

University of São Paulo  
“Luiz de Queiroz” College of Agriculture

Natural genetic variations from the tomato wild relative *Solanum pennellii*  
associated with domestication and drought resistance

**Mateus Henrique Vicente**

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

Piracicaba  
2019

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Bachelor in Agronomy

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

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1. Tomate 2. Linha de introgressão 3. Domesticação 4. Resistência à seca 5. Condutância hidráulica I. Título

To my father, mother, brother, nephew,  
And my love wife,  
For all unconditional love, help, friendship  
and support, I dedicate...

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“The mind that opens to a  
new idea never returns to its  
original size.”

Albert Einstein

“If you don’t like your destiny, don’t accept it.  
Instead, have the courage to change  
it the way you want it to be.”

Masashi Kishimoto



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## RESUMO

**Variações genéticas naturais do tomateiro selvagem *Solanum pennellii* associadas à domesticação e resistência à seca**

A domesticação das plantas levou a uma perda de variação genética em muitas culturas, devido à ênfase excessiva na seleção de órgãos comestíveis (raiz, folha, caule ou fruto) e a baixa pressão de seleção para outras características no ambiente cultivado. Essa "erosão genética" levou à perda de alelos associados à resistência de diversos estresses ambientais, como seca e salinidade, os quais, por sua vez, podem conduzir a perdas significativas na produtividade das plantas. Entretanto, no tomate (*Solanum lycopersicum* L.), é possível acessar um banco valioso de variação genética nas espécies selvagens relacionadas. Assim, a identificação de variantes genéticas associadas ao processo de domesticação do tomateiro e a mecanismos de resistência a estresses ambientais, os quais podem ter sido perdidos durante a domesticação, pode auxiliar em programas de melhoramento do tomateiro e de outras culturas de interesse comercial. Diante disso, no presente trabalho, o qual foi dividido em dois capítulos, realizamos cruzamentos entre a espécie selvagem, *S. pennellii*, e a cultivar miniatura de tomateiro Micro-Tom (MT) para criarmos duas linhas de introgressão (ILs), uma com tamanho de órgão reduzido e outra com maior tolerância à seca. No primeiro capítulo, relatamos a caracterização e mapeamento da IL denominada como *Tiny organs and reduced yield (Toy)*. O genótipo *Toy* carrega um segmento do genoma de *S. pennellii* no cromossomo 7 e apresenta uma considerável redução em órgãos vegetativos (folhas) e reprodutivos (frutos). Os resultados obtidos conduziram a uma discussão de como esse genótipo pode ser relevante para a domesticação do tomateiro, devido ao seu impacto no tamanho de diversos órgão. Por outro lado, no segundo capítulo, descrevemos o mecanismo de tolerância à seca da IL *Water Economy Locus em Lycopersicon (Well)*. Plantas *Well* carregam um segmento do genoma de *S. pennellii* no cromossomo 1 e exibem uma menor condutância hidráulica, possivelmente relacionada ao tamanho reduzido do vaso xilemático. A menor condutância hidráulica do genótipo *Well* conduz a perturbações no contínuo solo/planta/atmosfera levando a mudança no comportamento estomático, que, por sua vez, provavelmente está relacionado a maior resistência ao murchamento apresentada por esse material em condições de déficit hídrico.

Palavras-chave: Tomate; Linha de introgressão; Domesticação; Resistência à seca; Condutância hidráulica

## ABSTRACT

**Natural genetic variations from the tomato wild relative *Solanum pennellii* associated with domestication and drought resistance**

Plant domestication led to a loss of genetic variation in many crops, due to the excessive emphasis in the selection of edible organs (root, leaf, stem or fruit) and the low selection pressure for other traits in the cultivated environment. This ‘genetic erosion’ led to loss of alleles associated with resistance to environmental stresses, such as drought and salinity, which can in turn culminate in productivity losses. In tomato (*Solanum lycopersicum* L.), it is possible to tap into a reservoir of valuable genetic variation in its wild relatives. Identification of genetic variants associated with tomato domestication, and with stress resistance mechanisms which may have been lost during domestication, could be used to aid in breeding programs. In the present work, which was divided into two chapters, we carried out crosses between the wild species *S. pennellii* and the miniature tomato cultivar Micro-Tom (MT) and created two introgression lines (ILs), one with reduced organ size and another with increased drought tolerance. In the first chapter, we report the characterization and mapping of the IL denominated as *Tiny organs and reduced yield* (*Toy*). *Toy* harbors a *S. pennellii* genome segment on chromosome 7 and presents a considerable reduction in both vegetative (leaves) and reproductive (fruit) organs. We discuss how this could be a relevant trait underpinning tomato domestication. In the second chapter, we describe the drought tolerance mechanism of the IL *Water Economy Locus in Lycopersicon* (*Well*). *Well* harbors a *S. pennellii* genome segment on chromosome 1 and shows lower hydraulic conductance, possibly related to decreased xylem vessel size. The results shown suggest that this lower hydraulic conductance promotes a disturbance in the soil-plant-atmosphere hydraulic continuum leading to changes in stomatal behavior, which, in turn, are probably related to the delayed wilting of *Well* under conditions of water deficit.

Keywords: Tomato; Introgression line; Domestication; Drought resistance; Hydraulic conductance

# 1. A NATURAL GENETIC VARIATION LINKING LEAF AND FRUIT GIGANTISM IN THE TOMATO DOMESTICATION SYNDROME

## Abstract

The domestication process involved the selection of traits that differentiate the cultivated and the wild ancestor species. The set of resulting traits is referred to as the domestication syndrome, and includes alterations in growth habit, flowering time and organ size. Increased size of plant structures (gigantism) is evident in many crops. In tomato (*Solanum lycopersicum*), an obvious hallmark of domestication is the massive increase in fruit size in comparison to wild relatives. However, most studies neglect the importance of leaf size, which is also bigger in cultivated tomato cultivars. As a consequence, the genetic basis of leaf size increase during domestication remains relatively unexplored. Here, the tomato wild relative *S. pennellii* was crossed with the cultivated tomato cv. Micro-Tom, aiming to find a possible genetic component associated with leaf size. After successive backcrosses and phenotypic selection, we produced an introgression line (IL) with reduced leaves. The IL also present reduced ovary sizes and fruit weight compared to the MT plants. The IL was thus named *Tiny organs and reduced yield (Toy)* and mapped on chromosome 7. Anatomical determinations showed that the organ size reduction in *Toy* plants is due to a decreased cell division. Consistently, genes related to cell division, such as *CYCB2,1* and *FRUIT WEIGHT 2.2* had an heterochronic expression during ovary development in *Toy*. Together, the results presented here suggest that *Toy* may represent a major variation selected during tomato domestication, since it conciliates the increase of fruit size, which are strong sinks, with the necessary increase in the source of photoassimilates.

Keywords: Tomato; Introgression line; Domestication; Leaf size; Fruit size

## 1.1. INTRODUCTION

The domestication syndrome can be defined as the suite of phenotypic changes that occurred through artificial selection to transform wild species into crops (Evans, 1993). Some of commonly found traits are more vigorous growth, increased apical dominance, determinate growth, loss of natural seed dispersal and larger fruits or grains. (Frary and Doganlar, 2003; Meyer and Purugganan, 2013). Increased size of the whole plant or of certain organs, namely gigantism, is widespread in crops. Gigantism can be also a consequence of allometric alterations in the relative size of certain plant structures (Niklas, 2004). A prime example is the species *Brassica oleracea*, where multiple cultivated strains were produced through selection on the differential growth of edible organs such as stems (kohlrabi), buds (cabbage, Brussels sprouts), leaves (kale) and flowers (broccoli, cauliflower) (Lester, 1989; Gómez-Campo and Prakash, 1999; Prakash et al. 2011). Although increased organ size can be explained by either increased cell size or number, or a combination of both (Krizek, 2009), it also requires developmental alterations to transform larger organs into stronger photosynthetic sinks (Gifford and Evans, 1981).

In tomato (*Solanum lycopersicum* L.), gigantism during domestication is evidenced by the massive increase in fruit size when compared to its wild progenitor *S. pimpinellifolium*, which has

pea-sized fruits (Tanksley, 2004). The genetic basis of fruit gigantism has attracted considerable attention (reviewed in van der Knaap et al., 2014). Increased fruit size in tomato involved mutations in at least five major loci: *fruit weight 2.2 (fw2.2)*, *fruit weight 3.2 (fw3.2)*, *fruit weight 11.3 (fw11.3)*, *fasciated (fas)* and *locule number (lc)* (Tanksley, 2004). The *FW2.2* gene is a negative regulator of cell division (Guo and Simmons, 2011) and is responsible for up to 30% of the increase on fruit size when comparing lines harboring small- and large-fruit alleles (Frary et al., 2000). The *fw3.2* and *fw11.3* together explained 27% of the phenotypic variance in an F<sub>2</sub> population obtained by crossing the tomato cv. Yellow Stuffer and *S. pimpinellifolium* (van der Knaap and Tanksley, 2003). *FW3.2* and *FW11.3* were identified as a P450 enzyme of the CYP78A subfamily (*SKLUH*) and a *Cell Size Regulator (CSR)*, respectively (Chakrabarti et al., 2013; Mu et al., 2017). Unlike *fw2.2*, *fw3.2* and *fw11.3*, which mostly affect fruit size, *fas* and *lc* also control fruit shape. Hence, the domestication *fas* and *lc* alleles increase the number of carpels (and locules), often resulting in larger and wider fruits with pronounced ribbing (Lippman and Tanksley, 2001; van der Knaap and Tanksley, 2003). The *lc* mutant phenotype is caused by two single-nucleotide polymorphisms (SNPs) downstream the coding region of the *WUSCHEL* gene (Muños et al., 2011). The activity of *WUSCHEL* increases the number of stem cells (Mayer et al., 1998) in the meristem that will form the carpels. The *fas* mutation is a loss-of-function allele caused by an inversion in the regulatory region of the *CLAVATA3* gene (Xu et al., 2015), a negative regulator of *WUSCHEL*. Thus, the *fas* mutation also affects stem cell fate, resulting in an increased number of carpels that derive from the altered meristem. The genetic basis for the gigantism of vegetative organs, on the other hand, is hitherto unknown. Increased stem and leaf size could indirectly have affected fruit size and provided for the necessary balance between photosynthetic sources and sinks accompanying the allometric alterations driven by domestication.

In the carbon economy of the plant, larger fruits represent stronger ‘sinks’, as they require more photosynthate to achieve a greater size (Gustafson and Stoldt, 1936; Li et al., 2015; Osorio et al., 2014). This, in turn, would necessitate increased photoassimilate export from ‘source’ tissues, mainly leaves. In tomato, as in other crop plants, this can be achieved by increasing either photosynthetic rate or leaf size. As most domesticated tomato cultivars have bigger leaves than their wild relatives (Milla and Matesanz, 2017), the latter appears to be a more parsimonious possibility. In contrast to fruit size, however, the genetic basis of changes in vegetative organ size remain relatively unexplored in tomato. Given the recently established possibility of performing *de novo* domestication of wild species (Li et al., 2018; Zsögön et al., 2018), the discovery of alleles

responsible for vegetative gigantism would be a valuable addition to the repertoire of traits whose genetic basis is well understood.

We hypothesized that if such a genetic determinant exists, the corresponding non-domesticated alleles could be found through wide crosses between cultivated tomato with any wild relative species. Thus, we crossed *S. pennellii* with the cultivated tomato cv. Micro-Tom (MT). After successive backcrosses and phenotypic selection, we retrieved an introgression line (IL) that presents simultaneous reduction in leaf size and fruit weight, compared to the recurrent parental MT. We mapped this IL on chromosome 7 and named it as *Tiny Organs and Reduced Yield* (*Toy*). *Toy* presented as a single Mendelian locus. We also conducted a literature survey for described QTLs (Quantitative Trait Loci) for leaf size and fruit weight, and screened in the existent collection of introgression lines (ILs), where a defined genomic segment of *S. pennellii* replaces a homologous region in the cultivated tomato cultivar M82 background (Eshed and Zamir, 1994; 1995), and found a similar phenotype in the ILs corresponding to the *Toy* region in chromosome 7. We speculate on the impact of this pleiotropic locus in tomato domestication syndrome and discuss its potential exploitation for crop breeding.

## 1.2. CONCLUSION

Based on the analysis of natural genetic variation, we presented a potential genetic determinant for increased leaf size in cultivated tomato. Furthermore, our results could unveil a novel link in the genetic basis of fruit and leaf size in tomato. Fruit gigantism in tomato is a hallmark domestication trait, however, less attention has been paid to the increase in vegetative organ size, which represents a necessary source of photosynthates to supply larger fruits.

Further research should determinate the molecular identity of the gene(s) underlying the *Toy* phenotype. This would place one more piece in the tomato domestication puzzle and provide new information that could be harnessed to improve both tomatoes as well as other species, as gigantism is recurring theme in most extant crops. In addition, the pleiotropic effect presented by *Toy* plants, credits this locus as a good candidate for the implementation of the *de novo* domestication (Zsogon et al. 2017; 2018) in several species.

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## 2. THE WATER ECONOMY LOCUS IN LYCOPERSICON (WELL) HAS A LOWER HYDRAULIC CONDUCTANCE, WHICH IS LINKED TO ITS DELAYED WILTING AND FAST RECOVERY FROM DROUGHT

### Abstract

The search for drought-resistant plants is becoming increasingly relevant, especially in light of future predictions of climate change. Tomato (*Solanum lycopersicum*) is an important crop that presents a great source of drought resistance in its wild relatives, such as *S. pennellii*. However, studies exploring the genetic components and physiological mechanisms involved in this resistance are still incipient. In the present work the physiological mechanisms responsible for the drought resistance of an introgression line (IL) named *Water Economy Locus in Lycopersicon (Well)* were studied. *Well* was obtained by crossing *S. pennellii* and the miniature tomato cultivar Micro-Tom (MT), and further mapped on chromosome 1. This IL presents a noteworthy delay in wilting upon water withdrawal and a great control of water loss compared to MT. Measurements of stomatal conductance ( $g_s$ ) showed no significant difference between MT and *Well* plants under well-hydrated and moderate stress conditions, but a significant  $g_s$  reduction in *Well* plants was verified after the resuming of irrigation. *Well* plants also presented midday depressions in leaf patch pressure ( $P_p$ ) curve, which is inversely proportional to the leaf turgor pressure, as well as a smaller “wrong-way responses” (WWR) duration compared to MT. These results suggest that *Well* plants exhibit a differential stomatal behavior. *Well* stomatal behavior was associated with a lower hydraulic conductance ( $K$ ), which is probably ascribed to a decreased xylem vessel size observed in its stem. The further molecular identification of the *Well* gene will provide for our understanding of drought-resistance mechanisms and allows its faster use in breeding programs.

Keywords: Tomato; Introgression line; Drought resistance; Hydraulic conductance; Xylem vessel

### 2.1. INTRODUCTION

Water has a fundamental role in biomass accumulation in plants, namely photosynthesis, and its lack may beget irreparable losses in crop production (Boyer, 1982; Mueller et al., 2012; van Ittersum et al., 2013). The importance of water is undeniable for current agriculture. Hence, climate change has caused significant impacts on the global water cycle, leading to concerning prospects for food security, mostly due to changes in crop yield (Kang et al., 2009; Sun et al., 2012; Najafi et al. 2018). In this context, the investigation of the genetic basis and the physiological mechanisms that improve drought resistance or water-use efficiency (WUE, amount of carbon fixed per unit water transpired) in plants is required. In addition, the manipulation of such parameters might be essential for modern agriculture, which requires more sustainable crop production.

Noteworthy, the process of crop domestication has generated a bottleneck in genetic diversity (Doebley et al., 2006), which has made it difficult to search for putative genes and mechanisms involved in biotic and abiotic stress tolerance. However, wild progenitors and/or

related species of crops represent an excellent alternative for breeding, as they are a rich reservoir of natural genetic variation (Rick, 1973; Zamir, 2001; Koornneef et al., 2004; Juenger, 2013). Such genetic variation can directly contribute to improve crop performance under resource-limited conditions (e.g. low water availability). Among domesticated crops, the tomato (*Solanum lycopersicum* L.) stands out as a useful model for natural genetic variation studies (Rick, 1973; Zsögön et al., 2017). Tomato has a fully sequenced genome and a broad repository of natural genetic variation in the form of 12 closely related wild species (Rick, 1976, 1983; The Tomato Genome Consortium, 2012; Bolger et al, 2014). Each of these relatives presents peculiarities according to the conditions that they experience in their natural habitat, including water availability. The major genetic sources for drought resistance in tomato are *S. chilense* and *S. pennellii* (Rick, 1973). Although some features of *S. chilense* preclude its use in genetic and physiological studies (e.g. fertility barriers with cultivated tomato) (Zsögön et al, 2017), *S. pennellii* has attracted the interest of plant biologists around the world (Bolger et al, 2014).

*S. pennellii* exhibits a remarkable capacity of surviving, maintaining water status and growth in environments with low water availability (Yu, 1972; Rick, 1973; Martin and Thorstenson, 1988). Besides, a high-quality, fully annotated genome sequence of *S. pennellii* is already publicly available (Bolger et al., 2014). Thus, determination of the genetic basis of its drought resistance is highly desirable, and some progress has been made by identification and characterization of *S. pennellii*'s individual genes (Wei and O'Connell, 1996; Treviño and O'Connell, 1998; Ziaf et al., 2011; Loukehaich et al., 2012; Li et al., 2015; Li et al., 2018). Furthermore, *S. pennellii* has a higher WUE than cultivated tomatoes (Kebede et al., 1994; Martin et al., 1999; Xu et al., 2008).

Drought resistance is sometimes treated as synonymous with high WUE, and although drought can increase WUE (Franks et al 2015), they are not physiologically equivalent (Blum 2005; 2009). Different from WUE, drought resistance is more complex than the relationship between two physiological variables, transpiration and photosynthesis (Blum, 2005). Instead, it is a trait strongly linked to all aspects of plant development, including phenology and morphology. In line with this, Levitt (1972) pointed out that the mechanisms of drought resistance can be drought escape, dehydration avoidance and dehydration tolerance. Drought escape consists of phenological modifications that make plants capable of completing their life cycle before the onset of severe water stress, such as earlier flowering (Kooyers, 2015). Drought avoidance involves morphological adaptations such as deep root systems or stomatal modifications (Pinheiro et al. 2005; Basu et al. 2016). Both drought scape and avoidance comprise mechanisms that maintain plant tissues with high water potentials in dry environments (Levitt, 1972). In

dehydration tolerance, biochemical mechanism allows plants to withstand severe water scarcity through the protection of membranes and other cellular components (Serraj and Sinclair, 2002; Basu et al. 2016). Drought tolerance can lead to a growth reduction and thus lower yields. Therefore, despite the fact that drought resistance is a trait long sought by breeders, it is important to determine the kind of mechanism involved and its impact on productivity.

Herein, we use an introgression line (IL) produced by successive backcross between *S. pennellii* and the tomato model system Micro-Tom (MT), which was denominated as *Water Economy Locus in Lycopersicon (WELL)* (Zsögön, 2011). *WELL* was mapped on chromosome 1 and characterized as both drought resistant (Zsögön, 2011) and more effective in water use (Vicente et al., 2015). Probably, *Well* plants harbor one of the genetic components of *S. pennellii*'s drought resistance, whose physiological mechanism has not yet been explored. The aim of this work was to investigate the physiological mechanisms involved in the drought-resistance presented by the *Well* genotype. The data presented here indicate a putative mechanism of drought resistance in the *Well* plants involving decreased xylem conductance, which promotes changes in the stomatal behavior and dehydration avoidance.

## 2.2. CONCLUSION

In this work, we characterized the water relations in an introgression line with delayed wilting, greater recovery after a severe drought stress, and that had already been reported as more efficient in water use (Vicente et al., 2015). The lower hydraulic conductance found in *Well* plants may be a possible mechanism for such effects. We also presented evidences indicating that the greater stomatal sensitivity observed in *Well* plants occurs in response to lower hydraulic conductance, which correlates to the reduction in the size of the xylem vessels. The further molecular identification of the *Well* locus may favor its incorporation in breeding programs, aiming to improve water use efficiency and minimize plant losses due to drought stress in seedling transplant.

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