

Small and remarkable

The Micro-Tom model system as a tool to discover novel hormonal functions and interactions

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Hormones are molecules involved in virtually every step of plant development and studies in this field have been shaping plant physiology for more than a century. The model plant *Arabidopsis thaliana*, long used as a tool to study plant hormones, lacks significant important developmental traits, such as fleshy climacteric fruit, compound leaf and multicellular trichomes, suggesting the necessity for alternative plant models. An attractive option often used is tomato, a species also of major economic importance, being ideal to bring together basic and applied plant sciences. The tomato Micro-Tom (MT) cultivar makes it possible to combine the direct benefits of studying a crop species with the fast life cycle and small size required for a suitable biological model. However, few obscure questions are constantly addressed to MT, creating a process herein called “MT mystification”. In this work we present evidence clarifying these questions and show the potential of MT, aiming to demystify it. To corroborate our ideas we showed that, by making use of MT, our laboratory demonstrated straightforwardly new hormonal functions and also characterized a novel antagonistic hormonal interaction between jasmonates and brassinosteroids in the formation of anti-herbivory traits in tomato.

The Need of Alternative Models to Discover Novel Hormonal Functions and Interactions

Since the classical experiments with coleoptile tips by Darwin & Darwin¹ to

the molecular evidences of a novel hormone controlling shoot branching and mycorrhiza formation,² the study of plant hormones shaped plant physiology's history. Time has shown the importance of these molecules: from root/shoot development to stress response, from light perception to trichome formation, from stomatal closure to seed germination, virtually every step of plant development is regulated by one or several hormones at the same time.³ Since one hormone class can control various different processes and, conversely, a single process can be controlled by several hormones, the discovery of novel hormonal functions and interactions is relevant to understand plant development and its interplay with the environment.

Much of what we know about plant hormones came from studies with *Arabidopsis thaliana*. Its small size, fast life cycle, sequenced genome (see www.arabidopsis.org/), lenience to grow in controlled conditions and, most importantly, the identification and characterization of several hormonal mutants make *Arabidopsis* the first and most sensible choice when conducting research on plant hormones.

On the other hand, *Arabidopsis* lack many traits of great economic importance and that is when tomato (*Solanum lycopersicum* L.) thrives as an interesting plant model. Tomato can be used to address developmental processes difficult or impossible to be studied in *Arabidopsis*, such as photoperiod-independent sympodial flowering;^{4,5} formation of fleshy climacteric fruits, compound leaves and multicellular/glandular trichomes;^{6,7} mycorrhizal

association and agronomically relevant plant-insect and pathogen interactions.⁷⁻⁹

Tomato Genetics and a Remarkable Gizmo—Bringing Everything into One Cultivar

Tomato presents many characteristics of a biological model: it is an autogamous diploid species with a small genome (950 Mb) distributed in 12 chromosomes; it has a saturated genetic linkage map with numerous markers associated with traits of great economic and biological importance (solgenomics.net/). It belongs to a taxonomic group (Asterid clade) largely unexplored yet at the molecular level. Highly efficient protocols for transformation of tomato are already developed¹⁰ and rich germplasm collections are available, such as the Tomato Genetics Resource Center (tgrc.ucdavis.edu/). The tomato genome is currently being sequenced by the “International Solanaceae Genome Project” (SOL) consortium.¹¹ Tomato is also a potentially valuable tool for research on plant hormones, due to the availability of a vast number of hormonal mutants.⁷ And differently from *Arabidopsis*, the edible crop status of tomato makes it one of the best candidates to bring basic and applied sciences together.^{12,13}

However, two major problems are generally raised on plant hormone studies in tomato. Firstly, since many of the hormonal mutations are present in diverse cultivars, the information/comparison between them is poorly exchangeable. Secondly, due to the normal plant size of the species (>1-m tall), it requires considerable growing spaces, and the somewhat long generation time (~4 months) makes it a not-so-easy-to-handle plant. For these reasons, the dwarf tomato cultivar Micro-Tom (MT) is now becoming increasingly used as a model system and was described as the “laboratory tomato”.¹⁴ Because of its compact plant size (~15-cm tall) and red-ripened fruits, MT was originally described for ornamental purposes,¹⁵ but because its short life cycle (~10 weeks) and the ability to growth at high densities, it has also become a suitable genetic model system.¹⁴

MT is now being widely used in studies of fruit development,¹⁶ hormonal interactions,⁷ abiotic/biotic stress responses,^{17,18}

mycorrhizal colonization,⁸ small RNA gene regulation¹⁹ and even microgravity growth.²⁰ Several genetic and physiological tools are already available in the MT cultivar, such an efficient transformation protocol,¹⁰ comprehensive EMS and gamma-ray mutant collections,²¹ EST and SNP databases (www.kazusa.or.jp/jsol/microtom/index.html), and a “metabolite annotation” compilation.²² A hormonal mutant collection introgressed into the MT genotype is also publicly available,^{7,23} making it also an ideal model to characterize physiological and developmental functions of plant hormones and their interactions, as it has been proven recently.⁷

Despite its potential value, the complete acceptance of MT as a model plant has not taken off as yet because some confusing and obscure concerns still persist, creating what we called herein the “mystification of the MT cultivar”. Nonetheless, if looked closer, one will realize that once understanding the genetic concepts behind the MT and taking the necessary scientific approaches to avoid misleading conclusions, these concerns are vanished and MT becomes an ideal model system.

Demystifying Micro-Tom

Indeed, the MT cultivar harbours some distinctive mutations. The most well known mutant alleles are: *dwarf* (*d*), a brassinosteroid (BR)-related mutation²⁴ responsible for the small plant size, and *self-pruning* (*sp*), responsible for its determinate growth habit.^{4,25} The *miniature* (*mnt*) allele was also suggested to contribute to the MT small plant size,¹⁴ although this has not been proven yet. Additional alleles present in MT are *uniform ripening* (*u*), *Stemphylium resistance* (*Sm*) and *Immunity to Fusarium wilt* (*I*),¹⁵ which confer the absence of green shoulders in fruits and resistance to the pathogenic fungi *Stemphylium solani* and *Fusarium oxysporum* f. *lycopersici*, respectively. The presence of such mutations led to the pre-judgment that MT cannot be used in scientific studies because they can interfere with the results observed.

Scientists using crop species as genetic models should bear in mind that the domestication process itself was developed based on selection and recombination of

mutations. Thus, although the term “wild type” (WT) is adequate for non-domesticated animal and plants, it is not entirely accurate for domesticated models, such as tomato. Moreover, every cultivar holds many mutant alleles when compared to any other genotype and this genetic assortment is exactly what defines a cultivar. To exemplify, the cultivar M82 of tomato, used to study natural genetic variations²⁶ and induced mutagenesis,²⁷ holds the alleles *sp*, *obv*, *u*, *I* and *Vc* (tgrc.ucdavis.edu/). Even for cultivars with no apparent mutations, such as Ailsa Craig and MoneyMaker, the most used “non-dwarf” cultivars in genetic studies of tomato, allelic variations exist at least in quantitative trait loci (QTL) controlling fruit size,²⁸ since their fruits are larger than the fruits of the wild tomato (*Solanum lycopersicon* var. *cerasiforme*). If one denies the use of MT because of its mutations, one will come to the conclusion that no cultivar in any species can be used in scientific experiments. Moreover, it is worth noting that with an appropriate control, the presence of mutations or allelic variations in a cultivar does not preclude it from being used to study the effects of specific mutations. If the event under study is influenced by that particular mutation, the alternative of generating near isogenic lines (NILs) harboring the non-mutated allele as a control fulfills the requirement of an appropriate control in the scientific method. In the case of MT, NILs with indeterminate growth habit (MT-*Sp*), green-shoulder fruits (MT-*U*) and increased BR levels (MT-*D*) have recently been developed (Carvalho et al. in preparation).

Another concern commonly addressed to MT specifically in hormonal studies is associated to its BR-related mutation and a suggested gibberellin (GA) mutation, perceiving the genotype as unsuitable for hormone studies or to analyse GA and/or BR-dependent responses. Indeed, MT contains a weak mutation (*d*) related to BR biosynthesis, however, two points should be mentioned: (1) Although there is no “correct” level of BR that a plant should contain, *d* is not a severe BR mutation like *cu3* or *dpy*.²⁹ Additional evidence is that the *dpy* mutant was introgressed (as well as *cu3*) into MT and the resulting NIL was completely differently than MT itself

(Fig. 1A) and its original background; (2) a recent paper from our lab showed that MT can perfectly be used to study BR-dependent responses and how BR interacts with other hormones.⁷ The case of GA is even simpler. There is no indication for a GA mutation in MT since: (1) MT has normal seed germination and leaf development; (2) opposite GA mutations were introgressed into MT^{7,23} and their phenotypes were clearly observed, such as for the mutation in the DELLA repression domain *procera* (Fig. 1B) and the GA deficient *giberellin deficient 2* (Fig. 1C).^{30,31}

The Micro-Tom as a Model System to Discover Novel Hormonal Functions and Interactions

An example of the usefulness of the MT cultivar in hormonal functions and interaction investigation was demonstrated in the paper that is the object of this addendum.⁷ Novel hormonal functions were observed when comparing several different hormonal mutants in the MT cultivar. We found out that ethylene, GAs and auxin are capable of altering glandular and non-glandular trichome density in tomato leaves, showing a possible multi-hormonal control of this trait. However, the most remarkable finding was a BR-JA (jasmonic acid) interaction, where low BR levels positively control the formation of anti-herbivory traits by means of upregulating the JA pathway. This interaction seems to be evolutionary divergent in the plant kingdom, since the opposite is observed in Arabidopsis, in which high BR is capable of upregulating OPR3, an enzyme involved in JA biosynthesis.³² Additionally to these novel results, our work also proved that although MT harbors a BR mutation, it still can be employed to study plant hormones, even on BR action and interaction.

Conclusion

The late Charles Rick once said that “If Arabidopsis is the *Drosophila* of plant genetics, than tomato has become the mouse”.¹² If Rick was right, than MT is the best and fastest way to make tomato as widespread, as useful and perhaps as small as a mouse.

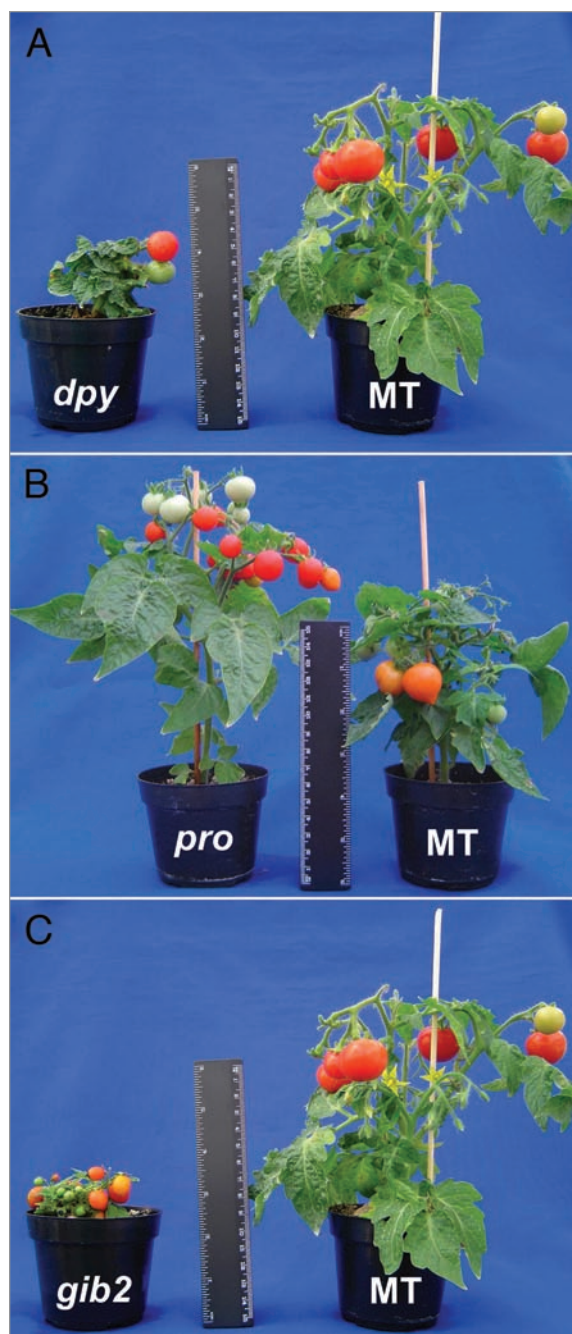


Figure 1. Comparison between Micro-Tom (MT) and near isogenic lines harboring hormonal mutations.^{7,23} (A) The brassinosteroid defective *dpy* has a severe dwarf phenotype and sessile, curled leaves. The phenotype of the BR-insensitive mutant *cu3* is indistinguishable from *dpy* (not shown). (B) *procera* is a mutant in the repressor domain of DELLA. *pro* plants are bigger than MT and their leaves are larger with smooth borders, typical of a GA⁺ mutant. (C) The *giberellin deficient 2* mutant is defective in GA production. It produces seeds that germinate only if exogenous GA is applied. *gib2* plants and leaves are also smaller than MT. This figure clearly shows that GA and BR mutations can be perfectly obtained in the MT background. Plants were cultivated in 150-ml pots and photos were taken at 80 days after germination. (ruler = 15 cm).

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