et al. (2020) "Major impacts of widespread structural variation on gene expression and crop improvement in tomato". **Cell** 182: 145–161.

Alpert, K. B., S. Grandillo, and S. D. Tanksley (1995) "Fw 2.2:A Major QTL Controlling Fruit Weight Is Common to Both Red- and Green-Fruited Tomato Species". **Theoretical and Applied Genetics** 91 (6–7): 994–1000. https://doi.org/10.1007/BF00223911.

Anwar, R.; Mattoo, A. K. and Handa, A. K. **Polyamines: A universal molecular nexus for growth, survival, and specialized metabolism**. Eds.; Springer: London, UK, 2015; pp. 267–302.

Anwar, Raheel, Shazia Fatima, Autar K. Mattoo, and Avtar K. Handa (2019) "Fruit Architecture in Polyamine-Rich Tomato Germplasm Is Determined via a Medley of Cell Cycle, Cell Expansion, and Fruit Shape Genes." **Plants** 8 (10): 1–19. https://doi.org/10.3390/plants8100387.

Azzi, Lamia, Cynthia Deluche, Frédéric Gévaudant, Nathalie Frangne, Frédéric Delmas, Michel Hernould, and Christian Chevalier (2015) "Fruit Growth-Related Genes in Tomato." **Journal of Experimental Botany** 66 (4): 1075–7086. https://doi.org/10.1093/jxb/eru527.

Blanca, José, Javier Montero-Pau, Christopher Sauvage, Guillaume Bauchet, Eudald Illa, María José Díez, David Francis, Mathilde Causse, Esther van der Knaap, and Joaquín Cañizares (2015) "Genomic Variation in Tomato, from Wild Ancestors to Contemporary Breeding Accessions." **BMC Genomics** 16 (1): 257. https://doi.org/10.1186/s12864-015-1444-1.

Boccalandro, Hernán E., Edmundo L. Ploschuk, Marcelo J. Yanovsky, Rodolfo A. Sánchez, Christiane Gatz, and Jorge J. Casal. (2003) "Increased Phytochrome B Alleviates Density Effects on Tuber Yield of Field Potato Crops." **Plant Physiology** 133 (4): 1539–46. https://doi.org/10.1104/pp.103.029579.

Campos, Marcelo Lattarulo, Rogério Falleiros Carvalho, Vagner Augusto Benedito, and Lázaro Eustáquio Pereira Peres (2010) "Small and Remarkable." **Plant Signaling & Behavior** 5 (3): 267–70. https://doi.org/10.1093/jxb/erp270.ormones.

Carvalho, Rogério F, Marcelo L Campos, Lilian E Pino, Simone L Crestana, Agustin Zsögön, and Joni E Lima (2011) "Convergence of Developmental Mutants into a Single Tomato Model System : ' Micro-Tom ' as an Effective Toolkit for Plant Development Research," **Plant Methods**, 7:18, 1–14.

Chakrabarti, Manohar, Na Zhang, Christopher Sauvage, Stéphane Muños, Jose Blanca, Joaquin Cañizares, Maria Jose Diez, et al. (2013) "A Cytochrome P450 Regulates a Domestication Trait in Cultivated Tomato." **Proceedings of the National Academy of Sciences of the United States of America** 110 (42): 17125–30. https://doi.org/10.1073/pnas.1307313110.

Cong, Bin, Luz S Barrero, and Steven D Tanksley (2008) "Regulatory Change in YABBY-like Transcription Factor Led to Evolution of Extreme Fruit Size during Tomato Domestication." **Nature Genetics** 40 (6): 800–804. https://doi.org/10.1038/ng.144.

Cong, Bin, Jiping Liu, and Steven D Tanksley (2002) "Natural Alleles at a Tomato Fruit Size Quantitative Trait Locus Differ by Heterochronic Regulatory Mutations." **Proceedings of the National Academy of Sciences of the United States of America** 99 (21): 13606–11. https://doi.org/10.1073/pnas.172520999.

D'Agostino, I B, J Deruère, and Joseph J. Kieber (2000) "Characterization of the Response of the Arabidopsis Response Regulator Gene Family to Cytokinin." **Plant Physiology** 124 (4): 1706–17. https://doi.org/10.1104/pp.1244.1706.

Dayan, Jonathan, Nickolay Voronin, Fan Gong, Tai-ping Sun, Peter Hedden, Hillel Fromm, and Roni Aloni (2012) "Leaf-Induced Gibberellin Signaling Is Essential for Internode Elongation, Cambial Activity, and Fiber Differentiation in Tobacco Stems." **The Plant Cell** 24 (1): 66–79. https://doi.org/10.1105/tpc.111.093096.

Dirzo, Rodolfo, and Peter H. Raven (2003) "Global State of Biodiversity and Loss." **Annual Review of Environment and Resources** 28 (1): 137–67. https://doi.org/10.1146/annurev.energy.28.050302.105532.

Duarte, Carlos M., Nùria Marbá, and Marianne Holmer (2007) "Rapid Domestication of Marine Species." **Science** 316 (5823): 382–83. https://doi.org/10.1126/science.1138042.

Falke, K. Christin, Shirin Glander, Fei He, Jinyong Hu, Juliette De Meaux, and Gregor Schmitz (2013) "The Spectrum of Mutations Controlling Complex Traits and the Genetics of Fitness in Plants." **Current Opinion in Genetics and Development** 23 (6): 665–71. https://doi.org/10.1016/j.gde.2013.10.006.

Fernie, Alisdair R., and Jianbing Yan (2019) "De Novo Domestication: An Alternative Route toward New Crops for the Future." **Molecular Plant** 12 (5): 615–31. https://doi.org/10.1016/j.molp.2019.03.016.

Forno, Ignacio Achon (2017) "Redefining the Hormonal Control of Tomato (Solanum Lycopersicum Cv. Micro-Tom) Fruit Development." University of São Paulo, "Luiz de Queiroz" College of Agriculture. http://www.teses.usp.br/teses/disponiveis/11/11140/tde-04012018-144113/en.php.

Frary, A, T C Nesbitt, S Grandillo, E Knaap, B Cong, J Liu, J Meller, R Elber, K B Alpert, and S D Tanksley (2000a) "Fw2.2: A Quantitative Trait Locus Key to the Evolution of Tomato Fruit Size." **Science** 289 (5476): 85–88. https://doi.org/10.1126/science.289.5476.85.

——— (2000b) "Fw2.2: A Quantitative Trait Locus Key To the Evolution of Tomato Fruit Size." **Science** 289 (5476): 85–88. https://doi.org/10.1126/science.289.5476.85.

Frary, Anne, and Sami Doganlar (2003) "Comparative Genetics of Crop Plant Domestication and Evolution Anne." **Turk J Agric For** 27: 59–69.

Freitas-astua, Juliana, Gustavo Astua-Monge, Jane Elisabeth Polston, and Ernest Hiebert (2003) "A Simple and Reliable Method for the Screening or Transgenic Tobacco Plants." **Pesquisa Atropecuaria Brasileira** 38 (7): 893–96.

Fuller, Dorian Q. (2007) "Contrasting Patterns in Crop Domestication and Domestication Rates: Recent Archaeobotanical Insights from the Old World." **Annals of Botany** 100 (5): 903–24. https://doi.org/10.1093/aob/mcm048.

Galati, V. C. et al. (2018). "Root-to-Shoot Communication to Modulate Source-Sink Relationship in Tomato Depends on Phytochromes" **Journal of Agricultural Science**, 10:8, p. 174.

Gasparini, K., Moreira J.R., Peres L.P.P., & Zsogon A. (2021) "De novo domestication of wild species to create crops with increased resilience and nutritional value". **Current Opinion in Plant Biology**, 60:102006

Giovannoni, J.(2018) "Tomato Multiomics Reveals Consequences of Crop Domestication and Improvement". **Cell**, 172:1–2, p. 6–8.

Gonzalo, Maria Jose, Marin Talbot Brewer, Claire Anderson, David Sullivan, Simon Gray, and Esther Van Der Knaap (2009) "Tomato Fruit Shape Analysis Using Morphometric and Morphology Attributes Implemented in Tomato Analyzer Software Program." **Journal of the American Society for Horticultural Science** 134 (1): 77–87. https://doi.org/10.21273/jashs.134.1.77.

Gonzalo, Maria Jose, and Esther Van Der Knaap (2008) "A Comparative Analysis into the Genetic Bases of Morphology in Tomato Varieties Exhibiting Elongated Fruit Shape." **Theoretical and Applied Genetics** 116 (5): 647–56. https://doi.org/10.1007/s00122-007-0698-7.

Huang, Zejun, and Esther van der Knaap (2011) "Tomato Fruit Weight 11.3 Maps Close to Fasciated on the Bottom of Chromosome 11." **Theoretical and Applied Genetics** 123 (3): 465–74. https://doi.org/10.1007/s00122-011-1599-3.

Jefferson, R A, T A Kavanagh, and M W Bevan (1987) "GUS Fusions: Beta-Glucuronidase as a Sensitive and Versatile Gene Fusion Marker in Higher Plants." **The EMBO Journal** 6 (13): 3901–7. https://doi.org/10.1073/pnas.1411926112.

Jenkins, J. A. (1948) "The Origin of Cultivated Plants." **Economic Botany** 2 (4): 379–92. https://doi.org/https://doi.org/10.1007/BF02859492.

Jiang, Ning, Dongying Gao, Han Xiao, and Esther Van Der Knaap (2009) "Genome Organization of the Tomato Sun Locus and Characterization of the Unusual Retrotransposon Rider." **Plant Journal** 60 (1): 181–93. https://doi.org/10.1111/j.1365-313X.2009.03946.x.

Jinek, Martin, Krzysztof Chylinski, Ines Fonfara, Michael Hauer, Jennifer A Doudna, and Emmanuelle Charpentier (2012) "A Programmable Dual-RNA – Guided" **Science** 337 (August): 816–22.

Kharshiing, Eros, and Shriravi Prasad Sinha (2016) "Deficiency in Phytochrome A Alters Photosynthetic Activity, Leaf Starch Metabolism and Shoot Biomass Production in Tomato." **Journal of Photochemistry and Photobiology B: Biology** 165: 157–62. https://doi.org/10.1016/j.jphotobiol.2016.10.026.

Koshioka, M., T. Nishijima, H. Yamazaki, Y. Liu, M. Nonaka, and L. N. Mander (1994) "Analysis of Gibberellins in Growing Fruits of Lycopersicon Esculentum after Pollination or Treatment with 4-Chlorophenoxyacetic Acid." **Journal of Horticultural Science** 69 (1): 171–79. https://doi.org/10.1080/14620316.1994.11515263.

Krizek, Beth A. (2009) "Making Bigger Plants: Key Regulators of Final Organ Size." **Current Opinion in Plant Biology** 12 (1): 17–22. https://doi.org/10.1016/j.pbi.2008.09.006.

Lippman, Zachary, and Steven D. Tanksley (2001) "Dissecting the Genetic Pathway to Extreme Fruit Size in Tomato Using a Cross between the Small-Fruited Wild Species *Lycopersicon Pimpinellifolium* and *L. Esculentum* Var. Giant Heirloom." **Genetics** 158 (1): 413–22.

Liu, Jiping, Joyce Van Eck, Bin Cong, and Steven D. Tanksley (2002a) "A New Class of Regulatory Genes Underlying the Cause of Pear-Shaped Tomato Fruit." **Proceedings of the National Academy of Sciences of the United States of America** 99 (20): 13302–6. https://doi.org/10.1073/pnas.162485999.

Liu, Jiping, Joyce Van Eck, Bin Cong, and Steven D Tanksley (2002b) "A New Class of Regulatory Genes Underlying the Cause of Pear-Shaped Tomato Fruit." **Proceedings of the National Academy of Sciences of the United States of America** 99 (20): 13302–6. https://doi.org/10.1073/pnas.162485999.

Livak, Kenneth J., and Thomas D. Schmittgen (2001) "Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-ΔΔCT Method." **Methods** 25 (4): 402–8. https://doi.org/10.1006/meth.2001.1262.

Luo, Ming, Brian Gilbert, and Michael Ayliffe (2016) "Applications of CRISPR/Cas9 Technology for Targeted Mutagenesis, Gene Replacement and Stacking of Genes in Higher Plants." **Plant Cell Reports** 35 (7): 1439–50. https://doi.org/10.1007/s00299-016-1989-8.

Martin, L. B. B. et al. (2017) "Cuticle biosynthesis in tomato leaves is developmentally regulated by abscisic acid" **Plant Physiology**, 174: 3, 1384–1398.

Matthiolus, PA. Anazarbos PDo. Di pedacio dioscoride anazerbeo libri cinque della historia, and material medical trodottie in language vulgare Italian *De materia medica,* 1544. Venetia de Bascaraini. *In*: Paran, Ilan, and Esther Van Der Knaap (2007) "Genetic and Molecular Regulation of Fruit and Plant Domestication Traits in Tomato and Pepper." **Journal of Experimental Botany** 58 (14): 3841–52. https://doi.org/10.1093/jxb/erm257.

Matsuo, Satoshi, Kaori Kikuchi, Machiko Fukuda, Ichiro Honda, and Shunsuke Imanishi (2012) "Roles and Regulation of Cytokinins in Tomato Fruit Methylation And." **Journal of Experimental Botany** 63 (15): 5569–79. https://doi.org/10,1093/jxb/ers207.

Meyer, Rachel S., Ashley E. Duval, and Helen R. Jensen (2012) "Patterns and Processes in Crop Domestication: An Historical Review and Quantitative Analysis of 203 Global Food Crops." **New Phytologist** 196 (1): 29–48. https://doi.org/10.1111/j.1469-8137.2012.04253.x.

Meyer, Rachel S., and Michael D. Purugganan (2013) "Evolution of Crop Species: Genetics of Domestication and Diversification." **Nature Reviews Genetics** 14 (12): 840–52. https://doi.org/10.1038/nrg3605.

Mu, Qi, Zejun Huang, Manohar Chakrabarti, Eudald Illa-Berenguer, Xiaoxi Liu, Yanping Wang, Alexis Ramos, and Esther van der Knaap (2017) "Fruit Weight Is Controlled by Cell Size Regulator Encoding a Novel Protein That Is Expressed in Maturing Tomato Fruits." *PLoS* **Genetics** 13 (8). https://doi.org/10.1371/journal.pgen.1006930.

Nagai, H. Tomate. In: Furlani, A.M.C.; Viegas, G.P. (eds.). O melhoramento de plantas no Instituto Agronômico. Campinas: Instituto Agronômico, p.195-203; 301-313. 1993.

Nesbitt, C. T., and Tanksley, S. D. (2002) "Comparative Sequencing in the Genus *Lycopersicon*: Implications for the Evolution of Fruit Size in the Domestication of Cultivated Tomatoes." **Genetics** 162 (1): 365–79.

Nesbitt, T. C., and S. D. Tanksley (2001) "Fw2.2 Directly Affects the Size of Developing Tomato Fruit, With Secondary Effects on Fruit Number and Photosynthate Distribution." **Plant Physiology** 127 (2): 575–83. https://doi.org/10.1104/pp.010087.

Nozue, K. et al. (2015) "Shade Avoidance Components and Pathways in Adult Plants Revealed by Phenotypic Profiling". **PLOS Genetics**, 11 (4): 1–26.

Paran, Ilan, and Esther Van Der Knaap (2007) "Genetic and Molecular Regulation of Fruit and Plant Domestication Traits in Tomato and Pepper." **Journal of Experimental Botany** 58 (14): 3841–52. https://doi.org/10.1093/jxb/erm257.

Pino, Lilian E, Simone Lombardi-Crestana, Mariana S Azevedo, Danielle C Scotton, Lucélia Borgo, Vera Quecini, Antonio Figueira, and Lázaro Ep Peres (2010) "The *Rg1* Allele as a Valuable Tool for Genetic Transformation of the Tomato 'Micro-Tom' Model System." **Plant Methods** 6: 23. https://doi.org/10.1186/1746-4811-6-23.

Piotto, Fernando Angelo, and Lázaro Eustáquio Pereira Peres (2012) "Base Genética Do Hábito de Crescimento e Florescimento Em Tomateiro e Sua Importância Na Agricultura." **Ciência Rural** 42 (11): 1941–46. https://doi.org/10.1590/S0103-84782012001100006.

Purugganan, Michael D., and Dorian Q. Fuller (2009) "The Nature of Selection during Plant Domestication." **Nature** 457 (7231): 843–48. https://doi.org/10.1038/nature07895.

Rodriguez, G. R., S. Munos, C. Anderson, S.-C. Sim, A. Michel, M. Causse, B. B. M. Gardener, D. Francis, and E. van der Knaap (2011) "Distribution of SUN, OVATE, LC, and FAS in the Tomato Germplasm and the Relationship to Fruit Shape Diversity." **Plant Physiology** 156 (1): 275–85. https://doi.org/10.1104/pp.110.167577.

Saath, Kleverton Clovis de Oliveira, and Arlei Luiz Fachinello (2018) "Crescimento Da Demanda Mundial de Alimentos e Restrições Do Fator Terra No Brasil." **Revista de Economia e Sociologia Rural** 56 (2): 195–212. https://doi.org/10.1590/1234-56781806-94790560201.

Sastry, KKS, and RM Muir (1963) "Gibberelin: Effect on Diffusible Auxin in Fruit Development." **Science** 140: 494–95.

Shan, Qiwei et al. (2013) "Targeted Genome Modification of Crop Plants Using a CRISPR-Cas System" **Nature Biottechnology** 31 (8): 8–10. https://doi.org/10.1038/nbt.2650.

Stepanova, A. N., J. Yun, A. V. Likhacheva, and J. M. Alonso (2007) "Multilevel Interactions between Ethylene and Auxin in Arabidopsis Roots." **The Plant Cell Online** 19 (7): 2169–85. https://doi.org/10.1105/tpc.107.052068.

Tang, Yun jia, and Johannes Liesche (2017) "The Molecular Mechanism of Shade Avoidance in Crops – How Data from Arabidopsis Can Help to Identify Targets for Increasing Yield and Biomass Production." **Journal of Integrative Agriculture** 16 (6): 1244–55. https://doi.org/10.1016/S2095-3119(16)61434-X.

Tanksley, Steven D. (2004) "The Genetic, Developmental, and Molecular Bases of Fruit Size and Shape Variation in Tomato." **Plant Cell** 16 181-190 (SUPPL.): 181–90. https://doi.org/10.1105/tpc.018119.

Thiele, Alexandra, Michael Herold, Ingo Lenk, Peter H. Quail, and Christiane Gatz (1999) "Heterologous Expression of Arabidopsis Phytochrome B in Transgenic Potato Influences Photosynthetic Performance and Tuber Development." **Plant Physiology** 120 (1): 73–81. https://doi.org/10.1104/pp.120.1.73.

Ulmasov, Tim, Jane Murfett, Gretchen Hagen, and Tom J Guilfoyle (1997) "Creation of a Highly Active Synthetic AuxRE." **Society** 9 (November): 1963–71. https://doi.org/10.1105/tpc.9.11.1963.

Van der Knaap, E. et al. (2014) "What lies beyond the eye: the molecular mechanisms regulating tomato fruit weight and shape". **Frontiers in plant science**, 5 (May): 227.

Van der Knaap, E.; Tanksley, S. D. (2003) "The making of a bell pepper-shaped tomato fruit: Identification of loci controlling fruit morphology in Yellow Stuffer tomato". **Theoretical and Applied Genetics**, 107 (1): 139–147.

Wang, Shucai, Ying Chang, Jianjun Guo, and Jin Gui Chen (2007) "Arabidopsis Ovate Family Protein 1 Is a Transcriptional Repressor That Suppresses Cell Elongation." **Plant Journal** 50 (5): 858–72. https://doi.org/10.1111/j.1365-313X.2007.03096.x.

Wang, Shucai, Ying Chang, Jianjun Guo, Qingning Zeng, Brian E. Ellis, and Jin Gui Chen (2011) "Arabidopsis Ovate Family Proteins, a Novel Transcriptional Repressor Family, Control Multiple Aspects of Plant Growth and Development." **PLOS ONE** 6 (8): e23896. https://doi.org/10.1371/journal.pone.0023896.

Wang, Yanping, Josh P. Clevenger, Eudald Illa-Berenguer, Tea Meulia, Esther van der Knaap, and Liang Sun (2019) "A Comparison of Sun, Ovate, Fs8.1 and Auxin Application on Tomato Fruit Shape and Gene Expression." **Plant and Cell Physiology** 60 (5): 1067–81. https://doi.org/10.1093/pcp/pcz024.

Wu, Shan, Han Xiao, Antonio Cabrera, Tea Meulia, and Esther van der Knaap (2011) "SUN Regulates Vegetative and Reproductive Organ Shape by Changing Cell Division Patterns." **Plant Physiology** 157 (3): 1175–86. https://doi.org/10.1104/pp.111.181065.

Xiao, Han, Ning Jiang, Erin Schaffner, Eric J. Stockinger, and Esther Van Der Knaap (2008) "A Retrotransposon-Mediated Gene Duplication Underlies Morphological Variation of Tomato Fruit." **Science** 319 (5869): 1527–30. https://doi.org/10.1126/science.1153040.

Xiao, Han, Cheryll Radovich, Nicholas Welty, Jason Hsu, Dongmei Li, Tea Meulia, and Esther Van Der Knaap (2009) "Integration of Tomato Reproductive Developmental Landmarks and Expression Profiles, and the Effect of SUN on Fruit Shape." **BMC Plant Biology** 9:1-21 . https://doi.org/10.1186/1471-2229-9-49.

Xu, Cao, Katie L. Liberatore, Cora A. MacAlister, Zejun Huang, Yi-Hsuan Chu, Ke Jiang, Christopher Brooks, et al. (2015) "A Cascade of Arabinosyltransferases Controls Shoot Meristem Size in Tomato." **Nature Genetics** 47 (7): 784–92. https://doi.org/10.1038/ng.3309.

Ying, L. X. et al. (2011) "Regulation of chloroplast ultrastructure, cross-section anatomy of leaves, and morphology of stomata of cher ry tomato by different light irradiations of light-emitting diodes". **HortScience** 46 (2): 217–221.

Zhang, Zengcui, Aili Li, Gaoyuan Song, Shuaifeng Geng, Bikram S. Gill, Justin D. Faris, and Long Mao (2020) "Comprehensive Analysis of Q Gene Near-Isogenic Lines Reveals Key Molecular Pathways for Wheat Domestication and Improvement." **Plant Journal** 102 (2): 299–310. https://doi.org/10.1111/tpj.14624.

Zhang, Zhi, Cuiyu Mao, Zheng Shi, and Xiaohong Kou (2017) "The Amino Acid Metabolic and Carbohydrate Metabolic Pathway Play Important Roles during Salt-Stress Response in Tomato" **Frontiers in Plant Science** 8 (July): Article 1231. https://doi.org/10.3389/fpls.2017.01231.

Zhu, Guangtao, Shouchuang Wang, Zejun Huang, Shuaibin Zhang, Qinggang Liao, Chunzhi Zhang, Tao Lin, et al. (2018). "Rewiring of the Fruit Metabolome in Tomato Breeding." **Cell** 172 (1–2): 249-261.e12. https://doi.org/10.1016/j.cell.2017.12.019.

Zhu, Mingku, Guoping Chen, and Jianling Zhang (2014) "The Abiotic Stress-Responsive NAC-Type Transcription Factor SlNAC4 Regulates Salt and Drought Tolerance and Stress-Related Genes in Tomato (Solanum Lycopersicum)" **Plant Cell Rep** 33:1851–63. https://doi.org/10.1007/s00299-014-1662-z.

Zsögön, Agustin, Tomáš Čermák, Emmanuel Rezende Naves, Marcela Morato Notini, Kai H. Edel, Stefan Weinl, Luciano Freschi, Daniel F. Voytas, Jörg Kudla, and Lázaro Eustáquio Pereira Peres (2018) "De Novo Domestication of Wild Tomato Using Genome Editing." **Nature Biotechnology** 36 (12): 1211–16. https://doi.org/10.1038/nbt.4272.

Zuo, Z. et al. Blue Light-Dependent Interaction of CRY2 with SPA1 Regulates COP1 activity and Floral Initiation in Arabidopsis. **Curr Biol.**, v. 21, n. 10, p. 841–847, 2011.

2. THE INFLUENCE OF THE DOMESTICATED ALLELE OF THE *FRUIT WEIGHT 11.3* (*FW11.3*) GENE ON TOMATO DEVELOPMENT

Abstract

Among the genes related to tomato domestication, the *FRUIT WEIGHT 11.3* (*FW11.3*) is one of the genetic determinants of the dramatic increase in fruit size. The protein coded by *FW11.3* was directly linked to cell expansion in the fruit mesocarp. Here we present evidences that the vegetative part of the plant is also influenced by different alleles of *FW11.3* obtained in a single genetic background, the cv Micro-Tom (MT). We observed that plants harbouring the domesticated *FW11.3* allele (*fw11.3*-D), which is linked to large fruits, underwent changes in both shoot and root systems, which were smaller compared to plants harbouring the wild type *FW11.3* allele (*fw11.3*-W). There was also a delay in flowering in MT-*fw11.3*-D, although it produces more ripe fruits at harvesting time. More importantly, *fw11.3*-D only increased the fruit size in situations where the number of fruits were controlled. When fruit set was not controlled, MT-*fw11.3*-D plants had smaller fruits, but the yield was increased due to the augmented number of fruits. Collectively, the results presented here point to a complex effect of *fw11.3*-D in different organs favoring productivity independent of fruit size.

Keywords: Tomato; Size fruit; Fruit weight;

2.1. Introduction

The domestication syndrome represents the characteristics by which cultivated species differ from their wild ancestors (Evans, 1996). Most of these characteristics are selected under the pressure of cultivation over the years (Fuller, 2007). This pressure triggered diversification, which led to the subsequent evolution of new varieties (Meyer and Purugganan, 2013). New varietis are thus produced by a pyramid of mutations and recombinants, providing a series of phenotypic changes and facilitating the reproduction, cultivation and storage of cultivars (Fernie & Yan, 2019).

In the Solanaceae family, tomatoes, whose closest wild ancestor is *Solanum pimpinellifolium* (Jose Blanca et al., 2012), are among the species that present the greatest diversity in the morphology of the fruits, and among the main characteristics selected specifically during the domestication is the fruit weight (Paran & Van Der Knaap, 2007b). This characteristic was strongly selected in *Solanum lycopersicum* var. *cerasiforme* in the Andean region of Ecuador and northern Peru even before the domestication of tomatoes in Mesoamerica (José Blanca et al., 2015). A recent study showed that the pre-domesticated form of *S. lycopersicum* var. *cerasiforme* originated in South America 78.000 years ago and spread to North America, originating, 7.000 years ago, the cultivated *S. lycopersicum* in Mexico (Razifard et al., 2020). In continuous selections, genes related to the size and shape of the fruit led to an increase of up to 100 times the weight of the fruits when compared to the ancestor (Tanksley, 2004). Many factors interfere with the size and final fruit shape throughout plant development. Fruit development begins during the formation of inflorescences and subsequent development of the floral meristem. At the anthesis, all floral organs are already formed and most cell division already occurred in tomato ovary (Tanksley, 2004). After pollination, both cell division and expansion contribute to the tomato fruit develops until it reaches maturation, a process that can last up to 7 weeks depending on the genotype (Xiao et al., 2009).

During the domestication of plant species, meristems were regulated in such a way that larger meristems tend to form large fruits (Rodríguez et al., 2011). However, there are other regulatory points that occur before or after anthesis that also interfere with fruit size and weight, such as cell division, organization and expansion (Krizek, 2009; van der Knaap et al., 2014). Therefore, fruit size is likely a polygenic characteristic, although it does not exclude the presence of "major genes" of great effect.

Among the major genes controlling fruit size, *FRUIT WEIGHT 11.3* (*FW11.3*) (Solyc11g071940) codes for a *CELL SIZE REGULATOR* (*CSR*) (Mu et al. 2017). The protein derived from this regulator has not yet been well characterized, but it is known that it is involved in cell expansion. The mutant allele, selected during domestication, encodes a shorter protein due to an exclusion of 1406 kb at the 3' end and an addition of 22 bp, resulting in a loss of 194 amino acids. This allele appeared in *S. lycopersicum* var *cerasiforme* and is found in many tomato cultivars, suggesting that this was a fundamental selection for the complete domestication of tomatoes from their intermediate ancestors (Mu et al., 2017). The domesticated allele of *FW11.3* (*fw11.3*-D) is partially dominant over the wild type allele (*fw11.3*-W) (Huang & van der Knaap, 2011).

The regulation of fruit size by the *FW11.3* gene takes place by increasing the pericarp areas, making it expanded and resulting in an increase in the size of the mesocarp cells, but there is no increase in the number of cell layers. The expression of this regulator is undetectable in the period of development of the floral meristem until the rapid cell proliferation stage right after anthesis. Although it is low, the expression can be detected during fruit growth and around vascular bundles, which coincides with the fruit maturation period, in which there is cell expansion increase (Mu et al., 2017).

Even though there are reports of the influence of *FW11.3* gene on fruit development (Mu et al., 2017), its influence on other parts of the tomato plant is hitherto unknow. Here we present evidences that the *FW11.3* gene and its different alleles affect not only the fruits, but also the shoot and root system, suggesting more pleiotropic effects of *FW11.3* that may have been selected during domestication.

2.2. Materials and methods

2.2.1 Plant material

The tomato cv. Micro-Tom (MT), which has the wild type allele for the *FW11.3* gene (Solyc11g071940) was use as the control in experiments and as the recurrent parental during the introgression of the domesticated *FW11.3* allele (*fw11.3*-D). For the introgression *fw11.3*-D, MT flowers were emasculated and fertilized with pollen from *S. lycopersicum* cv. Caqui using the same procedure described previously (Carvalho et al., 2011; Pino et al., 2010). Plants from the resulted F1 generation were use as pollen donors to pollinate MT flowers, originating the BC1 generation. The process was repeated until the BC6F2 generation was obtained. The cv Caqui also has the *fas* mutation (Solyc11g071380), which is linked to *fw11.3*-D, and was used as a morphological marker during the introgression process. MT-*fas* BC6Fn plants were crossed with MT and the resulted BC7 plants were allowed to self-pollinate. In BC7F2 and BC7F3, recombinant plants were screened for the absence of the *fas* phenotype and the presence of the *fw11.3*-D. CAPS/SCAR markers were used to confirm the presence of *fw11.3*-D in the homozygous form (fw11,3_Fwd CAATAGTCTCCATGCTCAACG and fw11,3_Rev CTGTCATAGAAACATATAAAAGG) and the absence of the *fas* allele (fas_clv3_Fw CCAATGATAATTAAGATATTGTGACG, and fas_clv3_P2 GTCACTCTGTCTTGTTACTGC and fas_clv3_Rv2 ATGGTGGGGTTTTCTGTTCA).

2.2.2 Characterization of the *fw11,3* mutant

2.2.2.1 Plant growth conditions

The plants were kept in a growth room with a photoperiod of 16h, luminous intensity of approximately 200 μmol m^{-2} s^{-1}, containing two LED lamps with blue and red light per shelf, and an average temperature of 28°C. For all experiments, ten plants of each genotype were used.

The seeds were germinated in 350 mL pots containing a mixture of commercial substrate Basaplant HT (Base Agro) and vermiculite (1: 1), enriched with 1 g / L of NPK 10:10:10 and 4 g / L of limestone. At 15 days the plants were individualized in 350 ml pots containing same mixture of commercial substrate and vermiculite, enriched with 2 g / L of NPK 20:20:20 and 8 g / L of limestone. The plants were kept in trays containing the same amount of water. The plants were fertilized again with NPK at the beginning of flowering and during fruit development.

2.2.2.3 Growth analysis

The measurements of the vegetative part were performed 40 days after seed germination. The height measurements were done measuring the distance from the base of the stem on the substrate to the top of the plant, which is composed, by the sympodial branch and the first inflorescence. The number of inflorescences, flowers, leaves up to the first inflorescence and the total number of leaves were evaluated, as well as the length of the largest root. The leaf area was determined using a leaf area meter (LI-3100C). The leaves, stem, fruits and roots were packed separately and placed in an oven with air circulation at 70°C until they reached constant weight, and then the dry mass was determined using an analytical balance. Flower time analyses were made in the same plants starting the observation 32 days after germination.

2.2.2.4 Fruit analysis

The productivity parameters were done in plants 116 days after germination. The frequency of green and ripe fruits at the time of harvest was determined. The fruit height and diameter were determined in 70 ripe fruits of each genotype using a caliper. The same fruits were weighed in analytical balance and their content of soluble solids (Brix index) were measured with the aid of a digital refractometer (Atago PR-101α, Bellevue, WA). The roots of the plants were measured with the aid of a ruler, and the leaf area was measured.

In order to observe whether the *fw11.3*-D interferes with fruit size or weight, a controlled fruit experiment was carried out. Plants were kept with 3, 6 and 9 fruits in each genotype. In order to do this, the first flowers of each inflorescence were pollinated manually and the rest discarded, trying to distribute an equal amount of pollinated flowers in each inflorescence of the plant. All the side shoots (buds) that developed were removed. The evaluation took place 92 days after germination. The fruits were weighed and their height and diameter were measured, as well as their content of soluble solids.

2.2.3 qRT-PCR

Gene expression analyzes were performed on young leaf, mature leaf and root, using a Rotor-Gene Q (Qiagen) real-time PCR cycler, the mix used was the Kapa Sybr Fast qPCR Master Mix (Kapa Biosystems). The specific primers used for *FW11,3* (Solyc11g071940) were: fw11,3_Fwd CAATAGTCTCCATGCTCAACGC and fw11,3_Rev CAAACTCTTCAACTTCATCCTCGC. The reactions were amplified for 2 min at 95°C, followed by 40 cycles of 95°C for 15 s and 60°C for 30 s. The threshold cycle (CT) was determined. The analysis of the fusion curve was performed with each set of primers to confirm the presence of only a single peak before the analysis of gene expression. Two technical replicates were analyzed for each of the three biological samples. The expression has been normalized for the ACTIN gene (Solyc04g011500) with the following primers: actin_Fwd GGTCCCTCTATTGTCCACAG and actin_Rev TGCATCTCTGGTCCAGTAGGA. The folding changes for each gene were calculated using the equation 2- ΔΔCT (Livak and Schmittgen, 2001).

2.3 RESULTS

The locus where *fw11.3*-D is located is very close to the locus where the *fasciated* mutation is found (Huang and van der Knaap, 2011). Initially, the *fasciated* mutation was was introgressed until the BC6Fn generation using the cv Micro-Tom (MT) as the recurrent parental. This genotype, called MT-fas BC6Fn, was crossed with MT producing BC7 plants that were allowed to self-pollinate, producing the BC7F2 generation. We tracked these BC7F2 plants for the absence of the faciated phenotype, which were self-pollinated producing the BC7F3 generation. BC7F3 plants were than screened, using molecular markers, for the selection of plants that contained only the *fw11.3-D* in the homozygous form and the absence of the *fas* allele (Fig.S1).

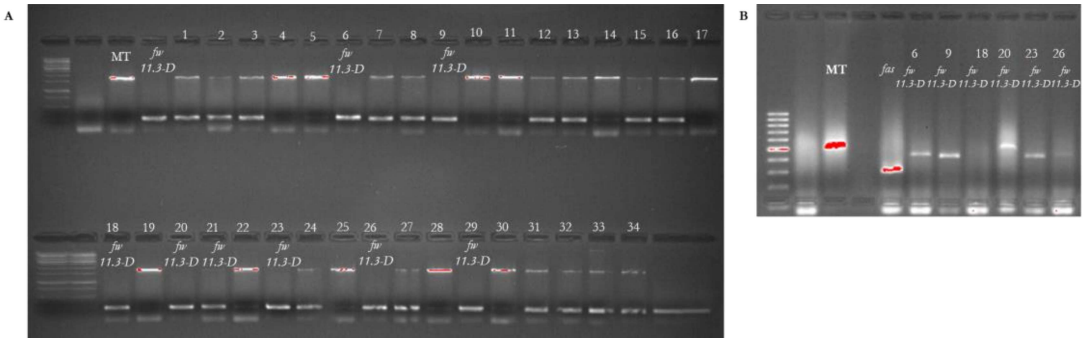

Figure S1. Micro-Tom plants (BC7F3) segregating for the *fw11.3*-D allele from *S. lycopersicum* cv. Caqui. A) Homozygous plants were indicated as *fw11.3-D*. B) *fw11.3*-D plants from A were screened for the absence of the *fas* allele.

The BC7F3 plants containing the *fw11.3*-D were selfed and the subsequent generations (BC6Fn) generations were used for phenotypic characterization comparing them with the near isogenic line (NIL) MT as a control. At 40

days after germination, *fw11.3*-D plants showed a significant reduction in plant height (Fig. 1), number of side shoots and number of leaves per plant (Tab. 1), as well as, root length (Tab. 1 and Fig. 1) in relation to the control MT, which contains the WT allele (*fw11.3*-WT). No significant differences were observed for plant height up to the first inflorescence, plant height up to the branching of the main stem, number of leaves in the main stem and total leaf area, although this last parameter tended to be reduced in MT-*fw11.3-D* plants (Tab. 1).

Figure 1. Shoot and root phenotype of Micro-Tom (MT) plants and plants harbouring the domesticated *FW11.3* allele (*fw11.3-D*) 40 days after germination (DAG). (A) Representative shoot system of MT and MT-*fw11.3*-D genotypes. (B) Representative root system of MT and MT-*fw11.3*-D genotypes. Scale bar = 1cm.

Table 1. Characterization of morphological parameters of wild type Micro-Tom (MT) and *fruit weight 11.3-D* plants *(fw11.3-D)* 40 days after germination (DAG). Asterisks indicate significant differences according to Student's t-test. $P < 0.05$ (*), $P<0.01$ (**) and $P < 0.001$ (***), (n=8).

	Plant height (cm)	Plant height 1st inflorescence (cm)	Branch height (cm)	Number of side shoots	Total leaves per plant	Leaves on the main stem	Total leaf area (cm^2)	Root length (cm)
MT	14,2	9,3	6,3	7,8	11,7	6,1	288,9	28,17
fw 11.3-D	13,0 *	9,5	7,2	6,4 **	10,2 ***	6,4	261,83	19,93 **

MT plants harbouring the domesticated *FW11.3* allele presented a delay in flowering and/or flower development, since they presented only 20% of plants in anthesis when the control MT have 70% of plants at this stage of flowering (Fig. 2).

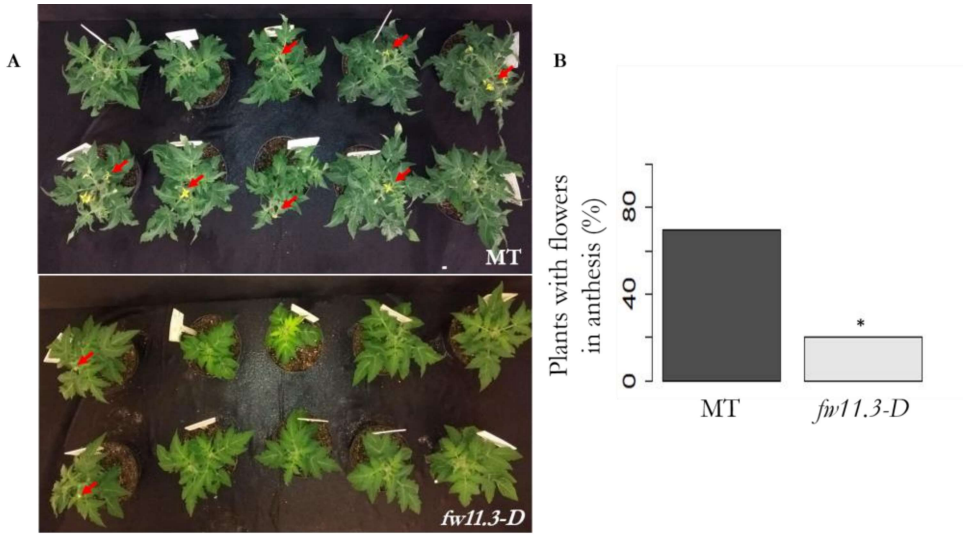

Figure 2. Plants with flowers in anthesis in Micro-Tom (MT) and MT-*fw11.3*-D plants. (A) Plant overview 32 days after germination (DAG) showing the first flowers in anthesis (red arrowhead). (B) Percentage of plants with flowers in anthesis. Asterisk indicates significant difference according to Student's t-test from Micro-tom (MT). P < 0.05 (*). Values are means ± standard error of 10 replicates. Scale bar= 1cm.

The dry mass of leaves, stem and roots of MT-*fw11.3-D* plants were significantly smaller than the control MT plants (Fig. 3), which is in accordance to their observed reduced plant size (Fig. 1).

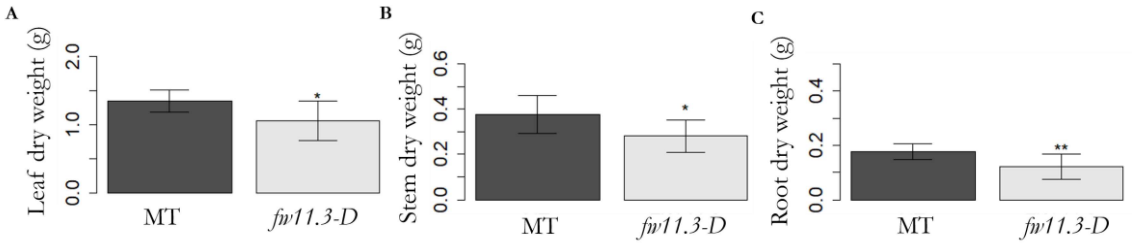

Figure 3. Dry weight of Micro-Tom (MT) plants harbouring the domesticated *FW11.3* allele (*fw11.3*-D) at 40 days after germination. (A) Total leaf dry weight. (B) Stem dry weight. (C) Root dry weight. Asterisks indicate significant differences according to Student's t-test. $P < 0.05$ (*), $P < 0.01$ (**). Values are means ± standard error of 8 replicates.

At 54 DAG, it is already possible to notice a significant decrease in the total leaf area of MT-*fw11.3-D* (Fig. S2), confirming a tendency already observed at 40 DAG (Tab. 1). The lower stem and root dry mass observed in MT-*fw11.3-D* at 40 DAG was also confirmed in plants at 54 DAG (Fig. S2). There was no significant differences for root length (Fig. S2B) and leaf dry mass (Fig. S2D).

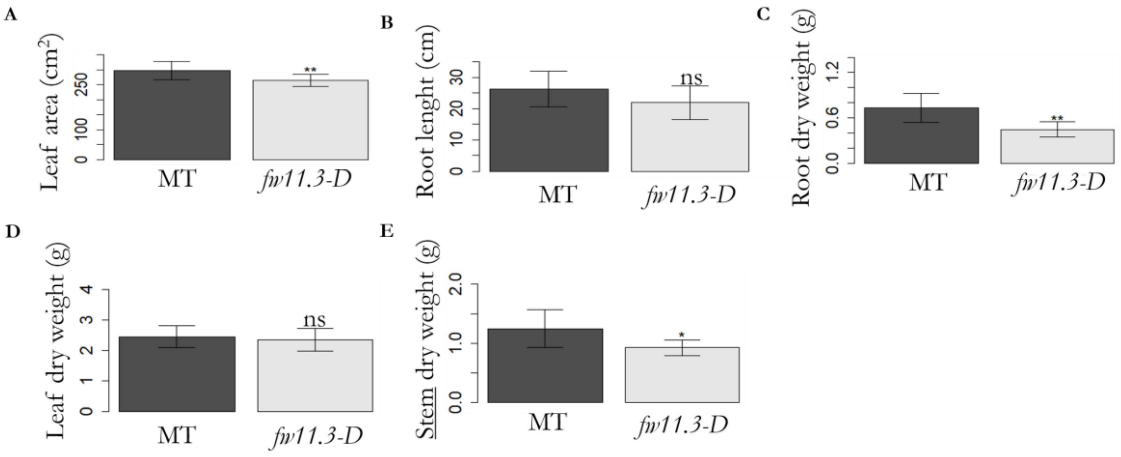

Figure S2. Growth parameters of Micro-Tom (MT) plants harbouring the domesticated *FW11.3* allele (*fw11.3*-D) at 54 days after germination (DAG). (A) Total leaf area. (B) Root length. (C) Root dry weight. (D) Total leaf dry weight. (E) Stem dry weight. Asterisks indicate significant differences according to Student's t-test. P <0.05 (*), P <0.01 (**). Values are means ± standard error of 8 replicates.

The reduced leaf area observed in MT-*fw11.3-D* at 56 DAG was confirmed in plants 116 DAG (Fig. 4). The shorter root length observed in MT-*fw11.3-D* plants at 40 DAG was also confirmed in plants at 116 DAG. Although MT-*fw11.3-D* plants presented reduced stem and root dry weight at both 40 and 56 DAG, these differences were no longer significant at 116 DAG, probably due to a buffering effect in older plants imposed by the size of the pot. On de other hand, MT-*fw11.3-D* plants presented a significant lower leaf dry weight at 116 DAG (Fig. 4), which was also seen at 40 DAG.

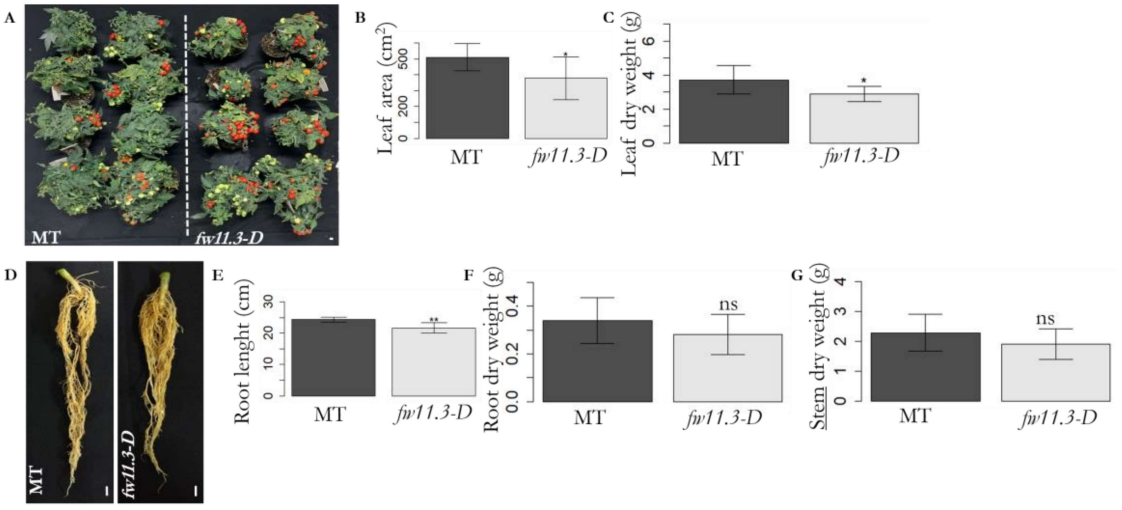

Figure 4. Growth parameters of Micro-Tom (MT) plants harbouring the domesticated *FW11.3* allele (*fw11.3*-D) at 116 days after germination (DAG). (A) General aspect of MT and MT- *fw11.3*-D plants. (B)Total leaf area (C) Total leaf dry weight (D) Root phenotype. (E) Root length. (F) Root dry weight. (G) Stem dry weight. Asterisks indicate significant differences according to Student's t-test. P < 0.05 (*), P < 0.01 (**). Values are means ± standard error of 8 replicates and 70 fruits for brix. Scale bar= 1cm.

MT-*fw11.3-D* showed a greater number of ripe fruits when compared to MT (Fig. 5). However, the mean fruit dry weight of MT-*fw11.3-D* fruits was smaller than MT, which could be either an intrinsic characteristic of MT-*fw11.3-D* fruits or the effect of source-sink limitations due to the increased amount of fruits. The Brix of MT-*fw11.3-*

D were also lower when compared to MT (Fig. 5F), favoring the hypothesis of a source-sink limitation. The total fruit dry weight of MT-*fw11.3-D* was increased when compared to MT, which evidences that the great number of fruits in this genotype (Fig. 5B and 5D) compensates for their individual small size (Fig. 5C and 5G).

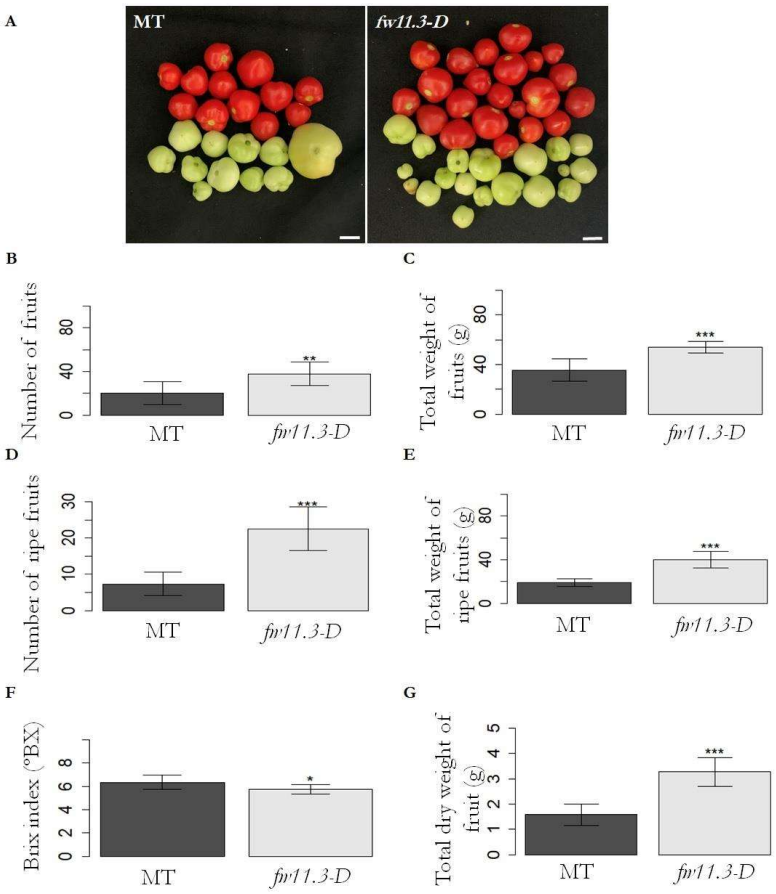

Figure 5. Fruit production of Micro-Tom (MT) and MT-*fw11.3*-D genotypes. (A) Fruit yield per plant. (B) Number of fruits per plant (ripe an unripe). (C) Total weight of fruits per plant (ripe and unripe). (D) Number of ripe fruits per plant. (E) Total weight of ripe fruits per plant. (F). Brix index (ºBrix). (G) Total fruit dry weight per plant. Fruits were collected from plants 116 days after germination (DAG). Asterisks indicate significant differences according to Student's t-test . $P < 0.001$ (***). Values are means ± standard error of 8 replicates. Scale bar= 1cm.

It is possible to observe a great heterogeneity of the size of the fruits in of MT-*fw11.3-D* (Fig. 5A), compared to MT. However, when analyzing the average diameter and height of selected fruits in each genotype, no significant differences were found between MT-*fw11.3-D* and MT (Fig. 6).

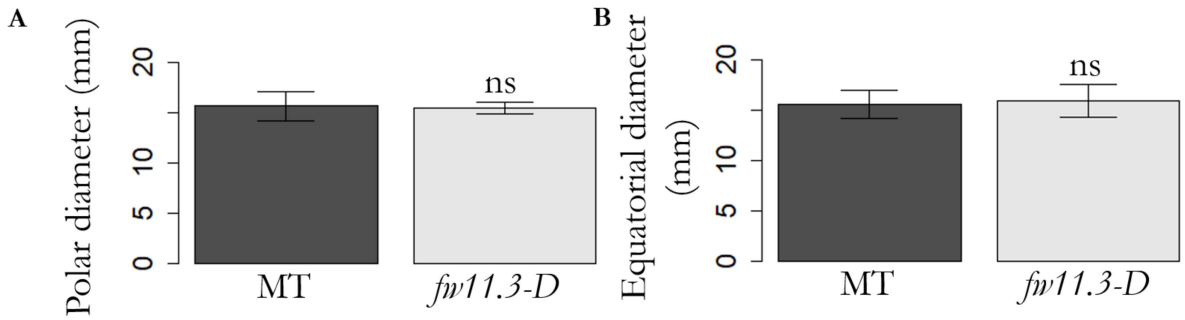

Figure 6. Fruit dimensions of ripe fruits of Micro-Tom (MT) and MT-*fw11.3*-D genotypes. (A) Polar diameter of the fruit. (B) Equatorial diameter of the fruit. Fruits were collected from plants 116 days after germination (DAG). ns= not significant differences according to Student's t-test from. Values are means ± standard error of 70 fruits (n=70).

To further study the impact of the *fw11.3-D* allele on the size of fruits, a controlled fruit experiment was conducted in which only 3, 6 or 9 fruits per plant were maintained (Fig. 7). No significant differences were observed for size, diameter, and fruit brix between *MT-fw11.3-D* and MT (Tab. 2). With the exception of the lower brix of *fw11.3-D* when with 3 fruits.

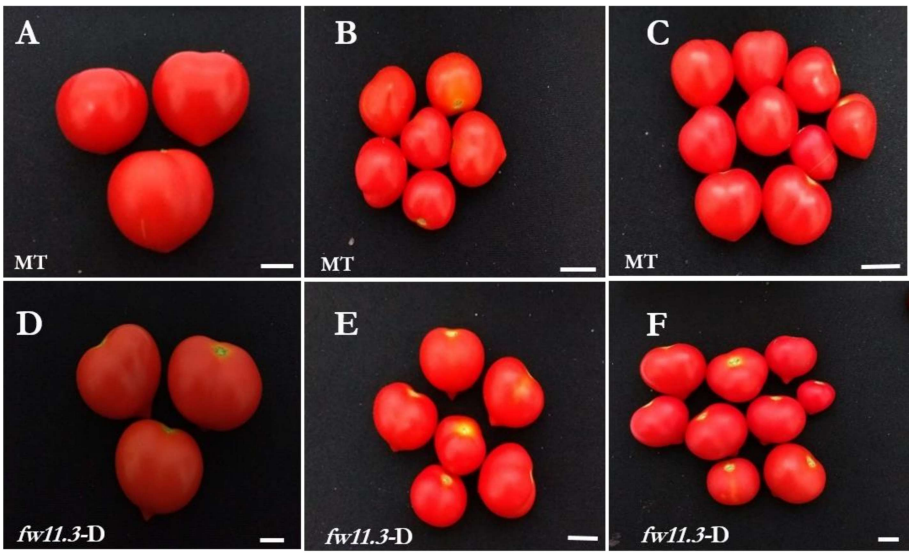

Figure 7. Fruit phenotype of Micro-Tom (MT) and MT-*fw11.3*-D genotypes in experiment with controled number of fruits. Number of fruits per MT plant: 3 (A), 6 (B) and 9(C). Number of fruits per MT-*fw11.3*-D plants: 3 (D), 6 (E) and 9 (F). Scale bars= 1cm. *92 days after germination.*

However, in the treatment where 9 fruits were maintained per plant, it can be seen that the biggest fruits of *fw11.3-D* were not only heavier in relation to MT, but also have greater height and larger diameter (Tab. 2).

Table 2. Fruit parameters of Micro-Tom (MT) and the MT line harbouring the *fw11.3-D* allele at different source-sink relationships. Three, six and nine were maintained per plant. The values represent the mean of the fruits, as well as the values of the biggest fruits of each group. Asterisks indicate significant differences according to Student's t-test. P < 0.05 (*), P<0.01 (**) and P < 0.001 (***), (n=8).

		Polar diameter fruit (mm)		Equatorial diameter fruit (mm)		Fruit weight (g)		Brix index (ºBrix)	
		Average fruits	Biggest fruit	Average fruits	Biggest fruit	Average fruits	Biggest fruit	Average fruits	Biggest fruit
3 Fruits	MT	19,76	22,41	21,62	24,65	5,26	6,97	5,44	5,32
	fw11.3-D	19,40	23,83	20,49	25,21	4,26	6,70	4,7 **	4,45 *
6 Fruits	MT	18,55	21,08	19,98	24,04	4,69	6,54	5,31	4,92
	fw11.3-D	19,06	22,45	19,08	23,15	3,5 *	5,78	5,41	4,67
9 Fruits	MT	17,36	20,65	18,43	22,19	3,52	5,17	5,09	4,73
	fw11.3-D	17,93	23,90 ***	18,14	24,76 **	3,48	6,85 **	5,31	4,63

Since the impact of the *fw11.3-D* allele was observed not only in fruits but also in vegetative parts such as leaves and roots, we sought to verify the expression of *FW11.3* gene in these organs. Both in young and mature leaves it is possible to observe a greater expression of the *FW11.3* gene in the MT-*fw11.3-D* plants compared to MT, but there was no significant gene expression differences in the roots (Fig. 8). This pattern of expression is very similar to the ones presented in public databases, which evidences the higher expression in mature leaves and very low expression in roots (Fig. S3).

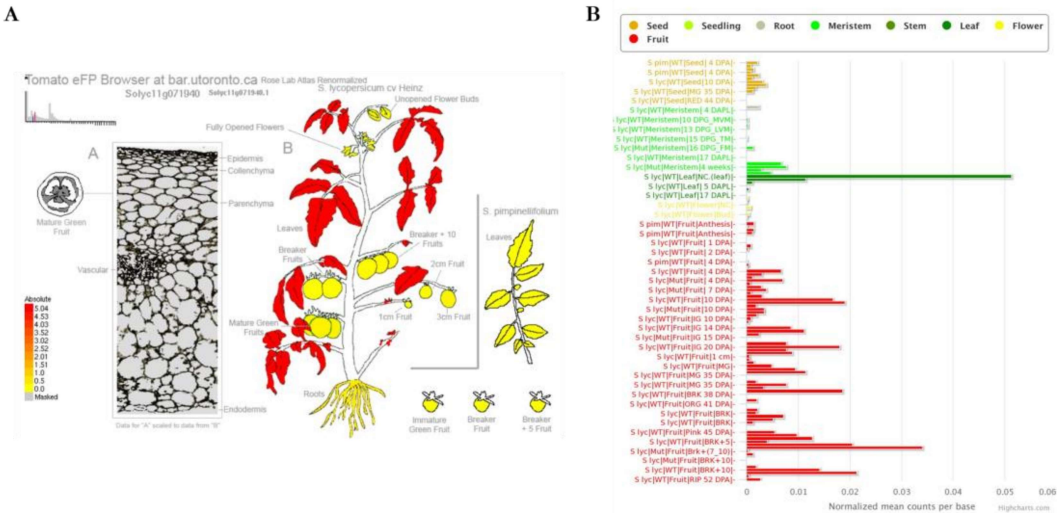

Figure S3. Expression of the *FW11.3* gene in different tomato organs from public databases. A) Data extracted from the eFP Browser (Tomato Genome Consortium, 2012) showing expression in *Solanum lycopersicum* cv. Heinz and *S. pimpinellifolium*. B) Data extracted from the TomExpress (Zouine et al., 2017) showing normalized expression in different genotypes and conditions.

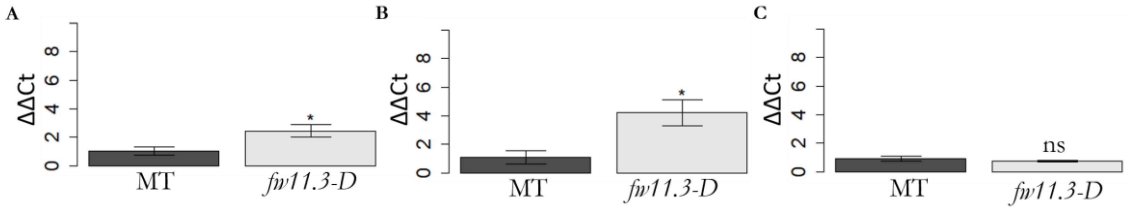

Figure 8. Expression of the *FW11.3* gene in leaves and root of Micro-Tom (MT) and MT-*fw11.3*-D genotypes. *(A)* Relative expression in young leaves. *(B)* Relative expression in mature leaves. (C) Relative expression in roots. Values are mean ± SD of three biological repetitions (n=3). Asterisks indicate significant differences according to Student's t-test. $P < 0.05$ (*). ns= not significant.

2.4. Discussion

In this work, we present evidences that the domesticated allele of the *FW11.3* gene (*fw11.3*-D), previously described as increasing fruit size (Mu et al., 2017), has an associated phenotype of small vegetative organs such as stem, leaves and roots. Regarding the smaller leaf area of plants harbouring the *fw11.3*-D allele, although it was not significant at 40 DAG, the differences became consistently significant along the plant development. This coincides with the onset of fruit development and suggests that source-sink relationships maybe involved. It is conceivable that smaller leaf areas can lead to less photoassimilate availability to other plant parts, including the fruits (Paul & Foyer, 2001). However, a recent report provided consistent evidences that photosynthesis, although positively related to Brix, can negatively affect fruit yield in tomato (Rowland et al., 2020). This reconciles the high yield presented by MT-*fw11.3*-D plants to their reduced leaf area, which could negatively affect photosynthesis. Consistently, MT-*fw11.3*-D plants tend to present lower Brix values. Since Brix and yield are long known to be negatively correlated (Beckles, 2011), the lower Brix of MT-*fw11.3*-D fruits can be explained by its higher yield in experiment where the number of fruits was not controlled. However, MT-*fw11.3*-D also presented lower Brix when only 3 fruits were kept per plant. In this situation, it is unlikely that there was a negative impact of yield on Brix, suggesting that other mechanisms could be also restricting Brix values in MT-*fw11.3*-D.

Similarly, the small root system of plants harbouring the *fw11.3*-D allele could be the direct effect of the gene or, alternatively, it could be via an indirect mechanism. Considering the lower expression of *FW11.3* in roots and the absence of gene expression difference between the genotypes, an indirect mechanism conditioning smaller roots is likely to occur. Since the function of *FW11.3* is not fully known, it is difficult to propose a possible mechanism. However, considering that *FW11.3* is highly expressed on mature leaves, which are active sources of substance that can translocate to the roots (Turgeon and Wolf, 2009), it is likely that a shoot-derived signal, mediated by *FW11.3*, could be the modulator of root growth. One obvious candidate is the hormone auxin, whose basipetal transport in the stele or through the phloem can control root growth (Delker et al., 2008). Further studies comparing the hormonal status in the NILs produced here will shed more light on the impact of the *FW11.3* gene on root growth.

Plants presenting the *fw11.3*-D allele had an increased yield even though their fruits were smaller when the number of fruits were not controlled. This can be explained by the augmented number of fruits and the higher number of ripe fruits at harvesting time. Therefore, it is likely that *fw11.3*-D can increase productivity by either increasing the number of fruits or their size. It is conceivable that such a mechanism, although complex, is likely to be a desirable target during domestication, since this kind of flexibility could provide for more stable yields (Evans, 1996).

Contrary to previous results (Mu et al., 2017), the weight of the fruits from plants harbouring the *fw11.3*-D allele was lower in conditions when the number of fruits were not controlled. When we carried out the experiment with different numbers of fruits per plant to verify if there was a difference in the partition of the photoassimilates, we found that there is no difference between the fruit averages for polar diameter, equatorial diameter and fruit weight. Nevertheless, there was a great variation in the sizes of the *fw11.3*-D fruits that was not seen in MT fruits, which had more homogeneous sizes. When we analyzed only the largest fruit of each plant, in plants with 9 fruits it was possible to observe that fruits of *fw11.3*-D plants were larger than the fruits of MT plants, both in diameter and in weight, which is consistent with the literature (Mu et al., 2017; Huang and van der Knaap, 2011).

These results, together with the observation that the *FW11.3* also controls the growth of other plant organs, strongly indicate that this domestication-related gene has complex functions whose allele effects can affect productivity independent of fruit size. Among the developmental processes, anthesis and fruit ripening seems to be altered in *fw11.3*-D. Although *fw11.3*-D delayed the time to anthesis, it presented more ripe fruits when compared to MT. Therefore, it would be interesting to investigate the time taken from the anthesis to fruit ripening in *fw11.3*-D. Since *fw11.3*-D influenced the root system and had no differences in gene expression compared to MT in this organ, it would be interesting to investigate the occurrence of compounds, such as auxin, that could be regulated by the expression of the allele in the leaves and translocate to influence root development.

REFERENCES

Alpert, K. B., S. Grandillo, and S. D. Tanksley (1995) "Fw 2.2:A Major QTL Controlling Fruit Weight Is Common to Both Red- and Green-Fruited Tomato Species". **Theoretical and Applied Genetics** 91 (6–7): 994–1000. https://doi.org/10.1007/BF00223911.

Anwar, Raheel, Shazia Fatima, Autar K. Mattoo, and Avtar K. Handa (2019) "Fruit Architecture in Polyamine-Rich Tomato Germplasm Is Determined via a Medley of Cell Cycle, Cell Expansion, and Fruit Shape Genes." **Plants** 8 (10): 1–19. https://doi.org/10.3390/plants8100387.

Arikita, Fernanda Namie, Mariana Silva Azevedo, Danielle Camargo Scotton, Maísa de Siqueira Pinto, Antonio Figueira, and Lázaro Eustáquio Pereira Peres (2013) "Novel Natural Genetic Variation Controlling the Competence to Form Adventitious Roots and Shoots from the Tomato Wild Relative *Solanum Pennellii*." **Plant Science** 199–200: 121–30. https://doi.org/10.1016/j.plantsci.2012.11.005.

Azzi, Lamia, Cynthia Deluche, Frédéric Gévaudant, Nathalie Frangne, Frédéric Delmas, Michel Hernould, and Christian Chevalier (2015) "Fruit Growth-Related Genes in Tomato." **Journal of Experimental Botany** 66 (4): 1075–7086. https://doi.org/10.1093/jxb/eru527.

Beckles D. M. (2011) "Factors affecting the postharvest soluble solids and sugar content of tomato (Solanum lycopersicum L.)" fruit". **Postharvest Biol. Technol.**,63, 129-140, doi:10.1016/j.postharvbio.2011.05.016

Blanca, José, Javier Montero-Pau, Christopher Sauvage, Guillaume Bauchet, Eudald Illa, María José Díez, David Francis, Mathilde Causse, Esther van der Knaap, and Joaquín Cañizares (2015) "Genomic Variation in Tomato, from Wild Ancestors to Contemporary Breeding Accessions." **BMC Genomics** 16 (1): 257. https://doi.org/10.1186/s12864-015-1444-1.

Boccalandro, Hernán E., Edmundo L. Ploschuk, Marcelo J. Yanovsky, Rodolfo A. Sánchez, Christiane Gatz, and Jorge J. Casal. (2003) "Increased Phytochrome B Alleviates Density Effects on Tuber Yield of Field Potato Crops." **Plant Physiology** 133 (4): 1539–46. https://doi.org/10.1104/pp.103.029579.

Campos, Marcelo Lattarulo, Rogério Falleiros Carvalho, Vagner Augusto Benedito, and Lázaro Eustáquio Pereira Peres (2010) "Small and Remarkable." **Plant Signaling & Behavior** 5 (3): 267–70. https://doi.org/10.1093/jxb/erp270.ormones.

Carvalho, Rogério F, Marcelo L Campos, Lilian E Pino, Simone L Crestana, Agustin Zsögön, and Joni E Lima (2011) "Convergence of Developmental Mutants into a Single Tomato Model System : ' Micro-Tom ' as an Effective Toolkit for Plant Development Research," **Plant Methods**, 7:18, 1–14.

Chakrabarti, Manohar, Na Zhang, Christopher Sauvage, Stéphane Muños, Jose Blanca, Joaquin Cañizares, Maria Jose Diez, et al. (2013) "A Cytochrome P450 Regulates a Domestication Trait in Cultivated Tomato." **Proceedings of the National Academy of Sciences of the United States of America** 110 (42): 17125–30. https://doi.org/10.1073/pnas.1307313110.

Cong, Bin, Luz S Barrero, and Steven D Tanksley (2008) "Regulatory Change in YABBY-like Transcription Factor Led to Evolution of Extreme Fruit Size during Tomato Domestication." **Nature Genetics** 40 (6): 800–804. https://doi.org/10.1038/ng.144.

Cong, Bin, Jiping Liu, and Steven D Tanksley (2002) "Natural Alleles at a Tomato Fruit Size Quantitative Trait Locus Differ by Heterochronic Regulatory Mutations." **Proceedings of the National Academy of Sciences of the United States of America** 99 (21): 13606–11. https://doi.org/10.1073/pnas.172520999.

D'Agostino, I B, J Deruère, and Joseph J. Kieber (2000) "Characterization of the Response of the Arabidopsis Response Regulator Gene Family to Cytokinin." **Plant Physiology** 124 (4): 1706–17. https://doi.org/10.1104/pp.1244.1706.

Dayan, Jonathan, Nickolay Voronin, Fan Gong, Tai-ping Sun, Peter Hedden, Hillel Fromm, and Roni Aloni (2012) "Leaf-Induced Gibberellin Signaling Is Essential for Internode Elongation, Cambial Activity, and Fiber Differentiation in Tobacco Stems." **The Plant Cell** 24 (1): 66–79. https://doi.org/10.1105/tpc.111.093096.

Delker C, Raschke A, Quint M.(2008) "Auxin dynamics: the dazzling complexity of a small molecule's message". **Planta**, 227, 929–941.

Dirzo, Rodolfo, and Peter H. Raven (2003) "Global State of Biodiversity and Loss." **Annual Review of Environment and Resources** 28 (1): 137–67. https://doi.org/10.1146/annurev.energy.28.050302.105532.

Duarte, Carlos M., Nùria Marbá, and Marianne Holmer (2007) "Rapid Domestication of Marine Species." **Science** 316 (5823): 382–83. https://doi.org/10.1126/science.1138042.

Evans, L. T (1996) Crop Evolution, Adaptation and Yield. Cambridge University Press.

Falke, K. Christin, Shirin Glander, Fei He, Jinyong Hu, Juliette De Meaux, and Gregor Schmitz (2013) "The Spectrum of Mutations Controlling Complex Traits and the Genetics of Fitness in Plants." **Current Opinion in Genetics and Development** 23 (6): 665–71. https://doi.org/10.1016/j.gde.2013.10.006.

Fernie, Alisdair R., and Jianbing Yan (2019) "De Novo Domestication: An Alternative Route toward New Crops for the Future." **Molecular Plant** 12 (5): 615–31. https://doi.org/10.1016/j.molp.2019.03.016.

Forno, Ignacio Achon (2017) "Redefining the Hormonal Control of Tomato (Solanum Lycopersicum Cv. Micro-Tom) Fruit Development." University of São Paulo, "Luiz de Queiroz" College of Agriculture. http://www.teses.usp.br/teses/disponiveis/11/11140/tde-04012018-144113/en.php.

Frary, A, T C Nesbitt, S Grandillo, E Knaap, B Cong, J Liu, J Meller, R Elber, K B Alpert, and S D Tanksley (2000a) "Fw2.2: A Quantitative Trait Locus Key to the Evolution of Tomato Fruit Size." **Science** 289 (5476): 85–88. https://doi.org/10.1126/science.289.5476.85.

——— (2000b) "Fw2.2: A Quantitative Trait Locus Key To the Evolution of Tomato Fruit Size." **Science** 289 (5476): 85–88. https://doi.org/10.1126/science.289.5476.85.

Frary, Anne, and Sami Doganlar (2003) "Comparative Genetics of Crop Plant Domestication and Evolution Anne." **Turk J Agric For** 27: 59–69.

Freitas-astua, Juliana, Gustavo Astua-Monge, Jane Elisabeth Polston, and Ernest Hiebert (2003) "A Simple and Reliable Method for the Screening or Transgenic Tobacco Plants." **Pesquisa Atropecuaria Brasileira** 38 (7): 893–96.

Fuller, Dorian Q. (2007) "Contrasting Patterns in Crop Domestication and Domestication Rates: Recent Archaeobotanical Insights from the Old World." **Annals of Botany** 100 (5): 903–24. https://doi.org/10.1093/aob/mcm048.

Galati, V. C. et al. (2018). "Root-to-Shoot Communication to Modulate Source-Sink Relationship in Tomato Depends on Phytochromes" **Journal of Agricultural Science** 10 (8):174.

Giovannoni, J.(2018) "Tomato Multiomics Reveals Consequences of Crop Domestication and Improvement". **Cell**, 172 (1–2): 6–8.

Gonzalo, Maria Jose, Marin Talbot Brewer, Claire Anderson, David Sullivan, Simon Gray, and Esther Van Der Knaap (2009) "Tomato Fruit Shape Analysis Using Morphometric and Morphology Attributes Implemented in Tomato Analyzer Software Program." **Journal of the American Society for Horticultural Science** 134 (1): 77–87. https://doi.org/10.21273/jashs.134.1.77.

Gonzalo, Maria Jose, and Esther Van Der Knaap (2008) "A Comparative Analysis into the Genetic Bases of Morphology in Tomato Varieties Exhibiting Elongated Fruit Shape." **Theoretical and Applied Genetics** 116 (5): 647–56. https://doi.org/10.1007/s00122-007-0698-7.

Huang, Zejun, and Esther van der Knaap (2011) "Tomato Fruit Weight 11.3 Maps Close to Fasciated on the Bottom of Chromosome 11." **Theoretical and Applied Genetics** 123 (3): 465–74. https://doi.org/10.1007/s00122-011-1599-3.

Jefferson, R A, T A Kavanagh, and M W Bevan (1987) "GUS Fusions: Beta-Glucuronidase as a Sensitive and Versatile Gene Fusion Marker in Higher Plants." **The EMBO Journal** 6 (13): 3901–7. https://doi.org/10.1073/pnas.1411926112.

Jenkins, J. A. (1948) "The Origin of Cultivated Plants." **Economic Botany** 2 (4): 379–92. https://doi.org/https://doi.org/10.1007/BF02859492.

Jiang, Ning, Dongying Gao, Han Xiao, and Esther Van Der Knaap (2009) "Genome Organization of the Tomato Sun Locus and Characterization of the Unusual Retrotransposon Rider." **Plant Journal** 60 (1): 181–93. https://doi.org/10.1111/j.1365-313X.2009.03946.x.

Jinek, Martin, Krzysztof Chylinski, Ines Fonfara, Michael Hauer, Jennifer A Doudna, and Emmanuelle Charpentier (2012) "A Programmable Dual-RNA – Guided" **Science** 337 (August): 816–22.

Kharshiing, Eros, and Shriravi Prasad Sinha (2016) "Deficiency in Phytochrome A Alters Photosynthetic Activity, Leaf Starch Metabolism and Shoot Biomass Production in Tomato." **Journal of Photochemistry and Photobiology B: Biology** 165: 157–62. https://doi.org/10.1016/j.jphotobiol.2016.10.026.

Koshioka, M., T. Nishijima, H. Yamazaki, Y. Liu, M. Nonaka, and L. N. Mander (1994) "Analysis of Gibberellins in Growing Fruits of Lycopersicon Esculentum after Pollination or Treatment with 4-Chlorophenoxyacetic Acid." **Journal of Horticultural Science** 69 (1): 171–79. https://doi.org/10.1080/14620316.1994.11515263.

Krizek, Beth A. (2009) "Making Bigger Plants: Key Regulators of Final Organ Size." **Current Opinion in Plant Biology** 12 (1): 17–22. https://doi.org/10.1016/j.pbi.2008.09.006.

Lippman, Zachary, and Steven D. Tanksley (2001) "Dissecting the Genetic Pathway to Extreme Fruit Size in Tomato Using a Cross between the Small-Fruited Wild Species *Lycopersicon Pimpinellifolium* and *L. Esculentum* Var. Giant Heirloom." **Genetics** 158 (1): 413–22.

Liu, Jiping, Joyce Van Eck, Bin Cong, and Steven D. Tanksley (2002a) "A New Class of Regulatory Genes Underlying the Cause of Pear-Shaped Tomato Fruit." **Proceedings of the National Academy of Sciences of the United States of America** 99 (20): 13302–6. https://doi.org/10.1073/pnas.162485999.

Liu, Jiping, Joyce Van Eck, Bin Cong, and Steven D Tanksley (2002b) "A New Class of Regulatory Genes Underlying the Cause of Pear-Shaped Tomato Fruit." **Proceedings of the National Academy of Sciences of the United States of America** 99 (20): 13302–6. https://doi.org/10.1073/pnas.162485999.

Livak, Kenneth J., and Thomas D. Schmittgen (2001) "Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-ΔΔCT Method." **Methods** 25 (4): 402–8. https://doi.org/10.1006/meth.2001.1262.

Luo, Ming, Brian Gilbert, and Michael Ayliffe (2016) "Applications of CRISPR/Cas9 Technology for Targeted Mutagenesis, Gene Replacement and Stacking of Genes in Higher Plants." **Plant Cell Reports** 35 (7): 1439–50. https://doi.org/10.1007/s00299-016-1989-8.

Martin, L. B. B. et al. (2017) "Cuticle biosynthesis in tomato leaves is developmentally regulated by abscisic acid" **Plant Physiology**, 174 (3): 1384–1398.

Matsuo, Satoshi, Kaori Kikuchi, Machiko Fukuda, Ichiro Honda, and Shunsuke Imanishi (2012) "Roles and Regulation of Cytokinins in Tomato Fruit Methylation And." **Journal of Experimental Botany** 63 (15): 5569–79. https://doi.org/10,1093/jxb/ers207.

Meyer, Rachel S., Ashley E. Duval, and Helen R. Jensen (2012) "Patterns and Processes in Crop Domestication: An Historical Review and Quantitative Analysis of 203 Global Food Crops." **New Phytologist** 196 (1): 29–48. https://doi.org/10.1111/j.1469-8137.2012.04253.x.

Meyer, Rachel S., and Michael D. Purugganan (2013) "Evolution of Crop Species: Genetics of Domestication and Diversification." **Nature Reviews Genetics** 14 (12): 840–52. https://doi.org/10.1038/nrg3605.

Mu, Qi, Zejun Huang, Manohar Chakrabarti, Eudald Illa-Berenguer, Xiaoxi Liu, Yanping Wang, Alexis Ramos, and Esther van der Knaap (2017) "Fruit Weight Is Controlled by Cell Size Regulator Encoding a Novel Protein That Is Expressed in Maturing Tomato Fruits." *PLoS* **Genetics** 13 (8). https://doi.org/10.1371/journal.pgen.1006930.

Nesbitt, C. T., and Tanksley, S. D. (2002) "Comparative Sequencing in the Genus *Lycopersicon*: Implications for the Evolution of Fruit Size in the Domestication of Cultivated Tomatoes." **Genetics** 162 (1): 365–79.

Nesbitt, T. C., and S. D. Tanksley (2001) "Fw2.2 Directly Affects the Size of Developing Tomato Fruit, With Secondary Effects on Fruit Number and Photosynthate Distribution." **Plant Physiology** 127 (2): 575–83. https://doi.org/10.1104/pp.010087.

Paran, Ilan, and Esther Van Der Knaap (2007) "Genetic and Molecular Regulation of Fruit and Plant Domestication Traits in Tomato and Pepper." **Journal of Experimental Botany** 58 (14): 3841–52. https://doi.org/10.1093/jxb/erm257.

Pino, Lilian E, Simone Lombardi-Crestana, Mariana S Azevedo, Danielle C Scotton, Lucélia Borgo, Vera Quecini, Antonio Figueira, and Lázaro Ep Peres (2010) "The *Rg1* Allele as a Valuable Tool for Genetic Transformation of the Tomato 'Micro-Tom' Model System." **Plant Methods** 6: 23. https://doi.org/10.1186/1746-4811-6-23.

Piotto, Fernando Angelo, and Lázaro Eustáquio Pereira Peres (2012) "Base Genética Do Hábito de Crescimento e Florescimento Em Tomateiro e Sua Importância Na Agricultura." **Ciência Rural** 42 (11): 1941–46. https://doi.org/10.1590/S0103-84782012001100006.

Purugganan, Michael D., and Dorian Q. Fuller (2009) "The Nature of Selection during Plant Domestication." **Nature** 457 (7231): 843–48. https://doi.org/10.1038/nature07895.

Rodriguez, G. R., S. Munos, C. Anderson, S.-C. Sim, A. Michel, M. Causse, B. B. M. Gardener, D. Francis, and E. van der Knaap (2011) "Distribution of SUN, OVATE, LC, and FAS in the Tomato Germplasm and the Relationship to Fruit Shape Diversity." **Plant Physiology** 156 (1): 275–85. https://doi.org/10.1104/pp.110.167577.

Rowland S. D, Zumstein K., Nakayama H., Cheng Z., Flores A. M., Chitwood D. H., Maloof J. N., Sinha N. R.(2020) Leaf shape is a predictor of fruit quality and cultivar performance in tomato. **New Phytologist** 226: 851–865.

Saath, Kleverton Clovis de Oliveira, and Arlei Luiz Fachinello (2018) "Crescimento Da Demanda Mundial de Alimentos e Restrições Do Fator Terra No Brasil." **Revista de Economia e Sociologia Rural** 56 (2): 195–212. https://doi.org/10.1590/1234-56781806-94790560201.

Sastry, KKS, and RM Muir (1963) "Gibberelin: Effect on Diffusible Auxin in Fruit Development." **Science** 140: 494–95.

Shan, Qiwei et al. (2013) "Targeted Genome Modification of Crop Plants Using a CRISPR-Cas System" **Nature Biottechnology** 31 (8): 8–10. https://doi.org/10.1038/nbt.2650.

Stepanova, A. N., J. Yun, A. V. Likhacheva, and J. M. Alonso (2007) "Multilevel Interactions between Ethylene and Auxin in Arabidopsis Roots." **The Plant Cell Online** 19 (7): 2169–85. https://doi.org/10.1105/tpc.107.052068.

Tang, Yun jia, and Johannes Liesche (2017) "The Molecular Mechanism of Shade Avoidance in Crops – How Data from Arabidopsis Can Help to Identify Targets for Increasing Yield and Biomass Production." **Journal of Integrative Agriculture** 16 (6): 1244–55. https://doi.org/10.1016/S2095-3119(16)61434-X.

Tanksley, Steven D. (2004) "The Genetic, Developmental, and Molecular Bases of Fruit Size and Shape Variation in Tomato." **Plant Cell** 16 181-190 (SUPPL.): 181–90. https://doi.org/10.1105/tpc.018119.

Thiele, Alexandra, Michael Herold, Ingo Lenk, Peter H. Quail, and Christiane Gatz (1999) "Heterologous Expression of Arabidopsis Phytochrome B in Transgenic Potato Influences Photosynthetic Performance and Tuber Development." **Plant Physiology** 120 (1): 73–81. https://doi.org/10.1104/pp.120.1.73.

Tomato Genome Consortium (2012) "The tomato genome sequence providesinsights into fleshy fruit evolution". **Natur**e 485:635–641.

Turgeon R. & Wolf S. (2009) "Phloem transport: cellular pathways and molecular trafficking". **Annual Review of Plant Biology** 60: 207–221.

Ulmasov, Tim, Jane Murfett, Gretchen Hagen, and Tom J Guilfoyle (1997) "Creation of a Highly Active Synthetic AuxRE." **Society** 9 (November): 1963–71. https://doi.org/10.1105/tpc.9.11.1963.

Van der Knaap, E. et al. (2014) "What lies beyond the eye: the molecular mechanisms regulating tomato fruit weight and shape". **Frontiers in plant science** 5 (May): 227.

Van der Knaap, E.; Tanksley, S. D. (2003) "The making of a bell pepper-shaped tomato fruit: Identification of loci controlling fruit morphology in Yellow Stuffer tomato". **Theoretical and Applied Genetics** 107 (1): 139–147.

Wang, Shucai, Ying Chang, Jianjun Guo, and Jin Gui Chen (2007) "Arabidopsis Ovate Family Protein 1 Is a Transcriptional Repressor That Suppresses Cell Elongation." **Plant Journal** 50 (5): 858–72. https://doi.org/10.1111/j.1365-313X.2007.03096.x.

Wang, Shucai, Ying Chang, Jianjun Guo, Qingning Zeng, Brian E. Ellis, and Jin Gui Chen (2011) "Arabidopsis Ovate Family Proteins, a Novel Transcriptional Repressor Family, Control Multiple Aspects of Plant Growth and Development." **PLOS ONE** 6 (8) e23896. https://doi.org/10.1371/journal.pone.0023896.

Wang, Yanping, Josh P. Clevenger, Eudald Illa-Berenguer, Tea Meulia, Esther van der Knaap, and Liang Sun (2019) "A Comparison of Sun, Ovate, Fs8.1 and Auxin Application on Tomato Fruit Shape and Gene Expression." **Plant and Cell Physiology** 60 (5): 1067–81. https://doi.org/10.1093/pcp/pcz024.

Wu, Shan, Han Xiao, Antonio Cabrera, Tea Meulia, and Esther van der Knaap (2011) "SUN Regulates Vegetative and Reproductive Organ Shape by Changing Cell Division Patterns." **Plant Physiology** 157 (3): 1175–86. https://doi.org/10.1104/pp.111.181065.

Xiao, Han, Ning Jiang, Erin Schaffner, Eric J. Stockinger, and Esther Van Der Knaap (2008) "A Retrotransposon-Mediated Gene Duplication Underlies Morphological Variation of Tomato Fruit." **Science** 319 (5869): 1527–30. https://doi.org/10.1126/science.1153040.

Xiao, Han, Cheryll Radovich, Nicholas Welty, Jason Hsu, Dongmei Li, Tea Meulia, and Esther Van Der Knaap (2009) "Integration of Tomato Reproductive Developmental Landmarks and Expression Profiles, and the Effect of SUN on Fruit Shape." **BMC Plant Biology** 9:1.21. https://doi.org/10.1186/1471-2229-9-49.

Xu, Cao, Katie L. Liberatore, Cora A. MacAlister, Zejun Huang, Yi-Hsuan Chu, Ke Jiang, Christopher Brooks, et al. (2015) "A Cascade of Arabinosyltransferases Controls Shoot Meristem Size in Tomato." **Nature Genetics** 47 (7): 784–92. https://doi.org/10.1038/ng.3309.

Zhang, Zengcui, Aili Li, Gaoyuan Song, Shuaifeng Geng, Bikram S. Gill, Justin D. Faris, and Long Mao (2020) "Comprehensive Analysis of Q Gene Near-Isogenic Lines Reveals Key Molecular Pathways for Wheat Domestication and Improvement." **Plant Journal** 102 (2): 299–310. https://doi.org/10.1111/tpj.14624.

Zhang, Zhi, Cuiyu Mao, Zheng Shi, and Xiaohong Kou (2017) "The Amino Acid Metabolic and Carbohydrate Metabolic Pathway Play Important Roles during Salt-Stress Response in Tomato" **Frontiers in Plant Science** 8 (July): Article 1231. https://doi.org/10.3389/fpls.2017.01231.

Zhu, Guangtao, Shouchuang Wang, Zejun Huang, Shuaibin Zhang, Qinggang Liao, Chunzhi Zhang, Tao Lin, et al. (2018). "Rewiring of the Fruit Metabolome in Tomato Breeding." **Cell** 172 (1–2): 249-261.e12. https://doi.org/10.1016/j.cell.2017.12.019.

Zhu, Mingku, Guoping Chen, and Jianling Zhang (2014) "The Abiotic Stress-Responsive NAC-Type Transcription Factor SlNAC4 Regulates Salt and Drought Tolerance and Stress-Related Genes in Tomato (Solanum Lycopersicum)" **Plant Cell Rep** 33:1851–63. https://doi.org/10.1007/s00299-014-1662-z.

Zouine M, Maza E, Djari A, Lauvernier M, Frasse P, Smouni A, Pirrello J and Bouzayen M. (2017) "TomExpress, a unified tomato RNA-Seq platform for visualization of expression data, clustering and correlation networks". **Plant Journal**. 92: 727–735.

Zsögön, Agustin, Tomáš Čermák, Emmanuel Rezende Naves, Marcela Morato Notini, Kai H. Edel, Stefan Weinl, Luciano Freschi, Daniel F. Voytas, Jörg Kudla, and Lázaro Eustáquio Pereira Peres (2018) "De Novo

Domestication of Wild Tomato Using Genome Editing." **Nature Biotechnology** 36 (12): 1211–16. https://doi.org/10.1038/nbt.4272.

Zuo, Z. et al. (2011) "Blue Light-Dependent Interaction of CRY2 with SPA1 Regulates COP1 activity and Floral Initiation in Arabidopsis". **Curr Biol.**, 10: 841–847.

3. EVIDENCE THAT THE *SUN* AND *OVATE* GENES INFLUENCE DIFFERENT HORMONAL PATHWAYS WITH EMPHASIS ON THE CYTOKININ

Abstract

The huge variety of tomato fruits on today´s supermarket shelves is the result of a long process of domestication and breeding. The *SUN* and *OVATE* genes are among the major controllers of tomato fruit shape diversity, contributing to more elongated fruits. The *SUN* gene codes for a positive growth regulator, and its mutated allele is a rare event of gene duplication from one chromosome to another that creates a gain-of-function mutation. The *OVATE* genes codes for a negative growth regulator, and the mutated allele comprises a premature stop codon probably creating a null allele. Both genes express in ovary at pre- and post-anthesis, but the highest expression is before anthesis for *OVATE* and after anthesis for *SUN*. Plant hormones are highly associated with changes in fruit shape, since they control the fundamental processes of cell division and expansion. Here we determined the expression of four hormonal classes (auxin, cytokinin, gibberellin and ethylene) at different stages of ovary/fruit development in the *ovate* and *sun* mutants. We find that the main hormonal change occurring during *ovate* fruit development was a reduction in the cytokinin status during the pre-anthesis and the early post-anthesis, a period of cell division in the ovary. There were also alterations in the auxin status in *ovate* in the period of the beginning of cell expansion in the ovary. In the case of *sun*, there was an early cytokinin peak in the period of cell division in ovaries at post-anthesis. These results indicate that at least part the effects of *sun* and *ovate* on fruit shape phenotype are mediated by cytokinin. The requirement of this hormone for cell division is likely a limiting factor favoring proximal-distal diameter and reducing equatorial diameter in *sun* and *ovate* mutants. In the specific case of ovate, it is suggested that auxin could also contribute to its distinct phenotype acting at cell expansion phase.

Keywords: Tomato; Fruit Shape; Plant hormones; Micro-Tom;

3.1. Introduction

The tomato, *Solanum lycopersicum*, is among the crops of great economic importance worldwide (Piotto and Peres, 2012). It represents the largest harvest of fruits and the most valuable vegetable in the world, contributing substantially to the human diet (G. Zhu et al., 2018). Tomato underwent a long domestication process to create the current varieties, in which the characteristics of greatest interest for human consumption, including fruit shape, were selected. (Anne Frary and Doganlar, 2003; Meyer and Purugganan, 2013).

The plant domestication process initiated more than 12,000 years ago, occurring at different times in different areas of the world (Meyer et al., 2012). There are already more than 250 species completely domesticated (Dirzo and Raven, 2003; Duarte, Marbá, and Holmer, 2007; Meyer and Purugganan, 2013), and several species share common domesticated traits (G. Zhu et al., 2018). The set of traits that distinguish domesticated crops from their wild ancestors is called domestication syndrome (Evans, 1996). A common theme in domestication is the occurrence of a great diversity of forms in a single species, reassembly that seen among different species in nature. Hence, the domestication provided a wide variety of fruits, which have different shapes and sizes. Most of this selection was made with the culinary use in mind, but ends up becoming a rich source of resources to study the molecular bases that allowed this diversity (Esther van der Knaap et al., 2014).

Alleles affecting the fruit shape were selected differently among tomato cultivars (Blanca et al., 2015). *OVATE* (Solyc02g085500) and *SUN* (Solyc10g079240) are genes related to fruit shape. Both control fruit elongation,

but *OVATE* is considered a negative growth regulator, while *SUN* is a positive growth regulator (Azzi et al., 2015). While the highest transcript levels of *SUN* is after anthesis (Xiao et al., 2008), the highest gene expression of *OVATE* is before anthesis (Liu et al., 2002), suggesting that they could alters fruit shape in independent pathways, acting in different stages of fruit development.

The occurrence of mutations in the *OVATE* gene in some accessions of *S. lycopersicum* var *cersiforme* suggests that these mutations appeared very early in the selection of cultivated tomatoes (Rodriguez et al., 2011). This gene was the first to be associated with the fruit shape in tomato, and belongs to the family of ovate proteins (OFP) whose function is not yet defined (Liu et al., 2002b; Wang et al., 2011). There is probably an epstasy between *OVATE* and other genes (Azzi et al., 2015), since the same *ovate* mutation generates variable fruit shapes, which may be more rounded, pear-shaped or more elongated (Gonzalo and Van Der Knaap, 2008). The classical *ovate* mutation is a loss-of-function in a putative negative growth regulator. This became clear when the pear-shaped *ovate* mutant was subjected to *OVATE* overexpression and has the rounded phenotype recovered (Azzi et al., 2015; Liu et al., 2002b). The allele responsible for the elongated fruit phenotype has a premature stop codon, which probably results in a null allele (Liu et al., 2002b).

The *SUN* gene has a much more pronounced effect on fruit elongation compared to *OVATE* (van der Knaap et al., 2014). The gene emerged from a highly unusual duplication of 24.7 kb from chromosome 10 to chromosome 7, encoding a protein in the IQD family, calmodulin-binding proteins (Jiang et al., 2009; Xiao et al., 2008). Unlike *OVATE*, in *SUN* there are no nucleotide differences in relation to the ancestral and the derived locus, with the exception of gene duplication (Rodríguez et al., 2011). In this case, the growth is regulated positively (XIAO et al., 2008). The duplication event was mediated by a Rider retrotransposon, placing the *SUN* under the control of the *DEFL1* promoter of the defensin gene, which leads to overexpression of the gene in fruits (JIANG et al., 2009; XIAO et al., 2008).

The gene overexpression in the *sun* mutant provides for an increased number of cells in the longitudinal direction and a reduction in the transverse direction of the fruit, resulting in the elongated shape (Wang et al., 2019). It was proposed that this difference in cell division could be regulated by plant hormones, such as auxin, or even by regulating the levels of secondary metabolites, affecting the fruit pattern (Xiao et al., 2008).

Although *sun* changes the fruit shape before anthesis, the most dramatic effect occurs after fertilization of the flower, during the stage of cell division of the ovary/fruit (Xiao et al., 2009). There is a cellular increase in the proximal-distal region and a decrease in the mid-lateral region, and it is possible to observe this difference seven few days after anthesis (Wu et al., 2011). These changes are probably related to the rate of cell division and not to the duration of this event, as there are no changes in the fruit ripening time (Esther van der Knaap et al., 2014).

Many of the genes related to fruit shape and fruit weight are linked to hormonal routes (Xiao et al., 2008; Anwar et al., 2019; Fernie and Yan, 2019; Zengcui Zhang et al., 2020; Wang et al., 2019). In view of thi,s we aim to investigate how the hormonal distribution of *sun* and *ovate* mutants occurs during fruit development.

3.2. Materials and methods

The experiments were conducted at the Laboratory of Hormonal Control of Plant Development, located in the Department of Biological Sciences of the College of Agriculture "Luiz de Queiroz" - ESALQ (University of São Paulo), Piracicaba-SP. For all experiments, ten plants of each genotype were used.

3.2.1 Plant material

The *ovate* and *sun* mutants were already available in the collection of mutants and transgenic plants maintained at ESALQ (www.esalq.usp.br/tomato) in the *Solanum lycorpersicum* cv. Micro-Tom (MT) genetic background. The mutants were originally introgressed into MT using the same procedures described previously (Carvalho et al., 2011) until the BC6Fn generation. The *sun* mutation was introgressed form the cv Long John (LA0791) and the ovate from a local market cultivar. In the present work, we crossed the mutants with MT again and then allowed the plants to self-pollinate obtaining the BC7Fn generation. MT transformed with report gene GUS fused with promoters responsive to auxin (*DR5::GUS*)(Ulmasov et al., 1997), cytokinin (*ARR5::GUS*) (D'Agostino, Deruère, and Kieber, 2000), gibberellin (*GA2OX::GUS*) (Dayan et al., 2012) and ethylene (*EBS::GUS*) (Stepanova et al., 2007) were already available in the same collection of mutants and transgenic plants. The transformation of MT with the mentioned constructions were performed as described previously (Pino et al., 2010). All transgenic plants are homozygous and in advances generations of self-pollination after transformation (Tn generation).

In order to obtain double mutants/transgene between *ovate* or *sun* and the GUS lines, the F1 plants were crossed again with *ovate* and *sun* mutants to obtain heterozygous genotypes for *GUS* and homozygous for *ovate* and *sun*. The heterozygous GUS plants were selected using Kanamycin spray (400 mg/L) as described previously (Pino et al., 2010), with exception made for EBS::GUS, whose construct has a hygromicin resistance gene. In the case of *EBS::GUS* leaf discs (Freitas-astua et al., 2003) were exposure to Ethrel 10μM, at room temperature and then exposure to x-gluc 37°C for 40 minutes. The screening for *sun* was performed by phenotypic analysis and the screening for ovate were made using CAPS markers: (V1031F: CGTGTGGAATTTGGAGAGGACAGA;V1031R:GTGGAGTTAGAAGTCCCATGAGCAAG). At the end of the process, 8 double mutants/transgene were obtained *ovate* x (*ARR5::GUS*; *EBS::GUS*; *GA2OX::gus* e *DR5::GUS*) and *sun* X (*ARR5::GUS*; *EBS::GUS*; *GA2OX::gus* e *DR5::GUS*).

As a control for comparing the hormonal distribution during fruit development, the transgenic *ARR5::GUS*; *EBS::GUS*; *GA2OX::GUS* e *DR5::GUS* were crossed with MT, obtaining hemizygous plants for *GUS* (F1), enabling comparison with the double mutants/transgenes ate the same gene dosage.

3.2.2 Plant growth conditions

The seeds were germinated in 350 mL pots containing a mixture of commercial substrate Basaplant Hortaliças (Base Agro) and vermiculite (1:1), enriched with 1 g/L of NPK 10:10:10 and 4 g/L of limestone. The plants were kept in a greenhouse under automatic irrigation (four times a day), average temperature of 28 ° C, photoperiod 11.5/13 h (summer/winter), and photosynthetically active radiation (RFA) of 250-350 μmol m^{-2} s^{-1} through the reduction of natural radiation using reflective mesh (Aluminet-Polysack Industrias Ltda; Leme, SP, Brazil). At 15 days

after germination (DAG) the plants were individualized in 350 mL pots containing a mixture of commercial substrate Basaplant Hortaliças (Base Agro) and vermiculite (1:1), enriched with 2 g/L of NPK 20:20:20 and 4 g/L of limestone. The plants were re-fertilized with NPK at the beginning of flowering and during fruit development.

For the phenotype recovery experiment, the plants were kept in a growth room with a photoperiod of 16h, luminous intensity of approximately 200 μmol $m^{-2} s^{-1}$ de, containing two LED lamps with blue and red light per shelf, and average temperature of 28°C. The plants were kept in trays containing the same amount of water, monitored daily.

3.2.3 Histochemical analyzes with b-glucoronidase (*GUS*)

Histochemical analyzes of *GUS* were performed using the protocol established by Jefferson; Kavanagh; Bevan, 1987. Since the highest transcript levels of *sun* is after anthesis (Xiao et al., 2008), we chose the following times for GUS expression analyses: 0 days after anthesis (DAA), 5 DAA, 10 DAA, 15 DAA, 20 DAA, 25 DAA and 30 DAA, totaling 7 times. Since the highest gene expression of *ovate* is before anthesis (Liu et al., 2002), we analyzed the times for GUS expression: 12 days before anthesis (DBA), 9 DBA, 6 DBA, 3 DBA, 0 DAA, 5 DAA and 10 DAA, totaling 6 times (Forno, 2017; Tanksley, 2004).

The ovary and fruits were collected, longitudinal cuts were made and immediately incubated in the oven at 37°C, in the dark, in a GUS staining solution [80 mM sodium phosphate buffer, pH 7.0; 8 mM EDTA; 0.4 mM potassium ferrocyanide; 0.05% Triton X-100; 0.8 mg ml-1 5-bromo-4-chloro-3-indolyl-b-D-glucuronide (X-Gluc); 20% methanol]. The reaction was stopped using 70% ethanol (Forno 2017). Image capture were performed using Leica Application Suite-LAS and with a camera. We analyzed 10 images for each treatment and the most representative image was chosen.

3.2.4 Phenotype recovery

In order to recover the *sun* phenotype, the flowers of the *sun* mutant and MT were marked at anthesis and subsequently dipped into solutions containing different concentrations of the cytokinin BAP (6-Benzylaminopurine) (0, 10 and 100 μM) plus two drops of tween per 100 mL. The flowers were immersed in the solutions daily for 20 days, at the same time, for 30". After 21 days, the fruits formed had their polar and equatorial diameters measured with the aid of a digital caliper.

3.3 Results

We found a conspicuous change in the cytokinin pathway for *sun*, compared to MT (Fig. 1). There was a higher *ARR5* expression at 5 and 10 DAA in *sun*, when compared to MT. The higher *ARR5* expression in *sun* decreased at 15 and 20 DAA, whereas the expression increased in MT at these same stages. At 30 DAA, the *ARR5* expression was high in both genotypes. However, contrary to MT, whose *ARR5* expression was already increased at 20 DAA, this increase was only observed at 25 DAA in *sun*. There were also minor variations regarding gibberellin pathway, with the higher expression of *GA2OX* in the seeds of both genotypes (Fig. 2). Apparently, GA2OX expression started earlier in MT (15 DAA) than *sun* (20 DAA). No obvious differences were observed in the ethylene pathway, which had a high *EBS* expression from 0 to 30 DAA in both genotypes (Fig. 3). Similarlly, in the auxin pathway, there was a continuous increase in *DR5* expression from 0 to 30 DAA in both genotypes (Fig. 4).

Figure 1. *ARR5::GUS* expression in MT and *sun* from 0 to 30 days after anthesis (DAA). (A) MT expressing the *ARR5::GUS* gene. Bar = 2mm from 0 to 10 DAA; Bar = 1cm from 15 to 30 DAA. (B) MT-*sun* expressing the *ARR5::GUS* gene. Bar = 2mm from 0 to 15 DAA; Bar = 1cm from 20 to 30 DAA.

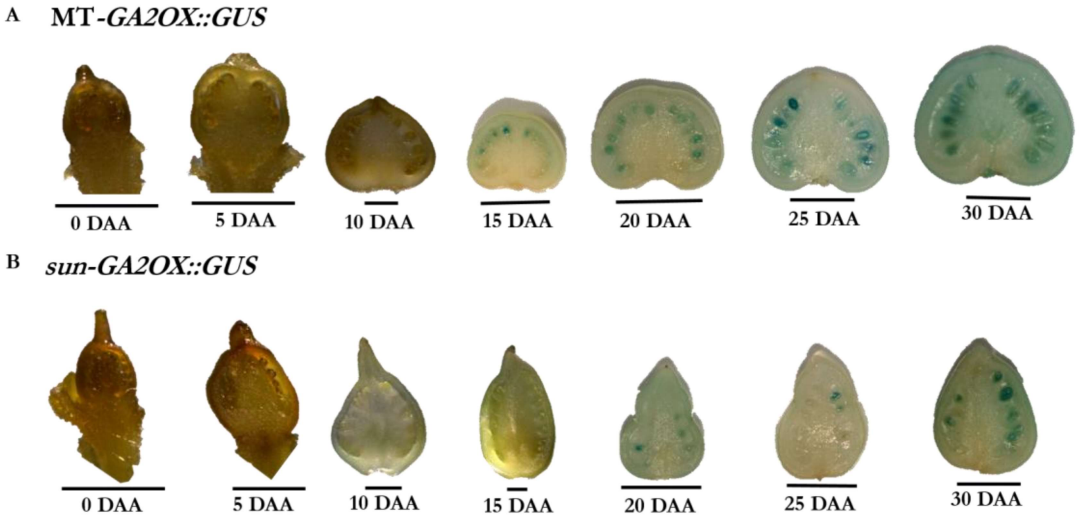

Figure 2. *GA2OX::GUS* expression in MT and *sun* from 0 to 30 days after anthesis (DAA). (A) MT expressing the *GA2OX::GUS* gene. Bar = 2mm from 0 to 10 DAA; Bar = 1cm from 15 to 30 DAA (B) MT-*sun* expressing the *GA2OX::GUS* gene. Bar = 2mm from 0 to 15 DAA; Bar = 1cm from 20 to 30 DAA.

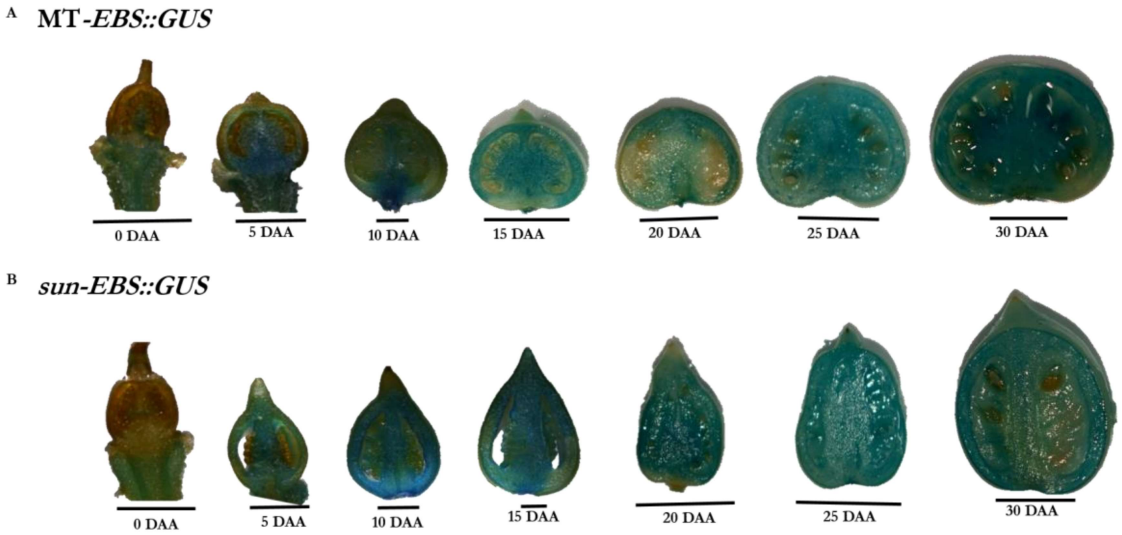

Figure 3. *EBS::GUS* expression in MT and *sun* from 0 to 30 days after anthesis (DAA). (A) MT expressing the *EBS::GUS* gene. Bar = 2mm from 0 to 10 DAA; Bar = 1cm from 15 to 30 DAA (B) MT-*sun* expressing the *EBS::GUS* gene. Bar = 2mm from 0 to 15 DAA; Bar = 1cm from 20 to 30 DAA.

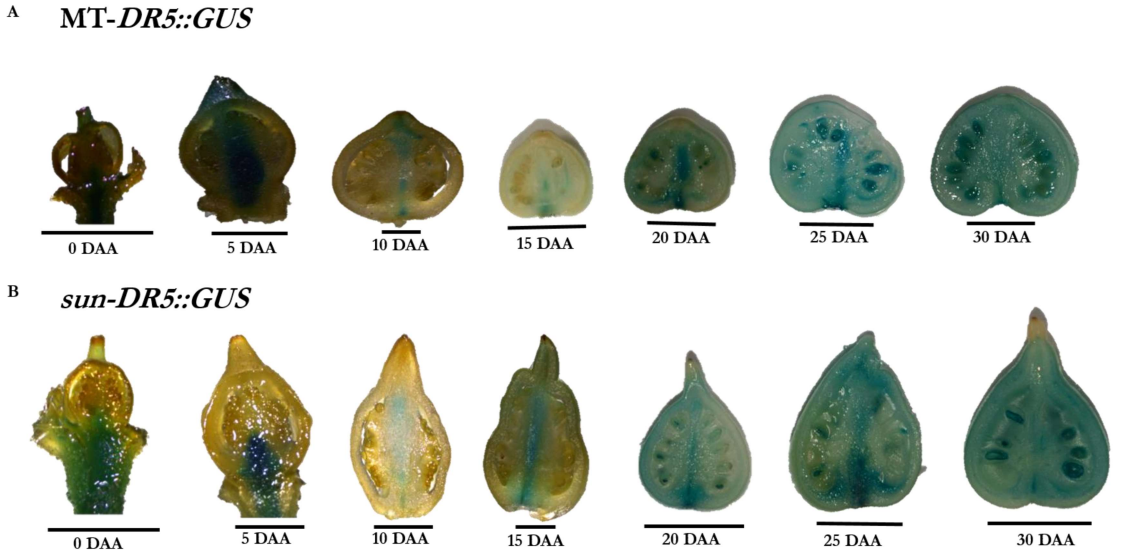

Figure 4. *DR5::GUS* expression in MT and *sun* from 0 to 30 days after anthesis (DAA). (A) MT expressing the *DR5::GUS* gene. Bar = 2mm from 0 to 10 DAA; Bar = 1cm from 15 to 30 DAA (B) MT-*sun* expressing the *DR5::GUS* gene. Bar = 2mm from 0 to 15 DAA; Bar = 1cm from 20 to 30 DAA.

Considering the *ovate* mutant, no obvious differences were found in the ethylene pathway compared to MT (Fig. 5). Hence, the expression of *EBS* was low before anthesis and increased after anthesis for both genotypes. For cytokinin pathway, there were dramatic differences in *ovate* ovaries when comparted to MT (Fig. 6). The *ARR5* expression in *ovate* was very low from 12 DBA to 5 DAA, compared to MT. At 10 DAA, the *ARR5* expression was low in both genotypes. Regarding the auxin pathway (Fig. 7), the *DR5* expression started early in MT than *ovate*. It was possible to observe an early *DR5* expression at 12 DBA and there is a more pronounced peak of *DR5* expression at 5 DAA, mainly in the fruit collumela. Although *ovate* also presented a high *DR5* expression at 5 DAA, it is likely that there was a delay in the peak of *DR5* expression, which was higher than MT at 10 DAA. For the gibberellin pathway

(Fig. 8), there was a high *GA2OX* expression in MT anthers at 3 DBA, which was not observed in *ovate*. The *GA2OX* expression was also high at 0 DAA in MT anthers and, in a lesser extent, in *ovate* anthers at this same stage.

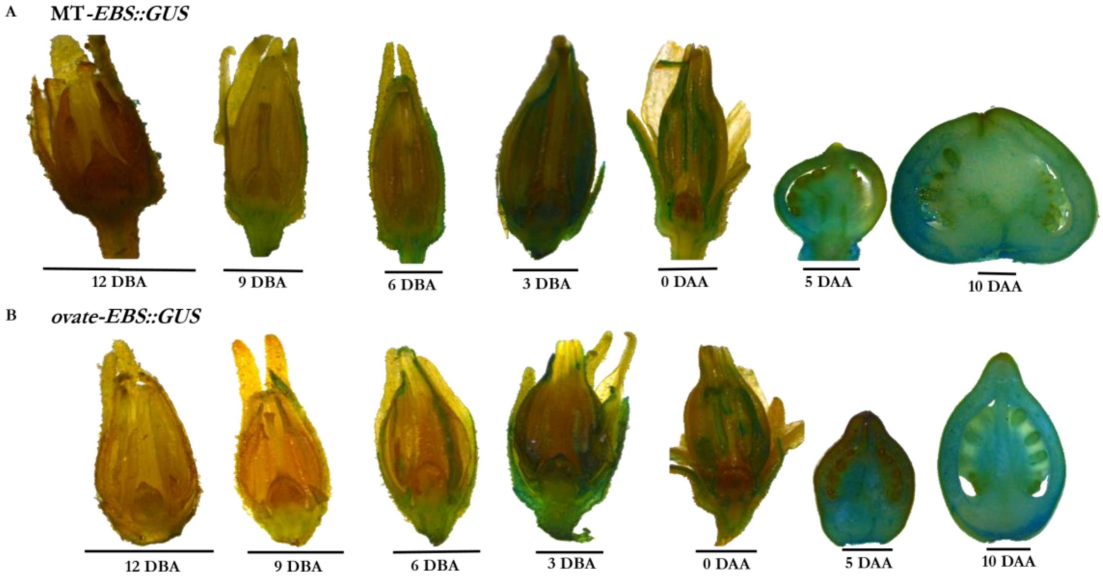

Figure 5. *EBS::GUS* expression in MT and *ovate* from 12 days before anthesis (DBA) to 10 days after anthesis (DAA). (A) MT expressing the *EBS::GUS* gene. (B) MT-*ovate* expressing the *EBS::GUS* gene. Bar = 2mm.

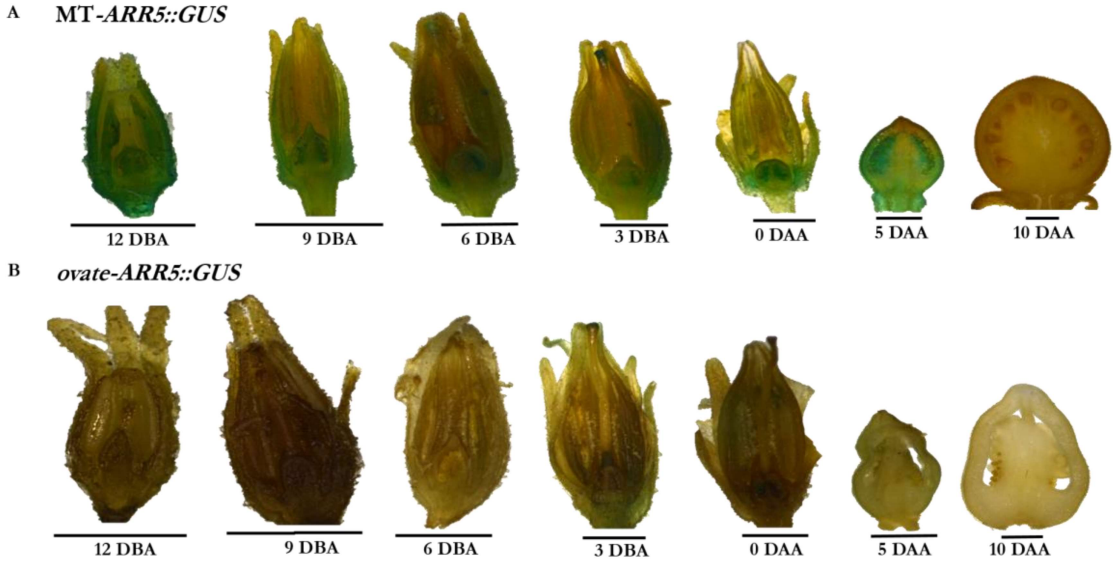

Figure 6. *ARR5::GUS* expression in MT and *ovate* from 12 days before anthesis (DBA) to 10 days after anthesis (DAA). (A) MT expressing the *ARR5::GUS* gene. (B) MT-*ovate* expressing the *ARR5::GUS* gene. Bar = 2 mm.

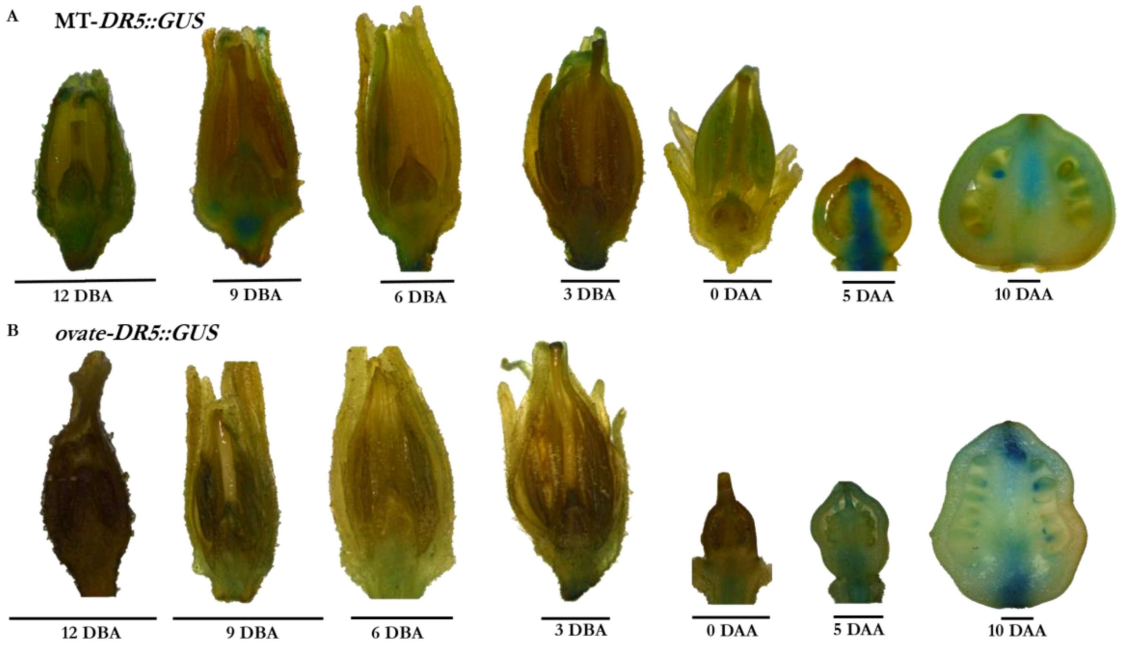

Figure 7. *DR5::GUS* expression in MT and *ovate* from 12 days before anthesis (DBA) to 10 days after anthesis (DAA). (A) MT expressing the *DR5::GUS* gene. (B) MT-*ovate* expressing the *DR5::GUS* gene. Bar = 2 mm.

Figure 8. *GA2OX::GUS* expression in MT and *ovate* from 12 days before anthesis (DBA) to 10 days after anthesis (DAA). (A) MT expressing the *GA2OX::GUS* gene. (B) MT-*ovate* expressing the *GA2OX::GUS* gene. Bar = 2 mm.

Due to the sudden change in the *ARR5* expression in *sun* mutants at 15 and 20 DAA, we performed an experiment providing BAP as an exogenous source of cytokinin for 20 days after anthesis. We observed that cytokinin application (10 and 100 □ M) had no effect in the equatorial diameters of both MT (Fig. 9) and *sun* (Fig. 10) fruits. However, there was a tendency of polar diameter decrease upon BAP treatments. This decrease was no significant for MT in both cytokinin concentrations but was significant when *sun* flowers were treated with 100 μM of BAP.

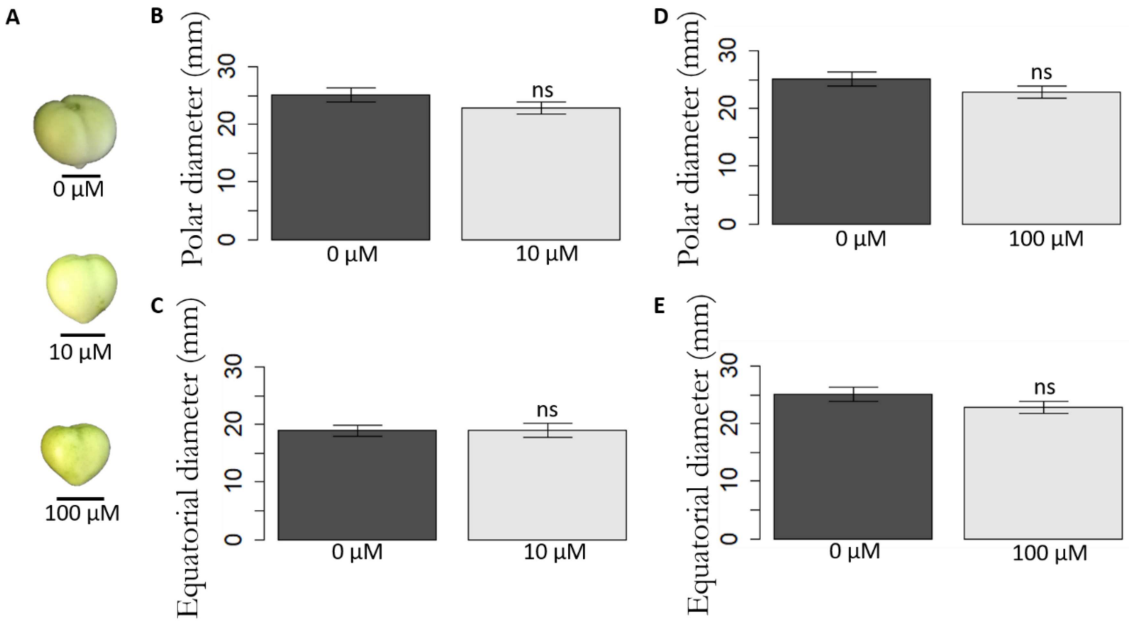

Figure 9. Measurements of Micro-Tom diameters after daily application of cytokinin (BAP) to flowers/fruits after anthesis. (A) MT fruit visual morphology at 21 days after BAP application in different concentrations. (B and D) MT fruit polar diameter. (C and E) MT fruit equatorial diameter. Bar=1cm.

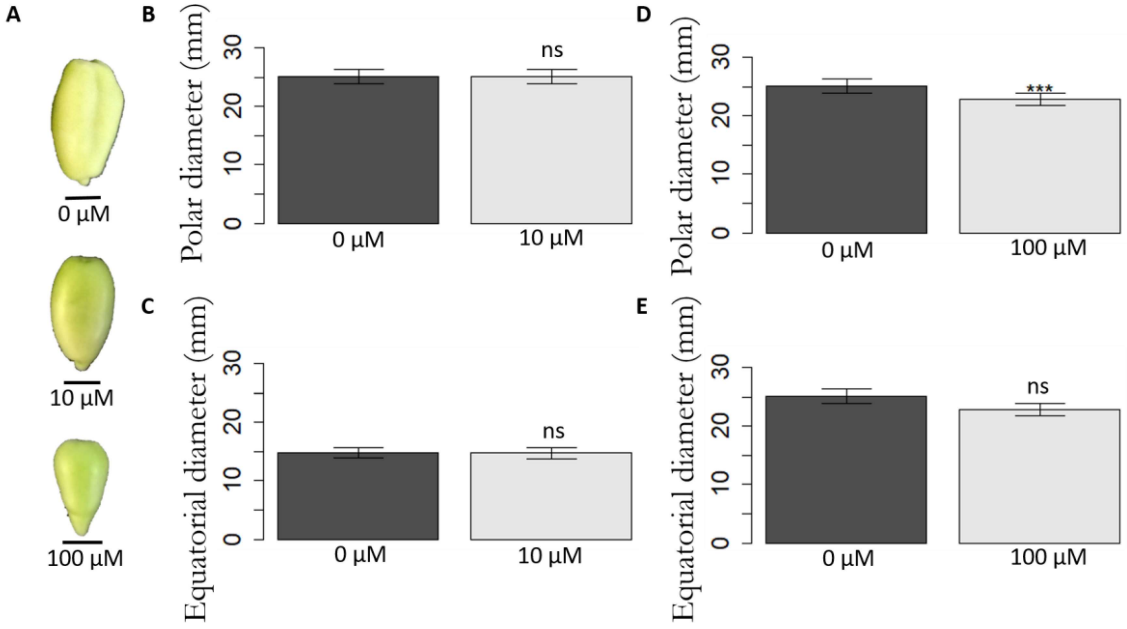

Figure 10. Measurements of *sun* diameters after daily application of cytokinin (BAP) to flowers/fruits after anthesis. (A) *sun* fruit visual morphology at 21 days after BAP application in different concentrations. (B and D) *sun* fruit polar diameter. (C and E) *sun* fruit equatorial diameter. Bar=1cm.

3.4. Discussion

Both *sun* and *ovate* mutations slenderizes the ovaries through increasing the proximal-distal diameter and decreasing the equatorial diameter. The increased ovary proximal-distal diameter in the *ovate* mutant is attributed primarily to the elongation of the proximal end (Wang et al., 2019). This could explain the occurrence of pear-shaped phenotypes, with elongated proximal ends, in some genetic backgrounds of *ovate*. In the case of *sun*, the increased proximal-distal diameter seems to be due to the elongation of the entire ovary (Wang et al., 2019), which could also contribute to the stronger elongated phenotype in *sun*, compared to *ovate*. In both *sun* and *ovate* the ovary cell number is significantly increased in the proximal-distal direction and decreased in the equatorial direction. It is long known that the main hormone class related to cell division is cytokinin (Amasino, 2005). Consistent with this, here we show that the cytokinin was the hormone class presenting the most conspicuous alteration in both mutants.

In the *sun* mutant, there was a higher *ARR5* expression at 5 and 10 DAA, compared to MT. These stages coincides with those related to cell division in the tomato ovary (Tanksley, 2004) and with the period of the highest *SUN* expression (Xiao et al., 2008). It is also known that cytokinin accumulates in tomato fruits at this period (Matsuo et al., 2012). The *ARR5* expression in *sun* decreased at 15 and 20 DAA, whereas the expression increased in MT at these same stages. Since the main phase of cell division in tomato ovary is before 15 DDA (Tanksley, 2004), it is likely that the early *ARR5* expression in *sun* could mean more cytokinin available in a phase that favors more cell division in the proximal-distal direction.

There were also alterations the gibberellin status in *sun*. Apparently, the action of gibberellin starts latter in *sun* (20 DAA) when compared with MT (15 DAA). Gibberellin accumulation was associated with the cell division phase in fruits (Kumar et al., 2014). However, in our analyses, there was no visible *GA2OX* expression in both *sun* and MT during the period related to cell division (from 0 to 10 DAA). Therefore, the differences in *GA2OX* expression at 15 and 20 DAA, which is considered the end of cell division and the beginning of cell expansion phase in tomato fruit development (Tanksley, 2004), should be attributed to the well-known effect of this hormone in cell expansion (Hedden and Sponsel., 2015). However, previous analyses provided evidences that fruit shape changes caused by *sun* are primarily due to alterations of cell number and not cell expansion (Wang et al., 2019).

Some members of the IQD family calmodulin-binding proteins plays a role in the transcript accumulation of a small group of cytochrome P450 genes that could be involved in auxin biosynthesis. Based on this, it was proposed that this hormone could be involved in the *sun* phenotype (Xiao et al., 2008). In our analysis, there was not differences between *sun* and MT regarding *DR5* expression. Instead, there was a continuous increase in *DR5* expression from 0 to 30 DAA in both genotypes. These results reinforce the importance of auxin for ovary development (Serrani et al., 2010). They are also in accordance to the fact that auxin is involved in both cell division and cell expansion phase (Kumar et al., 2014), since both process occur in the period from 0 to 30 DAA (Tanksley, 2004). Auxin and gibberellin interplay in fruit development (Kumar et al., 2014). For instance, auxin stimulates gibberellin biosynthesis in tomato fruits (KOSHIOKA et al., 1994). Consistent with this, in our results, the *DR5* expression occurred earlier than *GA2OX* in both *sun* and MT genotypes. However, the absence of differences in *DR5* expression between *sun* and MT argues against the hypothesis that auxin alterations could explain the *sun* elongated fruit phenotype.

In the *ovate* mutant, the *ARR5* expression was very low from 12 DBA to 5 DAA, compared to MT. At 10 DAA, the *ARR5* expression was low in both genotypes. This period parallels the expression of *OVATE*, which starts before anthesis and continues high until 8 DAA, but is considerably lower at 12 DAA (Liu et al., 2002). Since the period of cell division in tomato ovary coincides with *OVATE* expression (Tanskley, 2004), it is likely that the low

cytokinin status in *ovate* is limiting cell division in a way that the equatorial direction is more affected than the proximal-distal direction.

There were also alterations in the auxin status in *ovate*. The auxin action in *ovate* started much latter in relation to MT. Hence, in MT, it was possible to observe an early *DR5* expression at 12 DBA and there is a more pronounced peak of *DR5* expression at 5 DAA, mainly in the fruit collumela. Although *ovate* also presented a high *DR5* expression at 5 DAA, it is likely that there was a delay in the peak of *DR5* expression, which was higher than MT at 10 DAA. This suggests a role of auxin in *ovate* phenotype acting at the end of cell division phase and the beginning of cell expansion phase (Tanksley, 2004). Consistent with this, tomato flowers treated with exogenous auxin presented an elongation of the proximal end of the fruits, leading to the formation of a pear-shaped fruits (Wang et al., 2019).

We also observed differences between *ovate* and MT regarding the gibberellin pathway. However, the main alteration seems not be affecting fruit shape, since they occurred in the anthers and not in the ovaries. It is interesting to note that member of the Ovate Family Proteins (OFP) has been already linked to GA biosynthesis (Wang et al., 2007), which has also a role in male sterility (Zhou et al., 2019).

The increased early *ARR5* expression in *sun* in the phase of cell division led to the hypothesis that more cytokinin available in this mutant could contribute to its phenotype. However, we were unable to phenocopy the *sun* fruit upon exogenous cytokinin application in MT flowers. On the contrary, there was a tendency of polar diameter decrease upon BAP treatments in both MT and *sun*. This reduction in the proximal-distal direction actually makes fruits less elongated. It was already been reported that the application of synthetic cytokinin in tomato fruits causes a decrease in the fruit size, and that this reduction is due to the decrease in cell expansion and not cell division (Matsuo et al., 2012). This evidences the limitations of exogenous hormone treatment, which could interfere with other hormone classes and with the endogenous hormonal metabolism.

In summary, here we present evidences that the main hormone changes occurring during *sun* and *ovate* fruit development was associated to cytokinin. This is in accordance to former studies showing that fruit shape changes caused by *sun* and *ovate* are primarily due to the alterations in cell number (Wang et al., 2019). There were also alterations in the auxin status in *ovate* during the period of the end of cell division and the beginning of cell expansion in the ovary. This corroborates former evidences that exogenous auxin application can produce phenotypes similar to *ovate* (Wang et al., 2019). However, our results do not support the hypothesis that differences in the ovary auxin status could be involved in the *sun* phenotype (Xiao et al., 2008; Wang et al., 2009). Further studies involving gene expression of cytokinin and auxin pathways would shed more light in the findings presented here.

REFERENCES

Alpert, K. B., S. Grandillo, and S. D. Tanksley (1995) "Fw 2.2:A Major QTL Controlling Fruit Weight Is Common to Both Red- and Green-Fruited Tomato Species". **Theoretical and Applied Genetics** 91 (6–7): 994–1000. https://doi.org/10.1007/BF00223911.

Anwar, Raheel, Shazia Fatima, Autar K. Mattoo, and Avtar K. Handa (2019) "Fruit Architecture in Polyamine-Rich Tomato Germplasm Is Determined via a Medley of Cell Cycle, Cell Expansion, and Fruit Shape Genes." **Plants** 8 (10): 1–19. https://doi.org/10.3390/plants8100387.

Arikita, Fernanda Namie, Mariana Silva Azevedo, Danielle Camargo Scotton, Maísa de Siqueira Pinto, Antonio Figueira, and Lázaro Eustáquio Pereira Peres (2013) "Novel Natural Genetic Variation Controlling the Competence to Form Adventitious Roots and Shoots from the Tomato Wild Relative *Solanum Pennellii*." **Plant Science** 199–200: 121–30. https://doi.org/10.1016/j.plantsci.2012.11.005.

Azzi, Lamia, Cynthia Deluche, Frédéric Gévaudant, Nathalie Frangne, Frédéric Delmas, Michel Hernould, and Christian Chevalier (2015) "Fruit Growth-Related Genes in Tomato." **Journal of Experimental Botany** 66 (4): 1075–7086. https://doi.org/10.1093/jxb/eru527.

Blanca, José, Javier Montero-Pau, Christopher Sauvage, Guillaume Bauchet, Eudald Illa, María José Díez, David Francis, Mathilde Causse, Esther van der Knaap, and Joaquín Cañizares (2015) "Genomic Variation in Tomato, from Wild Ancestors to Contemporary Breeding Accessions." **BMC Genomics** 16 (1): 257. https://doi.org/10.1186/s12864-015-1444-1.

Boccalandro, Hernán E., Edmundo L. Ploschuk, Marcelo J. Yanovsky, Rodolfo A. Sánchez, Christiane Gatz, and Jorge J. Casal. (2003) "Increased Phytochrome B Alleviates Density Effects on Tuber Yield of Field Potato Crops." **Plant Physiology** 133 (4): 1539–46. https://doi.org/10.1104/pp.103.029579.

Campos, Marcelo Lattarulo, Rogério Falleiros Carvalho, Vagner Augusto Benedito, and Lázaro Eustáquio Pereira Peres (2010) "Small and Remarkable." **Plant Signaling & Behavior** 5 (3): 267–70. https://doi.org/10.1093/jxb/erp270.ormones.

Carvalho, Rogério F, Marcelo L Campos, Lilian E Pino, Simone L Crestana, Agustin Zsögön, and Joni E Lima (2011) "Convergence of Developmental Mutants into a Single Tomato Model System : ' Micro-Tom ' as an Effective Toolkit for Plant Development Research," **Plant Methods**, 7:18, 1–14.

Chakrabarti, Manohar, Na Zhang, Christopher Sauvage, Stéphane Muños, Jose Blanca, Joaquin Cañizares, Maria Jose Diez, et al. (2013) "A Cytochrome P450 Regulates a Domestication Trait in Cultivated Tomato." **Proceedings of the National Academy of Sciences of the United States of America** 110 (42): 17125–30. https://doi.org/10.1073/pnas.1307313110.

Cong, Bin, Luz S Barrero, and Steven D Tanksley (2008) "Regulatory Change in YABBY-like Transcription Factor Led to Evolution of Extreme Fruit Size during Tomato Domestication." **Nature Genetics** 40 (6): 800–804. https://doi.org/10.1038/ng.144.

Cong, Bin, Jiping Liu, and Steven D Tanksley (2002) "Natural Alleles at a Tomato Fruit Size Quantitative Trait Locus Differ by Heterochronic Regulatory Mutations." **Proceedings of the National Academy of Sciences of the United States of America** 99 (21): 13606–11. https://doi.org/10.1073/pnas.172520999.

D'Agostino, I B, J Deruère, and Joseph J. Kieber (2000) "Characterization of the Response of the Arabidopsis Response Regulator Gene Family to Cytokinin." **Plant Physiology** 124 (4): 1706–17. https://doi.org/10.1104/pp.1244.1706.

Dayan, Jonathan, Nickolay Voronin, Fan Gong, Tai-ping Sun, Peter Hedden, Hillel Fromm, and Roni Aloni (2012) "Leaf-Induced Gibberellin Signaling Is Essential for Internode Elongation, Cambial Activity, and Fiber Differentiation in Tobacco Stems." **The Plant Cell** 24 (1): 66–79. https://doi.org/10.1105/tpc.111.093096.

Dirzo, Rodolfo, and Peter H. Raven (2003) "Global State of Biodiversity and Loss." **Annual Review of Environment and Resources** 28 (1): 137–67. https://doi.org/10.1146/annurev.energy.28.050302.105532.

Duarte, Carlos M., Nùria Marbá, and Marianne Holmer (2007) "Rapid Domestication of Marine Species." **Science** 316 (5823): 382–83. https://doi.org/10.1126/science.1138042.

Falke, K. Christin, Shirin Glander, Fei He, Jinyong Hu, Juliette De Meaux, and Gregor Schmitz (2013) "The Spectrum of Mutations Controlling Complex Traits and the Genetics of Fitness in Plants." **Current Opinion in Genetics and Development** 23 (6): 665–71. https://doi.org/10.1016/j.gde.2013.10.006.

Fernie, Alisdair R., and Jianbing Yan (2019) "De Novo Domestication: An Alternative Route toward New Crops for the Future." **Molecular Plant** 12 (5): 615–31. https://doi.org/10.1016/j.molp.2019.03.016.

Forno, Ignacio Achon (2017) "Redefining the Hormonal Control of Tomato (Solanum Lycopersicum Cv. Micro-Tom) Fruit Development." University of São Paulo, "Luiz de Queiroz" College of Agriculture. http://www.teses.usp.br/teses/disponiveis/11/11140/tde-04012018-144113/en.php.

Frary, A, T C Nesbitt, S Grandillo, E Knaap, B Cong, J Liu, J Meller, R Elber, K B Alpert, and S D Tanksley (2000a) "Fw2.2: A Quantitative Trait Locus Key to the Evolution of Tomato Fruit Size." **Science** 289 (5476): 85–88. https://doi.org/10.1126/science.289.5476.85.

——— (2000b) "Fw2.2: A Quantitative Trait Locus Key To the Evolution of Tomato Fruit Size." **Science** 289 (5476): 85–88. https://doi.org/10.1126/science.289.5476.85.

Frary, Anne, and Sami Doganlar (2003) "Comparative Genetics of Crop Plant Domestication and Evolution Anne." **Turk J Agric For** 27: 59–69.

Freitas-astua, Juliana, Gustavo Astua-Monge, Jane Elisabeth Polston, and Ernest Hiebert (2003) "A Simple and Reliable Method for the Screening or Transgenic Tobacco Plants." **Pesquisa Atropecuaria Brasileira** 38 (7): 893–96.

Fuller, Dorian Q. (2007) "Contrasting Patterns in Crop Domestication and Domestication Rates: Recent Archaeobotanical Insights from the Old World." **Annals of Botany** 100 (5): 903–24. https://doi.org/10.1093/aob/mcm048.

Gonzalo, Maria Jose, Marin Talbot Brewer, Claire Anderson, David Sullivan, Simon Gray, and Esther Van Der Knaap (2009) "Tomato Fruit Shape Analysis Using Morphometric and Morphology Attributes Implemented in Tomato Analyzer Software Program." **Journal of the American Society for Horticultural Science** 134 (1): 77–87. https://doi.org/10.21273/jashs.134.1.77.

Gonzalo, Maria Jose, and Esther Van Der Knaap (2008) "A Comparative Analysis into the Genetic Bases of Morphology in Tomato Varieties Exhibiting Elongated Fruit Shape." **Theoretical and Applied Genetics** 116 (5): 647–56. https://doi.org/10.1007/s00122-007-0698-7.

Huang, Zejun, and Esther van der Knaap (2011) "Tomato Fruit Weight 11.3 Maps Close to Fasciated on the Bottom of Chromosome 11." **Theoretical and Applied Genetics** 123 (3): 465–74. https://doi.org/10.1007/s00122-011-1599-3.

Jefferson, R A, T A Kavanagh, and M W Bevan (1987) "GUS Fusions: Beta-Glucuronidase as a Sensitive and Versatile Gene Fusion Marker in Higher Plants." **The EMBO Journal** 6 (13): 3901–7. https://doi.org/10.1073/pnas.1411926112.

Jenkins, J. A. (1948) "The Origin of Cultivated Plants." **Economic Botany** 2 (4): 379–92. https://doi.org/https://doi.org/10.1007/BF02859492.

Jiang, Ning, Dongying Gao, Han Xiao, and Esther Van Der Knaap (2009) "Genome Organization of the Tomato Sun Locus and Characterization of the Unusual Retrotransposon Rider." **Plant Journal** 60 (1): 181–93. https://doi.org/10.1111/j.1365-313X.2009.03946.x.

Jinek, Martin, Krzysztof Chylinski, Ines Fonfara, Michael Hauer, Jennifer A Doudna, and Emmanuelle Charpentier (2012) "A Programmable Dual-RNA – Guided" **Science** 337 (August): 816–22.

Kharshiing, Eros, and Shriravi Prasad Sinha (2016) "Deficiency in Phytochrome A Alters Photosynthetic Activity, Leaf Starch Metabolism and Shoot Biomass Production in Tomato." **Journal of Photochemistry and Photobiology B: Biology** 165: 157–62. https://doi.org/10.1016/j.jphotobiol.2016.10.026.

Koshioka, M., T. Nishijima, H. Yamazaki, Y. Liu, M. Nonaka, and L. N. Mander (1994) "Analysis of Gibberellins in Growing Fruits of Lycopersicon Esculentum after Pollination or Treatment with 4-Chlorophenoxyacetic Acid." **Journal of Horticultural Science** 69 (1): 171–79. https://doi.org/10.1080/14620316.1994.11515263.

Krizek, Beth A. (2009) "Making Bigger Plants: Key Regulators of Final Organ Size." **Current Opinion in Plant Biology** 12 (1): 17–22. https://doi.org/10.1016/j.pbi.2008.09.006.

Lippman, Zachary, and Steven D. Tanksley (2001) "Dissecting the Genetic Pathway to Extreme Fruit Size in Tomato Using a Cross between the Small-Fruited Wild Species *Lycopersicon Pimpinellifolium* and *L. Esculentum* Var. Giant Heirloom." **Genetics** 158 (1): 413–22.

Liu, Jiping, Joyce Van Eck, Bin Cong, and Steven D. Tanksley (2002a) "A New Class of Regulatory Genes Underlying the Cause of Pear-Shaped Tomato Fruit." **Proceedings of the National Academy of Sciences of the United States of America** 99 (20): 13302–6. https://doi.org/10.1073/pnas.162485999.

Liu, Jiping, Joyce Van Eck, Bin Cong, and Steven D Tanksley (2002b) "A New Class of Regulatory Genes Underlying the Cause of Pear-Shaped Tomato Fruit." **Proceedings of the National Academy of Sciences of the United States of America** 99 (20): 13302–6. https://doi.org/10.1073/pnas.162485999.

Livak, Kenneth J., and Thomas D. Schmittgen (2001) "Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-ΔΔCT Method." **Methods** 25 (4): 402–8. https://doi.org/10.1006/meth.2001.1262.

Luo, Ming, Brian Gilbert, and Michael Ayliffe (2016) "Applications of CRISPR/Cas9 Technology for Targeted Mutagenesis, Gene Replacement and Stacking of Genes in Higher Plants." **Plant Cell Reports** 35 (7): 1439–50. https://doi.org/10.1007/s00299-016-1989-8.

Matsuo, Satoshi, Kaori Kikuchi, Machiko Fukuda, Ichiro Honda, and Shunsuke Imanishi (2012) "Roles and Regulation of Cytokinins in Tomato Fruit Methylation And." **Journal of Experimental Botany** 63 (15): 5569–79. https://doi.org/10,1093/jxb/ers207.

Meyer, Rachel S., Ashley E. Duval, and Helen R. Jensen (2012) "Patterns and Processes in Crop Domestication: An Historical Review and Quantitative Analysis of 203 Global Food Crops." **New Phytologist** 196 (1): 29–48. https://doi.org/10.1111/j.1469-8137.2012.04253.x.

Meyer, Rachel S., and Michael D. Purugganan (2013) "Evolution of Crop Species: Genetics of Domestication and Diversification." **Nature Reviews Genetics** 14 (12): 840–52. https://doi.org/10.1038/nrg3605.

Mu, Qi, Zejun Huang, Manohar Chakrabarti, Eudald Illa-Berenguer, Xiaoxi Liu, Yanping Wang, Alexis Ramos, and Esther van der Knaap (2017) "Fruit Weight Is Controlled by Cell Size Regulator Encoding a Novel Protein That Is Expressed in Maturing Tomato Fruits." *PLoS* **Genetics** 13 (8). https://doi.org/10.1371/journal.pgen.1006930.

Nesbitt, C. T., and Tanksley, S. D. (2002) "Comparative Sequencing in the Genus *Lycopersicon*: Implications for the Evolution of Fruit Size in the Domestication of Cultivated Tomatoes." **Genetics** 162 (1): 365–79.

Nesbitt, T. C., and S. D. Tanksley (2001) "Fw2.2 Directly Affects the Size of Developing Tomato Fruit, With Secondary Effects on Fruit Number and Photosynthate Distribution." **Plant Physiology** 127 (2): 575–83. https://doi.org/10.1104/pp.010087.

Paran, Ilan, and Esther Van Der Knaap (2007) "Genetic and Molecular Regulation of Fruit and Plant Domestication Traits in Tomato and Pepper." **Journal of Experimental Botany** 58 (14): 3841–52. https://doi.org/10.1093/jxb/erm257.

Pino, Lilian E, Simone Lombardi-Crestana, Mariana S Azevedo, Danielle C Scotton, Lucélia Borgo, Vera Quecini, Antonio Figueira, and Lázaro Ep Peres (2010) "The *Rg1* Allele as a Valuable Tool for Genetic Transformation of the Tomato 'Micro-Tom' Model System." **Plant Methods** 6: 23. https://doi.org/10.1186/1746-4811-6-23.

Piotto, Fernando Angelo, and Lázaro Eustáquio Pereira Peres (2012) "Base Genética Do Hábito de Crescimento e Florescimento Em Tomateiro e Sua Importância Na Agricultura." **Ciência Rural** 42 (11): 1941–46. https://doi.org/10.1590/S0103-84782012001100006.

Purugganan, Michael D., and Dorian Q. Fuller (2009) "The Nature of Selection during Plant Domestication." **Nature** 457 (7231): 843–48. https://doi.org/10.1038/nature07895.

Rodriguez, G. R., S. Munos, C. Anderson, S.-C. Sim, A. Michel, M. Causse, B. B. M. Gardener, D. Francis, and E. van der Knaap (2011) "Distribution of SUN, OVATE, LC, and FAS in the Tomato Germplasm and the Relationship to Fruit Shape Diversity." **Plant Physiology** 156 (1): 275–85. https://doi.org/10.1104/pp.110.167577.

Saath, Kleverton Clovis de Oliveira, and Arlei Luiz Fachinello (2018) "Crescimento Da Demanda Mundial de Alimentos e Restrições Do Fator Terra No Brasil." **Revista de Economia e Sociologia Rural** 56 (2): 195–212. https://doi.org/10.1590/1234-56781806-94790560201.

Sastry, KKS, and RM Muir (1963) "Gibberelin: Effect on Diffusible Auxin in Fruit Development." **Science** 140: 494–95.

Shan, Qiwei et al. (2013) "Targeted Genome Modification of Crop Plants Using a CRISPR-Cas System" **Nature Biottechnology** 31 (8): 8–10. https://doi.org/10.1038/nbt.2650.

Stepanova, A. N., J. Yun, A. V. Likhacheva, and J. M. Alonso (2007) "Multilevel Interactions between Ethylene and Auxin in Arabidopsis Roots." **The Plant Cell Online** 19 (7): 2169–85. https://doi.org/10.1105/tpc.107.052068.

Tang, Yun jia, and Johannes Liesche (2017) "The Molecular Mechanism of Shade Avoidance in Crops – How Data from Arabidopsis Can Help to Identify Targets for Increasing Yield and Biomass Production." **Journal of Integrative Agriculture** 16 (6): 1244–55. https://doi.org/10.1016/S2095-3119(16)61434-X.

Tanksley, Steven D. (2004) "The Genetic, Developmental, and Molecular Bases of Fruit Size and Shape Variation in Tomato." **Plant Cell** 16 181-190 (SUPPL.): 181–90. https://doi.org/10.1105/tpc.018119.

Thiele, Alexandra, Michael Herold, Ingo Lenk, Peter H. Quail, and Christiane Gatz (1999) "Heterologous Expression of Arabidopsis Phytochrome B in Transgenic Potato Influences Photosynthetic Performance and Tuber Development." **Plant Physiology** 120 (1): 73–81. https://doi.org/10.1104/pp.120.1.73.

Ulmasov, Tim, Jane Murfett, Gretchen Hagen, and Tom J Guilfoyle (1997) "Creation of a Highly Active Synthetic AuxRE." **Society** 9 (November): 1963–71. https://doi.org/10.1105/tpc.9.11.1963.

Van der Knaap, E. et al. (2014) "What lies beyond the eye: the molecular mechanisms regulating tomato fruit weight and shape". **Frontiers in plant science** 5 (May): 227.

Van der Knaap, E.; Tanksley, S. D. (2003) "The making of a bell pepper-shaped tomato fruit: Identification of loci controlling fruit morphology in Yellow Stuffer tomato". **Theoretical and Applied Genetics**, 107 (1): 139–147.

Wang, Shucai, Ying Chang, Jianjun Guo, and Jin Gui Chen (2007) "Arabidopsis Ovate Family Protein 1 Is a Transcriptional Repressor That Suppresses Cell Elongation." **Plant Journal** 50 (5): 858–72. https://doi.org/10.1111/j.1365-313X.2007.03096.x.

Wang, Shucai, Ying Chang, Jianjun Guo, Qingning Zeng, Brian E. Ellis, and Jin Gui Chen (2011) "Arabidopsis Ovate Family Proteins, a Novel Transcriptional Repressor Family, Control Multiple Aspects of Plant Growth and Development." **PLOS ONE** 6 (8) e23896. https://doi.org/10.1371/journal.pone.0023896.

Wang, Yanping, Josh P. Clevenger, Eudald Illa-Berenguer, Tea Meulia, Esther van der Knaap, and Liang Sun (2019) "A Comparison of Sun, Ovate, Fs8.1 and Auxin Application on Tomato Fruit Shape and Gene Expression." **Plant and Cell Physiology** 60 (5): 1067–81. https://doi.org/10.1093/pcp/pcz024.

Wu, Shan, Han Xiao, Antonio Cabrera, Tea Meulia, and Esther van der Knaap (2011) "SUN Regulates Vegetative and Reproductive Organ Shape by Changing Cell Division Patterns." **Plant Physiology** 157 (3): 1175–86. https://doi.org/10.1104/pp.111.181065.

Xiao, Han, Ning Jiang, Erin Schaffner, Eric J. Stockinger, and Esther Van Der Knaap (2008) "A Retrotransposon-Mediated Gene Duplication Underlies Morphological Variation of Tomato Fruit." **Science** 319 (5869): 1527–30. https://doi.org/10.1126/science.1153040.

Xiao, Han, Cheryll Radovich, Nicholas Welty, Jason Hsu, Dongmei Li, Tea Meulia, and Esther Van Der Knaap (2009) "Integration of Tomato Reproductive Developmental Landmarks and Expression Profiles, and the Effect of SUN on Fruit Shape." **BMC Plant Biology** 9:1-21. https://doi.org/10.1186/1471-2229-9-49.

Xu, Cao, Katie L. Liberatore, Cora A. MacAlister, Zejun Huang, Yi-Hsuan Chu, Ke Jiang, Christopher Brooks, et al. (2015) "A Cascade of Arabinosyltransferases Controls Shoot Meristem Size in Tomato." **Nature Genetics** 47 (7): 784–92. https://doi.org/10.1038/ng.3309.

Zhang, Zengcui, Aili Li, Gaoyuan Song, Shuaifeng Geng, Bikram S. Gill, Justin D. Faris, and Long Mao (2020) "Comprehensive Analysis of Q Gene Near-Isogenic Lines Reveals Key Molecular Pathways for Wheat Domestication and Improvement." **Plant Journal** 102 (2): 299–310. https://doi.org/10.1111/tpj.14624.

Zhang, Zhi, Cuiyu Mao, Zheng Shi, and Xiaohong Kou (2017) "The Amino Acid Metabolic and Carbohydrate Metabolic Pathway Play Important Roles during Salt-Stress Response in Tomato" **Frontiers in Plant Science** 8 (July) Article 1231. https://doi.org/10.3389/fpls.2017.01231.

Zhu, Guangtao, Shouchuang Wang, Zejun Huang, Shuaibin Zhang, Qinggang Liao, Chunzhi Zhang, Tao Lin, et al. (2018). "Rewiring of the Fruit Metabolome in Tomato Breeding." **Cell** 172 (1–2): 249-261.e12. https://doi.org/10.1016/j.cell.2017.12.019.

Zhu, Mingku, Guoping Chen, and Jianling Zhang (2014) "The Abiotic Stress-Responsive NAC-Type Transcription Factor SlNAC4 Regulates Salt and Drought Tolerance and Stress-Related Genes in Tomato (Solanum Lycopersicum)" **Plant Cell Rep** 33:1851–63. https://doi.org/10.1007/s00299-014-1662-z.

Zsögön, Agustin, Tomáš Čermák, Emmanuel Rezende Naves, Marcela Morato Notini, Kai H. Edel, Stefan Weinl, Luciano Freschi, Daniel F. Voytas, Jörg Kudla, and Lázaro Eustáquio Pereira Peres (2018) "De Novo Domestication of Wild Tomato Using Genome Editing." **Nature Biotechnology** 36 (12): 1211–16. https://doi.org/10.1038/nbt.4272.

4. CHARACTERIZATION OF MUTANTS *FW2.2*

Abstract

The increase in fruit weight was one of the most important events during the domestication of the tomato (*Solanum lycopersicum*). Although it is a polygenic characteristic, at least five major genes controlling it are known: *FRUIT WEIGHT 2.2 (FW2.2)*, *FRUIT WEIGHT 3.2 (FW3.2)*, *FRUIT WEIGHT 11.3 (FW11.3)*, *FASCIATED (FAS)* and *LOCULE NUMBER (LC)*. The *FW2.2* is related to the expression of a CELL NUMBER REGULATOR (CNR), in which the domesticated allele can promote an increase of up to 30% in the size of the fruit. Here we created a near isogenic line (NIL) harbouring the wild type allele of *FW2.2* (*fw2.2-W*) in the Micro-Tom (MT), which has the domesticated allele *(fw2.2-D)* for this gene. Besides the reduction in the fruit size, MT- *fw2.2-W* plants also presented smaller shoots, but did not alter the number of flowers and inflorescences.

Keywords: Tomato; Fruit Size; Fruit Weight; Micro-Tom

4.1. Introduction

Over the millennia, with the development and establishment of cultivation by man, the selection of phenotypes of interest in wild plant species, the domestication process, created a diversification of crops (Fernie and Yan, 2019). The process of domestication occurred independently in various areas of the world (Fuller, 2007; Meyer and Purugganan, 2013; Purugganan and Fuller, 2009). It started from local wild species with different botanical characteristics but that resulted in convergent phenotypes in their domesticated forms. The set of traits that distinguish domesticated crops from their wild ancestors is called the domestication syndrome (Evans, 1996). During the process, it was possible to amplify the desirable alleles, allowing the selection of cultures with favorable phenotypes, as well as disseminating the domesticated crops from their initial locations (centers of origin), promoting an adaptation to new environments and local preferences; maximizing productivity, allowing easy cultivation, uniformity and the quality of different cultures (Fernie and Yan, 2019; Meyer and Purugganan, 2013).

Tomato (*Solanum lycopersicum*) it a model to study the genetic basis of one of the main domestication syndromes, the gigantism of the fruits (Frary et al., 2000; Van der Knaap et al., 2014). This increase in organ size can be due to an increase in the number of cells and/or cell expansion in the ovaries, which provide to produce tomato varieties whose fruit weight is 100 heavier than the ancestral wild tomato (Zhu et al., 2018).

The increase in fruit weight is a polygenic characteristic with at least five major allelic variations whose genetic bases is already known: *fruit weight. 2.2* (*fw2.2*) (Solyc02g090730), *fruit weight 3.2* (*fw3.2*) (Solyc03g114940), *fruit weight 11.3* (*fw11.3*) (Solyc11g071940), *fasciated* (*fas*) (Solyc11g071380) and *locus number* (*lc*) (Solyc02g083950) (Cong, Barrero, and Tanksley, 2008; Cong, Liu, and Tanksley, 2002; Tanksley, 2004). *FW2.2* codes for a cell number regulator; *FW3.2* is a P450 monoozygenase from the CYP78A subfamily, homolog to the Arabidopsis *KLUH* and rice *PLASTOCHRON1*; *FW11.3* is a previously uncharacterized protein that was named *CELL SIZE REGULATOR* (*CSR*); while *FAS* and *LC* control the shape of the fruit by increasing the number of carpels, thus increasing fruit size (Lippman and Tanksley, 2001; Tanksley, 2004; Van der Knaap and Tanksley, 2003; Van der Knaap et al., 2014; Xu et

al., 2015). The *fas* mutation is a loss-of-function in the *CLAVATA3* gene, a negative regulator of the *WUSCHEL* gene, which increases the size of shoot meristem and, consequently, the number of carpels (locule) in fruits (Xu et al., 2015). The lc mutation is a gain-of-function in a *WUSCHEL* gene (Muños et al., 2011).

In the progeny of the cross between tomato cv. Yellow Stuffer and *S. pimpinellifolium*, *fw3.2* and *fw11.3* were considered the main loci responsible for the increase in fruit mass (Van der Knaap and Tanksley, 2003). A tomato cultivar with large fruits was transformed with a construction containing the *fw2.2* wild type allele (*fw2.2-W*) from *S. pennellii*, and there was a drastic reduction in the fruits size, proving that this gene can modify the fruit weight by up to 30% (Frary et al., 2000a).

FW2.2 is considered one of the key genes in the domestication process, since wild species of the *Solanum* genus present the small fruit allele, while domesticated tomato present the allele for large fruits (Fb et al., 2000a). However, although the large fruit allele, or domesticated allele (*fw2.2-D*), is already fixed in most cultivated tomatoes, estimates based on a molecular clock suggest that the large fruit allele appeared in tomato germplasm long before domestication (Nesbitt and Tanksley, 2002).

FW2.2 is located on chromosome 2 and regulates the cell cycle, being related to the initial events of increase in fruit size (Tanksley, 2004). The main difference between the *fw2.2-W* and the *fw2.2-D* alleles is their expression time (heterochronic allelic variation) and the total transcript level, resulting in a large phenotypic difference in fruit weight (Cong et al., 2002). Increased gene expression is associated with reduced cell division, the protein being located on the plasma membrane and containing a PLAC8 domain including two putative transmembrane motifs (Van der Knaap et al., 2014).

Quantitative loci are not always fully recessive or dominant, the *fw2.2* allele that characterizes small fruit for *S. pennellii* (*fw2.2-W*) is semi-dominant to the *S. lycopersicun* allele (*fw2.2-D*) that has large fruits (Alpert, Grandillo, and Tanksley, 1995). The protein regulates the number of cells, changing the cell layers in the wall of the ovary allowing that, in the case of the cultivated allele fruits, this organ is larger already at anthesis (Frary et al., 2000a).

It has already been observed in different isogenic tomato lines that the expression of the gene *FW2.2* is different, that is, the derived alleles result in changes in the level of expression of the gene (MU et al., 2017). *S. pimpinellifolium* when transformed with CRISPR/Cas9 vector for *FW2.2* knockout did not show changes in fruit sizes when compared to wild *S. pimpinellifolium*, reinforcing that perhaps the changes in *FW2.2* expression, and not the loss of function, affect the size of the tomato fruit (Zsögön et al., 2018).

The characteristics of interest during the domestication process are present in many species of different crops (Zhu et al., 2018). Such selections, which occurred over thousands of years of alleles that increased the fruit weight and changed their shape, provide a wealth of resources to study the molecular bases of this diversity. (Van der Knaap et al., 2014). In this context, tomato cv. Micro-Tom (MT) is considered a model plant for studies, as it has a relatively short genome (950Mb) distributed in 12 chromosomes (http://solgenomics.net/), it is a diploid species, with fast life cycle and fleshy fruits (Arikita et al., 2013). Although MT has small fruit, here we determined that it has the domesticated allele of FW2.2. Here we created a near isogenic line (NIL) harbouring the wild type allele of *FW2.2* in the Micro-Tom (MT). Besides the reduction in the fruit size, MT- *fw2.2-W* plants also presented smaller shoots, but did not alter the roots and the number of flowers and inflorescences.

4.2. Materials and methods

4.2.1 Plant material

The tomato cv. Micro-Tom (MT), which has the cultivated large-fruit allele for the *FW2.2* (Solyc02g090730), was use as a recurrent parental for the introgression of the wild type small-fruit allele of *Solanum galapagense* (*fw2.2*-WT). Was used as the control in experiments the MT and we compared it with *fw2.2*-WT. For the introgression *fw2.2*-WT, MT flowers were emasculated and fertilized with pollen from *S. galapagense* using the same procedure described previously (Carvalho et al., 2011; Pino et al., 2010). Plants from the resulted F1 generation were use as pollen donors to pollinate MT flowers, originating the BC1 generation. The process was repeated until the BC5F2 generation was obtained. In each generation of introgression, the presence of the *fw2.2*-WT allele was monitored using the following PCR-based molecular marker: CAPS/SCAR (fw2,2_Fwd GGTGGTGTTGATGTGGAGTGAGTG e fw2,2_Rev GGCAGATACATAGTGAGGAGGAAC.) during the introgression process.

4.2.2 Characterization of the *fw2.2* mutant

4.2.2.1 Plant growth conditions

The plants were kept in a growth room with a photoperiod of 16h, luminous intensity of approximately 200 μmol m^{-2} s^{-1}, containing two LED lamps with blue and red light per shelf, and an average temperature of 28°C. For all experiments, five plants of each genotype were used.

The seeds were germinated in 350 mL pots containing a mixture of commercial substrate Basaplant HT (Base Agro) and vermiculite (1: 1), enriched with 1 g/L of NPK 10:10:10 and 4 g/L of limestone. At 15 days the plants were individualized in 350 ml pots containing same mixture of commercial substrate and vermiculite, enriched with 2 g / L of NPK 20:20:20 and 8 g / L of limestone. The plants were kept in trays containing the same amount of water. The plants were fertilized again with NPK at the beginning of flowering and during fruit development.

4.2.2.3 Growth analysis

The measurements of the vegetative part were performed 46 days after seed germination. For height measurements, it was considered the distance from the base of the stem to the end of the plant. The inflorescences number, flowers of number, leaves number up to the first inflorescence were also evaluated, as well as the length of the largest root were determined. The leaf area was determined using a leaf area meter (LI-3100C). The leaves, stem, fruits and roots were packed separately and placed in an oven with air circulation at 70°C until they reached constant weight, and then the dry mass was determined using an analytical balance. Flower time analyses were made in the same plants starting the observation 32 days after germination.

4.2.2.4 Fruit analysis

The productivity parameters were done in plants 96 days after germination. To observe whether the *fw2.2*-WT interferes with fruit size or weight, a controlled fruit experiment was carried out, with 3 fruits per plant. The fruit height and diameter were determined of each genotype using a caliper. The same fruits were weighed in analytical balance and their content of soluble solids (Brix index) were measured with the aid of a digital refractometer (Atago PR-101α, Bellevue, WA).

4.2.3 qRT-PCR

Gene expression analyzes were performed on young leaves, mature leaves and roots, using a Rotor-Gene Q (Qiagen) real-time PCR cycler, the mix used was the Kapa Sybr Fast qPCR Master Mix (Kapa Biosystems). The specific primers used were FW2.2 (Solyc11g071940), fw2,2_Fwd CCTTGTATCACCTTTGGACAGATT e fw2,2_Rev CGGAGATAGCATTGTCAAAGTTA. The reactions were amplified for 2 min at 95°C, followed by 40 cycles of 95°C for 15 s and 60°C for 30s. The threshold cycle (CT) was determined. The analysis of the fusion curve was performed with each set of primers to confirm the presence of only a single peak before the analysis of gene expression. Two technical replicates were analyzed for each of the three biological samples. FW2.2 expression was normalized for the *ACTIN* gene (Solyc04g011500),actin_Fwd GGTCCCTCTATTGTCCACAG e actin_Rev TGCATCTCTGGTCCAGTAGGA. The folding changes for each gene were calculated using the equation 2- ΔΔCT (Livak; Schmittgen, 2001).

4.3 Results

The *fw2.2*-WT mutant had its allele, from the wild species *S. galapagense*, introduced in *Solanum lycopersicum* cv. Micro-Tom (MT), since this is considered a genetic model for studies with tomatoes. The MT genome was already sequenced and there is a genetic transformation protocol established (Pino et al., 2010). MT has also a small sized and a short life cycle that facilitates it use as a genetic model to study plant development (Carvalho et al., 2011). The selection of the *fw2.2*-WT was done successfully in each generation and in the BC5F2, homozygous plants were selected for the experiments (Fig. S1).

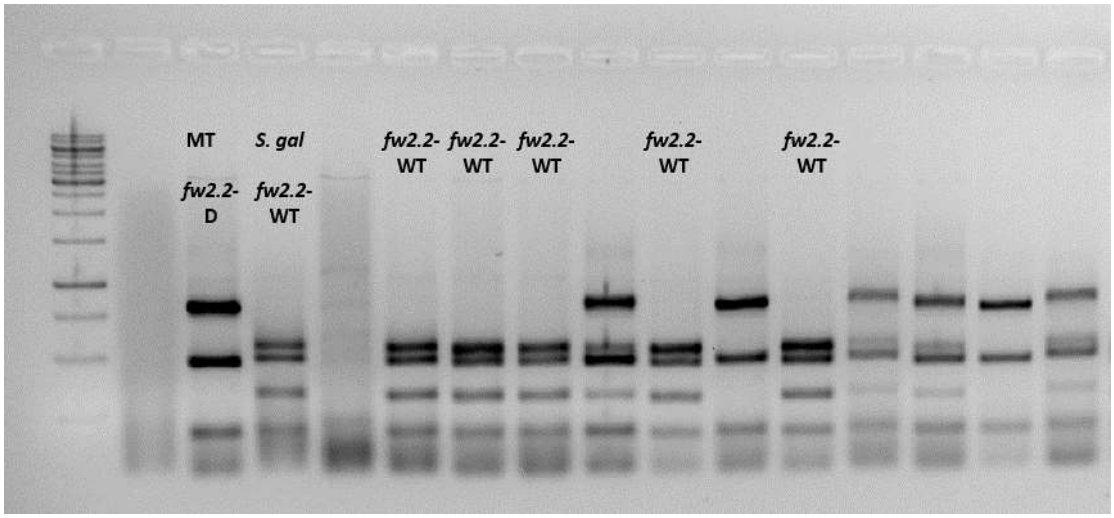

Figura S1. Micro-Tom (BC5F2) plants containing the *fw2.2* allele from *S. galapagense*.

The phenotypic characterization of the MT plant harbouring the *fw2.2*-WT allele at 46 DAG showed differences in the aerial part of the plant, but no difference was observed in the general aspect of root part (Fig. 2). All measures of the mutant aerial sizes, such as total height, height until the first inflorescence and height until branching were lower than the control MT, as well as the leaves number on the main stem (Tab. 1).

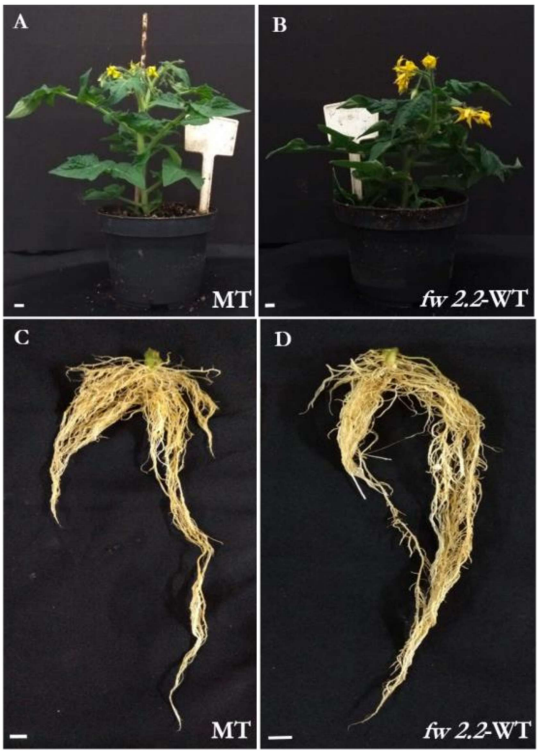

Figure 1. Shoot and root architecture of Micro-Tom (MT) and MT plants harbouring the wild type allele of *fruit weight 2.2* (*fw2.2*-WT). (A) Shoot of Micro-Tom (MT). (B) Shoot of *fw2.2*-WT (C) Root of Micro-Tom (MT). (D) Root of *fw2.2*-WT Shoot images were taken 46 days after germination (DAG). Root images were taken 96 days after germination (DAG). Scale bar = 1cm.

Table 1. Characterization of morphological parameters of Micro-Tom (MT) and MT plants harbouring the wild type allele of *fruit weight 2.2* (*fw2.2*-WT). Leaf area and root length were evaluated 96 days after germination (DAG). Other parameters were evaluated 46 DAG. Asterisk indicates significant difference by Student's t-test. P < 0.05 (*), P < 0.01 (**). Values are means ± standard error of 5 replicates (n=5).

	Plant height (cm)	Plant height 1st inflorescence (cm)	Branch height (cm)	Number of lateral buds	Number of leaves	Leaves on the main stem	Leaf area (cm^2)	Root lenght (cm)	Number of inflorescences	Number of flowers
MT	18,4	17,0	10,6	3,8	11,0	9,2	255,5	22,9	4,8	5,4
fw 2.2-WT	14,7 *	11,8 **	6,6 *	4,2	11,2	7,2 *	191,7	18,6	4,6	5,5

However, the total number of leaves did not differ between MT and the *fw2.2*-WT. There was also no significant difference between them regarding the total leaf area (Tab. 1). There was no changes in the reproductive part as well, with no significant differences in the inflorescences number and flowers number (Tab. 1). As much as the plants of *fw2.2*-WT have a smaller size in relation to MT, there were no significant differences between the dry weights of leaf, stem and root between the plants, although the values for *fw2.2*-WT tend to be lower (Fig. 2).

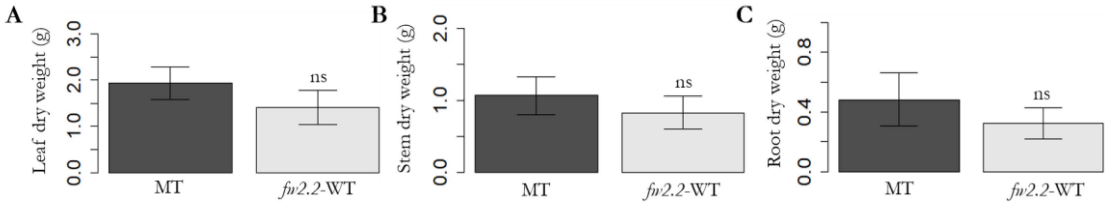

Figure 2. Dry weight characterization of Micro-Tom (MT) and MT plants harbouring the wild type allele of *fruit weight 2.2* (*fw2.2*-WT). No statistical significance was observed by Student's t-test (P < 0.05). ns=not significant.

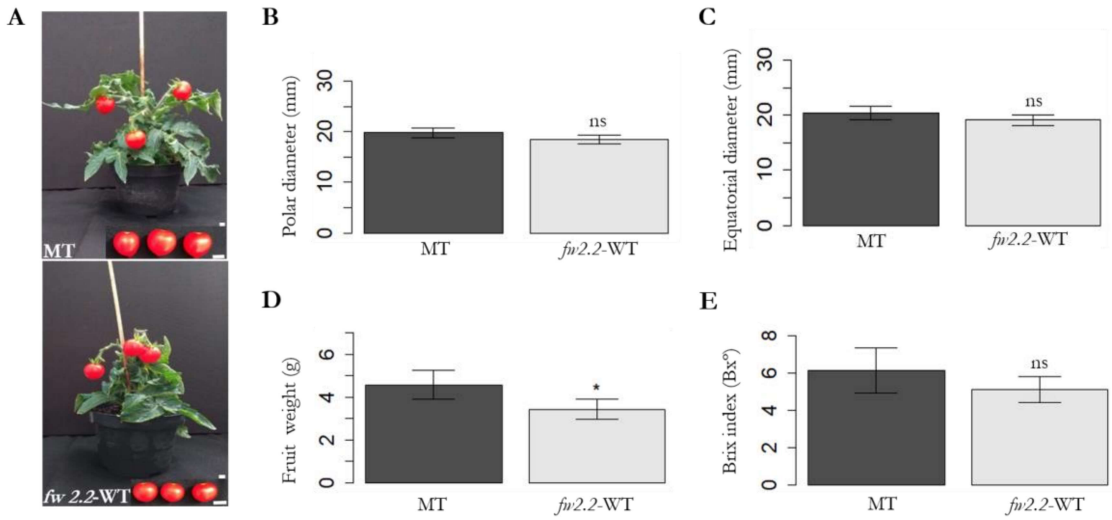

Figure 3. Fruit characterization of Micro-Tom (MT) and MT plants harbouring the wild type allele of *fruit weight 2.2* (*fw2.2*-WT) in a "controlled fruit experiment". (A) Phenotype of shoot and fruits of Micro-Tom (MT) and *fw2.2*-WT 96 days after germination (DAG). (B) Polar diameter and (C) equatorial diameter of the fruit. (D) Brix index. (E) Fruit fresh weight. Values are means ± standard error of 5 replicates (n=5). Asterisk indicates significant difference by Student's t-test. P < 0.05 (*). ns=not significant. Scale bar= 1cm.

When evaluating the fruits, there were no significant differences for equatorial diameter and polar diameter of the fruits, as well as for the brix index (Fig. 3). However, as described by Frary et al. (2000a) the fruits of *fw2.2*-WT were less heavy compared to MT (Fig. 3F).

Public data about *FW2.2* expression show that it has a great expression in roots (Fig. S2A), although more detailed and normalized data expression (Fig. S2B) show the greatest *FW2.2* in ovaries at 4 and 5 days post anthesis (DPA). Although there were low expression in leaves in both data sets, *FW2.2* express considerably in shoot meristems, which, together with its effect in cell division, could explain its impact in shoot growth.

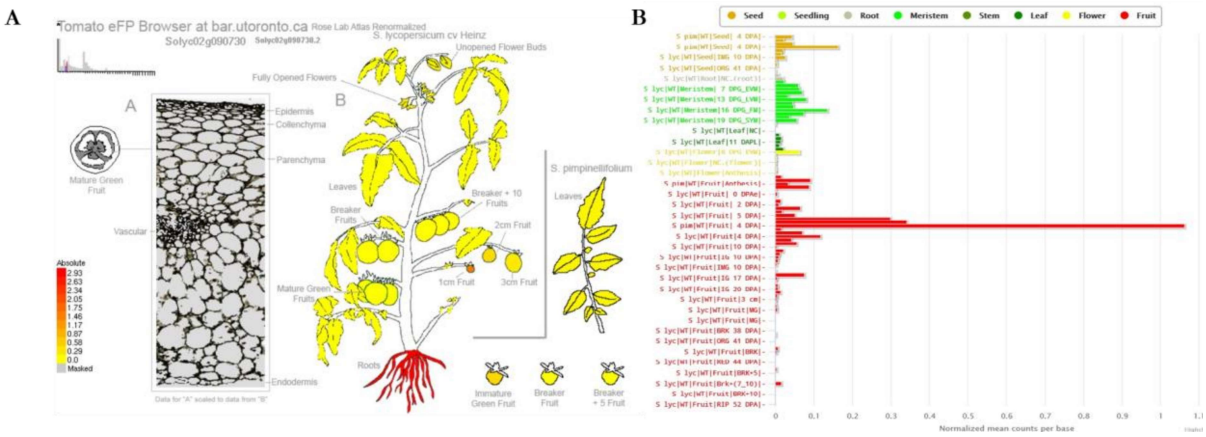

Figura S2. Expression of the *FW2.2* gene in different tomato organs from public databases. A) Data extracted from the eFP Browser (Tomato Genome Consortium, 2012) showing expression in *Solanum lycopersicum* cv. Heinz and *S. pimpinellifolium*. B) Data extracted from the TomExpress (Zouine et al., 2017) showing normalized expression in different genotypes and conditions.

4.4. Discussion

The *FW2.2*, found initially as a QTL controlling fruit weight in tomato (Paterson et al., 1988), was further isolated and considered one of the main genes selected during tomato domestication (Frary et al., 2000). It controls the size of the fruit, and plants with the wild type allele already present smaller ovary even before anthesis (Van der Knaap et al., 2014). This is in accordance to the pattern of expression of this gene in shoot meristems and the ovary (Fig. S2B, Cong et al., 2002). The expression of *FW2.2* in shoot meristem suggests that this gene could also have impact in other plant organs. Here we created NILs with different alleles of *FW2.2*, which allowed us to show that plants harbouring the *fw2.2*-WT allele were smaller than those harbouring the *fw2.2*-D allele. Since the shoots are very important for photosynthesis (Kharshiing and Sinha, 2016), the negative impact of *fw2.2*-WT reducing the plant size might account for the smaller fruits. Plants harbouring the *fw2.2*-WT allele also had fewer leaves on the main stem, but the number of total leaves did not change and there were no significant differences in the total leaf area. Moreover, there were no differences in the dry weight of leaves, stems and roots comparing *fw2.2*-WT and *fw2.2*-D plants. Therefore, it is unlike that the reduced plant size associated to the *fw2.2*-WT allele is affecting the fruit weight. Nevertheless, we could not rule out the possibility that the impact in plant size could be a limiting factor for plant productivity and affect fruit size in an indirect mechanism, for example, increasing the mutual shading of plants growing in high densities. The dry weight determinations also confirm that, despite the considerable expression of *FW2.2* in roots (Fig. S2A), there was no impact of different alleles on the size of these organs. In spite of this, it would be interesting to investigate in the future other possible implications for this high expression in roots. In this sense, it

is interesting to note that a member of the *FW2.2* gene family is essential for soybean nodule organogenesis (Libault et al., 2010).

It was proposed that the decrease in fruit size in plants harbouring the *fw2.2*-WT allele would be compensated by an increase in the number of fruits, mainly due to an increase in the inflorescences number (Nesbitt and Tanksley, 2001). However, in this work, there was no increase in inflorescences or flowers compared to MT. However further studies determining the total yield of fruits would help to clarify whether, as the plant development progresses, the wild type allele ends up investing more in the production of flowers and fruits. By removing the flowers, it was possible to control the differences in size and number of inflorescences, showing that the *FW2.2* acts directly in controlling the size of the ovary and the tomato fruit (Nesbitt and Tanksley, 2001). In agreement with this, here, the fruits of MT-*fw2.2*-WT were less heavy than the fruits of MT in an experiment where the number of fruits per plants were reduced to avoid source-sink limitation.

MT fruits are larger than wild *Solanum* spp fruits related to the cultivated tomato (Campos et al., 2010). Here, when introgressing the *fw2.2*-WT allele of *S. galapagense* it was possible to observe a 33.6% reduction in MT fruit size. The value found in our work corroborates that described by Frary et al. (2000a) and evidences the success of the introgression made here, which probably had no other chromosomal regions from *S. galapagense* interfering in the size of the fruit. However, this should be confirmed performing a mapping-by-sequencing of the produced NIL.

The decrease in the weight of the fruit upon the introgression of the *S. galapagense FW2.2* allele confirms that MT, despite its cherry-sized fruit, has the domesticated large-fruit *FW2.2* allele. The increase in weight by the domesticated allele is promoted by the enlargement of the regions of the placenta and columella of the fruit (Cong, Liu,and Tanksley, 2002; Gonzalo et al., 2009). However, in our work, this increase in weight did not increase the equatorial and polar diameters of the fruit, nor did it alter the increase in the Brix Index. Further studies will be necessary to access the impact of the smaller size of MT-*fw2.2-WT* plants in yield and Brix at different fruit number per plant. This will allow to determine whether the *fw2.2-D* allele was selected directly due to its impact on fruit size or, alternatively, due to a general impact on plant productivity.

REFERENCES

Alpert, K. B., S. Grandillo, and S. D. Tanksley (1995) "Fw 2.2:A Major QTL Controlling Fruit Weight Is Common to Both Red- and Green-Fruited Tomato Species". **Theoretical and Applied Genetics** 91 (6–7): 994–1000. https://doi.org/10.1007/BF00223911.

Anwar, Raheel, Shazia Fatima, Autar K. Mattoo, and Avtar K. Handa (2019) "Fruit Architecture in Polyamine-Rich Tomato Germplasm Is Determined via a Medley of Cell Cycle, Cell Expansion, and Fruit Shape Genes." **Plants** 8 (10): 1–19. https://doi.org/10.3390/plants8100387.

Arikita, Fernanda Namie, Mariana Silva Azevedo, Danielle Camargo Scotton, Maísa de Siqueira Pinto, Antonio Figueira, and Lázaro Eustáquio Pereira Peres (2013) "Novel Natural Genetic Variation Controlling the Competence to Form Adventitious Roots and Shoots from the Tomato Wild Relative *Solanum Pennellii*." **Plant Science** 199–200: 121–30. https://doi.org/10.1016/j.plantsci.2012.11.005.

Azzi, Lamia, Cynthia Deluche, Frédéric Gévaudant, Nathalie Frangne, Frédéric Delmas, Michel Hernould, and Christian Chevalier (2015) "Fruit Growth-Related Genes in Tomato." **Journal of Experimental Botany** 66 (4): 1075–7086. https://doi.org/10.1093/jxb/eru527.

Blanca, José, Javier Montero-Pau, Christopher Sauvage, Guillaume Bauchet, Eudald Illa, María José Díez, David Francis, Mathilde Causse, Esther van der Knaap, and Joaquín Cañizares (2015) "Genomic Variation in Tomato, from Wild Ancestors to Contemporary Breeding Accessions." **BMC Genomics** 16 (1): 257. https://doi.org/10.1186/s12864-015-1444-1.

Boccalandro, Hernán E., Edmundo L. Ploschuk, Marcelo J. Yanovsky, Rodolfo A. Sánchez, Christiane Gatz, and Jorge J. Casal. (2003) "Increased Phytochrome B Alleviates Density Effects on Tuber Yield of Field Potato Crops." **Plant Physiology** 133 (4): 1539–46. https://doi.org/10.1104/pp.103.029579.

Campos, Marcelo Lattarulo, Rogério Falleiros Carvalho, Vagner Augusto Benedito, and Lázaro Eustáquio Pereira Peres (2010) "Small and Remarkable." **Plant Signaling & Behavior** 5 (3): 267–70. https://doi.org/10.1093/jxb/erp270.ormones.

Carvalho, Rogério F, Marcelo L Campos, Lilian E Pino, Simone L Crestana, Agustin Zsögön, and Joni E Lima (2011) "Convergence of Developmental Mutants into a Single Tomato Model System : ' Micro-Tom ' as an Effective Toolkit for Plant Development Research," **Plant Methods**, 7:18, 1–14.

Chakrabarti, Manohar, Na Zhang, Christopher Sauvage, Stéphane Muños, Jose Blanca, Joaquin Cañizares, Maria Jose Diez, et al. (2013) "A Cytochrome P450 Regulates a Domestication Trait in Cultivated Tomato." **Proceedings of the National Academy of Sciences of the United States of America** 110 (42): 17125–30. https://doi.org/10.1073/pnas.1307313110.

Cong, Bin, Luz S Barrero, and Steven D Tanksley (2008) "Regulatory Change in YABBY-like Transcription Factor Led to Evolution of Extreme Fruit Size during Tomato Domestication." **Nature Genetics** 40 (6): 800–804. https://doi.org/10.1038/ng.144.

Cong, Bin, Jiping Liu, and Steven D Tanksley (2002) "Natural Alleles at a Tomato Fruit Size Quantitative Trait Locus Differ by Heterochronic Regulatory Mutations." **Proceedings of the National Academy of Sciences of the United States of America** 99 (21): 13606–11. https://doi.org/10.1073/pnas.172520999.

D'Agostino, I B, J Deruère, and Joseph J. Kieber (2000) "Characterization of the Response of the Arabidopsis Response Regulator Gene Family to Cytokinin." **Plant Physiology** 124 (4): 1706–17. https://doi.org/10.1104/pp.1244.1706.

Dayan, Jonathan, Nickolay Voronin, Fan Gong, Tai-ping Sun, Peter Hedden, Hillel Fromm, and Roni Aloni (2012) "Leaf-Induced Gibberellin Signaling Is Essential for Internode Elongation, Cambial Activity, and Fiber Differentiation in Tobacco Stems." **The Plant Cell** 24 (1): 66–79. https://doi.org/10.1105/tpc.111.093096.

Dirzo, Rodolfo, and Peter H. Raven (2003) "Global State of Biodiversity and Loss." **Annual Review of Environment and Resources** 28 (1): 137–67. https://doi.org/10.1146/annurev.energy.28.050302.105532.

Duarte, Carlos M., Nùria Marbá, and Marianne Holmer (2007) "Rapid Domestication of Marine Species." **Science** 316 (5823): 382–83. https://doi.org/10.1126/science.1138042.

Falke, K. Christin, Shirin Glander, Fei He, Jinyong Hu, Juliette De Meaux, and Gregor Schmitz (2013) "The Spectrum of Mutations Controlling Complex Traits and the Genetics of Fitness in Plants." **Current Opinion in Genetics and Development** 23 (6): 665–71. https://doi.org/10.1016/j.gde.2013.10.006.

Fernie, Alisdair R., and Jianbing Yan (2019) "De Novo Domestication: An Alternative Route toward New Crops for the Future." **Molecular Plant** 12 (5): 615–31. https://doi.org/10.1016/j.molp.2019.03.016.

Forno, Ignacio Achon (2017) "Redefining the Hormonal Control of Tomato (Solanum Lycopersicum Cv. Micro-Tom) Fruit Development." University of São Paulo, "Luiz de Queiroz" College of Agriculture. http://www.teses.usp.br/teses/disponiveis/11/11140/tde-04012018-144113/en.php.

Frary, A, T C Nesbitt, S Grandillo, E Knaap, B Cong, J Liu, J Meller, R Elber, K B Alpert, and S D Tanksley (2000a) "Fw2.2: A Quantitative Trait Locus Key to the Evolution of Tomato Fruit Size." **Science** 289 (5476): 85–88. https://doi.org/10.1126/science.289.5476.85.

——— (2000b) "Fw2.2: A Quantitative Trait Locus Key To the Evolution of Tomato Fruit Size." **Science** 289 (5476): 85–88. https://doi.org/10.1126/science.289.5476.85.

Frary, Anne, and Sami Doganlar (2003) "Comparative Genetics of Crop Plant Domestication and Evolution Anne." **Turk J Agric For** 27: 59–69.

Freitas-astua, Juliana, Gustavo Astua-Monge, Jane Elisabeth Polston, and Ernest Hiebert (2003) "A Simple and Reliable Method for the Screening or Transgenic Tobacco Plants." **Pesquisa Atropecuaria Brasileira** 38 (7): 893–96.

Fuller, Dorian Q. (2007) "Contrasting Patterns in Crop Domestication and Domestication Rates: Recent Archaeobotanical Insights from the Old World." **Annals of Botany** 100 (5): 903–24. https://doi.org/10.1093/aob/mcm048.

Galati, V. C. et al. (2018). "Root-to-Shoot Communication to Modulate Source-Sink Relationship in Tomato Depends on Phytochromes" **Journal of Agricultural Science**, 10(8):174.

Gasparini, K., Moreira J.R., Peres L.P.P., & Zsogon A. (2021) "De novo domestication of wild species to create crops with increased resilience and nutritional value". **Current Opinion in Plant Biology**, 60:102006

Giovannoni, J.(2018) "Tomato Multiomics Reveals Consequences of Crop Domestication and Improvement". **Cell**, 172 (1-2):6–8.

Gonzalo, Maria Jose, Marin Talbot Brewer, Claire Anderson, David Sullivan, Simon Gray, and Esther Van Der Knaap (2009) "Tomato Fruit Shape Analysis Using Morphometric and Morphology Attributes Implemented in Tomato Analyzer Software Program." **Journal of the American Society for Horticultural Science** 134 (1): 77–87. https://doi.org/10.21273/jashs.134.1.77.

Gonzalo, Maria Jose, and Esther Van Der Knaap (2008) "A Comparative Analysis into the Genetic Bases of Morphology in Tomato Varieties Exhibiting Elongated Fruit Shape." **Theoretical and Applied Genetics** 116 (5): 647–56. https://doi.org/10.1007/s00122-007-0698-7.

Huang, Zejun, and Esther van der Knaap (2011) "Tomato Fruit Weight 11.3 Maps Close to Fasciated on the Bottom of Chromosome 11." **Theoretical and Applied Genetics** 123 (3): 465–74. https://doi.org/10.1007/s00122-011-1599-3.

Jefferson, R A, T A Kavanagh, and M W Bevan (1987) "GUS Fusions: Beta-Glucuronidase as a Sensitive and Versatile Gene Fusion Marker in Higher Plants." **The EMBO Journal** 6 (13): 3901–7. https://doi.org/10.1073/pnas.1411926112.

Jenkins, J. A. (1948) "The Origin of Cultivated Plants." **Economic Botany** 2 (4): 379–92. https://doi.org/https://doi.org/10.1007/BF02859492.

Jiang, Ning, Dongying Gao, Han Xiao, and Esther Van Der Knaap (2009) "Genome Organization of the Tomato Sun Locus and Characterization of the Unusual Retrotransposon Rider." **Plant Journal** 60 (1): 181–93. https://doi.org/10.1111/j.1365-313X.2009.03946.x.

Jinek, Martin, Krzysztof Chylinski, Ines Fonfara, Michael Hauer, Jennifer A Doudna, and Emmanuelle Charpentier (2012) "A Programmable Dual-RNA – Guided" **Science** 337 (August): 816–22.

Kharshiing, Eros, and Shriravi Prasad Sinha (2016) "Deficiency in Phytochrome A Alters Photosynthetic Activity, Leaf Starch Metabolism and Shoot Biomass Production in Tomato." **Journal of Photochemistry and Photobiology B: Biology** 165: 157–62. https://doi.org/10.1016/j.jphotobiol.2016.10.026.

Koshioka, M., T. Nishijima, H. Yamazaki, Y. Liu, M. Nonaka, and L. N. Mander (1994) "Analysis of Gibberellins in Growing Fruits of Lycopersicon Esculentum after Pollination or Treatment with 4-Chlorophenoxyacetic Acid." **Journal of Horticultural Science** 69 (1): 171–79. https://doi.org/10.1080/14620316.1994.11515263.

Krizek, Beth A. (2009) "Making Bigger Plants: Key Regulators of Final Organ Size." **Current Opinion in Plant Biology** 12 (1): 17–22. https://doi.org/10.1016/j.pbi.2008.09.006.

Lippman, Zachary, and Steven D. Tanksley (2001) "Dissecting the Genetic Pathway to Extreme Fruit Size in Tomato Using a Cross between the Small-Fruited Wild Species *Lycopersicon Pimpinellifolium* and *L. Esculentum* Var. Giant Heirloom." **Genetics** 158 (1): 413–22.

Liu, Jiping, Joyce Van Eck, Bin Cong, and Steven D. Tanksley (2002a) "A New Class of Regulatory Genes Underlying the Cause of Pear-Shaped Tomato Fruit." **Proceedings of the National Academy of Sciences of the United States of America** 99 (20): 13302–6. https://doi.org/10.1073/pnas.162485999.

Liu, Jiping, Joyce Van Eck, Bin Cong, and Steven D Tanksley (2002b) "A New Class of Regulatory Genes Underlying the Cause of Pear-Shaped Tomato Fruit." **Proceedings of the National Academy of Sciences of the United States of America** 99 (20): 13302–6. https://doi.org/10.1073/pnas.162485999.

Livak, Kenneth J., and Thomas D. Schmittgen (2001) "Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-ΔΔCT Method." **Methods** 25 (4): 402–8. https://doi.org/10.1006/meth.2001.1262.

Luo, Ming, Brian Gilbert, and Michael Ayliffe (2016) "Applications of CRISPR/Cas9 Technology for Targeted Mutagenesis, Gene Replacement and Stacking of Genes in Higher Plants." **Plant Cell Reports** 35 (7): 1439–50. https://doi.org/10.1007/s00299-016-1989-8.

Martin, L. B. B. et al. (2017) "Cuticle biosynthesis in tomato leaves is developmentally regulated by abscisic acid" **Plant Physiology**, 174: 3, 1384–1398.

Matsuo, Satoshi, Kaori Kikuchi, Machiko Fukuda, Ichiro Honda, and Shunsuke Imanishi (2012) "Roles and Regulation of Cytokinins in Tomato Fruit Methylation And." **Journal of Experimental Botany** 63 (15): 5569–79. https://doi.org/10,1093/jxb/ers207.

Meyer, Rachel S., Ashley E. Duval, and Helen R. Jensen (2012) "Patterns and Processes in Crop Domestication: An Historical Review and Quantitative Analysis of 203 Global Food Crops." **New Phytologist** 196 (1): 29–48. https://doi.org/10.1111/j.1469-8137.2012.04253.x.

Meyer, Rachel S., and Michael D. Purugganan (2013) "Evolution of Crop Species: Genetics of Domestication and Diversification." **Nature Reviews Genetics** 14 (12): 840–52. https://doi.org/10.1038/nrg3605.

Mu, Qi, Zejun Huang, Manohar Chakrabarti, Eudald Illa-Berenguer, Xiaoxi Liu, Yanping Wang, Alexis Ramos, and Esther van der Knaap (2017) "Fruit Weight Is Controlled by Cell Size Regulator Encoding a Novel Protein That Is Expressed in Maturing Tomato Fruits." *PLoS* **Genetics** 13 (8). https://doi.org/10.1371/journal.pgen.1006930.

Nagai, H. Tomate. In: Furlani, A.M.C.; Viegas, G.P. (eds.). O melhoramento de plantas no Instituto Agronômico. Campinas: Instituto Agronômico, p.195-203; 301-313. 1993.

Nesbitt, C. T., and Tanksley, S. D. (2002) "Comparative Sequencing in the Genus *Lycopersicon*: Implications for the Evolution of Fruit Size in the Domestication of Cultivated Tomatoes." **Genetics** 162 (1): 365–79.

Nesbitt, T. C., and S. D. Tanksley (2001) "Fw2.2 Directly Affects the Size of Developing Tomato Fruit, With Secondary Effects on Fruit Number and Photosynthate Distribution." **Plant Physiology** 127 (2): 575–83. https://doi.org/10.1104/pp.010087.

Nozue, K. et al. (2015) "Shade Avoidance Components and Pathways in Adult Plants Revealed by Phenotypic Profiling". **PLoS Genetics**, 11(4): 1–26.

Paran, Ilan, and Esther Van Der Knaap (2007) "Genetic and Molecular Regulation of Fruit and Plant Domestication Traits in Tomato and Pepper." **Journal of Experimental Botany** 58 (14): 3841–52. https://doi.org/10.1093/jxb/erm257.

Pino, Lilian E, Simone Lombardi-Crestana, Mariana S Azevedo, Danielle C Scotton, Lucélia Borgo, Vera Quecini, Antonio Figueira, and Lázaro Ep Peres (2010) "The *Rg1* Allele as a Valuable Tool for Genetic Transformation of the Tomato 'Micro-Tom' Model System." **Plant Methods** 6: 23. https://doi.org/10.1186/1746-4811-6-23.

Piotto, Fernando Angelo, and Lázaro Eustáquio Pereira Peres (2012) "Base Genética Do Hábito de Crescimento e Florescimento Em Tomateiro e Sua Importância Na Agricultura." **Ciência Rural** 42 (11): 1941–46. https://doi.org/10.1590/S0103-84782012001100006.

Purugganan, Michael D., and Dorian Q. Fuller (2009) "The Nature of Selection during Plant Domestication." **Nature** 457 (7231): 843–48. https://doi.org/10.1038/nature07895.

Rodriguez, G. R., S. Munos, C. Anderson, S.-C. Sim, A. Michel, M. Causse, B. B. M. Gardener, D. Francis, and E. van der Knaap (2011) "Distribution of SUN, OVATE, LC, and FAS in the Tomato Germplasm and the Relationship to Fruit Shape Diversity." **Plant Physiology** 156 (1): 275–85. https://doi.org/10.1104/pp.110.167577.

Saath, Kleverton Clovis de Oliveira, and Arlei Luiz Fachinello (2018) "Crescimento Da Demanda Mundial de Alimentos e Restrições Do Fator Terra No Brasil." **Revista de Economia e Sociologia Rural** 56 (2): 195–212. https://doi.org/10.1590/1234-56781806-94790560201.

Sastry, KKS, and RM Muir (1963) "Gibberelin: Effect on Diffusible Auxin in Fruit Development." **Science** 140: 494–95.

Shan, Qiwei et al. (2013) "Targeted Genome Modification of Crop Plants Using a CRISPR-Cas System" **Nature Biottechnology** 31 (8): 8–10. https://doi.org/10.1038/nbt.2650.

Stepanova, A. N., J. Yun, A. V. Likhacheva, and J. M. Alonso (2007) "Multilevel Interactions between Ethylene and Auxin in Arabidopsis Roots." **The Plant Cell Online** 19 (7): 2169–85. https://doi.org/10.1105/tpc.107.052068.

Tang, Yun jia, and Johannes Liesche (2017) "The Molecular Mechanism of Shade Avoidance in Crops – How Data from Arabidopsis Can Help to Identify Targets for Increasing Yield and Biomass Production." **Journal of Integrative Agriculture** 16 (6): 1244–55. https://doi.org/10.1016/S2095-3119(16)61434-X.

Tanksley, Steven D. (2004) "The Genetic, Developmental, and Molecular Bases of Fruit Size and Shape Variation in Tomato." **Plant Cell** 16 181-190 (SUPPL.): 181–90. https://doi.org/10.1105/tpc.018119.

Thiele, Alexandra, Michael Herold, Ingo Lenk, Peter H. Quail, and Christiane Gatz (1999) "Heterologous Expression of Arabidopsis Phytochrome B in Transgenic Potato Influences Photosynthetic Performance and Tuber Development." **Plant Physiology** 120 (1): 73–81. https://doi.org/10.1104/pp.120.1.73.

Ulmasov, Tim, Jane Murfett, Gretchen Hagen, and Tom J Guilfoyle (1997) "Creation of a Highly Active Synthetic AuxRE." **Society** 9 (November): 1963–71. https://doi.org/10.1105/tpc.9.11.1963.

Van der Knaap, E. et al.(2014) " What lies beyond the eye: the molecular mechanisms regulating tomato fruit weight and shape". **Frontiers in plant science**, 5 (May): 227.

Van der Knaap, E.; Tanksley, S. D. (2003) "The making of a bell pepper-shaped tomato fruit: Identification of loci controlling fruit morphology in Yellow Stuffer tomato". **Theoretical and Applied Genetics**, 107 (1):139–147.

Wang, Shucai, Ying Chang, Jianjun Guo, and Jin Gui Chen (2007) "Arabidopsis Ovate Family Protein 1 Is a Transcriptional Repressor That Suppresses Cell Elongation." **Plant Journal** 50 (5): 858–72. https://doi.org/10.1111/j.1365-313X.2007.03096.x.

Wang, Shucai, Ying Chang, Jianjun Guo, Qingning Zeng, Brian E. Ellis, and Jin Gui Chen (2011) "Arabidopsis Ovate Family Proteins, a Novel Transcriptional Repressor Family, Control Multiple Aspects of Plant Growth and Development." **PLOS ONE** 6 (8) e23896. https://doi.org/10.1371/journal.pone.0023896.

Wang, Yanping, Josh P. Clevenger, Eudald Illa-Berenguer, Tea Meulia, Esther van der Knaap, and Liang Sun (2019) "A Comparison of Sun, Ovate, Fs8.1 and Auxin Application on Tomato Fruit Shape and Gene Expression." **Plant and Cell Physiology** 60 (5): 1067–81. https://doi.org/10.1093/pcp/pcz024.

Wu, Shan, Han Xiao, Antonio Cabrera, Tea Meulia, and Esther van der Knaap (2011) "SUN Regulates Vegetative and Reproductive Organ Shape by Changing Cell Division Patterns." **Plant Physiology** 157 (3): 1175–86. https://doi.org/10.1104/pp.111.181065.

Xiao, Han, Ning Jiang, Erin Schaffner, Eric J. Stockinger, and Esther Van Der Knaap (2008) "A Retrotransposon-Mediated Gene Duplication Underlies Morphological Variation of Tomato Fruit." **Science** 319 (5869): 1527–30. https://doi.org/10.1126/science.1153040.

Xiao, Han, Cheryll Radovich, Nicholas Welty, Jason Hsu, Dongmei Li, Tea Meulia, and Esther Van Der Knaap (2009) "Integration of Tomato Reproductive Developmental Landmarks and Expression Profiles, and the Effect of SUN on Fruit Shape." **BMC Plant Biology** 9:1.21. https://doi.org/10.1186/1471-2229-9-49.

Xu, Cao, Katie L. Liberatore, Cora A. MacAlister, Zejun Huang, Yi-Hsuan Chu, Ke Jiang, Christopher Brooks, et al. (2015) "A Cascade of Arabinosyltransferases Controls Shoot Meristem Size in Tomato." **Nature Genetics** 47 (7): 784–92. https://doi.org/10.1038/ng.3309.

Ying, L. X. et al. Regulation of chloroplast ultrastructure, cross-section anatomy of leaves, and morphology of stomata of cher ry tomato by different light irradiations of light-emitting diodes. **HortScience**, v. 46, n. 2, p. 217–221, 2011.

Zhang, Zengcui, Aili Li, Gaoyuan Song, Shuaifeng Geng, Bikram S. Gill, Justin D. Faris, and Long Mao (2020) "Comprehensive Analysis of Q Gene Near-Isogenic Lines Reveals Key Molecular Pathways for Wheat Domestication and Improvement." **Plant Journal** 102 (2): 299–310. https://doi.org/10.1111/tpj.14624.

Zhang, Zhi, Cuiyu Mao, Zheng Shi, and Xiaohong Kou (2017) "The Amino Acid Metabolic and Carbohydrate Metabolic Pathway Play Important Roles during Salt-Stress Response in Tomato" **Frontiers in Plant Science** 8 (July) Article 1231. https://doi.org/10.3389/fpls.2017.01231.

Zhu, Guangtao, Shouchuang Wang, Zejun Huang, Shuaibin Zhang, Qinggang Liao, Chunzhi Zhang, Tao Lin, et al. (2018). "Rewiring of the Fruit Metabolome in Tomato Breeding." **Cell** 172 (1–2): 249-261.e12. https://doi.org/10.1016/j.cell.2017.12.019.

Zhu, Mingku, Guoping Chen, and Jianling Zhang (2014) "The Abiotic Stress-Responsive NAC-Type Transcription Factor SlNAC4 Regulates Salt and Drought Tolerance and Stress-Related Genes in Tomato (Solanum Lycopersicum)" **Plant Cell Rep** 33:1851–63. https://doi.org/10.1007/s00299-014-1662-z.

Zouine M, Maza E, Djari A, Lauvernier M, Frasse P, Smouni A, Pirrello J and Bouzayen M. (2017) "TomExpress, a unified tomato RNA-Seq platform for visualization of expression data, clustering and correlation networks". **Plant J.**, 92: 727–735.

Zsögön, Agustin, Tomáš Čermák, Emmanuel Rezende Naves, Marcela Morato Notini, Kai H. Edel, Stefan Weinl, Luciano Freschi, Daniel F. Voytas, Jörg Kudla, and Lázaro Eustáquio Pereira Peres (2018) "De Novo Domestication of Wild Tomato Using Genome Editing." **Nature Biotechnology** 36 (12): 1211–16. https://doi.org/10.1038/nbt.4272.

Zuo, Z. et al. (2011) "Blue Light-Dependent Interaction of CRY2 with SPA1 Regulates COP1 activity and Floral Initiation in Arabidopsis". **Curr Biol.**, v (10), 841–847.