TESIS DOCTORAL

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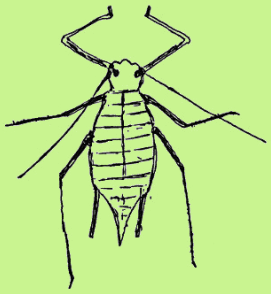
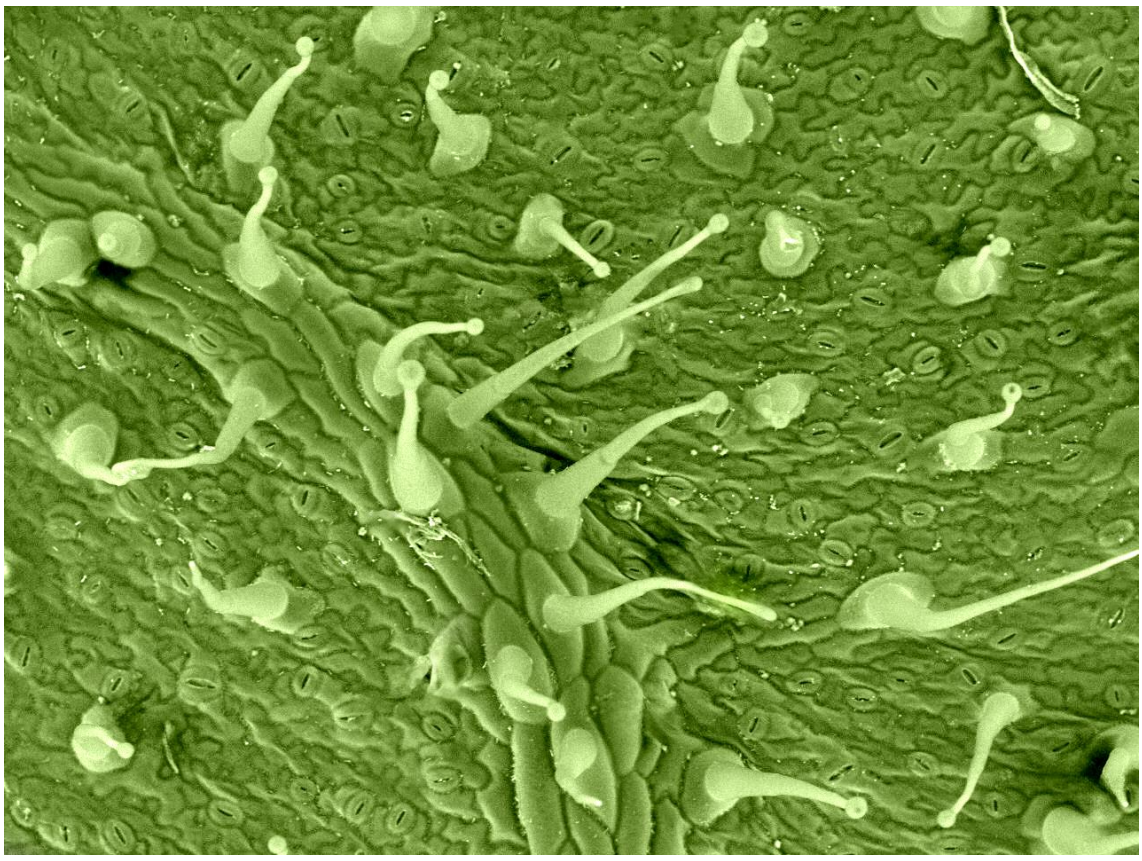


Lidia Blanco Sánchez

2023

Directores: Eduardo de la Peña Alonso y Juan Antonio Díaz Pendón



Universidad de Málaga – Facultad de Ciencias
Programa de Doctorado en Biotecnología Avanzada

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Evaluación experimental del papel de los tricomas glandulares tipo IV en la defensa del tomate frente al pulgón Macrosiphum euphorbiae

Lidia Blanco Sánchez

Directores: Eduardo de la Peña Alonso y Juan Antonio Díaz Pendón

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Realizada bajo la tutorización de EDUARDO DE LA PEÑA ALONSO y dirección de EDUARDO DE LA PEÑA ALONSO y JUAN ANTONIO DÍAZ PENDÓN (si tuviera varios directores deberá hacer constar el nombre de todos)

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Planelló, R., Llorente, L., Herrero, Ó., Novo, M., **Blanco-Sánchez, L**., Díaz-Pendón, J. A., Fernández-Muñoz, R., Ferrero, V. & de la Peña, E. (2022). Transcriptome analysis of aphids exposed to glandular trichomes in tomato reveals stress and starvation related responses. *Scientific Reports, 12*(1), 20154. *Impact factor (5-year): 5.52. Ranking: Multidisciplinary Sciences (Q1).*

Blanco-Sánchez, L., Planelló, R., Ferrero, V., Fernández-Muñoz, R., de la Peña, E., & Díaz Pendón, J. A. (2023). More than trichomes and acylsugars: the role of jasmonic acid as mediator of aphid resistance in tomato. *Journal of Plant Interactions, 18(1),* 2255597. *Impact factor (5-year)*: 5.1. *Ranking: Plant Sciences (Q2)*.

International conferences:

- **Oral presentation**: "Regulatory mechanisms of tomato glandular trichomes against the aphid *Macrosiphum euphorbiae*" (**Blanco L.,** Díaz-Pendón J. A., de la Peña E.) on the *70th International Symposium on Crop Protection* – Ghent (Belgium), 22nd May 2018.

- **Poster presentation** "Regulatory mechanisms of tomato glandular trichomes against the aphid *Macrosiphum euphorbiae*" (**Blanco L.**, Díaz-Pendón J. A., de la Peña E.) on the *41st New Phytologist Symposium* – Nancy (France), 11st - 13rd April 2018.
- **Oral presentation**: "Characterization and effect of glandular trichomes on plant aphid interactions in tomato" (**Blanco L.** and de la Peña E.) on the *3rd Hemipteran-Plant Interactions Symposium (HPIS)* – Madrid (Spain), 4th – 8th June 2017.

La ciencia y la vida cotidiana no pueden y no deben estar separadas – Rosalind Franklin

A mi madre

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INDEX

ABBREVIATIONS

16L:8D: Photoperiod of 16h light and 8h dark

ABL 10-4: Isogenic line to MM with enhanced glandular trichomes type IV and acylsucorses secretion

BLAST: Basic Local Alignment Search Tool.

cDNA: Complementary DNA

CM: *Solanum lycopersicum* L. var. Castlemart

CYP: Cytochrome P450

DAMP: Molecular Patterns Associated to Damage

DEG: Differential Expression Gene Analysis

DNA: Desoxyribonucleic acid

EcR: Ecdysone Receptor

EU: European Union

FAO: Food and Agriculture Organization of the United Nations

FDS: Frequency-Dependent Selection

GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase

GO: Gene Ontology

GPx: Glutathione Peroxidase

GST: Glutathione-S-Transferase

HAMP:Molecular Patterns of Herbivores

HSPs: Heat Shock Proteins

JA: Jasmonic Acid

MAMP: Molecular Patterns of Microbes

MeJA: Methyl Jasmonate

MM: *Solanum lycopersicum* L. var. Moneymaker

NahG: Salycilate Hydroxylase gene

PAMP: Molecular Patterns of Pathogens

phm: phantom

PI-I: Proteinase Inhibitor I

PI-II: Proteinase Inhibitor II

PINs: Proteinase inhibitor genes

PR: Pathogenesis-Related

PSM: Plant Seconday Metabolites

RNA: Ribonucleic Acid

RT: Room Temperature

RT-qPCR: Real Time Quantitative Chain Reaction

SA: Salicylic Acid

SAR: Systemic Acquired Resistance

SLC: *Solanum lycopersicum* L. var. Cerasiforme

SLL: *Solanum lycopersicum* L. var. Lycopersicum

SOD: Superoxide Dismutase

SP: *Solanum pimpinellifolium L.*

Spr2: JA biosynthetic mutant

TAGs: Triacylglycerols

UN: United Nations

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ABSTRACT

This thesis focuses on the study of various aspects related with the interaction between tomato *Solanum lycopersicum*, one of the most important crops worldwide, and the aphid *Macrosiphum euphorbiae*, one of its most common pests. To improve the understanding of this interaction and its implications in pest management and control, different experimental approaches to understand both ecological and molecular aspects were taken.

Firstly, it was investigated whether tolerance and resistance to different pests vary in a tomato domestication gradient. For this purpose, the response of 23 tomato genotypes, ranging from wild relatives to modern cultivars, was evaluated against *M. euphorbiae* and the caterpillars of *Spodoptera littoralis*. The results showed that the differences in genotype responses were not related to the degree of domestication or genetic proximity, since substantial variation among closely related wild tomato species were shown. This suggests the existence of complex genetic bases for resistance, implying that resistance traits have emerged at different stages and in unrelated tomato genetic lineages. However, it was observed that wild genotypes and early domesticated varieties showed higher resistance to aphids and greater tolerance to caterpillars compared to modern cultivars. These findings contribute to a better understanding of how domestication affects plant-pest interactions, highlighting the importance of tolerance in crop improvement.

Subsequently, a study was conducted to investigate the effect of type IV glandular trichomes on host plant choice behavior and on aphid population growth. To carry out these experiments, the tomato cultivar Moneymaker (MM), that lacks type IV glandular trichomes, and the quasi-isogenic line ABL 10-4, which has a high density of type IV trichomes and abundant production of acylsucroses, were used. In addition to examining the impact of glandular trichomes on host plant selection through choice tests under free-choice and no-choice conditions, population growth on the two genotypes was also compared. The results revealed that the presence of type IV glandular trichomes negatively affected population growth. Moreover, aphids showed

a clear aversion to the genotype with these glandular trichomes, actively avoiding it, and exhibited a very poor reproduction compared to the genotype lacking them.

Additionally, a study was conducted to determine if a previous infestation of *M. euphorbiae* or *S. littoralis* compromised the resistance conferred by type IV glandular trichomes against *M. euphorbiae*. The results demonstrated that the resistance mediated by type IV trichomes against aphids is effective regardless of the type of previous infestation. However, plants previously infested with *S. littoralis* were more resistant than control plants, indicating that a first attack by the chewing herbivore, which activates the jasmonic acid (JA) pathway, resulted in greater resistance against the aphid. Similarly, previous infestation of MM *with S. littoralis* led to an increase in resistance against *M. euphorbiae*. On the contrary, MM plants that had been previously infested with *M. euphorbiae* were more susceptible to a second attack by the same aphid.

Moreover, population growth of *M. euphorbiae* was determined in genotypes deficient in the jasmonic (JA) and salicylic acid (SA) pathways (i.e., with the s*pr2* and *NahG* lines). The results revealed that the *spr2* mutant, impaired in JA synthesis, was more susceptible than its wild-type counterpart (control genotype). In contrast, the transgenic tomato line *NahG*, deficient in SA accumulation, showed an increased resistance compared to the control genotype. These findings demonstrated that JA plays a relevant role in tomato defence against aphids.

Finally, the transcriptomic profiles of *M. euphorbiae* exposed to MM and ABL10-4 were compared using RNA seq. Firstly, a *de novo* transcriptome was generated, which proved to be a valuable genomic tool for identifying potential gene targets in *M. euphorbiae*. Additionally, a unique and distinctive gene expression profile related to the aphid's exposure to type IV glandular trichomes and their acylsucroses was identified for the first time. This analysis showed that tomato glandular trichomes and their associated secretions trigger stress-related responses in the aphid, demonstrating that their role in plant defence goes beyond the physical impairment of insect activity. Consequently, differentially expressed genes (DEGs) associated with carbohydrate, lipid, and xenobiotic metabolism, the immune system, oxidative stress response, and hormonal biosynthesis pathways were identified. Furthermore, the observed responses were

consistent with a starvation syndrome. Conserved biomarkers in arthropods (critical for their survival) affected by glandular trichomes and their secretions were also identified. Therefore, the results of this study suggest that other functional groups (e.g., pollinators or natural enemies of insect pests) may also experience similar responses when exposed to these defensive structures.

RESUMEN

Esta tesis aborda el estudio de diversos aspectos relacionados con la interacción entre el tomate (*Solanum lycopersicum*), uno de los cultivos más importantes a nivel mundial, y el pulgón *Macrosiphum euphorbiae*, una de sus plagas más comunes. Con este propósito, se han planteado enfoques experimentales tanto para comprender aspectos ecológicos como moleculares, con el fin de mejorar la comprensión de esta interacción y sus implicaciones en el manejo y control de plagas.

En primer lugar, se investigó si los niveles de tolerancia y resistencia a diferentes plagas varían en un gradiente de domesticación del tomate. Para ello, se evaluó el comportamiento de 23 genotipos de tomate, que abarcaban desde parientes silvestres hasta cultivares modernos, frente a *M. euphorbiae* y la oruga de *Spodoptera littoralis*. Los resultados obtenidos demostraron que las diferencias observadas en el comportamiento de los genotipos no estaban relacionadas ni con el grado de domesticación ni con la proximidad genética. Esto sugiere la existencia de una base genética compleja para la resistencia, lo cual implica que los rasgos de resistencia han surgido en diferentes etapas y en linajes genéticos no relacionados. No obstante, se observó que los genotipos silvestres y las primeras variedades domesticadas presentaban una mayor resistencia a los pulgones y una mayor tolerancia a las orugas en comparación con los cultivares modernos. Estos hallazgos contribuyen a una mejor comprensión de cómo la domesticación afecta las interacciones entre las plantas y las plagas, resaltando la importancia de la tolerancia en el mejoramiento de los cultivos.

Posteriormente, se investigó el efecto de los tricomas glandulares de tipo IV en el comportamiento de elección de planta huésped y en la dinámica de crecimiento poblacional del pulgón. Para llevar a cabo estos experimentos, se empleó el cultivar de

tomate Moneymaker (MM), que carece de tricomas glandulares tipo IV, y la línea cuasi-isogénica ABL 10-4, que presenta una alta densidad de tricomas IV y una producción abundante de acilsacarosas. Además de examinar el impacto de los tricomas glandulares en la selección de la planta hospedera mediante ensayos de elección en condiciones de libre elección y sin elección, también se comparó el crecimiento poblacional sobre los dos genotipos. Los resultados revelaron que la presencia de tricomas glandulares de tipo IV afectan negativamente la dinámica de crecimiento poblacional. Los pulgones mostraban una clara aversión hacia el genotipo que presentaba estos tricomas glandulares, evitándolo activamente, y presentaban una reproducción deficiente en comparación con el genotipo que carecía de ellos.

Por otro lado, se llevó a cabo un estudio para determinar si una infestación previa de *M. euphorbiae* o de *S. littoralis* comprometía la resistencia conferida por los tricomas glandulares de tipo IV de la línea ABL 10-4 a *M. euphorbiae*. Los resultados obtenidos demostraron que la resistencia basada en tricomas glandulares tipo IV es eficaz frente a los pulgones, independientemente del tipo de infestación previa. Sin embargo, las plantas previamente infestadas con *S. littoralis* fueron más resistentes que las plantas de control, lo que indica que un primer ataque del herbívoro masticador, que activa la vía del ácido jasmónico (JA), resultó en una mayor resistencia frente a *M. euphorbiae*. Del mismo modo, la infestación previa de MM con *S. littoralis* condujo a un incremento en la resistencia contra *M. euphorbiae*. Por el contrario, las plantas MM que habían sido infestadas previamente con *M. euphorbiae* resultaron ser más susceptibles a un segundo ataque de este mismo pulgón. Además, se determinó la dinámica de crecimiento poblacional del áfido *M. euphorbiae* en genotipos con deficiencia en las vías del JA y del ácido salicílico (SA) (líneas *spr2* y *NahG*, respectivamente). Los resultados revelaron que el mutante *spr2*, impedido en la síntesis del JA, es más susceptible que su contraparte silvestre (genotipo control). En cambio, la línea de tomate transgénica *NahG*, deficiente en la acumulación de SA, mostró una menor susceptibilidad en comparación con el genotipo control. Estos hallazgos demostraron que el JA juega un papel relevante en la defensa del tomate contra el pulgón.

Por último, se compararon los perfiles transcriptómicos de *M euphorbiae* expuestos a MM y ABL10-4 utilizando RNA seq. En un primer lugar, se generó un transcriptoma *de*

novo que resultó ser una valiosa herramienta genómica para la detección de potenciales dianas génicas en *M. euphorbiae*. Además, se logró identificar por primera vez un perfil de expresión génica único y distintivo relacionado con la exposición del pulgón a los tricomas glandulares de tipo IV y a sus acilsacarosas. Este análisis mostró que los tricomas glandulares del tomate y sus secreciones asociadas desencadenan respuestas relacionadas con el estrés en el pulgón, y demuestra que su papel en la defensa de la planta va más allá del impedimento físico de la actividad del insecto. De este modo, se identificaron genes diferencialmente expresados (DEGs) asociados con los metabolismos de carbohidratos, lípidos y xenobióticos, el sistema inmunitario, la respuesta al estrés oxidativo y las vías hormonales de biosíntesis. Por otro lado, las respuestas observadas fueron compatibles con un síndrome de inanición. También se identificaron biomarcadores conservados en artrópodos (fundamentales para su supervivencia) afectados por los tricomas glandulares y sus secreciones y, en consecuencia, los resultados de es este estudio sugieren que otros grupos funcionales (por ejemplo, polinizadores o enemigos naturales de las plagas de insectos) también podrían experimentar respuestas similares cuando se exponen a estas estructuras defensivas.

INTRODUCTION

I. INTRODUCTION

Agriculture is essential in a society where a growing population and shifting diets are projected to increase the demand of food in the following years. However, the production of crops grown for human consumption is continuously threatened by the attack of insect pests. Insects are the most ubiquitous, diverse and abundant group of animals (Arora, 2017). They cause direct damages on plants and can transmit plant diseases that entail considerable crop losses worldwide (Oerke, 2006; Sharma *et al.*, 2017). In this context, the big challenge for the agriculture of the 21st century is to produce more food to meet the global demand but adopting more efficient and sustainable production methods. Which goes in line with the global Sustainable Development Goals (SDGs) proposed for the next 2030 (UN, 2015).

One of the most important means of increasing agricultural production involves reducing pre-harvest losses produced by agricultural pests. Traditionally, arthropod pest management has been achieved by using different kinds of chemical pesticides that not only pose health and environmental hazards (Alewu & Nosiri, 2011) but that often leads to pest resistance (Bass *et al.*, 2015). Besides other problems such as nontarget effects on benefitial organisms, pest resurgence, emergence of secondary pests and high costs associated with both active ingredients and application (Ekstrom & Ekbom, 2011). Since 2009, the European Union is aiming at reducing their use (European directive 2009/128/EC) and nowadays, one of the major challenges is to develop a sustainable management production strategy.

In this context, this PhD dissertation studies in detail the interaction between tomato and an important pest of this crop i.e., the aphid *Macrosiphum euphorbiae* (Hemiptera: Aphididae), by focusing on the particular role and impact of plant defences on the insect. Ecological and molecular approaches are combined to analyze, on one hand, plant resistance and the impact of the glandular trichomes on the behavior and performance of the aphid; and on the other, the hormonal pathways underlying the defence response. The results of this research provide information that leads to a better understanding of plant-pest relationships and allows to develop more sustainable management strategies that can be transferred to other crops.

A Plant defences against herbivores

Plants, as sessile organisms, continuously face attacks from a myriad of pests and pathogens. However, they have evolved different mechanisms to recognize the diverse biotic stresses and translate this perception into an adaptive and accurate response (Howe & Jander, 2008; Dodds & Rathjen, 2010). These differential responses involve complex signaling pathways that regulates numerous cellular reactions that contribute to the overall response (War *et al*., 2012).

A.1 Tolerance and resistance as the main defence mechanisms

Originally, three basal strategies of defence were proposed: deterrence (antixenosis), resistance (antibiosis) and tolerance (buffers negative impact) (Painter, 1951). Deterrence traits can emanate from colours, ordours or textures (such as hairs) that demotivate an herbivore from feeding on the plant or that signify the absence of feeding stimuli for the attacker. Resistance traits are those that impair herbivore performance by either killing it or slowing its development and reproduction. Tolerance comes from those traits that do not limit damage but buffers its negative effect, allowing a perfectly tolerant plant to achieve equal fitness when damaged or undamaged (Strauss & Agrawal, 1999; Little *et al*., 2010). However, as pointed by Stout (2013), this trichotomous framework does not encompass all known mechanisms of resistance, and the antixenosis and antibiosis categories are ambiguous and difficult to separate in practice. Which has currently led to to a more simplistic approach to understanding arthropod resistance in crop plants by considering a dichotomous scheme with a major division between resistance (all strategies that reduce herbivore damage) and tolerance (strategies that compensate the herbivore damage).

Under resistance are all the strategies that deter herbivore feeding and thus reduce the amount of damage experienced by the plant. Which can be achieved through physical and chemical traits that either deter or impair the performance of the insect. Whereas tolerance is understood as those mechanisms that reduce the negative impact caused by the herbivore on the crop yield. Mainly by exerting changes in photosynthesis, growth, phenology (delayed growth, flower and fruit production) and the storage of nutrients (Kant *et al*., 2015; Mitchell *et al*., 2016).

In most analysed plant-pest systems, resistance and tolerance coexist at the same time and some authors propose that, under this scenario, trade-offs can occur between resistance and tolerance in natural ecosystem, i.e. the resistance to a certain pest goes at the cost of the resistance/tolerance to a different pest (Fineblum & Rausher, 1995; Pagán & García-Arenal, 2018). For crops, the amount of experimental work is rather limited and empirical evidence is still ambiguous, largely because most studies only considered single genotypes or very few pest species, and they focussed essentially on cultivars only (Whitehead *et al*., 2017). How selection and what ecological conditions favour the allocation of available resources to one strategy over the other are crucial to understand the evolution of plant defence and their implications in plant-insect 'arms-race' (Núñez-Farfán *et al*., 2007; Garrido *et al*., 2016).

In cultivated plants, the selection pressures maintaining trade-offs are expected to be alleviated because cultivated plants are artificially protected from pests by the use of phytosanitary products. Moreover, increased yield is expected to reduce levels of resistance and tolerance in crops compared with their wild relatives (Welter & Steggall, 1993; Rosenthal & Dirzo, 1997) and this could affect the magnitude of the trade-offs between resistance and tolerance (Leimu & Koricheva, 2006). One way to test for the changes in the resistance-tolerance trade-offs in the course of domestication is through a comparative approach using a gradient from wild to increasingly domesticated species. Previous meta-analyses comparing herbivore resistance and plant defence traits between crops and their wild relatives revealed that resource allocation trade-offs may occur and affect overall plant defence strategies in crops (Whitehead *et al*., 2017).

A.2 Resistance against herbivores

Resistance to herbivores largely depends on physical and chemical plant defences that deter or inhibit feeding, oviposition and development of larvae or adults (Kessler & Baldwin, 2002). Within plant responses against herbivores, we distinguish between constitutive defences, constantly expressed by the organism, and inducible ones, those that appear in response to an attack. Constitutive defences are always activated, but not always needed, which require high costs for the plant (Karban, 2011). On the other side, inducible defences are activated when the surveillance system of the plant recognizes

oral secretions or injured cells and a signaling pathway is triggered (Howe & Jander, 2008; Steinbrenner *et al*., 2020). After that, a systemic response via mobile signals (such as hormones) is activated, and will eventually, display direct or indirect defences that interfere with herbivore feeding, growth and development, fecundity and fertility (Kant *et al*., 2015). Since induced defences are only activated in the presence of the attaker, the plant theory suggests that inducible resistance has evolve to reduce the costs of constitutive defences (Cipollini & Heil, 2010).

Direct defences refer to those defences that by themselves affect the susceptibility of host plants to insect attacks. Plant traits that confer mechanical protection to plants (e.g., hairs, trichomes, thorns, spines, and thicker leaves) or with the ability to produce toxic chemicals (such as terpenoids, alkaloids, anthocyanins, phenols, and quinones) that either kill or damage the development of the herbivores. While indirect defences, include plant traits that by themselves do not affect the susceptibility of host plants, but can serve as attractants to natural enemies of the attacking insect (Kessler & Baldwin, 2002).

A.2.1 Induced defences

Inducible defences include defensive mechanisms activated upon insect attack and comprise three steps i.e., surveillance, signal transduction, and the production of defensive chemicals (Kessler & Baldwin, 2002; Walling, 2000). Depending on the mode of herbivore feeding and the degree of tissue damage at the feeding site, the downstream genes activated after the attack vary, and different types of induced resistance are triggered (Mewis *et al*., 2005; Howe & Jander, 2008). On one hand, we have insects that do not cause extensive damage on the plant and are perceived as pathogens. Most aphids, mealy bugs, leafhoppers, psyllids, and whiteflies insert their specialized stylets between cells to establish a feeding site in the phloem (Miles 1999; Raven 1983). On the other hand, there are those who cause a greater damage on leaves and activate the wound-response pathway. Leaf-eating beetles (Coleoptera) or caterpillars (Lepidoptera) have mouthparts evolved for chewing, snipping, or tearing (Kesser & Baldwin, 2002) and leafminers develop and feed in the soft tissue between epidermal cell layers that also cause a greater damage on the leaves.

The genetic and physiological regulation of plant signalling pathways ensure that the most effective defence strategy to minimize current and future pest and disease damages is displayed without sacrificing vegetative growth and reproductive success (Karban and Baldwin 1997; Walters & Heil, 2007). Induced defences, those that appear in response to an attack, have lower resource allocation costs than constitutive resistance traits, those that are always present, and for this reason they have presumably evolved in environments with temporary variation of herbivores (Karban, 2011). It has also been described that the pathways involved in their upregulation seem to be conserved across many plant taxa (Núñez-Farfán *et al.*, 2007). Nevertheless, there are still some costs that arise when these defences are activated. Mostly due to the allocation of resources to defence or due to the plant's interaction with beneficial organisms and away from plant growth or development (Walters & Heil, 2007).

A.2.2 Main hormonal pathways involved in plant defence against pests.

Plants usually respond to external stimulus by the simultaneous activation of signaling networks that involve hormone-activated targeting of transcriptional regulators that control many developmental and physiological responses. Comparative analysis of hormone signaling pathways in many species beyond model plants points to important differences in the functions played by these hormones that affect the outcome response.

Phloem-feeders, as aphids and whiteflies, produce little injury to plant foliage and are perceived by plants similarly to pathogens activating the salicylic acid (SA)-dependent signaling pathway. Insect chewers, such as caterpillars and beetles, or mining herbivores such as mites and thrips, cause more extensive tissue damage and lead to the wounding response mediated by the jasmonic acid (JA) (Walling, 2000). In addition to local resistance, many of these phytohormones also induce defence responses in systemic tissues, vital for the plant outcome. Each hormone activates a specific pathway and these pathways act individually, synergistically or antagonistically, depending on the pathogen involved. In essence, the SA pathway is primarily induced by and effective in resistance against biotrophic pathogens (successful groups of plant pathogens that require living plant tissue to survive and complete their life cycle) while the JA pathway is primarily induced by and effective in mediating resistance against herbivores and

necrotrophic pathogens (Glazebrook, 2005). However, some other hormones such as abscisic acid, auxin, brassinosteroids, cytokinins, ethylene and gibberellic acid may participate also, modulating this major response (Robert-Seilaniantz *et al*., 2011).

It has been evidenced that SA is the endogenous signal that promotes the induction of the systemic acquired resistance (SAR). Which involves the spreading of the local resistance throughout the plant's tissues after a pathogen attack and confers a broad-range resistance to pathogens (Bostock 1999; Dempsey *et al*., 1999). This resistance is long-lasting and characterized by the increased expression of a large number of pathogenesis-related genes (PR genes) (Durrant & Dong, 2004). The work with transgenic plants, in particular, the *NahG* ones, allowed to elucidate the key role of the SA in plant defence mechanism. *NahG* is a bacterial gene encoding salycilate hydroxylase gene, that converts SA to catechol and therefore *NahG* transgenic plants are unable to accumulate SA (Gaffney *et al*., 1993; Delaney *et al*., 1994).

Jasmonates have been proposed as the "master regulators" of plant defences because of their role in multiple routes. Among others, they mediate the wound-related response which is critical in the outcome of plants against herbivores that inflict various types of tissue damage. However, this response is not only locally deployed on the plant, but also systematically, where unwounded and distal leaves can also activate it. The systemic response has been extensively studied in tomato, *Solanum lycopersicum* (Solanales: Solanaceae). Basically, systemin and its precursor, prosystemin, are upstream components that leads to the synthesis of JA through the "octadecanoid" (C18-fatty acids) pathway, which in turn activates the expression of a subset of target genes including those encoding defensive *Proteinase Inhibitor* genes (PINs). PINs represent one of the main types of wound-induced genes. Two non-homologous subfamilies are found in Solanaceae species like tomato: *PI-I* and *PI-II* (Ryan, 1990). In response to wound herbivory proteinase inhibitors are accumulated to high levels and impair the herbivore performance by inhibiting insect digestive enzymes (Zhang *et al*., 2004). The work with a JA biosynthetic mutant (*spr2*) and a JA response mutant (*jai-1*) has allowed to unravel the crucial role of JA acting as a transmissible wound signal (Li *et al*., 2002) (*Fig. I1*). Evidencing this way, the significant insight that mutants can offer in order to unravel gen functions.

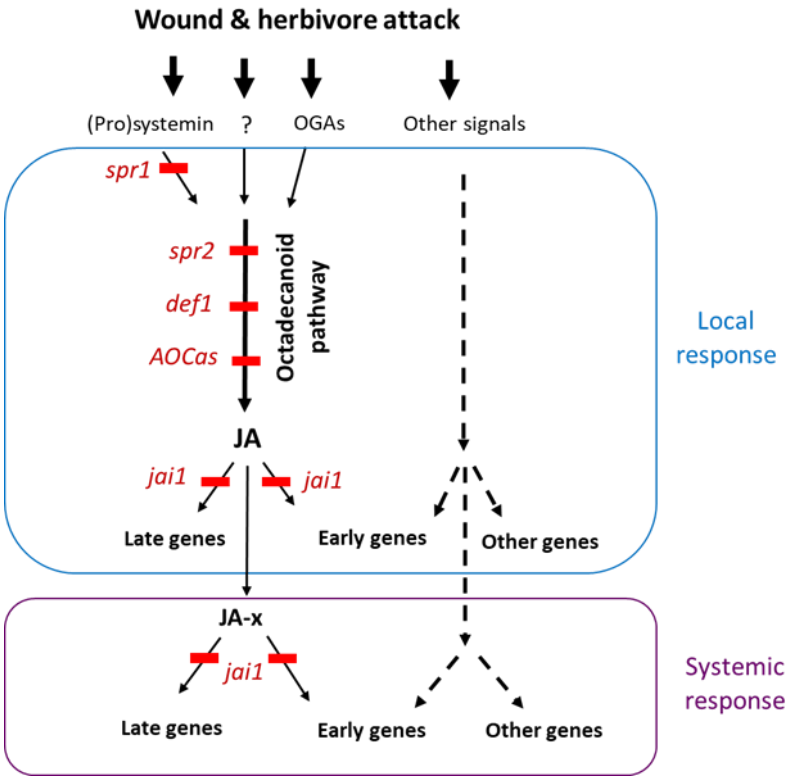


***Fig. I1.* Model of wound signaling based on genetic studies in tomato**. Wound-induced signals including systemin, OGAs, and putative unidentified compounds (?) activate the octadecanoid pathway for JA biosynthesis in response to mechanical wounding or herbivory. Production of JA mediates local and systemic activation of Early and Late response genes (solid arrows). Mutations that block various steps in the wound-signaling pathway are indicated (red bars). Grafting experiments conducted with these mutants support the hypothesis that JA or a derivative thereof (JA-x) functions as a systemic signal. Wounding also activates JA-independent signaling pathways (hatched lines) that regulate local and systemic expression of Early genes, as well as other genes (for example, WIPK) whose expression is completely independent of JA and systemin. AOCas, antisense suppression of allene oxide cyclase. Modified from Howe, 2004.

A.2.3 Primed responses

Expressing full resistance in response to a first contact with an herbivore would have the risk of allocating resources to a defence that ultimately may not be required. Therefore, plants have strategies to fine-tune their resistance according to these requirements. After an attack, plants are able to reach an enhanced state of defence called *primed state* from where they can respond with a faster and stronger activation of defences against a subsequent stress (Conrath *et al.*, 2006).

Plants firstly recognize the molecular patterns of microbes (MAMPs), pathogens (PAMPs), herbivores (HAMPs) or those associated to damage (DAMPs), then diverse defence pathways are triggered, and eventually, they can switch to a primed state.

However, some natural or synthetic chemicals can also induce the primed state on plants (Conrath *et al.*, 2015). Primed plants can store the information of the priming stimulus, until the eventual exposure to a triggering stimulus (Martínez-Medina *et al.*, 2016). Furthermore, defence priming is not only developed on the tissue exposed to the PAMP, MAMP, HAMP, DAMP, effector, or chemical compound, but also, in the systemic, unharmed, or untreated parts of the plant (Beckers and Conrath, 2007; Conrath *et al.*, 2006; Conrath, 2009; Jung *et al.*, 2009). As a result, after an exposure to herbivore feeding or harm at any part of the vegetable, the plant will develop a faster and more effective defence response in future attacks. Consequently, priming becomes as one of the key mechanisms in plant defence against herbivores.

Diverse studies have shown that sequential attacks from herbivores with different feeding type trigger different signalling pathways that can result in a crosstalk between these hormonal pathways (Pieterse *et al*. 2006; Erb *et al*., 2012). Which implies that early-arriving attackers may determine the magnitude and sign of effects on subsequent ones (Pieterse *et al*. 2006; Stout *et al*., 2006; Thaler, *et al*., 2012; Ali & Agrawal, 2014). One of the best characterized interactions in defence related signalling is the cross-talk between the JA and SA response pathways. Although the crosstalk hypothesis predicts interference between plant signaling pathways when initial and subsequent attackers induce defences associated with different pathways (Pieterse *et al*. 2006; Erb *et al*., 2012), synergistic interactions have been described as well (Moreira *et al*., 2018). A meta-analysis on plant-mediated effects of initial attackers on performance of subsequent attackers showed that a JA-inducing initial insect drove a reduction in the performance of both JA and SA- inducing subsequent herbivores, whereas initial herbivores inducers of SA pathway did not significantly affect the performance of either JA- or SA-inducing subsequent herbivores (Moreira *et al*., 2018). However, interactions due to sequential attacks of herbivores are not perfectly determined and further studies with diverse feeding strategies are required.

B Tomato

B.1 Importance as a model crop system

Tomato (*S. lycopersicum*) is one of the most important crops on a global scale with a worldwide production above 189 million of tonnes per year (Faostat, 2021). In the last decade its production has followed a lineal raising of an overall of 2% (*Fig. I2*) and nowadays is the 10th vegetable crop in the world in terms of annual production.

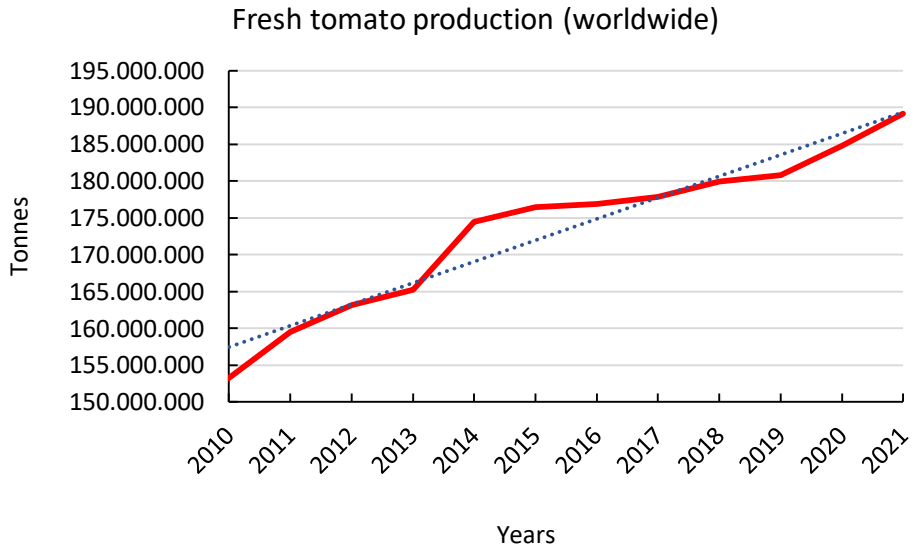


***Fig. I2.* Worldwide production of fresh tomato over the last 10 years (Datasource: FAOSTAT).** The red solid line represents the average tons per year. The blue dotted line represents the linear tendency followed.

It is a diploid specie (2n=2x=24) with a small genome size, a short generation time and simple growing requirements; which has promoted the recognition of its potential in scientific research for years (Mueller *et al.*, 2005; Labate *et al.*, 2007). The understanding of the mating systems and the possibility of controlled hybridization within and among species have also contributed to great advances in tomato genetics. Recent developments in molecular biology, plant biotechnology and modern genetic engineering of this organism, have led tomato as the perfect candidate for plant-insects research (Bai & Lindhout, 2007; de Vos *et al.*, 2011; Bergougnoux, 2014).

B.2 Tomato taxonomy

Tomato is a dicotyledon shrub that belongs to the genus *Solanum* L. from the nightshade family Solanaceae (division Magnoliophyta, class Magnoliopsida, subclass Asteridae and order Solanales). This diverse family comprises *ca.* 4000 species occurring in diverse habitats and showing diverse morphologies, among which, there are valuable crops such as eggplant (*S. melongena L.*), potato (*S. tuberosum L.*), sweet cucumber (*S. muricatum Ait*) and species used for medicinal or ornamental purposes. *Solanum* contributes to the 75 % of all species in Solanaceae being not only the largest genus of the family but also one of the largest genera of the angiosperms with around 2000 species (Symon, 1981; Knapp, 2002; Kaunda & Zhang, 2019). This hyperdiversity in one genus, which mostly occurs in South America and is concentrated in the Andes (Knapp, 2002), is unusual in angiosperms and makes *Solanum* very interesting from an evolutionary point of view.

Tomato taxonomy and nomenclature has been subject of debate for years and different classifications have been followed (*Fig. I3*). Nowadays molecular data and phylogenetic studies have unambiguously shown tomatoes to be deeply nested within *Solanum*. Cultivated tomato currently belongs to *Solanum* genus into the section *Lycopersicon* (Liedl, 2013) and the nomenclature used is the one originally suggested by Linnaeus (1753), *S. lycopersicum.* The current classification done by Peralta *et al*., in 2008 recognizes a clade with 12 close-related wild tomatoes and the cultivated tomato, all native to western South America, from Ecuador to northern Bolivia and Chile, with two endemic species in the Galapagos Islands. Besides these nominal 13 species, four closely related are also considered i.e., *S. juglandifolium*, *S. lycopersicoides*, *S. ochranthum* and *S. sitiens* (Peralta *et al*., 2008).

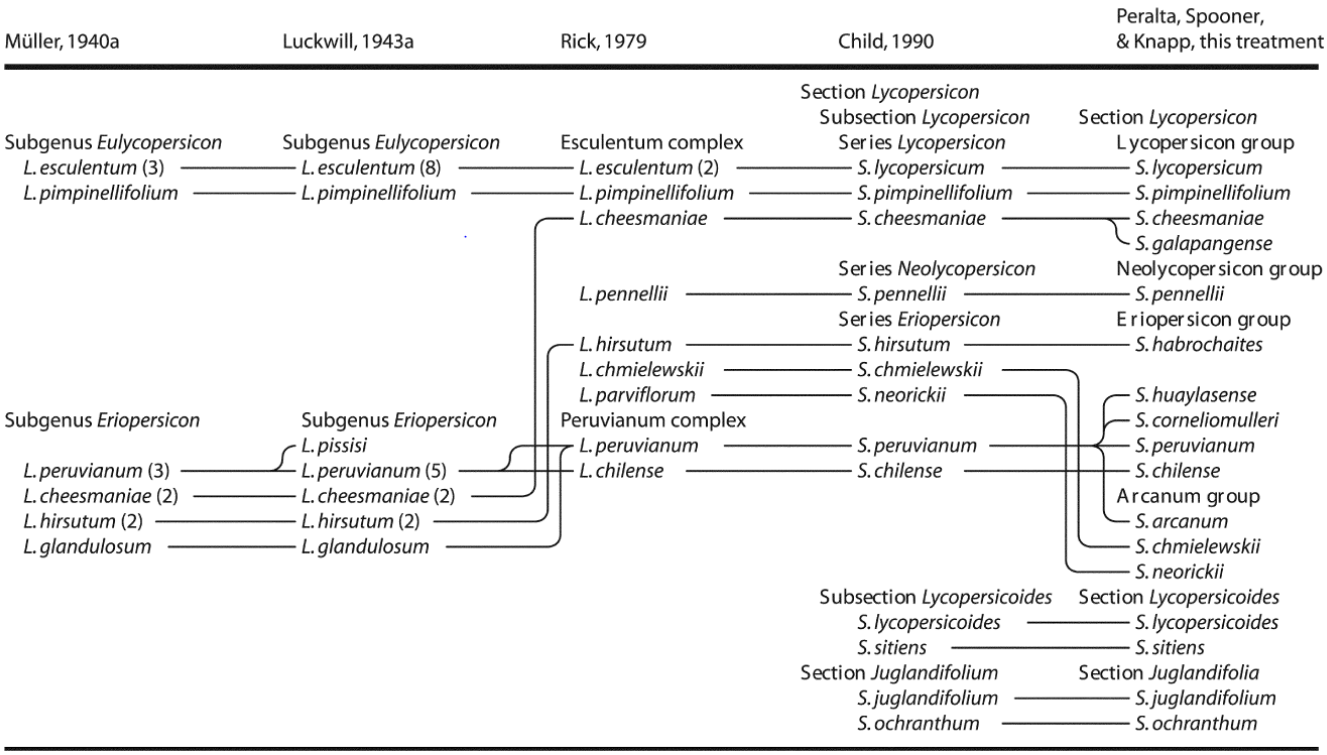


Fig. I3. **Chronological flow chart of hypotheses of species boundaries and relationships of *Solanum*** sect. *Lycopersicon,* sect. *Juglandifolia,* and sect. *Lycopersicoides* as recognized by Müller (1940), Luckwill (1943). Child (1990), and Peralta *et al*., 2008. The numbers in parentheses represent the number of intraspecific taxa recognized by these authors. Taken from Peralta *et al*., 2008.

B.3 Tomato domestication and research

Tomato has been artificially selected for traits beneficial for human consumption. The origin and the early events of tomato domestication are still unclear. It has been proposed that tomato has experienced a two-step transitions in domestication history i.e., a pre-domestication from wild *S. pimpinellifolium* L. (SP) to a semidomesticated intermediate, *S. lycopersicum* L. var. *cerasiforme* (SLC) that occurred in the Andean region, in South America (Ecuador and Northern Peru); and a second transition, where domestication was completed, from the SLC to fully domesticated *S. lycopersicum* L. var. *lycopersicum* (SLL) in Mesoamerica (Ranc *et al*., 2008; Blanca *et al*., 2012; Lin *et al.,* 2014). Subsequently, the Spanish explorers transferred these Mesoamerican varieties to Europe in the 16th century from where they were exported to the rest of the world (Blanca *et al.,* 2012). Therefore, the origin of the current commercial cultivars is most

likely placed in the Mesoamerican varieties of *S. lycopersicum* var. cerasiforme (SLC) which are phylogenetically related with *S. pimpinellifolium* (Peralta & Spooner 2005; Ranc *et al*., 2008; Blanca *et al*., 2012). This hypothesis is further supported by genetic analyses which confirm that some cerasiforme accessions resulted from the hybridisation between *S. pimpinellifolium* and local Mesoamerican *S. lycopersicum* whereas several Ecuadorian and Mexican accessions are similar, genetically, to *S. lycopersicum* (Nesbitt & Tanksley, 2002; Ranc *et al*., 2008; Blanca *et al*., 2012).

However, genomic analysis carried by Razifard and colleagues (2020) suggested that the origin of SLC may predate domestication, and that many traits considered typical of cultivated tomatoes arose in South American SLC, but were lost or diminished once these partially domesticated forms spread northward. These traits were then likely reselected in a convergent fashion in the common cultivated tomato, prior to its expansion around the world. That is that SLC diverged from SP as a fully wild species and later, human selection gave rise to domesticated SLC populations that have largely replaced the wild SLC.

Besides the origin, some of the most important features that were selected during domestication are the intrinsic qualities of the fruit such as size, shape, color, fruit firmness and shelf-life. Compared with their wild relatives, cultivated *S. lycopersicum* bear fruit that is much larger in size and exhibits an array of shapes. The presumed ancestor of cultivated tomato, *S. pimpinellifolium* has small fruits which weight barely reaches a few grams made to propagate the species. However, modern cultivated tomatoes, that feed humans, after the domestication and breeding pressure have developed bigger fruits around the 500 g each (*Fig. I4*). Wild tomatoes bear fruits that are almost invariably round, while cultivars available today show a wide variety of shapes: round, oblate, pear-, torpedo- and bell-shaped (Tanksley, 2004). Selection for increased fruit size may have led to phenotypic changes in fruit shape by pleiotropic effects of the fruit size loci or expression of 'hidden' fruit shape alleles that have little or no visible effect on fruit shape in a small-fruited background (Tanksley *et al*., 1996; Grandillo *et al*., 1999).

Fig. I4. **Fruits from different varieties of *Solanum lycopersicum* and *Solanum pimpinellifolium*.** **Left**: Fruits from different varieties of *Solanum lycopersicum* and *S. pimpinellifolium*. **Right**: fruits from *S. pimpinellifolium* (acc. TO-937-15).

A frequent hypothesis explaining the high susceptibility of many crops to pests and diseases is that domestication has unintentionally entailed the loss of defensive genes and traits against pests and diseases (Khush 2001; Whitehead *et al*., 2017). The strong selection for traits of agronomic importance have encompassed the loss of many other traits such as resistance to diverse herbivores. However, thanks to the current knowledge and technology in tomato plants it has been possible to transfer some defence traits from wild into cultivated tomatoes and enhance their protection against herbivores (Willits *et al*., 2005; Bleeker *et al*., 2012). Nevertheless, the major drawback of developing pest resistant cultivars is the fact that plant resistance to insects is, in general, a quantitative inherited trait, and identification and selection for quantitative resistance traits is strongly influenced by variations of the environment and the genetic variability of insects (Yencho *et al.,* 2000; de Morais & Pinheiro, 2012). Modern plant breeders have taken advantage of the variation found within wild species, available in germplasm banks, to develop resistant varieties. Improving cultivars in tomato plants is usually done by backcrossing. This technique is used to incorporate simply inherited traits from a donor parent into a recipient line and involves repeated cycles of crossing to the recipient line (recurrent parent), followed by selection of the trait being transferred (Kenaschuk, 1975). Which results in the so-called *introgressed line* (IL). Backcross breeding programs are less complicated when only one or a few number of

genes have to be introgressed into a commercial variety (Broekgaarden *et al*., 2011). Several sets of these ILs have been developed from wild relatives, including *S. habrochaites* (Monforte & Tanksley, 2000; Finkers *et al*., 2007), *S. pimpinellifolium* (Tanksley *et al*., 1996; Bernacchi *et al*., 1998; Kinkade & Foolad, 2013; Barrantes *et al*., 2016) and *S. pennellii* (Eshed & Zamir, 1994). ILs that carry a single introgressed region from one parent, are identical for the rest of their genome to the other parent. As a result, the phenotypic variation in these lines can be associated with individual introgression segments. Which could be, for example, a transferred disease-resistance gene into cultivated tomato that afterwards express this selected trait.

C Trichomes

In general, wild tomatoes produce an array of metabolites such as alkaloids, phenolic compounds and terpenes that are toxic or repellent to insects and play a key role in plant defence (Walling, 2000; Kennedy, 2003, Mirnezhad *et al*., 2010). However, for obvious reasons, artificial selection and breeding has selected those plant genotypes that show low toxicity or low concentrations of defence compounds detrimental for human consumption (Benrey *et al*., 1998; Wittkop *et al*., 2009; Bleeker *et al*., 2012). As a result, cultivated genotypes have become more attractive to pests compared to their wild counterparts. These toxic and repellent metabolites are mostly produced in glandular trichomes, epidermal hairs specialized in efficient production, storage, and release of defence compounds (Kennedy, 2003). Glandular trichomes are found on the surface parts of about 30% of all vascular plants (Fahn, 2000) and their density and production of secondary metabolites can be altered through the JA-related pathway (Glass *et al*., 2012; Escobar-Bravo *et al*., 2016, Chen *et al*., 2018), previously detailed (A.3 section). Not only has been described that the JA induces the presence glandular trichomes type IV on tomato leaves but also that this pathway can alter the production and storage of allelochemicals (Boughton *et al.*, 2005; Maes & Goossens, 2010; Escobar-Bravo *et al*., 2016), showing therefore, the importance of this route in the resistance against insects.

Trichomes are mostly present on leaves and stem, although they may also occur on petals, petioles, peduncles and seeds. They are widely conserved across the plant kingdom (Levin, 1973) and in tomatoes appear as the first line of defence. In tomato plants, according to their function, trichomes of tomato plants are classified in two groups (Luckwill, 1943): (1) Non-glandular trichomes (types II, III and V), that act as physical barriers blocking the movements of the insect pests and preventing their access to leaf and stem tissues, but also, causing post-ingestive damages (Kariyat *et al*., 2017); and (2) glandular trichomes (types I, IV, VI, VII), which in addition to these same functions, have glands producing compounds (volatile or not) that can repel, irritate and/or intoxicate arthropods, as well as mediate in the indirect defence against natural enemies (Wagner, 1991; Alba *et al*., 2009; Glas *et al*., 2012). They not only differ in the number of stalk and secretory cells (see *Table I1* for a description of trichome morphology and distribution), but also in their chemical contents and secretions that vary among species. The main content of glandular trichomes type I in *S. lycopersicum* are acylglucoses while for example, type VI trichomes contain mostly terpenoids. Type IV and VI across *Solanum* species express many of the genes necessary for acylsugar, flavonoid and terpenoid production. Type VII are less abundant, and in studies with *S. habrochaites* it has been suggested that their function is most related with synthesis of protease inhibitors and biosynthesis and storage of alkaloids rather than biosynthesis of secondary metabolites. Among all of them, type IV are particularly relevant since it has been shown to be responsible for the partial resistance against numerous herbivores such as whiteflies (Rodríguez-López *et al*., 2011), spider mites (Alba *et al*., 2009), leafminers (Hawthorne *et al*., 1992; de Resende *et al*., 2022), caterpillars (Juvik *et al*., 1994), thrips (Mirnezhad *et al*., 2010) and aphids (Goffreda *et al*., 1990). The main metabolites produced on glandular trichomes type IV, acylsugars (McDowell *et al*., 2011), are viscous polyesters that include an acyl chain on sucrose or glucose backbones. Acylsugars are related with movement impairment on insects and consequently, with a reduced access to leaf epidermis for feeding or oviposition (Simmons & Gurr, 2005).

Nonetheless, the type of predominant trichomes may change remarkably between genotypes. For example, and without being exhaustive, adult phases of cultivated tomato (*S. lycopersicum*) lack trichomes type IV (Tian *et al*., 2012; Escobar-Bravo, 2013),

while in other species such as *S. pimpinellifolum*, *S. galapagense* and *S. pennellii* type IV glandular trichomes are pervasive and involved in the constitutive defence against different pests. Cultivated tomato lacks many of these secondary metabolites and its glandular trichomes produce insufficient levels of anti-herbivore substances making them relatively susceptible to a wide range of pests (Besser *et al*., 2009; McDowell *et al*., 2011).

However, breeding of hybrid lines of domestic tomato (*S. lycopersicum*) and other species with glandular trichomes (*S. pimpinellifolum*, *S. galapagense*, *S. pennellii*) has allowed to introgress this type of defence mechanism into commercial varieties (Rodríguez-López *et al*., 2011). As a result, these inbred lines become a great model to study the effect of glandular trichomes type IV on herbivore's performance. Especially relevant for this study is the backcrossing line developed by Escobar-Bravo and colleagues (2016) that results as the introgression of some glandular trichomes type IV traits from an accession of *S. pimpinelifollium* into the tomato cultivar Moneymaker (*S. lycopersicum*) which do not display these traits. Several studies have shown a detrimental effect of this and similar lines (also introgressed ones with glandular trichomes into Moneymaker background) on the performance of spider mites (Fernández-Muñoz *et al*., 2003; Alba *et al*., 2009) and whiteflies (Rodriguez López *et al*., 2011; Escobar-Bravo *et al*., 2016). However, up to date, the mechanisms by which glandular trichomes affect herbivorous insects are not fully understood. While there is considerably evidence of the physical impairment of the exudates of glandular trichomes on herbivore feeding and movement, the effect on the physiology of the insect remains unclear.

Table I1. **Trichome description** according to Luckwill (1943) and distribution among the section *Lycopersicon* of the genus *Solanum*. Modified from Glas *et al*., 2012.

Trichome		Description	Species
I		Thin glandular trichomes consisting of 6–10 cells and 2–3 mm long. Globular and multicellular base with a small and round glandular cell in the trichome tip.	*S. habrochaites, S. lycopersicum, S. pennellii, S. pimpinellifolium, S. peruvianum, S. arcanum, S.corneliomuelleri, S.huylasense*
II		Similar to trichome I but non-glandular and shorter (0.2–1.0 mm). Globular and multicellular base.	*S. pimpinellifolium, S. peruvianum, S. arcanum, S. corneliomuelleri, S. huylasense*
III		Thin non-glandular trichome consisting of 4–8 cells and 0.4–1.0 mm long with a unicellular and flat base. External walls lack intercellular sections.	*S. habrochaites, S. lycopersicum*
IV		Similar to trichome I but shorter (0.2–0.4 mm) and with a glandular cell in the tip. Trichome base is unicellular and flat.	*S. habrochaites, S. pennellii, S. pimpinellifolium*
V		Very similar to type IV with respect to height and thickness but non-glandular.	*S. lycopersicum, S. cheesmaniae, S. galapagense, S. pimpinellifolium, S. peruvianum, S. arcanum, S. corneliomuelleri, S. huylasense, S. chilense, S. chmielewski, S. neorickii*
VI		Thick and short glandular trichomes composed of two stalk cells and a head made up of 4 secretory cells.	*S. habrochaites, S. lycopersicum, S. pennellii, S.pimpinellifolium, S. peruvianum, S. arcanum, S. corneliomuelleri, S. huylasense, S. chilense, S. chmielewski, S. neorickii*
VII		Very small glandular trichomes (0.05 mm) with a head consisting of 4–8 cells	*S. habrochaites, S. lycopersicum, S. pennellii, S. pimpinellifolium, S. peruvianum, S. arcanum, S.corneliomuelleri, S.huylasense, S. peruvianum, S. arcanum, S. corneliomuelleri, S. huylasense*
VIII		Non-glandular trichome composed of one basal and thick cell with a leaning cell in the tip.	*S. lycopersicum, S. chilense*

D Aphids as crop pests

Among the most damaging pests of vegetable crops are aphids. These insects are important herbivores of both cultivated and wild crops that use their specialized mouthparts, stylets, to pierce plant tissue and establish feeding sites in phloem sieve elements (SEs) (Blackman & Eastop, 2000; *Fig. I5*). Although they do not inflict a significant structural harm to a plant, they consume substantial amount of photoassimilates and also act as vectors of important viral diseases that entail massive losses to crops (Martin *et al*., 1997; Ng & Perry, 2004; Quisenberry & Ni, 2007). In addition, aphids are able to reach high densities of population fast because they reproduce parthenogenically and their generation time is short. Features that promote the plant infestation and can also conduct to the growth of saprophytic fungi such as sooty mold. Their ability to produce winged morphs when conditions are adverse

facilitates aphid's dispersal and colonization of new plants. Which added to their resistance to diverse insecticides turn aphids into key pest of tomato plants.

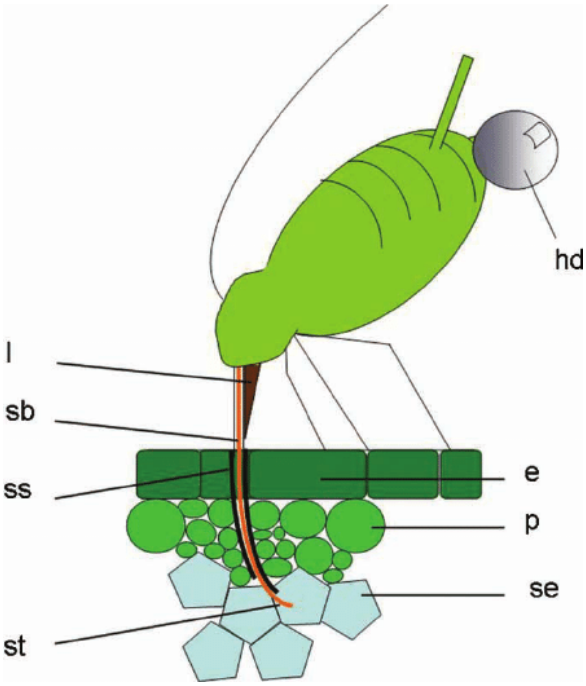


***Fig. I5*. Schematic representation of a feeding aphid** (Guerrieri & Digilio 2008).

- e: epidermis
- hd: honeydew droplet
- l: labium, not participating to the piercing activity (brown)
- p: parenchyma
- sb: stylet bundle (orange)
- se: sieve elements (blue)
- ss: stylet sheaths (black)
- st: stylet tip.

Since finding a suitable host is very important for their survival, aphids use olfactory and visual cues (Döring, 2014). They monitor plant surface features and make short probes to evaluate sample cell contents (Pettersson *et al*., 2017). At this point, physical barriers like trichomes and metabolites or volatiles released by glandular trichomes can be crucial to the repellence and deterrence of the insects. Although there are a few highly generalist aphid species, most aphids specialize on just one or a few closely related plant species (Emden & Harrington, 2017). This co-evolutionary process has facilitated the feeding since the aphid's stylet length matches with the phloem depth. But also, during the process, aphids have become able to tolerate some toxins and suppress host plant defences by manipulating the plant's own hormonal crosstalk (Züst and Agrawal, 2016).

D.1 Macrosiphum euphorbiae

The potato aphid, *M. euphorbiae*, is one of the most frequent pests of tomato plants and also well adapted to Solanaceae. This aphid prefers to colonize the tips of the plant and its feeding often results in stunted growth, shoot dieback, malformation of the leaves and terminal growth, chlorosis and necrosis of young leaves that leads to significant yield and quality losses in fresh-market tomatoes (Walgenbach, 1997; Emden & Harrington 2007). However, it causes not only direct damages on the plant, but also transmits multiple persistent and non-persistent viruses (Kok-Yokomi, 1978; Lange & Bronson, 1981; Emden & Harrington, 2007; Teixeira *et al*., 2016). Furthermore, the potato aphid infestation also promotes the growth of sooty mold on the leaves, increases the risk of weather damage to the fruit, and attracts other damaging hemipteran insects (Lange & Bronson, 1981; Flint, 1990; Walgenbach, 1997).

M. euphorbiae is one of the few cosmopolitan aphid pests of field crops unequivocally originated in North America that became spread to the continental Europe (Eastop, 1958). It is a medium-sized to large, spindle-shaped aphid, usually green but sometimes pink or magenta (being this form increasingly common in Europe, *Fig. I6*). The adult apterae often rather shiny in contrast to the immature stages, which have a light dusting of greyish-white wax (Emden & Harrington 2007). These individuals need to ingest large quantities of phloem to meet their nutritional requirements, which entails an overabundance of sugars in their diet and a challenge to cope with all the chemical defences mentioned previously. To deal with it, aphids have evolved morphological adaptations to exude large quantities of sugars (in honeydew), often together with plant secondary metabolites.

Fig. I6. Macrosiphum euphorbiae. **A**. Apterous female. **B**. Group of apterous females with molting exoskeletons. **C.** Winged adult on *S. lycopersicum.* Images taken from Joseph Berger, Bugwood.org.

D.2 Aphid avoidance of plant defences

Aphids need to overcome a number of plant responses in order to keep feeding from the floem. The bypass of the defences range from the prevention of sieve tube occlusion and inactivation of phytohormone-signalling pathways to avoid expression of anti-insect molecules. After puncturing, aphids modulate the signals related with the oxidative stress and calcium which are the initial steps of plant responses. They prevent the influx of apoplastic Ca^{2+} and the obstruction of the food canal through coagulation proteins by sealing the sieve elements with sheat saliva. Moreover, their secretions appear fully involved in plant reprogramming, while the underlying mechanisms remain partially unknown. It has been described that through this transcriptional reprogramming, aphids can induce morphological changes on the plant host, they can modify resource allocations and also vary local and systemic responses involved in defence mechanisms that could either limitate following infestations or promote the feeding of other aphids (Giordanengo *et al*., 2010). Although strategies to avoid the early responses and defence pathways of the plant are well studied, the mechanisms of adaptation of herbivorous insects to plant defences are not entirely understood.

D.3 Aphid adaptation to plant defences

The phytochemical co-evolution theory suggests that plant secondary metabolites, extensively recognized for their role in plant defence against hervibores, are likely the most important mediators of plant-herbivore interactions. In which both plants and insect herbivores generate selective forces that lead to the evolution of plant defence (i.e., plant secondary metabolites, PSM) and herbivore offense (i.e., detoxification ability) in the so-called co-evolutionary arms race (Steinberg, 2012). As seen before, metabolites present in tomatoes have been subject of many studies, especially when analyzing their particular role in the resistance against arthropods. But despite most works have shown their clear biocidal or deterrent effect on aphids, there are only a handful studies addressing how this affects insect's physiology, let alone of the aphid *M. euphorbiae*.

Insects have the ability to adapt to the plant defensive responses through physiological processes, metabolism and behavior to offset the adverse effects of the host plants' defence systems (War *et al*., 2020). They display a battery of biological responses that produce alterations on the main transcriptional profiles at molecular and cellular level regulating their physiological adaptation to the plant environment and, ultimately, their survival. The early detection of variations in the pattern of genetic activity is a very useful approach to analyze the effects of exposure to stress factors (Planelló *et al*., 2015; Herrero *et al*., 2015; Herrero *et al*., 2018). The recent proliferation of 'omics' technologies has allowed a more mechanistic assessment of the sublethal effects of hazardous components, and therefore promotes, a greater understanding of the mechanisms that trigger insect herbivores to select or not certain plant species. Together with classical toxicological tools, they lead to more solid approaches to decipher the complexity of the mechanisms of action of plant secondary metabolites regarding plant-pest insect interactions.

At the forefront of the physiological adaptation of insects to their environment are those enzymatic activities related to energy production and those involved in the biotransformation and detoxification of plant defensive compounds.

- Celullar stress response

Arthropods react to stress conditions by activating expression of Heat Shock Proteins (HSPs), also known as stress proteins and molecular chaperones. HSPs are a set of evolutionary conserved polypeptides (King & MacRae, 2015) that act in essential activities such as correct protein folding, localization and degradation of other proteins (Gupta, 2010). HSPs represent a primordial mechanism of intracellular self-defence and can also be related with multiple biotic stressors such as plant defences (Zhao & Jones, 2012; Kanzaki *et al*., 2003; Maimbo, 2007). These proteins are classified by family based on molecular size, such as HSP90, HSP70 and HSP27/28 (Schelling & Jones, 1996). Altough the heat shock protein 70 kDa family (HSP70) for insects is the best characterized regarding its function and molecular weight (Feder &Hoffmann, 1999; Neven, 2000) and also its role is a protein well documented in insects, no studies have been conducted studying its role in plant resistance interactions.

- Detoxification process

A complete avoidance of PSM is typically not possible due to the ubiquity and diversity of these chemicals. Thus, once the insect has been exposed to the plant's defensive toxic compound, if its inmediate excretion by transporter proteins is not possible, a metabolic biotransformation of the compound is triggered so that the organism is not intoxicated.brillo The involvement of the detoxifying enzymes in insect adaptation/tolerance to plant toxins depends on host diet composition and insect species and can involve glycosylation, glutathionation, sulfation, or deacylation (Salminen *et al*., 2004; Ferreres *et al*., 2008; Schramm *et al*., 2012).

Lots of PSM are lipophilic and the only way to drive them out is by making them more soluble in water. In this sense, the detoxification process activates a battery of chain reactions that increase the water solubility of the xenobiotic to ease the excreation. The mechanism consists on three phases: modification (I), conjugation (II) and excretion (III) that are mediated by a wide range of enzymes (Amiard-Triquet *et al*., 2013). In Phase I, the compound to metabolise undergoes a chemical modification by introducing polar functional groups through different chemical reactions. Hydrophilicity of the xenobiotic compound increases, mainly through the activity of cytochrome P450 (CYP) enzymes that act as the the primary catalytic system in this first step. If Phase I metabolites are polar enough, they are excreted in the feces. However, most of these products are difficult to eliminate, and they undergo Phase II biotransformation reactions. Here, Phase II transferases link metabolites from phase I with endogenous substrated increasing even more their water solubility. On of the most important reactions is the one due to GSTs (mainly cytosolic phase II enzymes) which catalyze conjugation products to GSH. The GSH-xenobiotic conjugate is too hydrophilic to diffuse freely from the cell and must be pumped out actively by a transmembrane ATPase (phase III) (Steinberg, 2012; Amiard-Triquet *et al*., 2013).

Relevance of *CYP* genes relies on the fact that they form one of the oldest and largest families of genes, with representatives in all living organisms: bacteria, protists, plants, fungi and animals (Werck-Reichhart & Feyereisen, 2000).

- Ecdysone pathway

On the other side, the endocrine system of insects become essential in the interaction between the environment and various physiological and developmental events for the aphid. The ecdysone receptor (EcR) is a nuclear receptor (a ligand-activated transcription factor), which controls development and contributes to other processes such as reproduction (Laudet, 1997; Riddiford *et al*., 2000). Hence, this pathway becomes crucial to the population progression on the plant. EcR acts as a ligand-dependent transcription factor (Mangelsdorf *et al*., 1995; Freedman, 1997). The functional ecdysone receptor is a heterodimer of EcR with another member of the nuclear receptor (NR) superfamily, Ultraspiracle (USP; NR2B4 according to Auwerx *et al*., 1999) (Yao *et al*., 1992, 1993). The DNA-binding domains of EcR:USP heterodimer recognize specific short DNA secuences and mediate the binding of the heterodimer to ecdysone response elements (ECREs) in the promoters of ecdysone-responsive genes. The first gene to be activated are called early response genes. This is a code for proteins that are themselves gene regulatory factors either alone or in combination with hormones. They activate late-response genes that code for the proteins that actually cause structural changes, cell differentiation or apoptosis (programmed cell death). Ultimately, the activation cascade causes physiological changes that result in ecdysis (moulting) (Henrich, 2005).

- Energy metabolism

Furthermore, the GADPH enzyme is a protein that plays a key role in energy production during glycolysis, critical for the insect survival, that recently has also been shown to be involved in other functions at multiple subcellular compartments (Glaser *et al*., 2002; Kosova *et al*., 2017). Therefore, is expressed at constitutively high levels in most tissues and cells and has been widely used as a reference gene for quantitative and semiquantitative PCR analyses, as well as in real-time PCR studies (Carnahan *et al*., 2013; Sirakov *et al*., 2013).

Recently, new GAPDH roles have been discovered. It is a protein with multiple functions in cytoplasm, reticulum, mitochondria and nucleus involved in common cellular processes like DNA repair (Kosova *et al*., 2017), membrane fusion (Glaser *et al*., 2002),

nuclear RNA export (White and Garcin, 2016), activation of neurons transcription (Morgenegg *et al*., 1986), apoptosis, oxidative stress, regulation of the cytoskeleton, and transport among others (Tristan, 2011; Nicholls *et al*., 2012; Nakajima *et al*., 2017). This implication in numerous processes from metabolic to physiological pathways in which GADPH is regulated under diverse conditions makes it a gene reference for plant-insect interactions.

Consequently, the analysis of the effects of tomato plant defences on the activity of genes coding for heat shock proteins (HSPs), biotransmormation enzymes, ecdysone pathway and energy-metabolism become the most relevant in order to understand the effect of tomato plants on *M. euphorbiae* individuals, where modulations are expected regarding aphid adaptation to plant defences. However, to date, the information available on molecular biomarkers in phytophagous insects exposed to secondary metabolites of host plants is limited. In addition, the lack of a described transcriptome for the aphid *M. euphorbiae* difficults the screening of other potential gene targets involved in oxidation-reduction pathways, transport, energy metabolism and development. Which alteration would not only imply their key role in the physiological adaptation of *M. euphorbiae* to tomato plants but also provide information on the subtler effects of plant defences on the aphid's organism.

In this context, this PhD addresses the interaction between tomatoes and one of the main pests of this crop, the aphid *M. euphorbiae*, by paying special attention to the role of the natural defences and type IV glandular trichomes in particular. In order to do that, it is necessary to unravel the defence mechanisms operating in both, tomatoes and aphids in this interaction. As a first step, it is important to evaluate differences among genotypes of tomato in terms of tolerance and resistance against pests. Therefore, this works begins by analyzing the resistance of different tomato genotypes (with varying defence mechanisms) against herbivores with contrasting feeding strategies; and follows with a more detailed analysis of the role of the glandular trichome type IV in particular, in the performance of *M. euphorbiae*. Afterwards, to unravel the mechanisms underlying this defence trait, the hormonal pathways and the effect of priming on plant defence was also studied. Moreover, the responses that the aphid undergoes to overcome tomato defences were also evaluated. In this sense, the present work finishes

by producing a *de novo* transcriptome and analysing the gene expression of potential biomarkers key for the aphid's survival.

OBJECTIVES

II. OBJECTIVES

Extensive studies have been conducted on the glandular type IV trichomes in tomato, revealing their crucial role as an insect resistance against pests such as whiteflies and spider mites. However, the impact of these trichomes on the aphid *Macrosiphum euphorbiae*, one of the most common pests affecting tomato plants, remains poorly understood. Therefore, the general objective of this work is to enhance our understanding of the role of glandular type IV trichomes in tomato-*M. euphorbiae* interactions and contribute to the development of effective pest management strategies in tomato crops. To accomplish this overarching goal, the following specific objectives were established:

1. **To determine whether the trade-offs between tolerance and resistance against two functionally different herbivores depends on the level of tomato domestication or on the contrary, are influenced by phylogenetic structure.**

Firstly, the genetic relationships of the 23 tomato genotypes selected (that cover a domestication gradient i.e., from wild relatives to modern cultivars) were assessed. Then, the performance of the differet genotypes with varying resistance was evaluated by using different pests with contrasting feeding strategies, i.e., the aphid *M. euphorbiae* and the caterpillar *Spodoptera littoralis*.

2. **To investigate the impact of glandular trichomes type IV and acyl-sucrose secretions on the performance and behavior of *M. euphorbiae*.**

The cultivar 'Moneymaker' (MM) that lacks glandular trichomes type-IV and its near-isogenic line ABL 10-4 with high density of trichomes type-IV were selected to conduct further comparisons and evaluate the specific contribution of glandular trichomes type IV in conferring the resistance of tomato plants against *M. euphorbiae*. Firstly, their leaf morphology was examined through optical and electronical microscopy. Afterwards, the content of the principal metabolite, acylsugars (reported to be involved in pest defence mechanisms), was measured. Finally, the performance of *M. euphorbiae* was analysed

in both genotypes. Evaluating, on one side, the choice preference between genotypes and on the other, studying the population's growth on MM and ABL 10-4 plants.

3. **To identify the role of jasmonic acid and salicylic acid defence pathways on the performance of *M. euphorbiae*.**

Firstly, it was evaluated the impact of a priming stimulus due to a previous attack of pests with contrasting types of herbivory on the resistance outcome of tomato plants against *M. euphorbiae*. Secondly, the growth of the aphid *M. euphorbiae* on plant genotypes defective in the JA or SA hormonal pathway, the two main signalling defence routes, was monitored to infer the implications of both hormonal routes on the defensive response.

4. **To unravel the physiological mechanisms of *M. euphorbiae* involved in counter adaptation to trichome-based resistant mechanisms in tomato.**

The effect of the secretions of tomato type IV glandular trichomes on *M. euphorbiae* was studied by means of a transcriptomic study. Transcriptomic profiles of *M. euphorbiae* reared in MM and ABL10-4 plants were compared using RNA seq. Additionally, a wide range of genes were identified as biomarkers. These genes were involved in the regulation of several routes including detoxification of plant secondary metabolites, fundamental metabolism and ecdysone pathways; all of them pivotal for insect adaptation to phyto-chemicals and insect survival.

It is worth noting that each objective is fully developed into a standalone peer-reviewed publication. Objectives 1 to 3 have been already published while 4 is currently under review (all published or under-review drafts are included in the Annex 3, page 205).

MATERIAL AND METHODS

III. MATERIAL AND METHODS

A Plant material

A.1 Genotypes

A.1.1 Tolerance and resistance assays

For the evaluation of the resistance and tolerance of diverse tomato genotypes against pests, 23 accessions of tomato (genus *Solanum* L., section *Lycopersicon*) from different geographic origins, were used. All of them represent putatively different stages in the domestication of tomato and show marked differences in fruit and growth traits (which underlies a clear artificial selection background).

Accessions were classified into four tomato types:

- **Wild relatives**: *S. habrochaites* and *S. pimpinellifolium.*
- **Early domesticated**: *S. lycopersicum* var. *cerasiforme.*
- **Traditional local** landraces: *S. lycopersicum* genotypes resulting from local adaptation to its natural environment, traditional agricultural practices and the isolation from other populations of the species.
- **Commercial** cultivars: *S. lycopersicum* genotypes selected for desirable characters that have been maintained during propagation.

All seeds were obtained from the germplasm bank at the Instituto de Hortofruticultura Subtropical y Mediterránea 'La Mayora' (IHSM-UMA-CSIC). Detailed descriptions of the accessions are shown in the supplementary *Table S1* (Annex 1).

➢ Selection of the plant material

After the introduction of tomato in Europe, a relatively wide range of landraces were traditionally bred in different geographic areas attending to fruit traits (form and colour), organoleptic quality, adaptation to local environmental conditions and phenology. This progressive breeding yielded later to a few commercial varieties selected for certain plant architecture and habit, high yield, earliness in fruit ripening,

external quality of fruits (shape, colour, homogeneity), internal quality of fruits (flavour, sweetness, juiciness), long shelf life, adaptation to growing systems and resistance to biotic and abiotic stresses (Ruiz *et al*., 2005a-c) Although commercial varieties – especially those cultivated in greenhouse – were introgressed with a high number of genes related with resistance mainly to fungi and viruses (Laterrot, 2000), domestication led to a drastic reduction of genetic diversity in cultivated tomato due to several bottlenecks over ages (Bauchet & Causse, 2012). The low genetic diversity of modern tomato cultivars is reflected by a poor level of polymorphism for proteins, isoenzymes, and most types of DNA markers (Bredemeijer *et al*., 1998).

Still, in South-eastern Spain several traditional local cultivars or heirlooms are cultivated by local farmers in small orchards being renowned for their high quality. Landraces such as 'Muchamiel', "De la Pera', 'Valenciano', 'Morunos', and 'Flor de Baladre' types are very popular for their organoleptic fruit quality reaching in the market three to five times the price of the hybrid varieties. Heirlooms are also known to maintained considerable levels of diversity for micronutrient content (Ruiz *et al*., 2005a) and volatile aromas (Ruiz *et al*., 2005b).

Finally, cultivars are plants selected for desirable characters that are maintained during propagation. Usually for this process the tomato 'Moneymaker' has been chosen to be introgressed with resistance genes. The accession Edkawy from North African, which shows salinity resistance (Moghaieb *et al*., 2001) was also selected for this study.

A.1.2 Trichome IV studies

The recurrent commercial variety of *S. lycopersicum* selected for this work is the fresh market cultivar 'Moneymaker' (MM) which produces medium-sized, round and red fruits. MM has been widely used in different studies of fruit development, ripening, physiology and phytopathology that have provided a pool of accessible genetic resources such as EMS mutant collections and TILLING databases (e.g., http://wwwurgv.versailles.inra.fr/tilling/tomato.htm) and collections of nearly isogenic lines containing disease-resistance genes (Laterrot, 1997), among other plant genetic resources.

Its near-isogenic line ABL 10-4 was generated from the initial cross *S. lycopersicum* cv. Moneymaker × *S. pimpinellifolium* acc. TO-937 followed by five cycles of combined recurrent crosses toward 'Moneymaker' and subsequent selfing steps with selection for high type IV trichome density and acylsugar production, plus two additional final selfing steps. ABL 10-4 exhibits trichomes type IV, which are not usually present in *S. lycopersicum* but they appear in another related species. The presence of these trichomes is associated to the production of acyl-sucroses and, therefore, the defence against white flight (*Bemisia tabaci*) and other herbivores (Escobar-Bravo *et al*., 2016). However, the fully expression of acylsucrose's production in glandular trichomes comes after the 10-leaf growth stage (Rodríguez-López *et al*., 2012).

Since both materials have the same genetic background (as revealed by QTL analysis and NGS), comparisons between MM and ABL 10-4 assure that differences found can be ascribed to the absence or presence of type IV glandular trichomes and acylsucroses and not to any other trait.

A.1.3 Studies of the molecular pathways underlying the tomato-aphid defence

Different tomato mutants and their wild relatives were used to evaluate the molecular pathways underlying the defence response of tomato plants against the aphid *M. euphorbiae*.

To study the role of the SA-dependant pathway, comparisons were made between MM and the decreased SA transgenic line *NahG* (with a MM genetic background; Oldroyd & Staskawicz, 1998). On the other side, to evaluate the implication of the JA-dependent pathway, were used the mutant line *spr2* impaired for the synthesis of JA and the expression of the JA-responsive *proteinase inhibitor II* (*PI-II*) gene (a marker of induced resistance to insects) and their corresponding wild background *S. lycopersicum* cv Castlemart (CM).

A.1.4 *M.euphorbiae*'s transcriptome

To have a representative sample for the transcriptome analysis, aphids were reared on different cultivars. On one hand, as tomato cultivars (*S. lycopersicum*), MM and ABL 10-4 genotypes were selected. While, on the other, bell peppers *Capsicum annum* cv. California Wonder were used. *Table M1* summarizes all the genotypes used in this work.

Table M1. Plant genotypes used in this work, grouped by the assays they were used in. Genotypes in bold type were used in multiple studies.

Studies	Crop	Specie	Genotype
1. Resistance and tolerance	**Tomato**	*Solanum pimpinellifolium*	TO 93715
			LA1589
		Solanum habrochaites	PI134418
		Solanum lycopersicum	**ABL 10-4**
			Moneymaker (MM)
			Kalohi
			Melillero
			Muchamiel
			Valenciano
			Flor Baladre
			Marmande
			San Marzano
			Moruno
			Quicena
			Cazorla
			Huevo de Toro
			De Penjar
			Pontevedra
			Periana
			Edkawi
			Monita
		Solanum lycopersicum var. cerasiforme	PE-55
			Mex 89
2. Trichomes IV	**Tomato**	*Solanum lycopersicum*	**ABL 10-4**
			MM
3. Defence signallying pathways	**Tomato**	*Solanum lycopersicum*	*NahG* (transgenic SA)
			MM (wild-type)
			spr2 (mutant JA)
			Castlemart (CM) (wild-type)
4. *Macrosiphum euphorbiae*'s transcriptome	**Tomato**	*Solanum lycopersicum*	**MM**
			ABL 10-4
	Pepper	*Capsicum annum*	California Wonder

A.2 Growth conditions

Seeds were sterilized with an aqueous solution of 50% of household bleach (35 g/L of active chlorine, NaClO <5%) for 30 min, rinsed two times with distilled water and sown on wet filter paper in Petri dishes. Ten days after germination, seeds were put in seedbeds with autoclaved soil mixture (45% peat (Emuflor), 45% coconut fiber (NutriCrop), and 10% perlite (Projar)), and tomato plantlets were grown in a greenhouse (25 ± 5 °C). Twenty days later, plants were transplanted to 18 cm diameter pots filled with the same sterile soil mixture.

Plants were watered twice a week. Water-soluble NPK (SO3) [1.98–3, 41–20, (4.46)] fertilizer mixture (Fertiluq®) and watersoluble N (Ca Mg) [4.32 (6.51–4.02)] fertilizer mixture (Fertiluq®) with micronutrients were applied twice a week every 2 weeks.

B Herbivores

Pest species used in this study were selected for their particularly damaging impact on tomato production. In addition, herbivores with contrasting feeding strategies that are known to trigger different plant defensive responses via different physiological defensive hormonal pathways (Kuć, 1982; Ryals *et al*., 1996; Bhattarai *et al*., 2008; Fujimoto *et al*., 2011), were used in these expperiments.

B.1 Macrosiphum euphorbiae

A clonal population of the aphid *M. euphorbiae* was derived from a single wingless viviparous female obtained from an infested tomato field in Aranjuez (Madrid, Spain). The clonal population was reared at low density on 3 months-old Moneymaker plants. In an insect chamber (47.5x47.5cm) (*Fig. M1*) at 22-25°C, 65% of relative humidity (HR) and a photoperiod of 16L:8D hours during at least six generations.

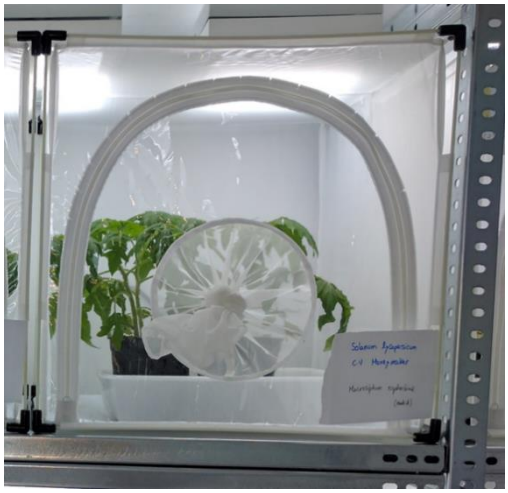


Fig. M1. Insect chamber used to rear the aphids *Macrosiphum euphorbiae.*

B.2 *Spodoptera littoralis*

Caterpillars of genus *Spodoptera* (Lepidoptera: Noctuidae), are among the most destructive and widely distributed Lepidopteran pests of many important crops, including tomato (Bhonwong *et al.,* 2009). The cotton leafworm, *Spodoptera littoralis*, is a generalist herbivore that attacks tomato leaves and fruits (Wakil *et al.,* 2017) with a chewing feeding strategy that cause extensive tissue damage.

S. littoralis pupae supplied by Dra. Carme Quero (Research Unit on Bioactive Molecules, IQAC, CSIC) were reared in boxes (18.5 cm x 7 cm x 29.5 cm) with mesh on the top and filter paper on the base in a climatic chamber at 25 °C day and 18 °C night, 70% HR, with a 16L:8D photoperiod. Fresh laid eggs were hand-picked and transferred into new boxes (10 cm x 13 cm x 4.5 cm). Freshly hatched larvae were reared in artificial diet (Navon, 1985) until the larvae developed into second instars.

One of the clearest differences in defence responses to herbivores exists between chewing caterpillars and phloem-sap-sucking aphids. Therefore, choosing *M. euphorbiae* and *S. littoralis* as study models in plant-insect interaction trials allow to evaluate and compare the outcome of both responses.

C Molecular techniques

C.1 Genotyping by sequencing

C.1.1 Basis

Progress of next-generation DNA sequencing (NGS) technologies has led to the development of rapid genome-wide Single Nucleotide Polymorphism (SNP) detection applications in various plant species. Recent improvements in sequencing throughput combined with an overall decrease in costs per gigabase of sequence is allowing NGS to be applied to not only the evaluation of small subsets of parental inbred lines, but also the mapping and characterization of traits of interest in much larger populations. Such an approach, where sequences are used simultaneously to detect and score SNPs, therefore bypassing the entire marker assay development stage, is known as genotyping-by-sequencing (GBS) (Deschamps *et al*., 2012; *Fig. M2*). Analysis of these DNA polymorphisms, can therefore, be used to evaluate relationships between individuals in populations (Rafalski, 2002). In this work, to assess the genetic relationships among our selected 23 accessions (previously described in the point 1.1) a genotyping-by-sequencing (GBS) approach was made.

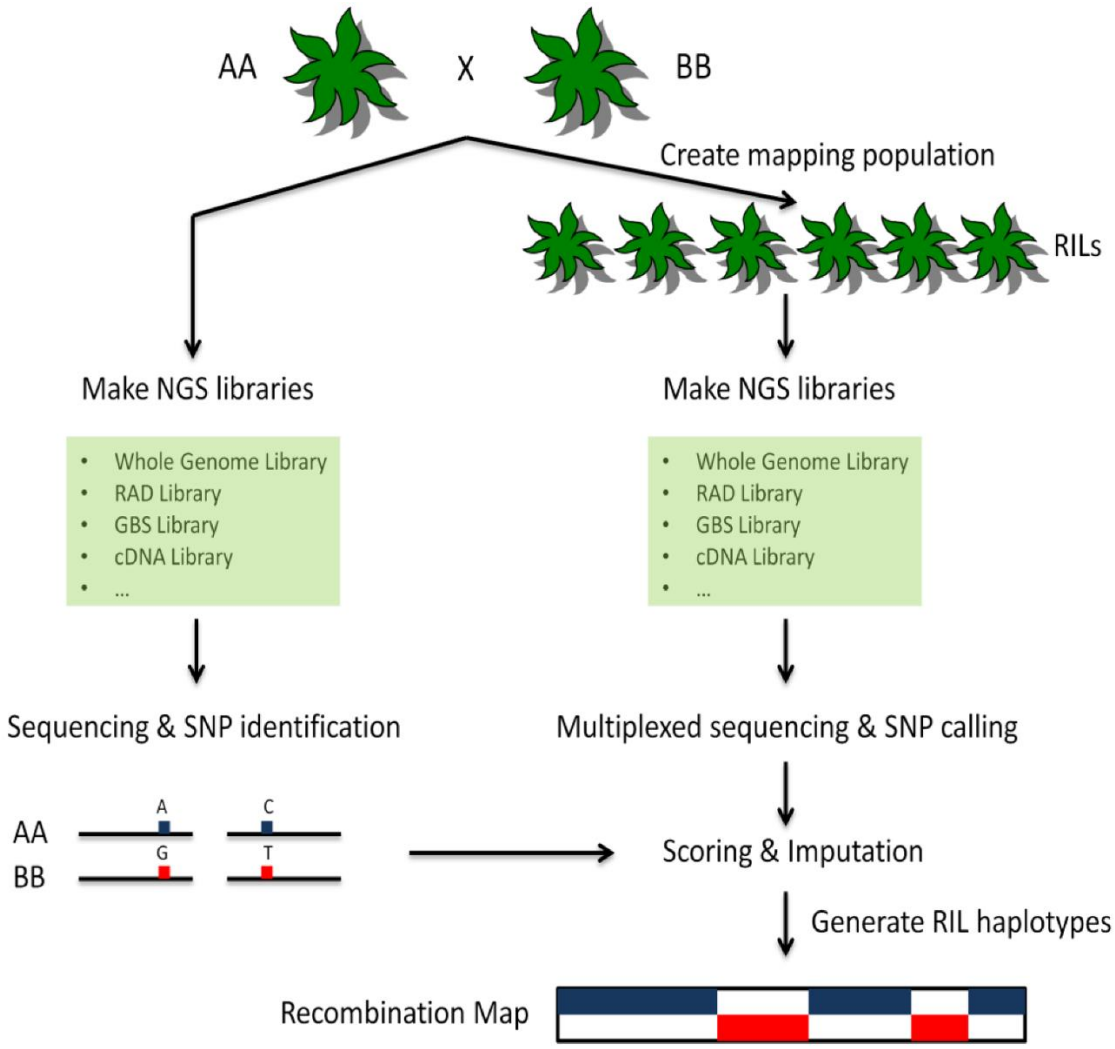


***Fig. M2*. Schematic diagram of a representative GBS procedure**. Two parents (AA and BB) are selected to create a mapping population. The parents are deeply sequenced using NGS technologies. SNPs and other variations between them are identified. The RILs are prepared using the same library construction strategy as the two parents (see text for details) and sequenced at lower coverage using NGS technologies. The resulting sequences are used to determine allelic diversity for each individual. Genotypes are assigned based on parental information. Haplotypes and recombination maps are created for each RIL. Blocks of haplotypes can be used directly as markers for mapping applications. Figure taken from Deschamps *et al.*, 2012.

C.1.2 Procedure

Genomic DNA samples were extracted from fresh leaf tissues of all accessions used in the glasshouse experiment. The DNeasy Plant Mini Kit (Qiagen, CA, USA) was used to obtain high-quality DNA at a concentration of 20 ng/µl. The quantity and quality of the genomic DNA was checked on a 1.5% agarose gel and determined using a NanoDrop Spectrophotometer (ND-1000; ThermoFisher, MA, USA). RAD sequencing library preparation was performed by Floragenex, Inc. (Ore., USA). Genomic DNA from each genotype was digested with the restriction endonuclease *PstI* and processed into multiplexed RAD libraries following the methods described in previous studies (Baird *et al*., 2008; Stölting *et al*., 2013). *PstI* adapters, each containing a unique 6-bp multiplex sequence index (barcode), were affixed to digested templates, polished and amplified via a polymerase chain reaction. RAD libraries were barcoded by individual and sequenced on an Illumina Genome Analyser IIx at Floragenex, Inc. (single end sequencing, 96 bp). Restriction site remnants of the reads were trimmed and low-quality sequences (mean quality < 25) and sequences containing N's were removed using PRINSEQ-LITE v.0.20.4 (Schmieder & Edwards, 2011), resulting in 90-bp RAD fragments. These trimmed fragments were then mapped to a reference tomato genome sequence (v.SL3.0) from the Sol Genomics Network (Fernández-Pozo *et al*., 2015) using BWA-MEM v.0.7.8 (Li & Durbin, 2010). SAMTOOLS v.1.2.115 (Li *et al*., 2009) was used to retain only reads with unique mapping positions and with a mapping quality of minimum 20. Variants were called using the Genome Analysis Toolkit (GATK) v.3.7 (McKenna *et al*., 2010). Resulting variants and genotype calls were subsequently filtered using VCFTOOLS v.0.1.14 (Danecek *et al*., 2011), keeping only biallelic SNPs with a minimum average depth of 10, and genotypes with a minimum quality of 30. The resulting VCF file was subsequently used for phylogenetic analysis using SNPHYLO with an LD threshold of 1, a MAF threshold of 0, a missing rate of 0 (no missing data allowed), and with 100 bootstraps (Lee *et al.*, 2014), The tree was plotted with FIGTREE v.1.4.3. www.tree.bio.ed.ac.uk/software/figtree). All sequence data analysed here are available as FASTQ files from NCBI's Short Read Archive under the project PRJNA591728.

C.2 Acylsucrose cuantification

C.2.1 Basis

Sugars esters, biologically active components of trichrome exudates, have been shown to be an important factor in insect resistance among different species (Goffreda *et al.,* 1990). In this sense, the concentration of sugar esters on the leaf surface of tomato plants with and without glandular trichomes type IV was assessed to evaluate the role of this metabolite in tomato plant defence against pests.

C.2.2 Procedure

Epicultural leaf acylsucroses were extracted following the method described in Goffreda *et al*., 1990. The third youngest fully expanded leaf of MM or ABL 10-4 (depending on the test) was placed on a high-density polyethylene scintillation vial (20 mL), 5 mL of chilled dichloromethane were added and after 15 seconds shaking, the leaflet was removed. The extracts were washed twice with sterile distilled water. After that, dichloromethane was evaporated and the extracted acylsugars were re-dissolved in 2 mL of methanol. Once they were concentrated twice and saponified with NaOH phosphorylated by adenosine triphosphate (ATP) in the reaction catalyzed by hexoquinase. The resulting glucose-6-phosphate is oxidized to 6-phosphogluconate in the presence of nicotinamide adenine dinucleotide (NAD) in a reaction catalyzed by glucose-6-phosphate dehydrogenase (Sigma-Aldrich, ref.) 0.04N, free sucrose was hydrolyzed to glucose and fructose by adding invertase (Sigma-Aldrich), and then phosphorylated by adenosine triphosphate (ATP) in the reaction catalyzed by hexoquinase. The increase in absorbance at 340 nm is recorded and initial sucrose quantities are determined by using a sucrose standard curve in the range of 0 mM to 5.84 mM and expressed as nmol of sucrose esters per cm^2 of leaf area (ΔT Area Meter MK2; Cambridge, UK). Quantification was made for five random samples per genotype.

C.3 Leaf morphology and trichomes' density studies

C.3.1 Basis

Epidermal structures of plants have complex shapes and functions that vary among species. Variation in trichome types can play a key role in resistance against pests, but also, also provide insight into the evolutionary relationships within and among species (Payne, 1978; Lucatti *et al*., 2013). In many plant groups, trichomes are frequently present, easily observable, and often possess variable patterns that can be study through microscopy methods. In this sense, the morphology and density of tomatoes' trichomes were analyzed with a Scanning Electron Microscope (SEM) and an optical microscope.

C.3.2 Procedure

Density of trichomes is known to be affected by leaf age (because of leaf expansion) and environmental conditions (Nihoul, 1993; Wilkens *et al*., 1996). But in order to decrease interference from these effects, all trichome counts were made over leaflets at the same position and developmental stage. Eleven-leaf stage ABL 10-4 and MM tomato plants were used in our study. Fresh discs (2 cm of diameter) from the first pair (starting from terminal position) of opposite leaflet of the third leaf below the apex, were fixed in formalin, alcohol and acetic acid solution (FAA) made up of distilled water: 95% ethanol: formalin: glacial acetic acid (28:17:6:2) (Johansen, 1940). For the morphology study of the leaf trichomes, suitable portions were mounted onto SEM stubs using double-sided adhesive tape. Both adaxial and abaxial surfaces were examined with a TM-1000 (Hitachi High-Technologies, Tokyo, Japan) scanning electron microscope (*Fig. M3*) at an acceleration voltage of 15 kV. The resulting images were captured digitally. Counts to calculate density of glandular trichomes type IV on the adabaxial surfaced of ABL 10-4 leaflet discs were conducted by using a dissecting microscope (40X magnification over five random sample areas of 3.14 mm^2.

Fig. M3. **Left.** Main unit of the scanning electronic microscope Hitachi TM-1000. **Right**. Detail of the electron column.

C.4 RNA extraction for gene expression analysis

C.4.1 Basis

Understanding of molecular mechanisms underlying plant–pest interactions is of primary importance in devising strategies to crop management. For this purpose, gene expression analysis is massively applied, and therefore, extractions of a large quantity of high-quality RNA are required. In this study, depending on the origin of the sample (aphids or tomato leaves) two similar methods were used.

C.4.2 Procedure

- RNA extraction from tomato leaves

RNA of tomato leaves was extracted following a combined LiCl-Trizol protocol in order to obtain more quality RNA fragments. The third and fourth leaf from 3 replicates of each genotype were harvested and frozen in liquid nitrogen and stored at −80 °C until the processing.

Tomato leaves were grinded in liquid nitrogen with a pestle and mortar and then the powder was mixed with 400 µL of extraction buffer and 400 µL of phenol. After that, roughly vortex. For the phase separation 400 µL of chloroform were added (1:1 with phenol) and this mix centrifuged at 13000 rpm for 15 min and RT. Aqueous phase was

collected and transferred to a new tube. To enhance RNA purity, a second wash with chloroform was made. Then, 1/3 of LiCl 8M (final concentration 2M) was added and well mixed. The mix was incubated overnight at 4 ˚C for the precipitation of the RNA and then centrifuged for 15 min at 4 ˚C, 13000 rpm. The resultant pellet was washed with 1 mL of 70% ethanol and resuspended with 100 µL of H_2Od. 50 µL were saved at -80°C and the other 50 µL continued the procedure. 500 µL of trizol were added (250 µL of trizol per 50 µL of chloroform) and it was incubated for 5 min at RT. Afterwards, 100 µL of chloroform were added, vortex for 2-3 min at RT and centrifuged 13000 rpm for 15°C at 4°C. Aqueous phase was collected and transferred to a new tube. RNA was precipitated by adding 250 µL of isopropanol (500 µL/1mL Trizol), incubated for 10 min at RT and then centrifugated for 15 min at 4°C. Aqueous phase was discarded and the pellet washed with ethanol 70% (1 mL), mixed and centrifuged for 15 min at 4°C, 13000 rpm. The pellet was dried and resuspended in 50 µL H_2O.

The RNA suspension was treated with DNase-Free RNase (Roche) to avoid possible DNA contaminations and then, purified with NucleoSpin®RNA Clean-up (Macherey-Nagel, Germany) following the manufacturer's procedure.

This combined method for the RNA extraction, revealed intense RNA bands through gel electrophoresis and a nanodrop spectrophotometer detected ratios of ≥ 2 and 1.8 for A260/A230 and A260/A280, respectively, which points to a good quality RNA.

Samples were stored at -80°C.

- RNA extraction from aphids

RNA from aphids were extracted to analyse the expression of diverse genes when fed in different tomato genotypes. Parthenogenetic aphid females coming from the population growth bioassays (see below, 4.3) were used. Three biological replicates containing 25 adult females were used per condition (gen studied). Total RNA of aphid samples reared in MM and ABL 10-4 tomato genotypes, respectively, was extracted using TRIzol Reagent (Invitrogen, Germany), aphids were homogenized in 0.5 mL of isopropanol and 1 mL of TRIzol followed by an incubation of 10 min at room temperature and a centrifugation for 4 min at 12000 g and 4 °C. After that, RNA pellets were treated with RNase-free DNase (Roche, Germany) and purified with phenol:chloroform:isoamyl

alcohol (Fluka, Germany) using 5PRIME Phase Lock Gel Light tubes (Quantabio, USA). Purified RNA was resuspended in RNase free water, quantified by spectrophotometry at 260 nm using a BioPhotometer (Eppendorf, Germany), and stored at −80 °C.

C.5 cDNA synthesis and quantitative Real-Time PCR (RT-qPCR)

C.5.1 Basis

Reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) is a principal/foremost molecular biology tool and a powerful method for quantification of gene expression levels in real-time (Vandesompele *et al*., 2002). Among the different methods, RT-qPCR is postulated as the best method available for determining changes in gene expression, due to its higher sensitivity, specificity, and broad quantification range (reviewed in Bustin, 2002). In this study, we quantified mRNA transcripts to elucidate on one hand, the expression of genes involved in plant hormonal defence pathways, and on the other, of aphid's gene targets.

C.5.2 Procedure

For each condition and sample, 500 ng of isolated RNA were used for retrotranscription using BioRAD iScript™ cDNA Synthesis Kit, following manufacturer's instructions, in a reaction volume of 20 µl. The obtained cDNA was stored at −80 °C and used as the template for subsequent quantitative PCR analyses.

Quantitative PCR (qPCR) were done using as template 1 µl of cDNA using TAKARA SYBR Green PCR kit on a CFX96 Real-time PCR detection system (Bio-Rad, USA). Cycling conditions were i.e., 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min. Genes encoding actin and the 26S ribosomal subunit were used as endogenous references for the analysis of aphid's genes while relative quantification of specific RNAs from tomato leaves were normalized to the elongation factor 1-α (SlEF) gene (López-Ráez *et al*., 2010). Relative quantifications were measured by using the comparative Ct method, as described by Livak and Schmittgen (2001), using mock samples as a calibrator.

Primers used in the analysis of the expression profile of the selected genes are detailed in *Table M2.*

***Table M2*. Primers used for real-time qPCR amplification of the studied genes in *Macrosiphum euphorbiae* and *Solanum lycopersicum*.** 5'-3' forward (Fw) and reverse (Rv) sequences, length of amplified fragments and reference to the original published paper when corresponds.

Organism	Gene	Primer sequence (5'-3')	Fragment size	Origin
Macrosiphum euphorbiae	*EcR*	Fw: AGTACGGCAACAACTGCGAAA Rv: CATTGGTTCACCGCATTCAA	245 bp	
	ERR	Fw: CTCAGCAAGTAAGGAGGAG Rv: CGTCTAATAATGTGATCGG	200 bp	*(Morales et al., 2014)*
	E74	Fw: TCTTACTGAAACTTCTTCAAG Rv: GCTTTGAGACAGCTTTGGAAT	120 bp	*(Morales et al., 2014)*
	ftz-f1	Fw: CACCGACTCAAAGCCCAGAC Rv: GCGAAACAGACAGCGAGGA	219 bp	
	HR38	Fw: CAGTTTTTCGGATTGCTCACG Rv: CGTATCGGCGTATTTGGTCCT	168 bp	
	HR4	Fw: TCCATTACGGCATTATCACTTGC Rv: TGACGGACTTCTCCATTTTTACTTG	300 bp	
	hsp70	Fw: CATGTGAACGAGCCAAGAGA Rv: TCGAGTTGATCCACCAACAA	280 bp	(Lee *et al.,* 2006)
	hsp17	Fw: GGAAGACGAATTTGGCCATATTG Rv: GGGTTCATAGTTGGTGGC	140 bp	(Martín-Folgar *et al*., 2015)
	hsp10	Fw: CGTCCAAAGAGCTGAGGCAT Rv: TTTCGCGAGGATATCGCCTT	210 bp	(Martín-Folgar *et al*., 2018)
	Phm	Fw: CCAGGTCCTTGGGGTGTTC Rv: GCGGTCTGCCAGTTCGTT	196 bp	(Wu *et al*., 2018)
	GAPDH	Fw: GGTATTTCATTGAATGATCACTTTG Rv: TAATCCTTGGATTGCATGTACTTG	100 bp	(Martínez-Paz *et al*., 2013)
	Actin	Fw: GATGAAGATCCTCACCGAACG Rv: CGGAAACGTTCATTACCG	205 bp	(Martínez-Guitarte *et al.,* 2007)
	26S	Fw: TTCGCGACCTCAACTCATGT Rv: CGCATTCAAGCTGGACTTA	220 bp	(Planelló *et al.,* 2011)
Solanum lycopersicum	*SlEF*	Fw: GATTGGTGGTATTGGAACTGTC Rv: AGCTTCGTGGTGCATCTC	130 bp	(Rotenberg *et al*., 2006)
	PI-II	Fw: GAAAATCGTTAATTTATCCCAC Rv: ACATACAAACTTTCCATCTTTA	92 bp	(Uppalapati *et al*., 2005)

C.6 De novo transcriptome of *M. euphorbiae*

C.6.1 Basis

The production of a reference transcriptome and quantification of transcript abundances of *M. euphorbiae* fed in nearly-isogenic tomato lines with and without resistance traits based on type IV leaf glandular trichomes, provides insights into insect gene expression in response to these different plant tomato genotypes and their trichome secretions. Which prior to this work wasn't described.

C.6.2 Procedure

- Design and workflow followed to produce the *M. euphorbiae* transcriptome

Four aphid populations were used for the reference transcriptome: three colonies reared on tomato (two on susceptible tomato "Moneymaker" -named as MM and MEM, respectively- and one on resistant (ABL10-4) and a colony maintained on susceptible pepper (MEM-CW) to maximize the genome coverage of the *M. euphorbiae* expressed genes (*Fig. M4*, *Table M3*). A total of 12 libraries for three biological replicates (25 insects each) for each population were prepared. Plants received 3 adult females, apterous and viviparous, that came from a single aphid clone. This set-up was replicated 15 times per tomato genotype. The population build-up was monitored every day, and we collected 25 adult individuals for transcriptome analysis after 5 weeks on the plants. *Fig. M4* show in detail different steps followed in this last part of the study where the transcriptome is obtained and the differentially expressed genes, analysed.

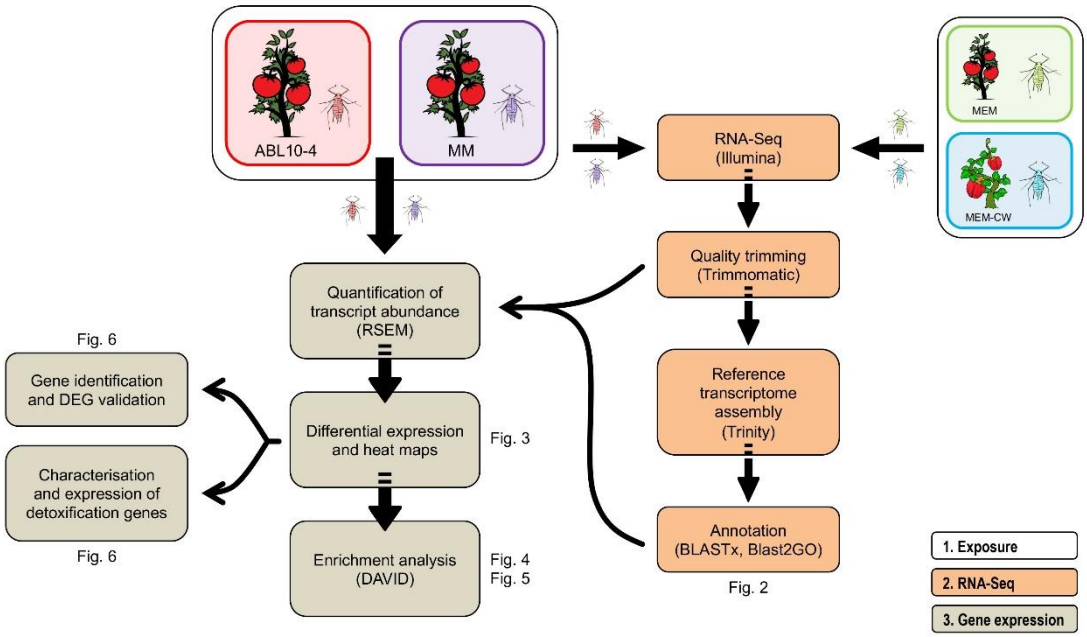


Fig. M4. Workflow followed to produce the *Macrosiphum euphorbiae* transcriptome and analyse the differential gene expression of aphids reared in diverse genotypes.

Table M3. **Aphids samples used for *Macrosiphum euphorbiae de novo* transcriptome**. Type of population and read counts per sample after trimming.

Population group	Host	Sample	Read Count	GC (%)
MM	Tomato cultivar "Moneymaker"	MM-P1	53175708	43.56
		MM-P2	53291090	44.44
		MM-P3	58748458	43.61
ABL10-4	Tomato isogenic line ABL10-4	ABL10-4 -P1	21725650	40.27
		ABL10-4 -P2	44579956	47.08
		ABL10-4 -P3	37381678	42.93
MEM	Tomato cultivar "Moneymaker"	MEM1	42960032	48.62
		MEM2	39617224	44.27
		MEM3	40539508	45.25
MEM-CW	Pepper cultivar	MEM-CW1	45487942	44.23
		MEM-CW2	48825974	44.57
		MEM-CW3	52505780	43.65

➢ *De novo* transcriptome assembly and annotation

The raw reads (100pb) were assessed for quality using FastQC4 (Andrews, 2010). Further, low-quality (Phred value<33) and adaptor sequences from the raw data were removed using the Trimmomatic (v0.32) tool. These clean reads were then assembled into contigs with Trinity software (Grabherr, 2011) using default parameters. Next, all unigenes >200 bp were searched using BLASTx against Metazoa, with the following protein sequences databases: UNIPROT (v20170706), Kyoto Encyclopedia of Genes and Genomes (KEGG_v20170706) and GO (v20150407) (e-value<10−5), to identify proteins with high sequence similarity and to assign putative functional annotations. Subsequently, Gene Ontology (GO) annotations of the unigenes were obtained using the Blast2GO program (Conesa *et al*., 2005).

➢ Gene characterization

The nucleotide coding sequences for relevant genes involved in diverse key pathways for the aphid were identify from the *M. euphorbiae reference* transcriptome and used to design primer oligos to characterize their expression levels.

A number of genes known to be involved in ecdysone synthesis and response pathway (*EcR, E74, ftz-f1, HR38, HR4*), cell stress response (*hsp70*), energetic metabolism (*GAPDH*), and genes related with biotransformation and detoxification pathways (*cyp4g15, cyp6a13-like, cyp6k1-like, cyp4c1*, Cu-Zn superoxide dismutase (SOD), glutathione S transferase (GST) and glutathione peroxidase (GPx)) were used to design primer oligos to characterize their expression levels. These genes' coding sequences were identified from a *de novo* transcriptome for the first time (Project number PRJEB35133).

For this purpose, a total of 12 RNA-Seq libraries constructed from four clones-populations of aphids maintained on tomato (MM and ABL10-4), sweet pepper and melon plants Cucumis melo. cDNA libraries were constructed by Macrogen following Tru-Seq Stranded mRNA (Illumina) protocol and sequenced on Illumina Hi-Seq 4000 using a 100 cycles paired-ended protocol generating 698.5 million paired-end reads. The reads were de novo assembled using with Trinity software (Grabherr *et al*., 2011) using default parameters. All unigenes>200 bpwere searched using BLASTx with the following

protein sequences databases: UNIPROT (v20170706), Kyoto Encyclopedia of Genes and Genomes (KEGG_v20170706) andGO(v20150407) (e-value < 10–5), to identify proteins with high sequence similarity, and to assign putative functional annotations. Subsequently, Gene Ontology (GO) annotations of the unigenes were obtained using Blast2GO (Conesa *et al*., 2005).

A number of genes also related to the above-mentioned categories (*phm*, *ERR*, *hsp17*, and *hsp10*) were not present in de novo *M. euphorbiae* transcriptome and then they had to be amplified by using published primers from related insect species (*Table M2**Table S2***, *Annex 1*).

In all cases, the identification of each gene amplified was verified by sequencing and analysis through the Basic Local Alignment Search Tool (BLAST). Sequence alignments were performed with Clustal X Version 2 and MAFFT Version X. SnapGene (GSL Biotech LLC), BLAST protein tool, and DOG V.2 software were used to identify and characterize genes.

D Bioassays – experimental design

D.1 Resistance and tolerance assays

In a glasshouse experiment, tomato tolerance and resistance against pest with contrasting feeding strategies was tested. For tolerance, the growth of the different selected genotypes was compared among a control group and two herbivory treatments. While resistance was analysed through pest performance. The experiment comprised 1035 experimental pots, that is 23 genotypes, three treatments (control, aphid and caterpillar) and 15 replicates per treatment and genotype.

One month old seedlings were transplanted to 3.6 l pots (18 cm diameter) filled with the same sterile substrate. The experimental pots were set in a glasshouse that was kept at 23°C (with a minimum temperature of 16°C at night) and natural illumination. Experimental plants were distributed in four blocks and plants were randomised in each block. All plants from all treatments were caged with tulle-mesh (i.e., confined within a cage of 70 cm x 30 cm x 30 cm) (*Fig. M5*).

Fig. M5. **Experimental design for tolerance and resistance assays.** Tomato plants transplanted to the 18cm pots used (left). Plants covered in tulle-mesh and randomly set among the blocks in the glasshouse (right).

For the aphid treatment, plants were artificially infested with three viviparous adult female aphids reared in tomato (MM). For the caterpillar treatment, two *Spodoptera* larvae (2nd instar caterpillars reared in artificial diet) were transferred to the experimental plants 7 days after hatching. Each caterpillar was weighted before placement in the plants (*Fig. M6* (A)).

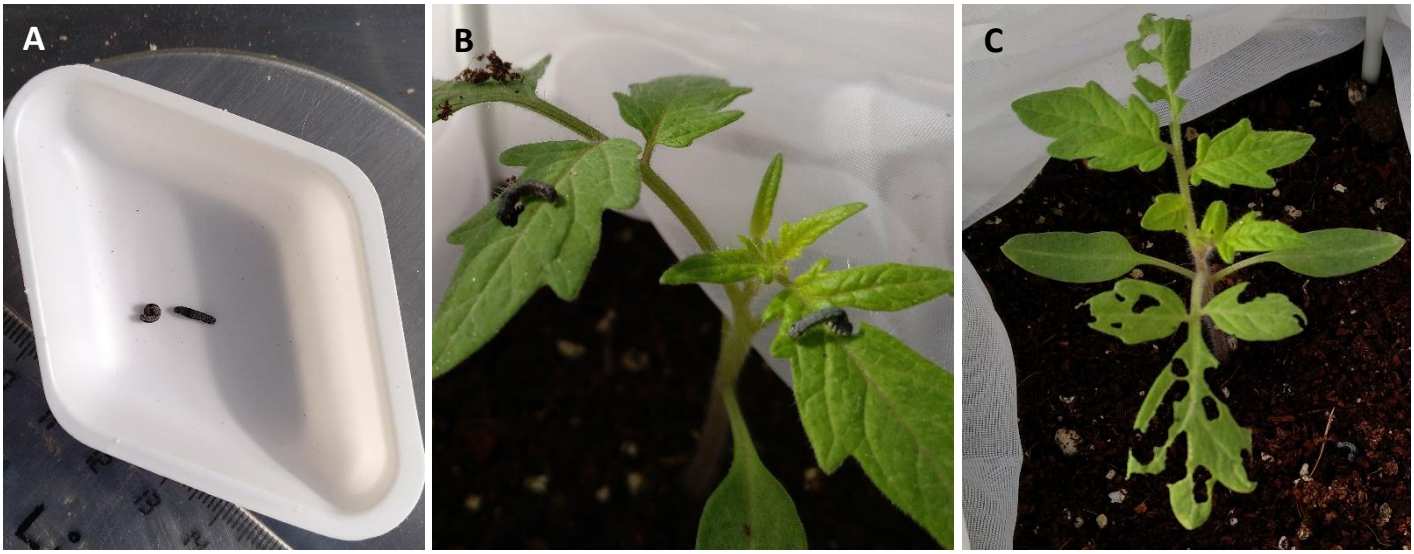


Fig. M6. **Individuals of *Spodoptera littoralis.* (A)** 2nd instar caterpillars weighed before their placement on tomato plants. **(B)** Caterpillars used as priming stimulus feeding on tomato plants. **(C)** Tomato plants after removing caterpillars.

Plants were left to grow for 5 weeks and then harvested individually. Plant biomass aboveground was obtained after oven drying for 48 h at 80°C. For the aphid treatment, the total number of aphids was counted. For the caterpillar treatment, *Spodoptera* individuals were collected and weighed. Increase in caterpillar weight d–1 was calculated by subtracting the initial from the final caterpillar weight and dividing by the total number of days infested.

D.2 Choice assays

M. euphorbiae's preference among MM and ABL 10-4 was evaluated by means of host-choice bioassays under free-choice and no-choice conditions. Bioassays were carried out in a plastic Petri dish of 9 cm diameter. One apterous female adult of *M. euphorbiae* was placed equidistantly to two tomato leaflets, one of MM and the other ABL 10-4 in free-choice tests; or either both leaflets of MM or ABL 10-4 in no-choice tests (*Fig. M7*). Tomato leaflets came from detached leaflets from tomato plants of MM and ABL 10-4 taken at 10-leaf growth stage. Insects were left to visit the leaflets and their position was checked after 20, 40 and 60 min.

Each plate, both in choice and no-choice experiments, was used up to three times. For the free-choice experiment 60 aphids were tested accounting for 165 aphid choices at the end of the experiment. For the no-choice experiment 60 aphids per genotype were tested accounting for 360 aphid choices. Bioassays were carried out on a lab bench with room temperature conditions (22 ± 2 °C, 65% HR). The Petri dishes' relative orientation on the bench was changed in every trial to avoid bias due to changes in the light incidence or unnoticed environmental factors.

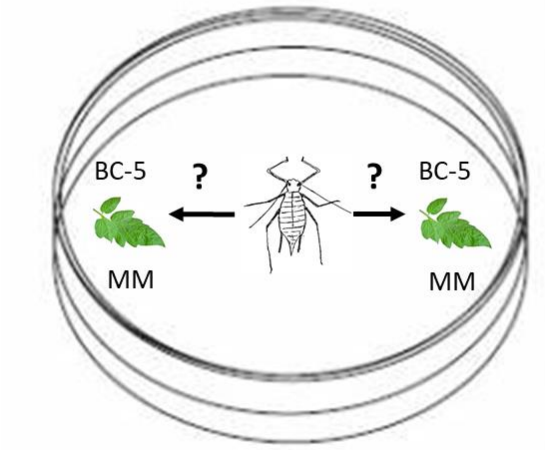


Fig. M7. **Experimental design of choice assays.** Scheme of a 9 cm diameter Petri dish in which an aphid is placed equidistantly among either two MM/ABL 10-4 leaflets (no-choice) or one MM and other ABL 10-4 leaflets (choice).

D.3 Population growth assay

The performance of *M. euphorbiae* in MM and ABL 10-4 genotypes was evaluated following the population growth of the aphid over 5 weeks on the tomatoes with and without glandular trichomes type IV. Plants were manually infested, transferring 3 apterous female adults into four leaf-stage tomato plants. The experiment comprised 20 plants per genotype that were set following a random design. All plants were caged with tulle-mesh (i.e., confined within a cage of W30 × D30 × H70 cm). The experimental pots were set in a glasshouse that was kept at 23°C (with a minimum temperature of 16°C at night) and natural illumination.

D.4 Priming assay

It was evaluated to what extent a short pulse of herbivory could prime tomato plants (i.e., indice plant defences) and in consequence, affect aphid performance in a second aphid attack. The assay comprised 104 experimental pots with 4 weeks old tomatoes, that is 2 genotypes (MM and ABL 10-4) and 13 replicates per genotype and treatment (control, MeJA, aphids and caterpillars).

For the aphid treatment, plants were artificially infested with three viviparous adult female aphids that had been reared in tomato before the establishment of the experiment (see below). For the caterpillar treatment, two *Spodoptera* larvae (2nd instar caterpillars reared in artificial diet) were transferred to the experimental plants 7 days after hatching (*Fig. M6*). Each caterpillar was weighted before placement in the plants. For 5 days, plants were left to grow and afterwards both the aphids and the caterpillars were removed from the plant. The weight of the caterpillar and the number of aphids were examined (data not shown). Then the plants were left to grow for a week with no herbivores at all and after that, all plants were infested again with aphids (3 apterous female *M. euphorbiae* per plant). Plants were left to grow for 20 days and the number of aphids was count. A detailed diagram of the experimental design is shown in the *Fig. M8.*

For the MeJA treatment, tomatoes were sprayed with a 0.5mM MeJA (Sigma-Aldrich, Germany) in 0.8% ethanol and 0.1% tween-20® (Merck, Germany) aqueous solution until the point of run-off. A mock treatment with 0.8% ethanol (etOH) and 0.1% tween-20® (Merck, Germany) aqueous solution was used as control.

48h after tomatoes were treated (either sprayed with a MeJA solution or herbivores placed on them), the third and fourth leaf from 3 replicates of each genotype were harvested and frozen in liquid nitrogen in order to evaluate gene expression of the proteinase inhibitor II (*PI-II*), a marker gene of the jasmonic signaling pathway known to be involved in the plant resistance to *S. littoralis* and wound responses. Which was also helpful to verify that the response due to the hormonal spray was triggered. The third and fourth leaf from 3 replicates of each genotype were harvested and frozen in liquid nitrogen and stored at −80 °C until further processing for RT-qPCR (see C.5).

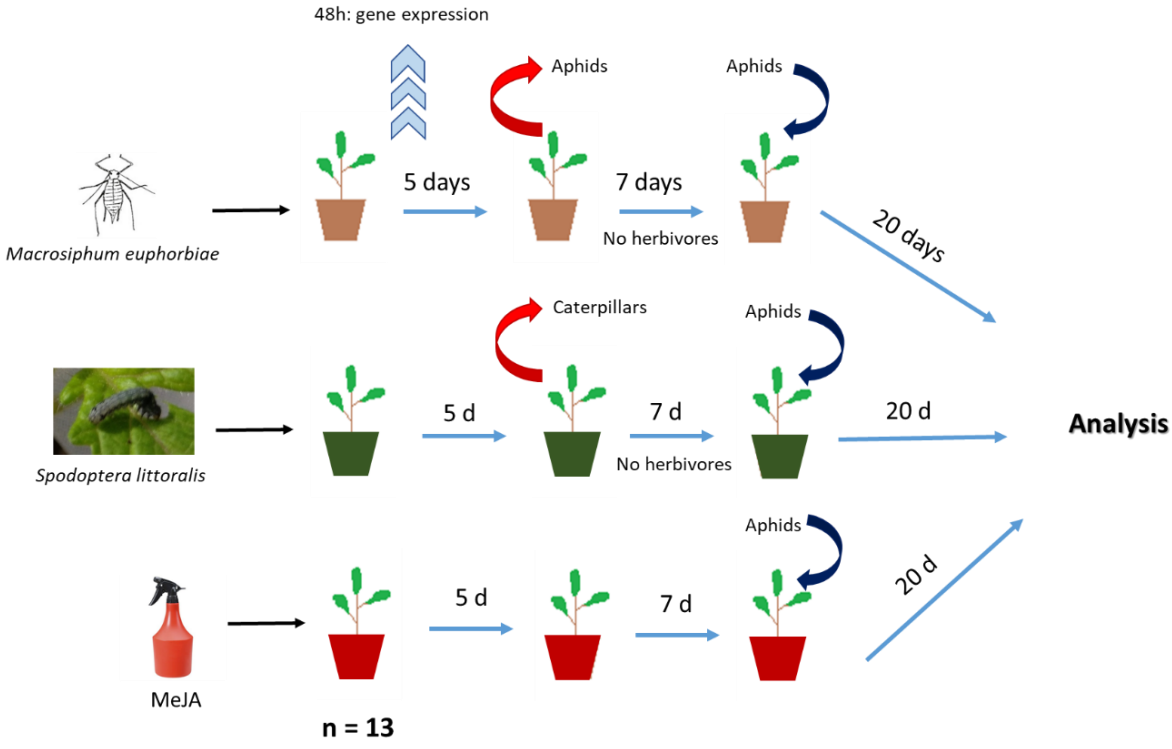


***Fig. M8*. Scheme of the experimental design of the priming experiment.** Tomato plants were infested either with aphid or caterpillars or treated with a MeJA solution. After 5 days of herbivory, insects were removed, plants were then left 1 week without herbivores and after that, all tomatoes were infested with 3 apterous female *Macrosiphum euphorbiae* individuals. 18 days later, the number of aphids were counted. d: days.

D.5 Assays with hormone impaired genotypes

Multiplication of *M. euphorbiae* was compared on mutant and transgenic tomato genotypes, that are impaired for the main defence signalling pathways and hence, challenged to induce plant defences. In a first assay, aphid multiplication was compared in the mutant *Spr2*, impaired for the synthesis of JA and their wild type CM. Then in a second assay, aphid multiplication was evaluated on *NahG*, a transgenic line that prevents the accumulation of SA, and MM (its wildtype). 13 replicates per genotypes were used.

The two assays were conducted in a greenhouse with cooling capacities and with a temperature ranging from 25 ± 2 °C during the day and 20 ± 2 °C at night, and with a relative humidity of 60–70% and a 16L:8D photoperiod. In both assays, for each genotype, ten 3-week-old plants were manually infested with three aphids and every five days the number of aphids was noted down. All plants from all treatments were caged individually with tulle-mesh (i.e., confined within a cage of W30 × D30 × H70 cm). The experiments were kept for 20 days after plants received the aphids. Control plants were not infested (*Fig. M9*).

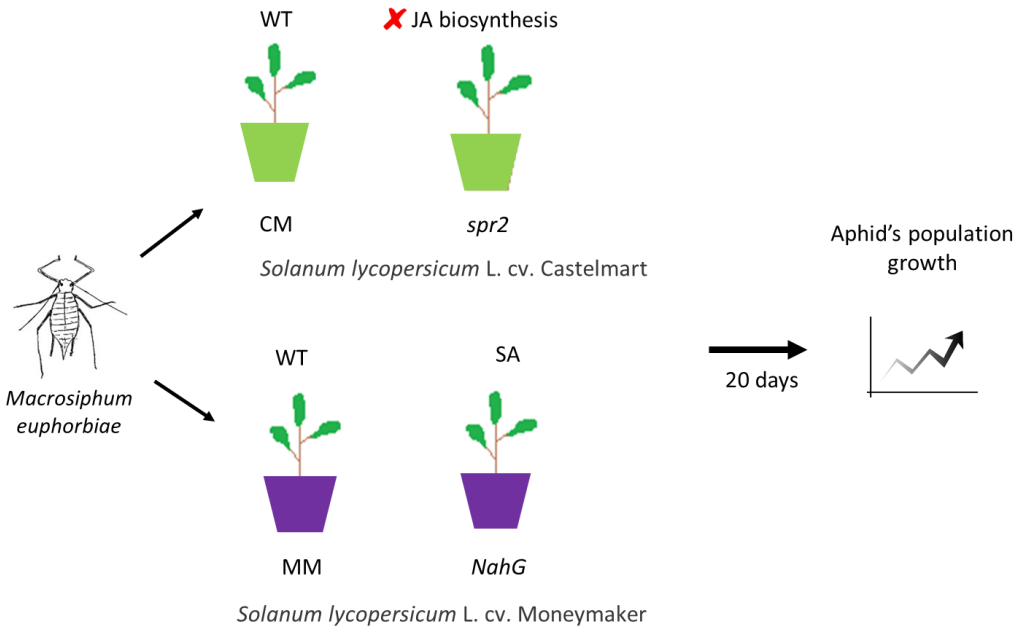


***Fig. M9*. Experimental design of hormone impaired assays**. Three apterous females of *Macrosiphum euphorbiae* were, on one side, placed on the impaired genotype for the JA biosynthesis (*spr2*) its wild type *Solanum lycopersicum* var. Castlemart, and on the other, on the transgenic tomato unable to accumulate SA (*NahG*). The population was left to grown. After 18 days, the number of aphids per plant was observed.

E Statistical analysis

All statistical analyses were carried out with the RStudio software (version 4.1.0) using the 'lme4', 'brms', 'nlme', 'emmeans', 'rstan', "lsmeans", "lmperm", "permuco", "performance" and "GGTREE" packages when required. Plots were designed using "ggplot2". Model assumptions (i.e., homogeneity of variance, collinearity, normality of random effects and residuals) were checked for all models. Plant distribution in all bioassays was randomized.

E.1 Phylogenetic studies

The pattern of the similarity in the trait values, called phylogenetic signal (Blomberg *et al*., 2003), was quantified through the *k statistic* of Blomberg and colleagues (2003) in plant biomass (measured in the control plants of the glasshouse experiment) and the herbivory responses (number of aphids and caterpillar's weight gain per mg). In this case, R library picante (Kembel *et al*., 2010) was used. There was no phylogenetic signal in any of the data (K = 0.014 for the biomass; K = 0.006 for the aphid number; K = 0.010 for caterpillar's weight gain); for this reason, the phylogenetic dependence across species in the subsequent analyses was not accounted for it. An annotated phylogenetic tree was drawn with the GGTREE (Yu *et al.,* 2018) package.

E.2 Tolerance and resistance assays

To evaluate whether tolerance and/or resistance decreases along the gradient from wild genotypes, over early domesticated to local landraces and finally cultivars, two different analyses were performed:

- To infer differences in **tolerance** between the four tomato types and two herbivore treatments, first, it was calculated an effect size for the treatment effects as the log response ratio between the biomass in the treated plants and control plants (Hedges & Olkin, 1985). Then, this effect size was related to tomato type, herbivore treatment and their interaction using generalised linear multilevel models fitted with MCMC methods for generalised linear mixed models (MCMCglmm package; Hadfield, 2010). Tomato accession and block were added as random effects. Within a particular tomato accession, the effect

of one herbivore type might not be independent from the effect of another type (e.g., a tomato that is sensitive to one type can also be sensitive to another), so the herbivore treatment effects to (co)vary within tomato accessions were allowed. The models were fitted with commonly applied inverse Wishart priors for the parameters (V = 1 and nu = 0.002, with V the expected variance if the degree of believe parameter nu goes to infinity). It was ran one chain, set to 20 000 iterations, after a burn-in of 10 000, and a thinning interval of 10 (resulting in 1000 samples) and report posterior modes ± 95% highest posterior density intervals in the results.

- (2) To test for **resistance**, it was examined the effect of tomato type on the number of aphids and caterpillar's weight gain as dependent variables. These two models included tomato accession and block as random effects again and also plant biomass. Biomass was included in the analyses for resistance because a larger plant size could be expected to favour greater number of aphids or having larger caterpillars (i.e., larger increases in size). Priors and sampling settings were defined as before. All response variables were $\log_e$ transformed and the numbers of aphids and caterpillar weight gain were also scaled to centre to zero and a standard deviation of one. Similarly, to compare resistance and tolerance among accessions the two previous analyses were repeated excluding type of tomato as a fixed factor. The output of these analyses was used to construct density curves with the posterior values for each accession.

E.3 Choice assays

For choice assays (free-choice and no-choice), Bayesian generalized mixed linear models were conducted using the 'brms' package (Bürkner., 2018) where priors define the type of error structure (e.g., binomial, *Poisson*, etc.) and Markov chain Monte Carlo (MCMC) is used for model parameters estimation and inference. In the no-choice assay, differences in aphid choice were evaluated by means of a binomial logistic regression (with a logit link function), and in particular considering a Bernoulli distribution in the response variable choice (where 1 = selection by an aphid of the genotype; 0 = no selection). Therefore, in the model, aphid choice was set as the response variable, genotype was included as fixed factor and aphid, plate and time were included as

random factors. A similar model built-up was used for the free-choice assay. For all Bayesian generalized mixed models, two MCMC chains with 2000 iterations each were run. Default priors for a binomial (Bernouilli) distribution (with logit link function) were allowed. Posterior differences according to genotype were assessed by testing the hypothesis of having equal probabilities for aphid choice within a CI of 95% for both genotypes (MM and ABL 10-4).

E.4 Population dynamic of *M. euphorbiae*

To assess differences of *M. euphorbiae* population growth dynamics in MM and ABL 10-4 plants, a linear mixed model for repeated measures was generated with log10 (nº. aphids+1) as response variable and days as a covariate (because of its linear or quadratic behavior) to correct for overdispersion. Plant was introduced in the model as a random variable. Pairwise differences on the number of aphids for each genotype were based on a LSmeans analysis.

E.5 Priming assays

For the priming assay, differences on aphid multiplication due to the priming stimulus were again evaluated by means of a GLMM (adjusting for a *Poisson* error structure using log link function). Again, the number of aphids was set as the response variable, genotype and treatment (i.e., different priming stimuli) were included as fixed factors. Greenhouse bench and date were included as random factor.

For the analysis of expression levels and given that the dependent variable did not fulfil the assumptions for a GLMM (i.e., homogeneity of variance and normality of residuals), a two-way permutational analysis of variance (aovp) was conducted using the R package "lmperm". Aovp is an analysis of variance that makes use of permutation tests instead of normal theory tests to calculate statistics. In this case, Log_fold value (as proxy for *PI-II* expression) was included as dependent variable and treatment and genotype as fixed factors. For the estimation of model parameters 5000 permutations were run.

E.6 Assays with hormone impaired genotypes

Aphid multiplication on the different mutants (i.e., for CM and *spr2,* in the first assay, and for MM and *NahG* in the second assay) was compared by means of two generalized

linear mixed models (GLMMs) where the number of aphids was the dependent variable with a *Poisson* error structure (with a log link function) and tomato genotype was used as fixed factor. As experimental pots were placed in different benches in the greenhouse and aphids on plants were measured on four different occasions, bench and date (day of measurement) were included in both models as a random factor.

E.7 Gene expression analysis

Normality and homoscedasticity of data were assessed with the Shapiro-Wilk's test and the Levene's test, respectively. Since data were not homogeneous or normally distributed, Mann-Whitney U test was used ($P < 0.05$). The relative expression of each target gene was calculated using a one-way nested analysis of variance (ANOVA) and Duncan's new multiple range tests.

RESULTS

IV. RESULTS

A Genetic relationship of different tomato accessions and their resistance to pests.

A.1 Genotyping-by-sequencing of tomato accessions

The genetic relationships among the selected accessions was assessed through a genotyping-by-sequencing (GBS) method. The final high-quality dataset of biallelic SNPS had 40 703 sites, but only 30 887SNPs were retained after removing SNPs where at least one individual had missing data. This GBS showed that the number of SNPs per sample varied from 345 (in the local San Marzano) to 27 683 (in the wild PI-134418). The number of SNPs varied greatly between *S. lycopersicum* related taxa (i.e., *S. lycopersicum* var. cerasiforme PE-55 (1262*), S. pimpinellifolium* (TO-937-15 (4344), LA1589 (4454)) and the rest of accessions (ranging from 352 to 573). The cultivar ABL 10-4, the near-isogenic line to 'Moneymaker', had 611 SNPs and Mex 89, the other *S. lycopersicum* var. cerasiforme, only showed 361 SNPs. The number of homozygous SNPs was highest in wild and early domesticated accessions: PI-134418 (97.2%), TO-937-15 (96.5%), PE-55 (92.3%) and LA1589 (92.1%). The percentage of heterozygous SNPs was highest for the locals, as for example Pera (13.7%) followed by Periana (13.0%) and Flor de Baladre (12.8%).

The ML tree produced by SNPHYLO that included 100 bootstraps, clearly separated the three species. *S. habrochaites, S. pimpinellifolium* and the *S. lycopersicum* varieties, *with S. lycopersicum* PE-55 placed between *S. pimpinellifolium* and the *S. lycopersicum* accessions. The rest of *S. lycopersicum* accessions was almost indistinguishable and grouped with 100% bootstrap support. The cultivars ABL 10-4 and Monita constituted a clade together with Moneymaker, from which they are derived (*Fig. R1*).

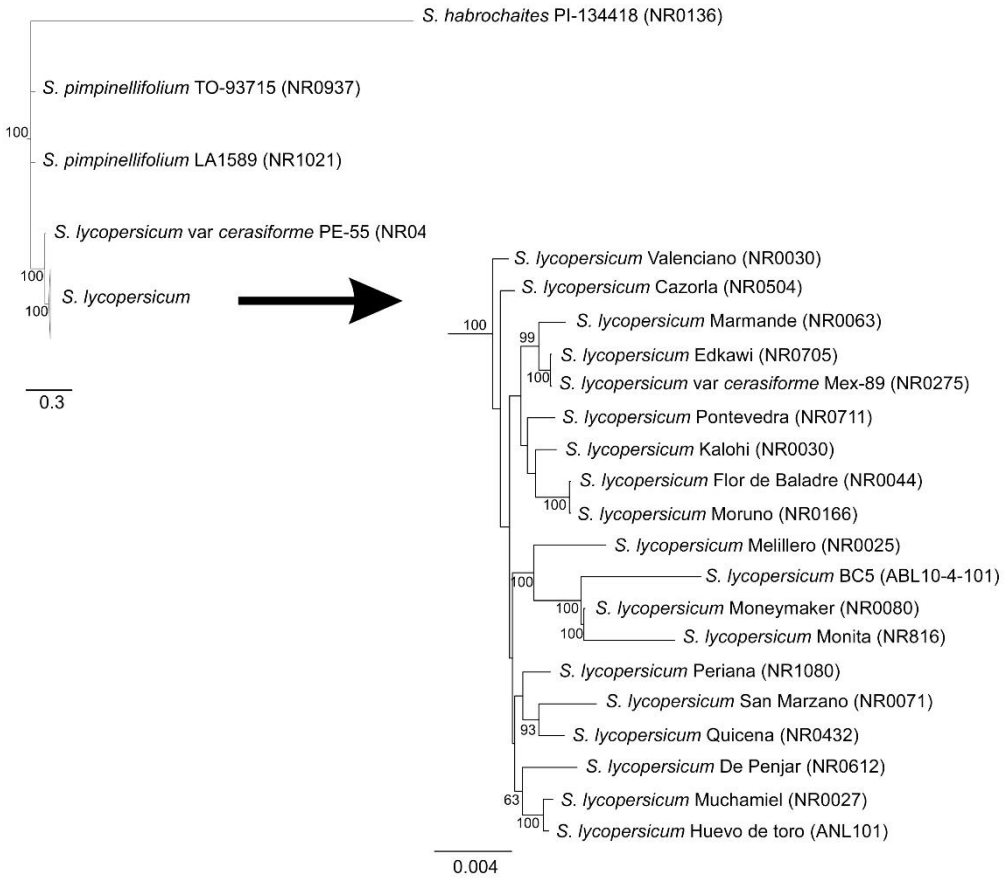


Fig. R1. **ML tree for tomato accessions.** Maximum likelihood tree for tomato accessions including 100 bootstraps. The tree was midpoint rooted. Accessions correspond to three species: *Solanum habrochaites* (PI-134418); *Solanum pimpinellifolium* (TO-93715 and LA1589) and *Solanum lycopersicum* (the rest of accessions). Only bootstrap values greater than 90 are shown.

A.2 Tolerance and resistance assay among the 23 tomato accessions.

Once the relationships among genotypes were settle, the tolerance and resistance against pest of these tomato plants that vary on their domestication degree, were studied. Tolerance was evaluated through the analysis of the biomass produced after the herbivory of insects with contrasting feeding strategies. On the other side, pest performance was examined to infer the resistance of tomato genotypes. The biomass values, number of aphids and caterpillar's weight gain varied among species independently of their genetic relatedness (values of Z statistic are shown in the previous Materials and Methods section and the annotation of the phylogenetic tree in *Fig. R2*).

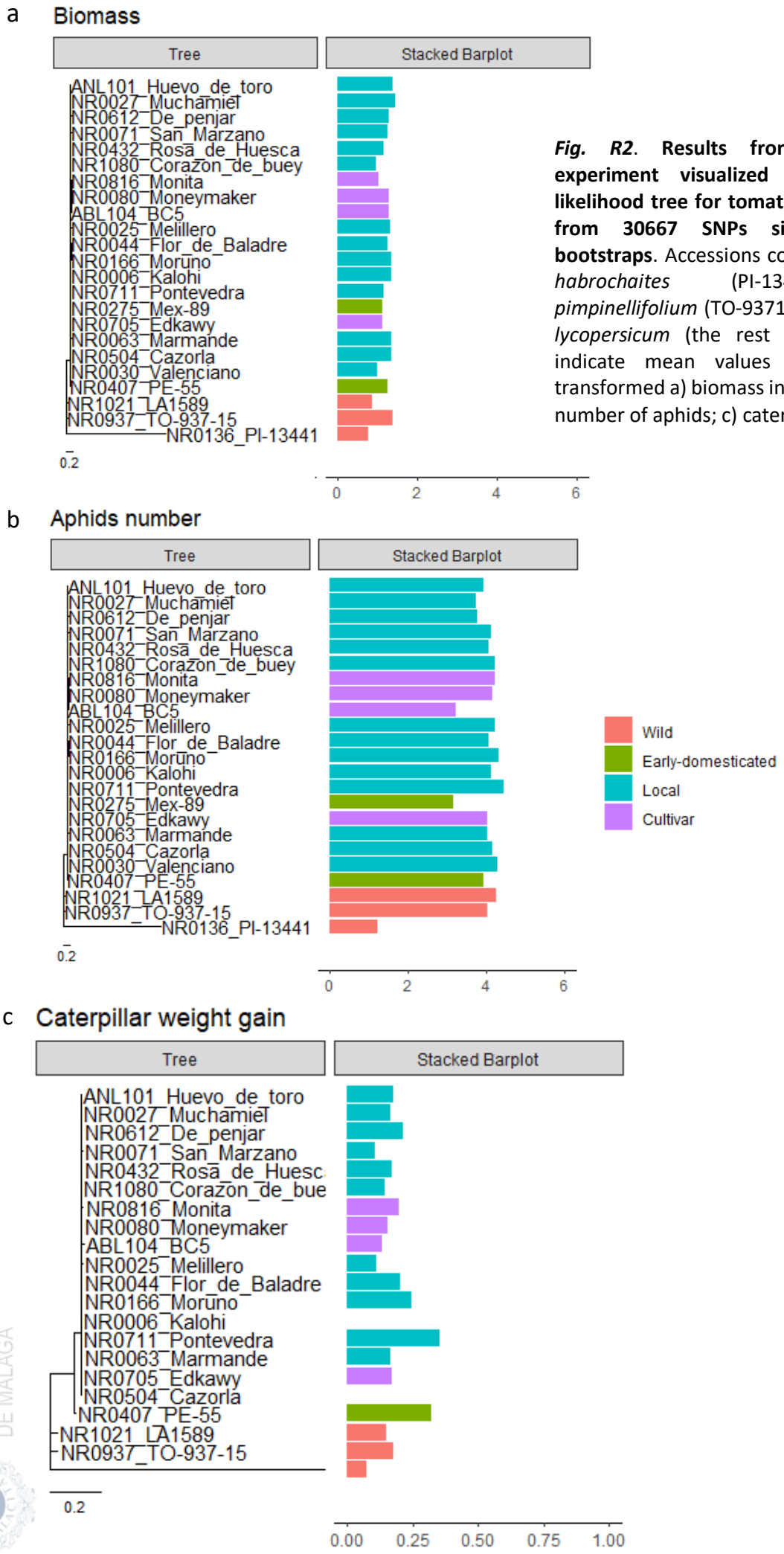


***Fig. R2*. Results from the greenhouse experiment visualized on the maximum likelihood tree for tomato accessions inferred from 30667 SNPs sites including 100 bootstraps**. Accessions correspond to *Solanum habrochaites* (PI-134418); *Solanum pimpinellifolium* (TO-93715 and LA1589) and *S. lycopersicum* (the rest of accessions). Bars indicate mean values of the logarithmic transformed a) biomass in control treatment; b) number of aphids; c) caterpillars' weight gain.

For the comparisons of tolerance among tomato types (wild, early domesticated, local landraces and commercial cultivars), it was found that aphids and caterpillars reduced plant biomass relative to the control (*Fig. R3*; *Table S2* and *Table S3*). The largest negative effect of herbivory on biomass was caused by the caterpillars with reductions in biomass of 40% and more (based on an effect size = -0.5). Furthermore, in this treatment the wild relatives and the early domesticated tomatoes experienced a smaller reduction in biomass after caterpillar herbivory than the other two types of accessions. There were no differences found in the effect of aphids according to the degree of domestication (*Fig. R3,* Supplementary *Table S2*).

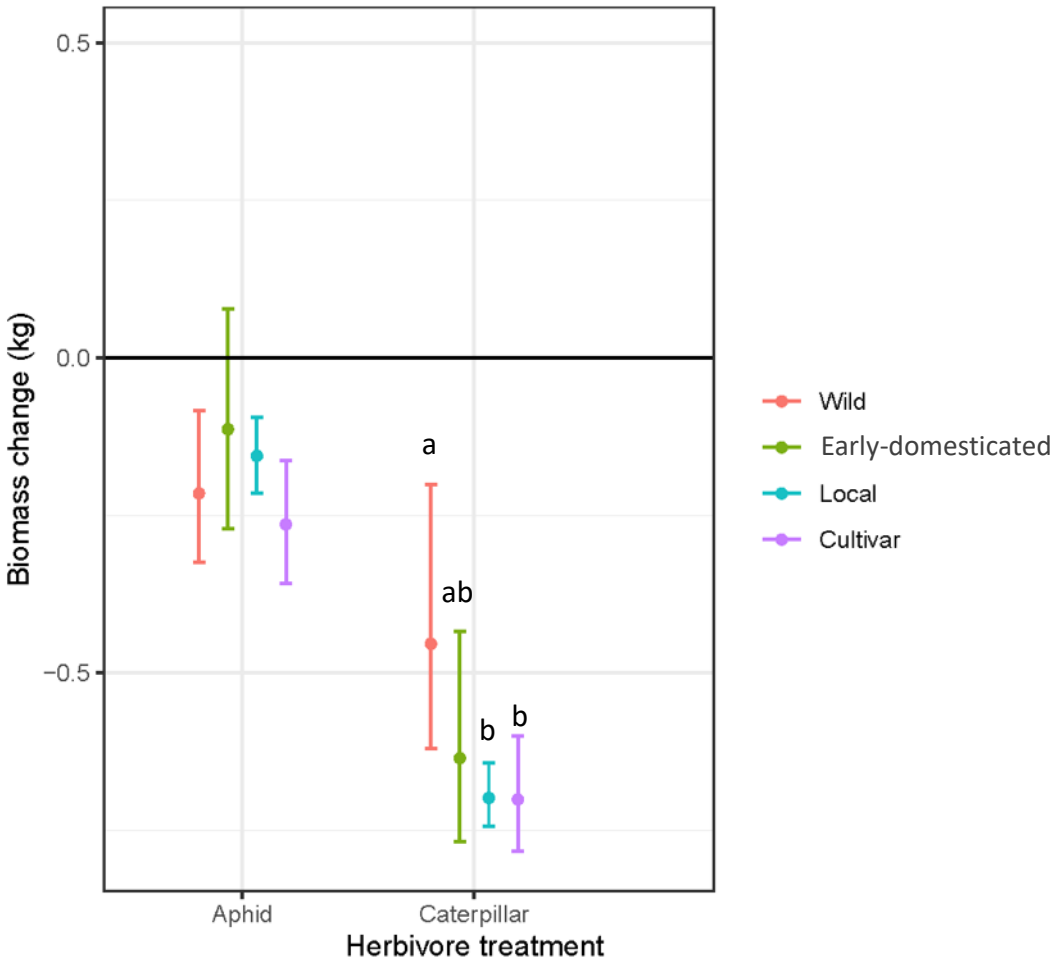


***Fig. R3.* Differences in plant biomass of herbivory treatments compared with the control.** The response is expressed as an effect size (log response ratio), so that negative values indicate the herbivory treatment decreased biomass relative to the control. Tomato accessions represent four types according to their degree of domestication: wild, early domesticated, local and cultivar. Different letters indicate significant differences between the tomato types within a treatment, based on the 95% credible intervals (indicated by the bars) around the effect sizes.

For the comparisons of resistance among type of accessions, there were differences in the number of aphids at harvest (*Fig. R4;* detailed data in *Table S3*). Aphids multiplied better on local varieties than on the other types of accessions (i.e., wild, early domesticated and cultivars). For the increase in caterpillars' weight, there were significant differences between wild and early domesticated tomatoes (greater weight gain for the early domesticated). Plant biomass was positively related to the increase in weight of the caterpillars, that is caterpillars ate more in larger plants. Results for these three models are summarised in Supplementary *Table S2*.

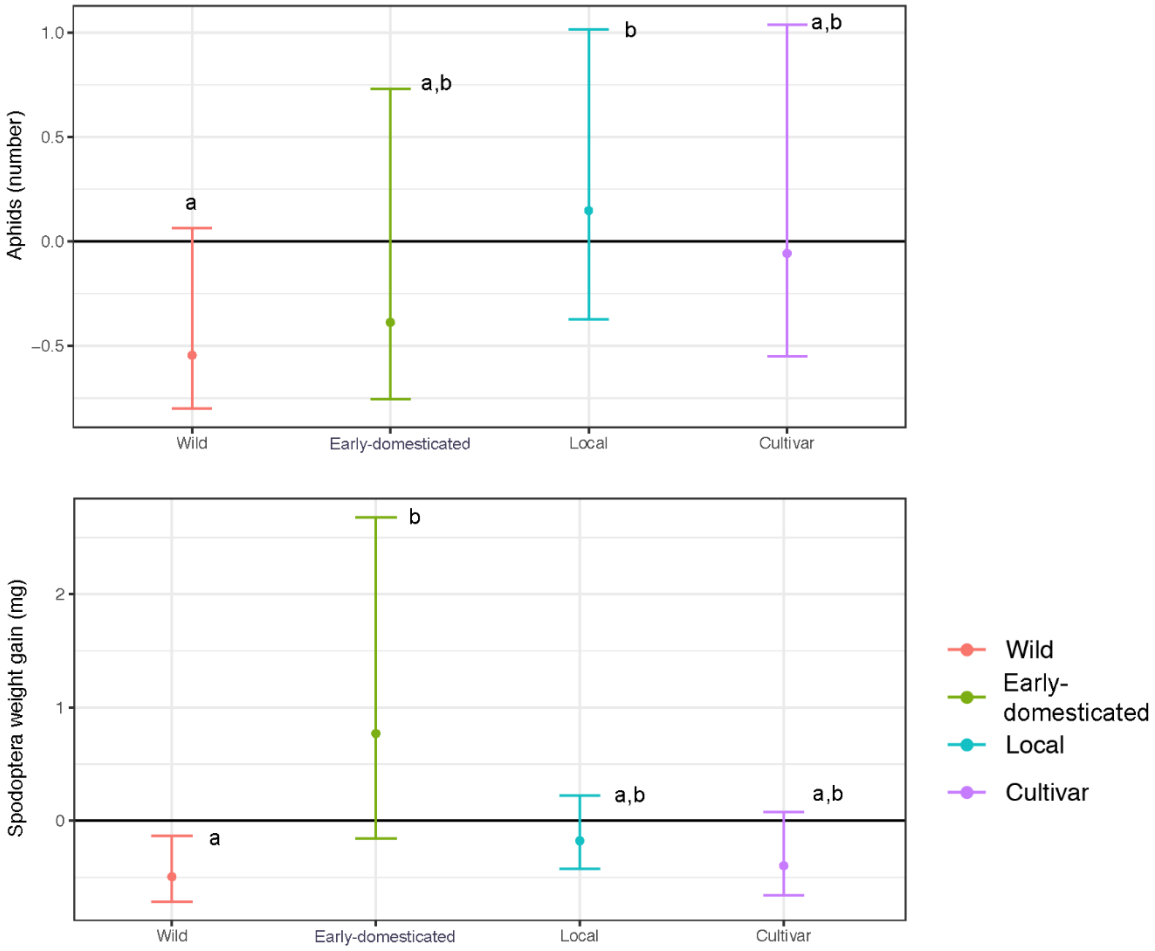


Fig. R4. **Estimates of resistance in the different tomato types**: number of aphids (Aphids (number)) and caterpillar weight gain (Spodopera weight gain (mg)). Tomato accessions represent four types according to their degree of domestication: wild, early domesticated, local and cultivar. The response variables were normalised, so that the zero line indicates average resistance and positive (negative) values higher (lower) than average resistance. Different letters indicate significant differences between types of tomato within a treatment, based on the MCMC models (Supplementary *Table S1*). Types for which the intervals do not overlap zero, show higher or lower than average resistance. Bars indicate 95% credible intervals.

When comparing tolerance among accessions, local varieties responded differently (from no effect to large decrease in biomass) in all treatments (*Fig. R5*). The largest effect of herbivores on biomass reduction was observed in the local Quicena (NR0432) in all herbivory treatments. The accession least affected by aphids was a local, Moruno (NR0166) whereas the lowest effect of caterpillars was for the wild LA1589 (NR1021). In the caterpillar treatment, no single plant of the local Valenciano (NR0030) survived the treatment and two more accessions had only one or two individuals remaining and, thus, were excluded from analyses (the wild PI134418 (NR0136) and the early domesticated Mex 89 (NR0275)). The great mortality in these accessions occurred because the caterpillars drop off the leaves and ate the stem of the plants, killing them.

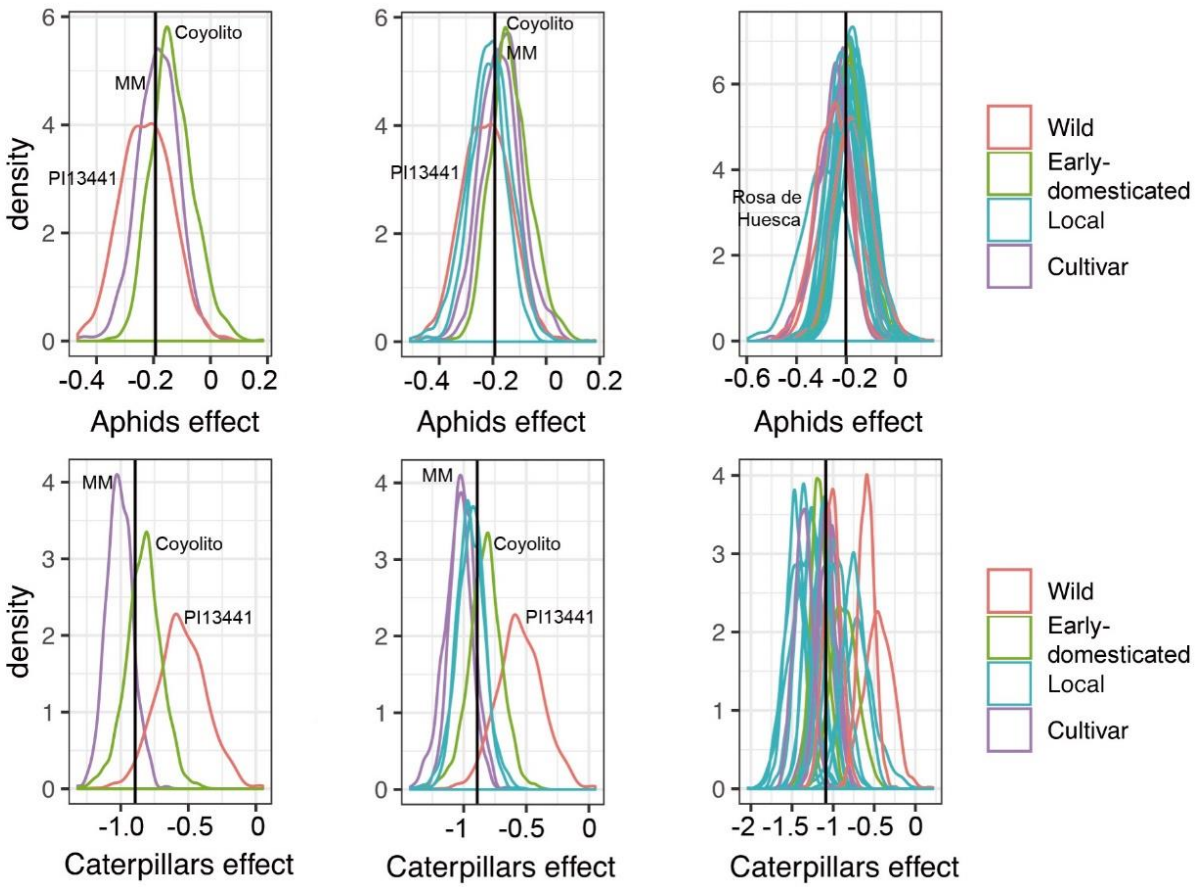


***Fig. R5*. Density plots for changes in plant biomass after herbivory treatments**. Density plots for changes in plant biomass after herbivory treatments for each tomato accession depending on their degree of domestication. Results report posterior density intervals. The vertical black line indicates the mean value of the variable for all the accessions. Herbivory treatments consisted in manual infestation with aphids and caterpillars. From left to right it is shown an increase in the number of accessions included in the analyses. In the graphs some names are displayed to show their relative position when more local accessions are added: Coyolito, Mex-89; MM, Moneymaker; Rosa de Huesca, Quicena; PI134418, *Solanum habrochaites*.

For the detailed comparison of the resistance of the accessions, similarly to tolerance, local varieties responded differently in all the resistance treatments (*Fig. R6*; and detailed data in *Table R1*). The accession with the smallest number of aphids was the wild PI134418 (NR0136) followed by the early domesticated Mex 89 (NR0275) and the cultivar ABL 10-4 (BC5). The weight gained by Spodopteras was consistently similar across the genotypes compared with only apparent differences between the local Pontevedra (NR0711) and the early domesticated tomato (PE-55).

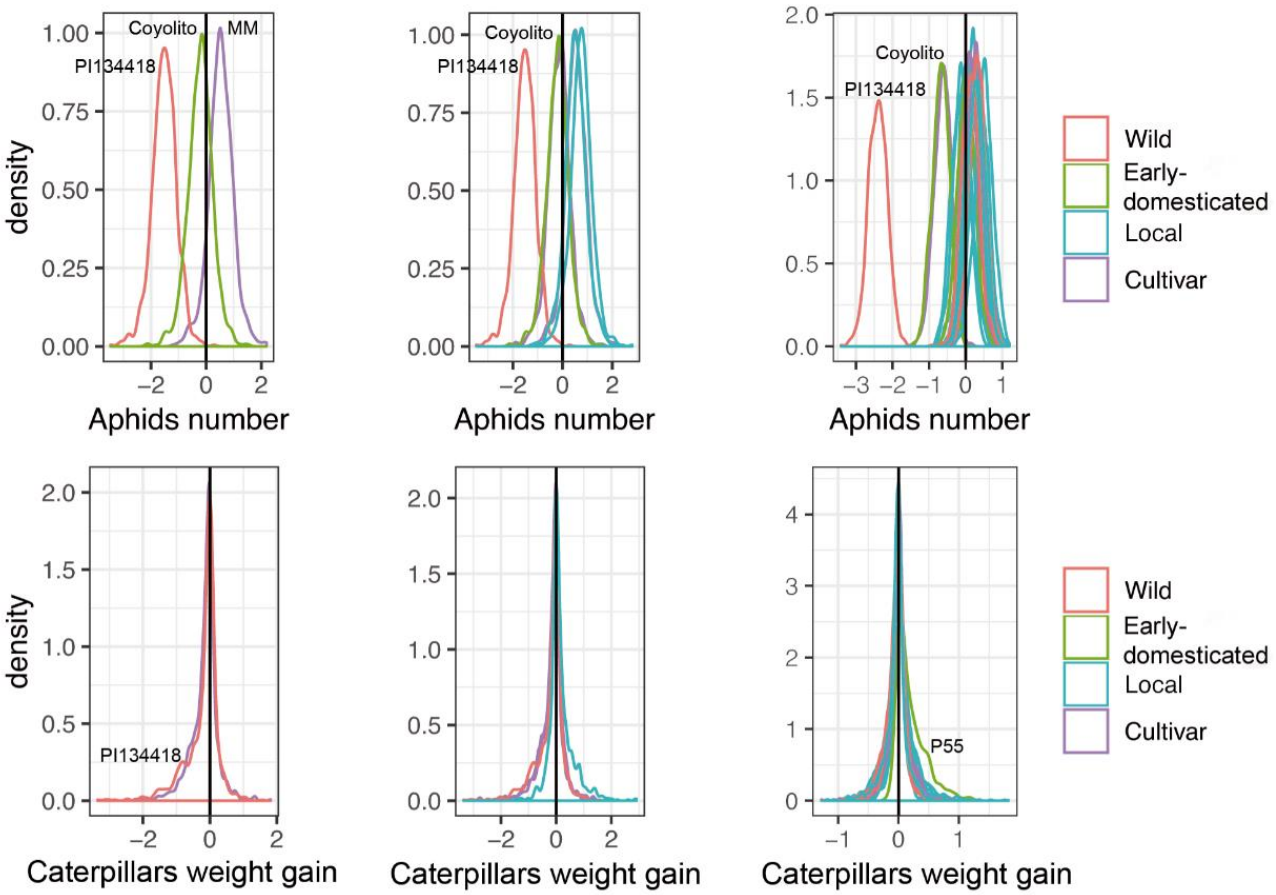


Fig. R6. **Density plots for changes herbivores' performance.** Density plots for changes in the number of aphids and Caterpillar's weight gain for each tomato accession depending on their degree of domestication after herbivory treatments. Results report posterior density intervals. The vertical black line indicates the scaled mean value of the variable for all the accessions. From left to right it is shown an increase in the number of accessions included in the analyses. In the graphs some names are displayed to show their relative position when more local accessions are added: Coyolito, Mex-89; MM, Moneymaker; PI134418, *Solanum habrochaites.*

B Role of glandular trichomes type IV on tomato resistance against *M. euphorbiae*

B.1 Performance of *M. euphorbiae* on different tomato species with and without glandular trichomes type IV

From the previous resistant assays, the number of aphids at harvest of representative lines from each specie in terms of presence and absence of glandular trichomes type IV were compared more in detail. ABL 10-4, its parental lines (MM and TO-937), the two wild tomatoes and the intermediate *S. lycopersicum* var. cerasiforme were selected to evaluate the effect of these glandular trichomes type IV in the resistance against *M. euphorbiae* (*Fig. R7*).

In the wild PI-134418, *S. habrochaites*, the mean ± standard error (SE) number of aphids per plant could not reach the ten (5.71 ± 1.82) being significantly lower than the rest of accessions. Among the wild *S. pimpinellifolium*, TO-937 with glandular trichomes type IV had less aphids per plant (68.60 ± 12.16) than LA1589 with no glandular trichomes type IV (77.93 ± 8.26). The early-domesticated *S. lycopersicum* var. cerasiforme tomato (PE-55), also without glandular trichomes type IV had a mean of 58.38 ± 10.91 aphids per plant. Less than the cultivar *S. lycopersicum* Moneymaker (74.79 ± 10.49), without glandular trichomes as well, although differences were not statistically significant. However, the introgressed line ABL 10-4 (red bar) also a cultivar *S. lycopersicum* but with glandular trichomes type IV, had less aphids, 32.86 ± 12.66 per plant, not only than the other cultivar MM (*t-statistic*: 3.29, df:19.96, *P*=0.03), but also less than the rest of genotypes except for the *habrochaites* PI134418 (glm *Poisson* distribution, LSmeans).

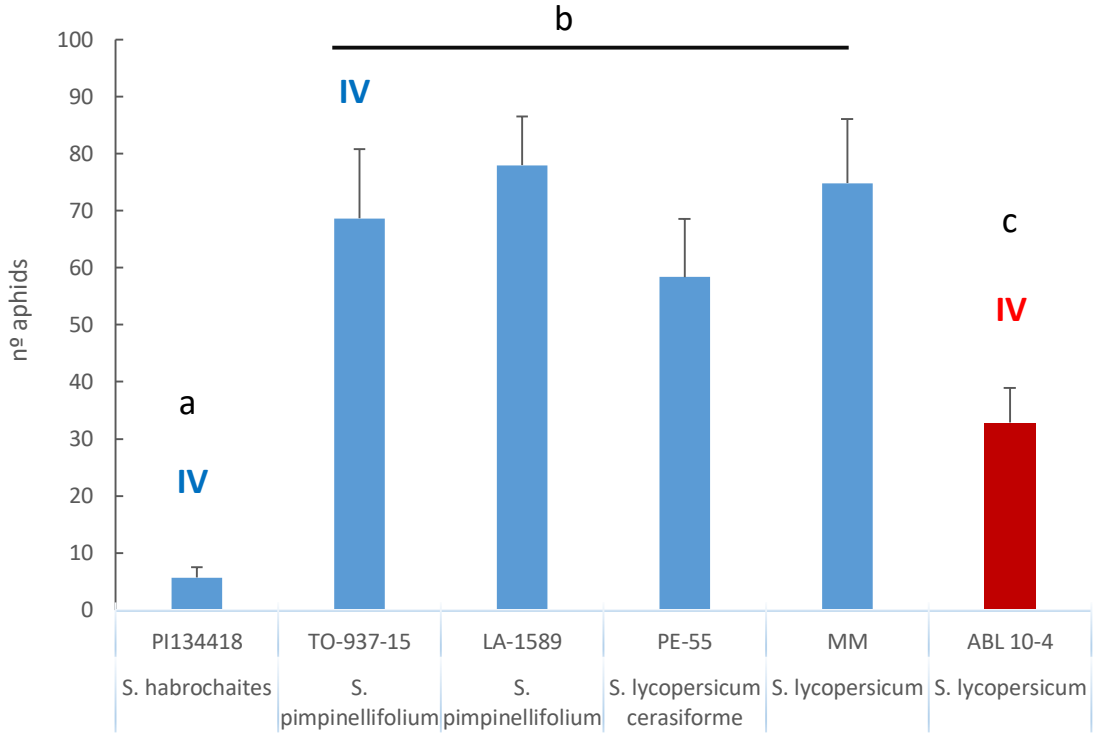


Fig. R7. **Aphid performance on different tomato accessions with and without type IV trichomes.** Comparison of the number of aphids per plant after 3 weeks of infestation on *Solanum. habrochaites* (PI-134418), *Solanum pimpinellifolium* (TO-93715 and LA1589), *Solanum lycopersicum* var. *cerasiforme* (PE-55) and *Solanum lycopersicum* (ABL 10-4 and MM). IV indicate accessions with glandular trichomes type IV. Bars represent the mean number of aphids per plant ± standard error. Different letters indicate statistical differences between genotypes calculated by least square means (LSMeans or Marginal Means) after a glm with Poisson distribution (*P<0.05*).

B.2 Production of acylsucroses in ABL 10-4 and MM

Following this idea of unravelling the importance of the type IV glandular trichomes and their secretions on the resistance of tomato plants against the aphid *M. euphorbiae*, further analysis of the plant material regarding this defence were made. Quantification of acylsucroses produced per cm^2 and measured at an absorbance of 550 nm was 14.54 times higher in ABL 10-4 leaves than in MM (*Fig. R8*). A difference statistically significant (t = 0.32, df =4.14, *P* = 0.02).

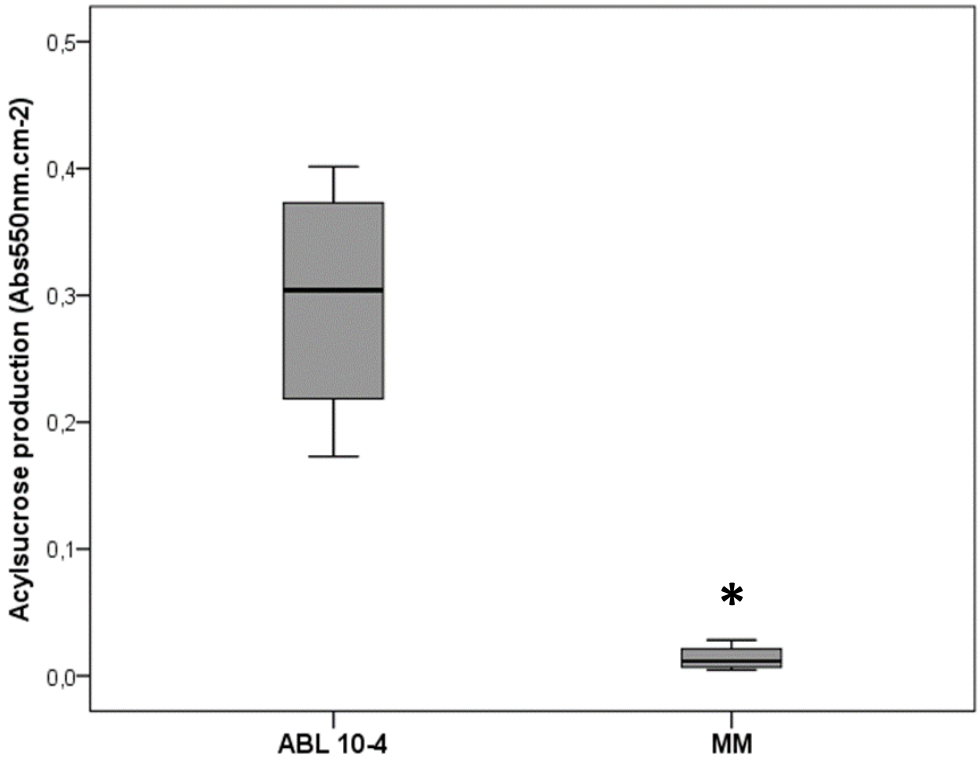


***Fig. R8*. Epicultural leaf acylsucroses of ABL and MM tomato plants**. Each box-plot represents the mean ± the maximum and mininimun values. Statistical differences between genotypes were calculated by t-test. *$P < 0.05$.

B.3 Microscopy studies

Observations made with the Scanning Electron Microscope (SEM) revealed that the morphology and disposition of trichomes on the leaf surface differed between MM and ABL 10-4 genotypes (*Fig. R9*). While in ABL 10-4 the majority of trichomes covering the abaxial face of the leaf are type IV (179 per mm2) (*Fig. R9* (A),(B),(E)), none of this trichomes were found and the type V, non-glandular, were the most representative (*Fig. R9 (C),(D),(F),(G)*). Glandular trichome type I (*Fig. R9 G*) was present in both accessions, but it was uniformly low on the abaxial and adaxial leaf surfaces. Among glandular trichomes, type II and III were ignored because they have not been associated with insect resistance.

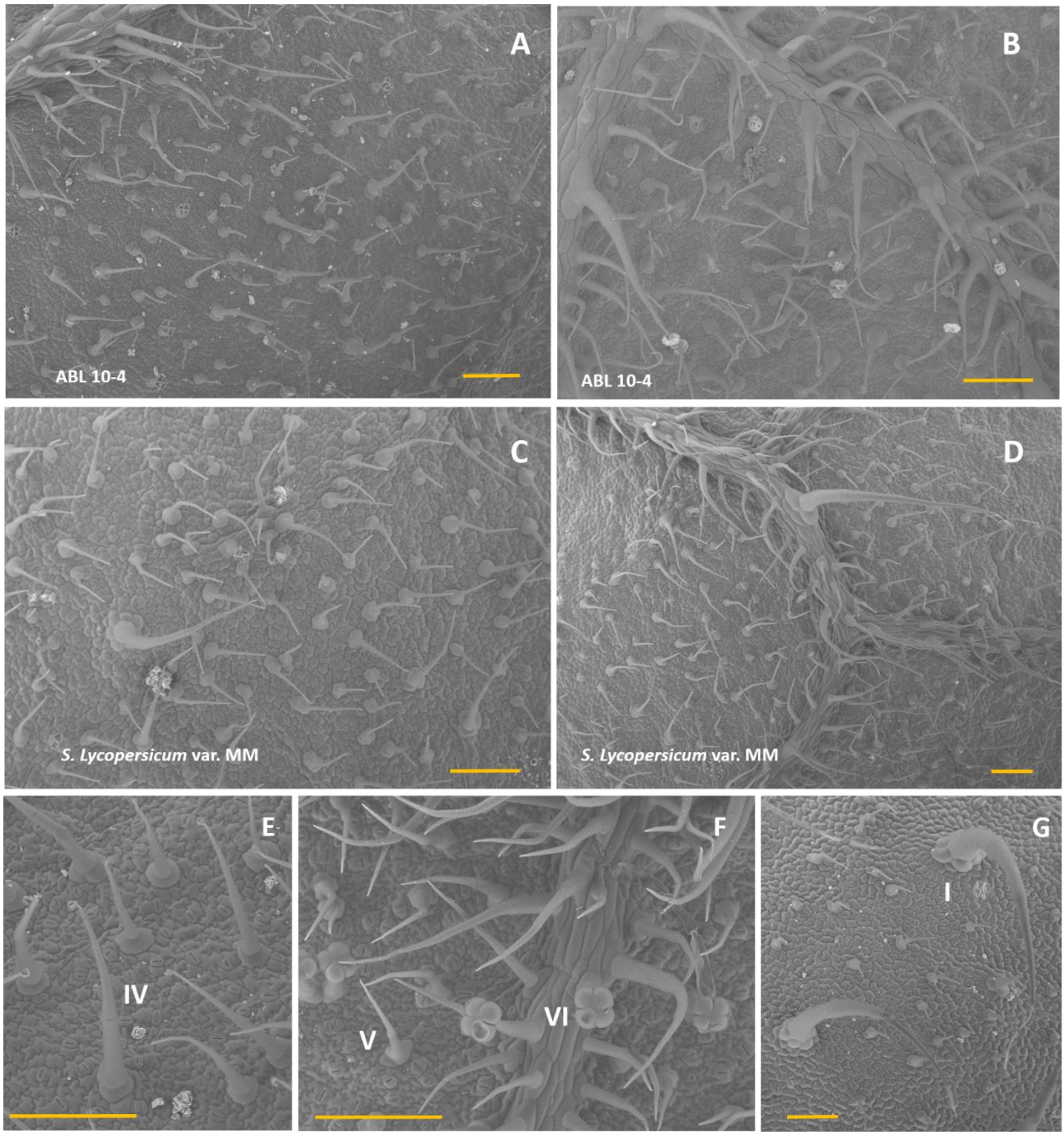


Fig. R9. **Morphological study of tomato leaves**. Scanning electron microscope (SEM) of ABL 10-4 (A, B) and MM (C, D) abaxial tomato leaf surface. Detailed SEM observation of the trichome's type and morphology on ABL 10-4 abaxial (E) and MM abaxial (F) and adaxial (G) tomato leaf surface. Scale bar: 0.2 mm.

B.4 Choice assays

Preference of aphids between ABL 10-4 and MM genotypes was evaluated through some choice and no-choice assays. Firstly, in the no-choice assay, aphids had to choose between two leaflets of the same genotype (either MM or ABL 10-4). The genotype here had a significant effect on aphid's choice. The estimated probability of aphid choice for MM was 0.69 (Credible Interval of 0.87–0.53; *Fig. R10* (A)); while for ABL 10-4 the probability of an aphid choosing any ABL 10-4 leaflet was 0.30 (Credible Interval 0.493–0.145; *Fig. R10* (A)). Testing the hypothesis of an equal probability for aphid choice in MM and ABL 10-4 revealed that this probability was equal or less than 0.05.

Under free-choice conditions, aphids could choose either MM or ABL 10-4 leaflets on the same experimental arena. In this case, most aphids (i.e., 83.64%) remained on the arena. However, for those aphids that made a choice (16.36%), the Bayesian binomial logistic regression showed a clear effect of genotype on the selection rate. Aphid choice for MM was estimated at 0.82 (Credible Interval 0.93–0.67) while for ABL 10-4, aphid choice rate was 0.47 (Credible Interval 0.69–0.26; *Fig. R10* (B)). Testing the hypothesis of an equal probability for aphid choice in MM and ABL 10-4 revealed that this probability was equal or less than 0.05.

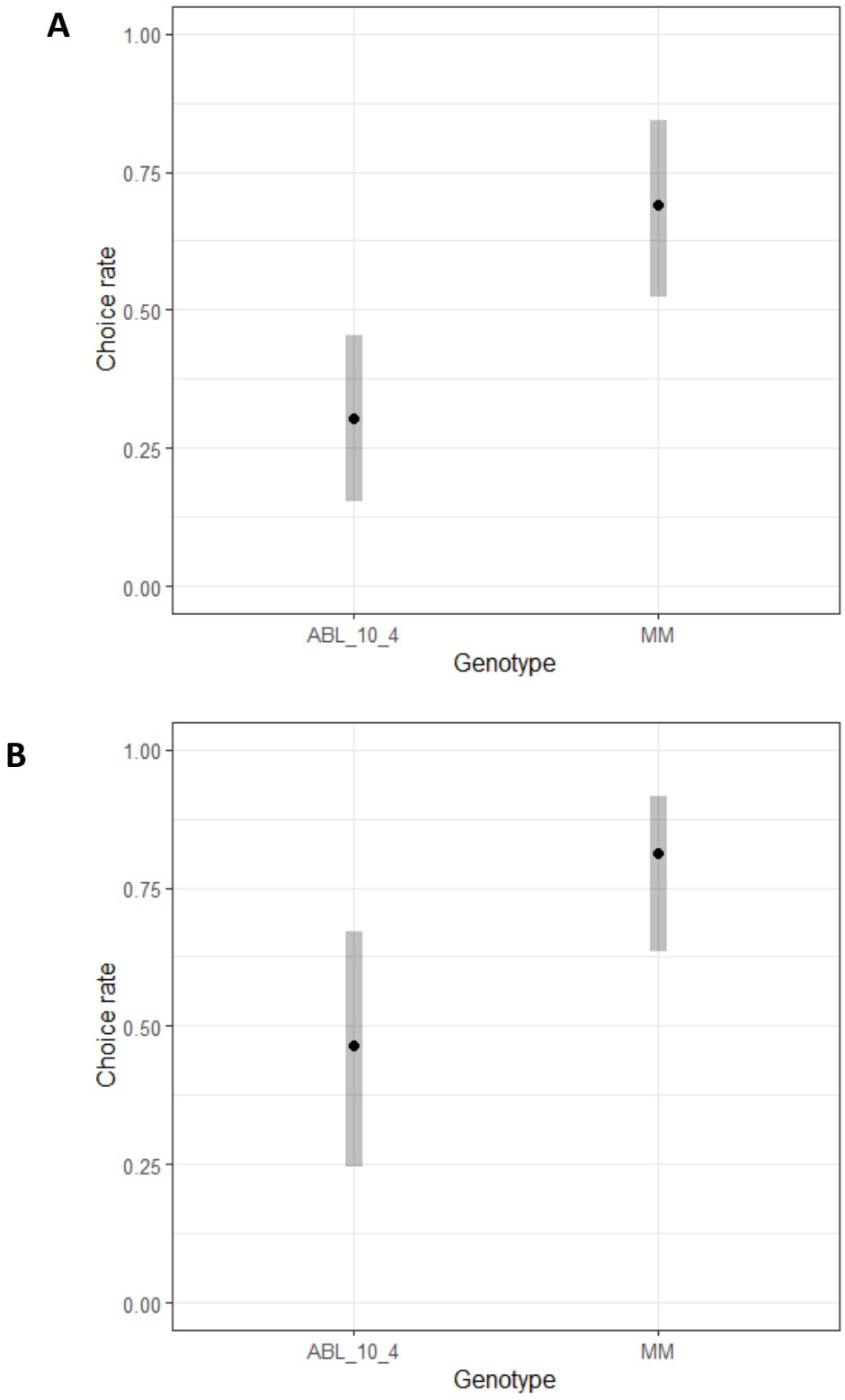


Fig. R10. ***M. euohorbiae*'s choice and no choice assay.** Probability of aphid choice for ABL 10-4 or MM leaflets in no-choice (A) and choice assays (B). The black dot represents the estimated point value (median), a gray band represents the highest posterior density (HPD) interval (HPD Credible interval probability = 0.95, i.e., probability to hold the parameter of interest i.e., estimated aphid choice).

B.5 Population growth assay

To compare the performance of the aphid between ABL 10-4 and MM plants, the population growth was monitored for 5 weeks (*Fig. R11***¡Error! No se encuentra el origen de la referencia.**). Genotype had a strong effect on aphids' population growth ($F_{1,360}$= 65.30, $P \leq 0.0001$) and this effect was already noticeable seven days after aphid set-up on experimental plants ($F_{1,360}$=120.62, $P \leq 0.0001$). In ABL 10-4 plants (with glandular trichomes type IV), aphid numbers remained nearly constant, and it did not exceed an average of 34 ± 9 (mean ± SE) aphids per plant after 5 weeks. However, in MM, after 14 days, population growth experienced an exponential increase (R^2 =0.96) that led to an average of 382 (±63) aphids per plant after 5 weeks. Significant differences were found in the number of aphids between MM and ABL 10-4 populations from the 7th day onwards (*t.ratio*= -2.178; P = 0.0321 for the 7th day; LSmeans contrasts for the rest of days not shown).

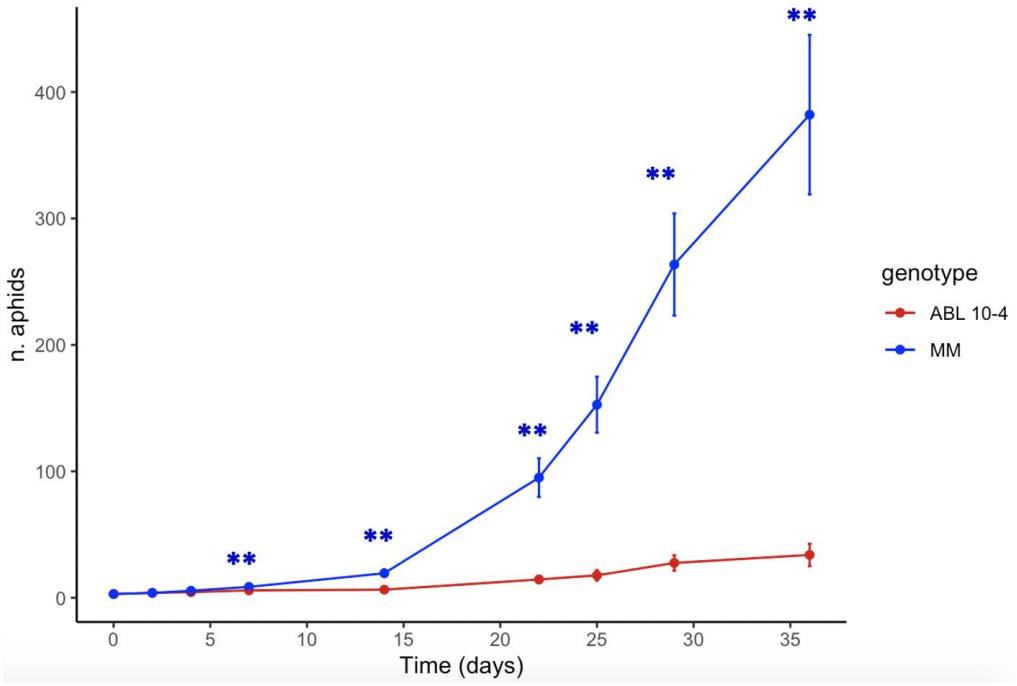


***Fig. R11. M. euphorbiae* dynamics.** Population growth of *Macrosiphum. euphorbiae* on *Solanum lycopersicum* cv. Moneymaker (MM) and ABL 10-4 genotypes. Each point represents mean values of aphids' number ± standard error. Statistical differences between genotypes were calculated by least square means (LSMeans or Marginal Means) significant differences at each counted interval are indicated with a double asterisk **($P \leq 0.01$).

C Underlying pathways in the tomato defence mechanism against *M. euphorbiae*

On the other side, two experiments were conducted to unravel the hormonal signalling pathway that drives the induced defence mechanism of tomato plants against *M. euphorbiae*. Firstly, it was evaluated the effect of a priming stimulus over the following defence response against *M. euphorbiae*. Secondly, to further test and corroborate the role of the pathway involved on the defence response, a bioassay with mutant and transgenic plants impaired for the main signalling pathways was carried out.

C.1 Priming effect on tomato resistance against *M. euphorbiae*

Priming assays revealed that both, the presence of glandular trichomes type IV and a previous herbivore attack, have an effect on the aphid's population growth after 20 days of infestation. The mean number of aphids was significantly lower in ABL 10-4 in comparison with MM ($Z\text{-ratio}_{ABL10\text{-}4\ vs.\ MM}$= -26.04, df= 1, $P \leq 0.0001$, *Fig. R12*). While in ABL-10-4 the average number of aphids after 20 days was 14.4 ± 3.4 (mean ± standard error), in MM aphid number reached 61.5 ± 14.3. The assay revealed that for both genotypes, a previous herbivore attack had a strong effect on aphid multiplication: prior caterpillar feeding resulted in the lowest aphid multiplication with an average of 7.95 ± 2.1 aphids on ABL-10-4 and 47.68 ± 11.3 on MM. In contrast, previous aphid feeding resulted for both genotypes in the highest aphid multiplications; with an average of 20.6 ± 5 in ABL10-4 and 76.2 ± 18 in MM. Control plants, those that did not have any pre-treatment, showed intermediate values in both cases, i.e., with an average of 18.4 ± 4.5 aphids per plant in ABL10-4 and 64 ± 15.1 in MM. The interaction term showed that the effect of treatment differed between the two compared genotypes; while there were no significant differences between the pretreatment with aphids and control plants for ABL10-4 ($Z\text{-ratio}_{\ aphids\ vs.\ control}$ = -1.19, df= 1, p = 0.38), for MM there were clear differences ($Z\text{-ratio}_{\ aphids\ vs.\ control}$ = -3.55, df= 1, $P \leq 0.0008$, *Fig. R12*).

The analysis of expression of the *proteinase inhibitor II* gene (*PI-II*) indicated that although there were significant differences between the compared genotypes (Parametric F-value= 12.37, df=1, P = 0.0054) with average expression values slightly higher for MM of 1.91 ± 0.06 than for ABL10-4; with and average values of 1.58 ± 0.07.

However, and more importantly, the treatment response was consistent for both genotypes, as the interaction term was not significant (Parametric F-value= 2.44, df= 3, *P*= 0.11). In other words, the proteinase inhibitor was only expressed after 48h when plants were either treated with MeJA or with caterpillars, showing no statistical differences between these treatments and with a similar response in both genotypes (Parametric F-value=566.88, df=3, p=0.0002, *Fig. R13*). In contrast, there was no expression of *PIN-II* when plants were treated with aphids or in the control plants, i.e., those that had received a mock treatment (*Fig. R13*).

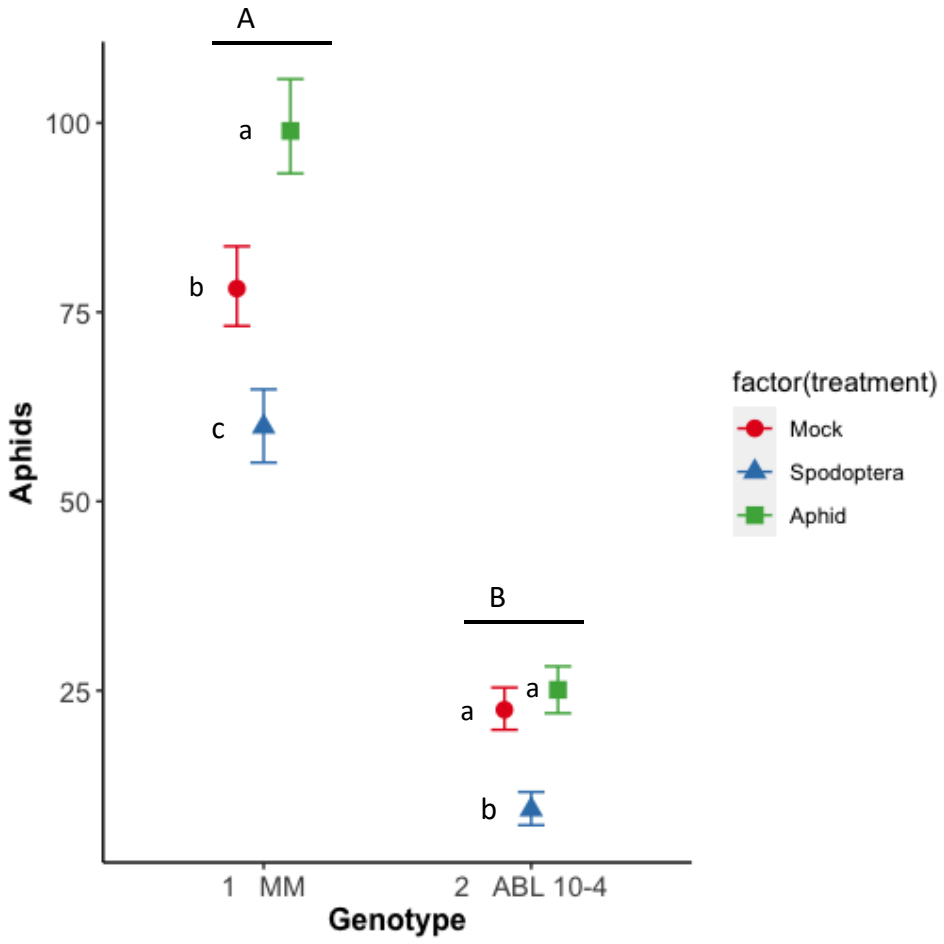


***Fig. R12. M. euphorbiae* population dynamics after priming.** Aphids (mean ± standard error) after 20 days of infestation in plants of *Solanum lycopersicum* cv. Moneymaker (MM, left) and ABL 10-4 (right), previously primed with caterpillars of *Spodoptera littoralis* (Spodoptera) or *M. euphorbiae* (Aphids) or with no previous infestation (Control). Capital letters indicate significant differences between the two genotypes, letters in lowercase indicate differences among treatments within a genotype according to a Generalized Linear Mixed Model $P \leq 0.001$, n=10.

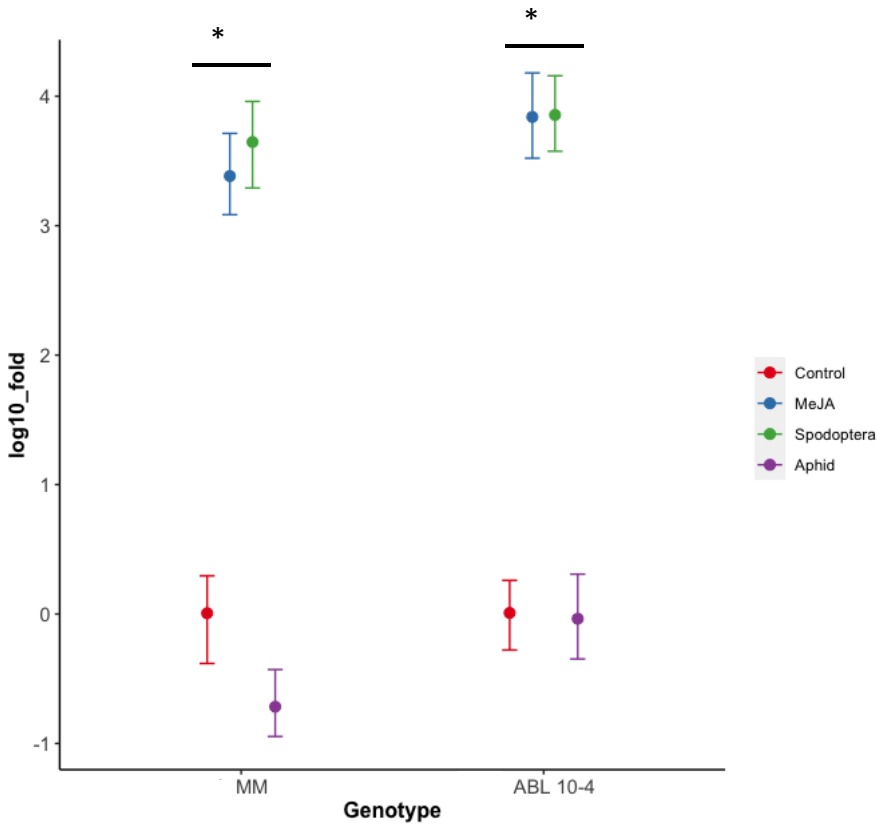


***Fig. R13*. Relative expression of *PI-II* 48h after the priming stimulus.** Relative expression of the Jasmonate-responsive defence gene Proteinase inhibitor II (*PI-II*), i.e., estimated median for the $\log_{10}$ of the *PI-III*'s fold change (mean ± standard error) in *Solanum lycopersicum* cv. Moneymaker (MM, left) and ABL 10-4 (right) 48h after being infested with the caterpillar *Spodoptera littoralis* (Spodoptera), the aphid *M. euphorbiae* (Aphid)), sprayed with a Methil-Jasmonate (MeJA) or a mock solution as Control. Asterisks indicate significant differences of MeJA and Spodoptera treatments respect to Contol and Aphid treatments, according to a 2-way permutational ANOVA, $P \leq 0.001$, n=10.

C.2 Performance of *M. euphorbiae* on hormone impaired genotypes

The number of aphids differed significantly between the two compared genotypes (*spr2* and CM) (*Fig. R14*). The *spr2* genotypes impaired in the JA hormonal pathway showed an enhanced susceptibility to the aphid in comparison to the wild type (CM) (*Fig. R14*). After 20 days, the maximum multiplication of aphids was significantly higher on *spr2* compared to CM; while on *spr*2 we found 194.1 ± 22 (mean ± standard error) aphids per plant; on CM, values averaged 116 ± 22.9 per plant ($Z\text{-ratio}_{CM \text{ vs. } spr\text{-}2} = -11.78$, df= 1, $P \leq 0.0001$, *Fig. R14*).

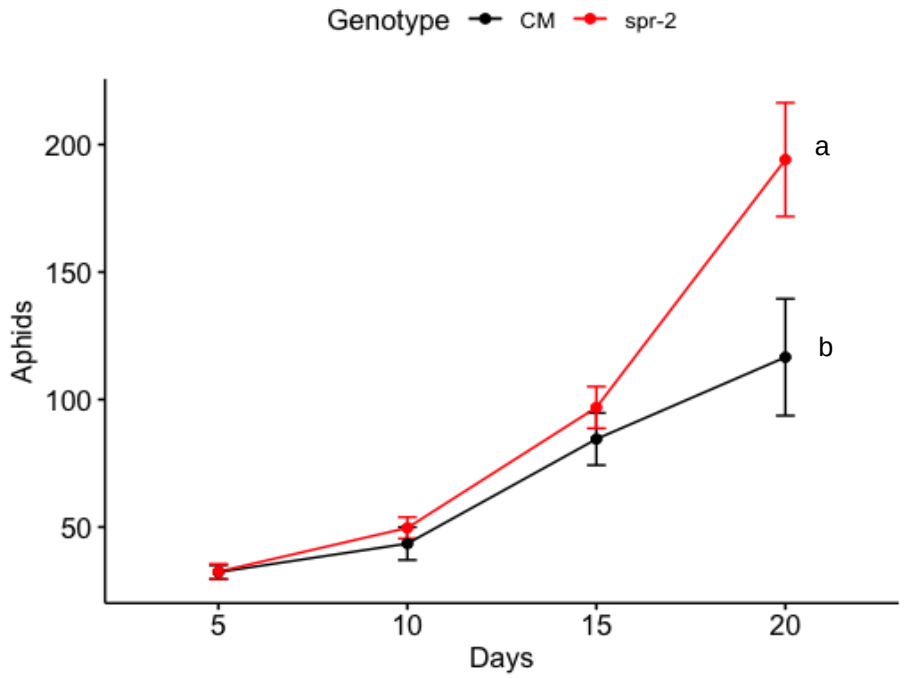


***Fig. R14*. Comparison on *Macrosiphum euphorbiae* dynamics on JA-impaired genotype vs. wild-type.** Population built-up (mean ± standard error) of *M. euphorbiae* on Solanum lycopersicum cv. Castlemart (CM, black line) and spr2 (red line). Aphid multiplication was monitored for 20 days. Different letters indicate significant differences among the compared genotyopes in aphid multiplication according to a Generalized Mixed Linear Model with a *Poisson* error structure, $P \leq 0.001$.

The comparison of aphid multiplication on MM and *NahG* revealed that aphid performance was significantly different between the compared genotypes ($Z\text{-ratio}_{MM\ vs.\ NahG} = -3.76$, df= 1, $P \leq 0.0002$, *Fig. R15*). The number of aphids on *NahG* plants was considerably lower than in wild-type MM. The number of aphids after 20 days of infestation in MM plants was 90.8 ± 15.15 while in *NahG* plants was 62± 8.5 (*Fig. R15*).

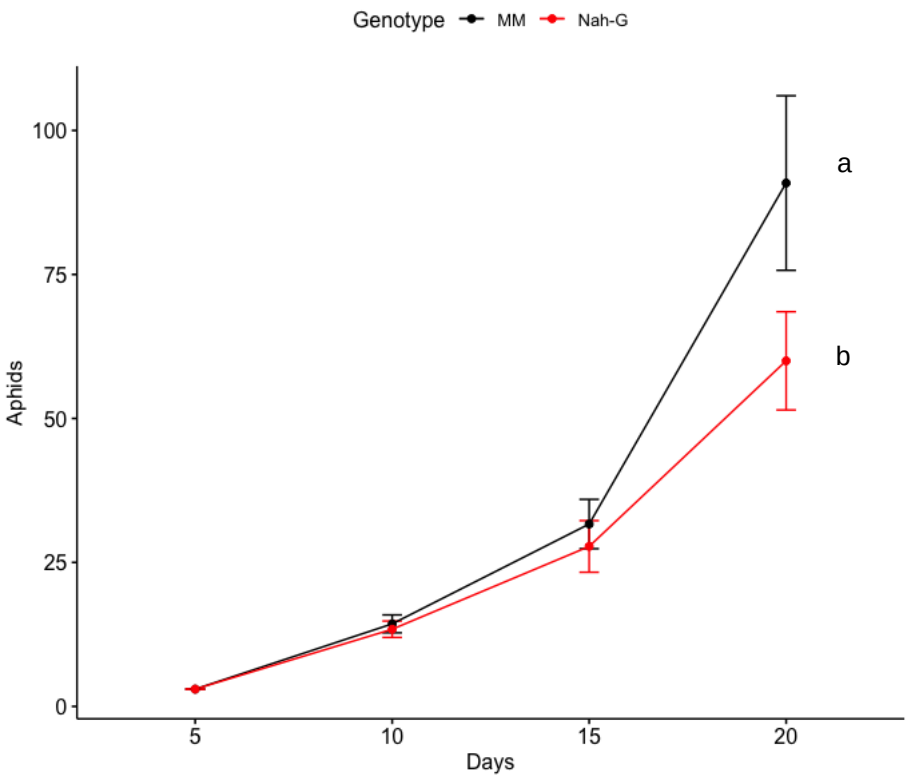


Fig. R15. **Comparison on *M. euphorbiae* dynamics on SA-impaired genotype vs. wild type.** Population built-up (mean ± standard error) of *M. euphorbiae* on *Solanum lycopersicum* cv. Monemymaker (MM, black line) and *NahG* (red line). Different letters indicate significant differences in aphid multiplication according to a Generalized Mixed Linear Model with a *Poisson* error structure, $P \leq 0.001$, n=10.

D Physiological mechanisms of the aphid to cope with tomatoes' glandular trichomes type IV

The last part of the study was focused on the physiological changes of *M. euphorbiae* in response to glandular trichomes type IV tomato defences and their secretions. To this extent, the transcriptome of the aphid *M. euphorbiae* was produced for the first time. And moreover, comparisons of the whole transcriptome gene expression of the aphid grown on two the two isogenic lines that only differ in the presence of type IV glandular trichomes and acylsucrose production, were made.

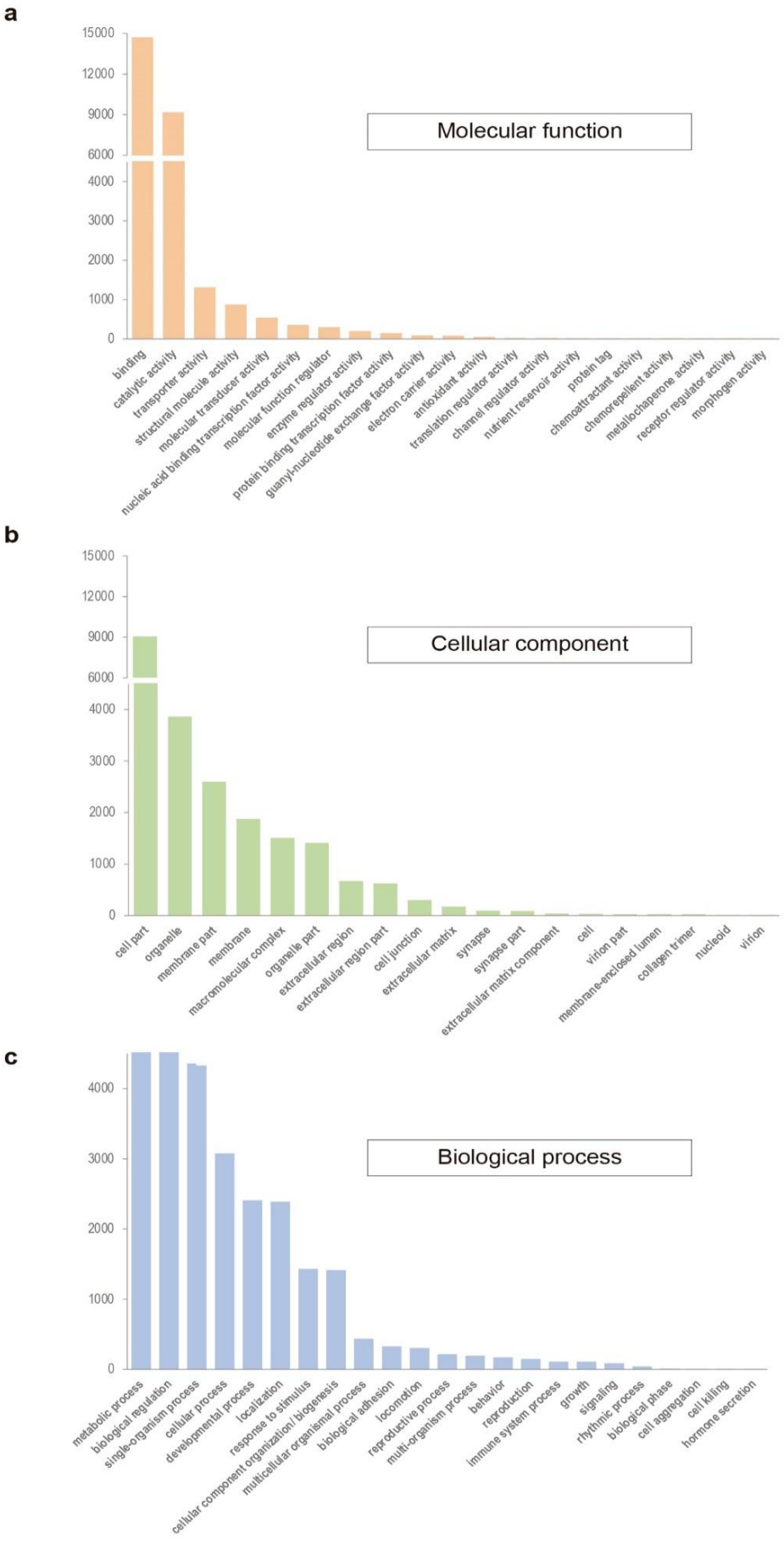
D.1 *De novo* reference transcriptome of *M. euphorbiae*: sequencing, assembly and analysis of RNA seq data

An extensive transcriptome of *M. euphorbiae* was assembled by pooling the data from the 12 libraries coming from four different populations (adapted to different host plants as detailed in *Table M3,* Material and methods), built from the whole body-RNA of apterous and viviparous adult females. After trimming, a total of 698,5 million reads were used to construct the reference transcriptome of *M. euphorbiae* and reads per sample ranged from 21 to 58 million. All reads are deposited in the European Nucleotide Archive (ENA) project number PRJEB35133.

After removing low-quality adaptors and reads, high-quality reads were assembled into 240,067 transcripts and 189,229 unigenes, with N50 lengths of 771 and 635 nucleotides, respectively (Supplementary, *Table S4*) and with 29128 contigs longer than 1,000 bp. BUSCO (*Seppey et al*., 2019) showed 96.8 % completeness and high duplication (37.4% complete and single, 59.4% complete and duplicated). Fragmentation was found in 2.2% of genes and 1% were missing. The number of contigs successfully annotated was 87,781 out of 240,067, although the number of unique hits against different ptotein sequence database was 32,601.

Among them, the categorisation by Gene Ontology (GO) identified 63 GO Terms within three categories: molecular function (21), cellular component (19), and biological process (23). In addition, GO categorisation shows the predominance of binding and catalytic activity for ‘Molecular function’, cell parts (including organelle and membrane) for ‘Cellular component’ and binding and catalytic activity as the most enriched terms for ‘Biological process’ (*Fig. R16*).

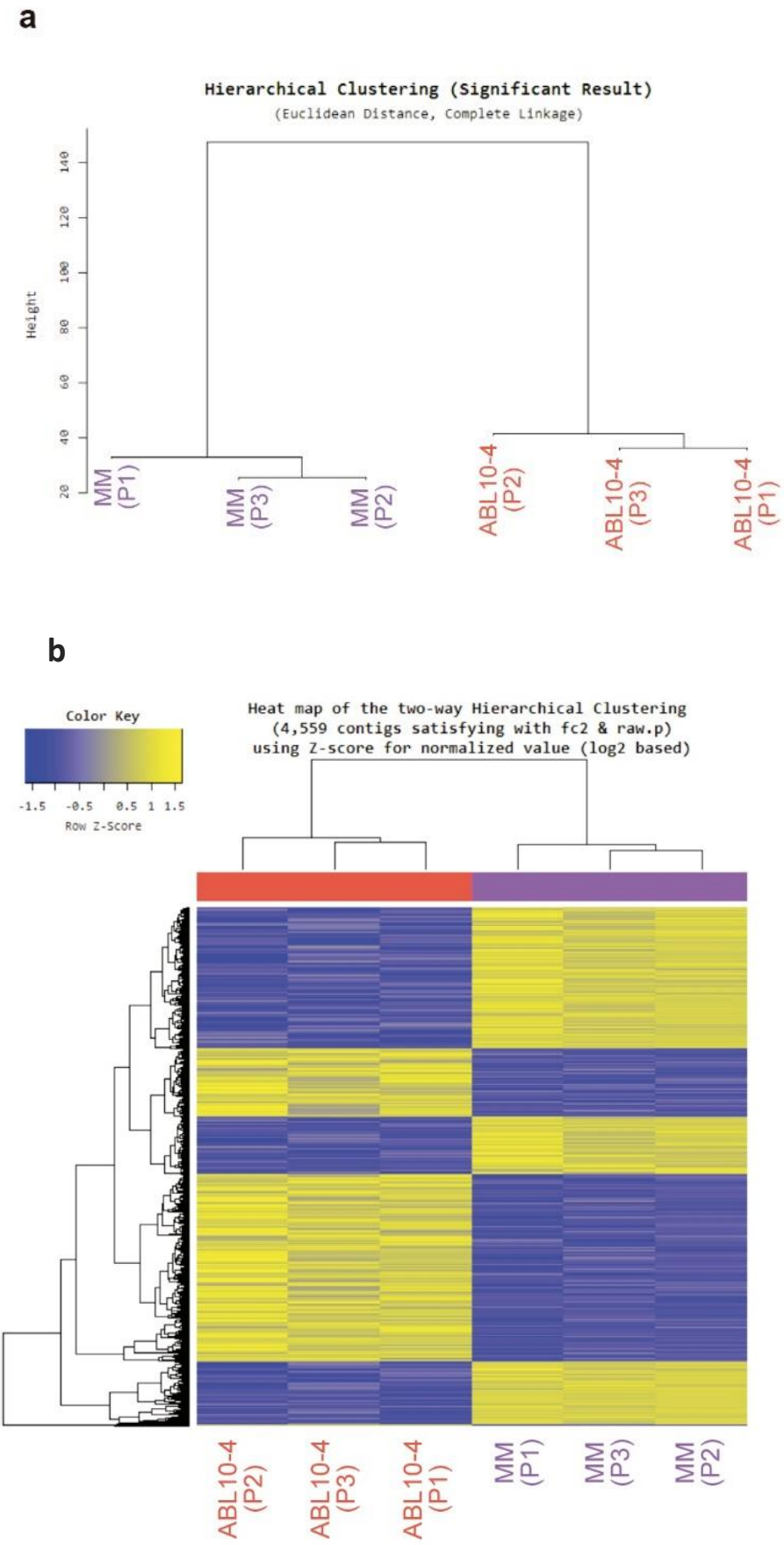
Fig. R16. **Gene Ontology (GO) classification of transcripts of *M. euphorbiae***. Bar chart describes the distribution of *M. euphorbiae* transcripts into GO categories. Transcripts were annotated in three domains: (a) molecular function, (b) cellular component, and (c) biological process. The y-axis indicates the number of sequences in each category.



D.2 Differential gene expression analyses

To explore the potential effect of glandular trichomes type IV in *M. euphorbiae*, data from six DNA libraries of MM (N=3) and ABL10-4 (N=3) samples to perform an analysis of the differentially expressed genes (DEGs) was used (*Fig. R17*). Out of the 240,067 contigs, 23,181 passed the filter following our criteria for FPKM 0 values. Those were, therefore, the basis for DEG analyses. In total, 4,559 contigs were differentially expressed following our criteria FC ≥ 2 and $P < 0.05$) in the aphids reared on ABL10-4 (with type IV glandular trichomes) compared with aphids on MM (without type IV glandular trichomes). Out of the 4,559 contigs, 2,711 showed a blast hit, but only 1,817 were unique. Paired comparisons between ABL10-4 and MM highlighted the up-regulation of 658 unigenes and down-regulation of 1,159 unigenes. To illustrate functional differences on aphids reared on MM and ABL10-4 plants, GO enrichment analysis of gene clusters of DEGs were characterised to explore relevant biological functions (Rivals *et al*., 2007). The significantly enriched GO terms ($P < 0.05$; FDR 5%) were identified both globally and separately for up-regulated and down-regulated genes in aphids reared in ABL10-4 comparing to those reared in MM (*Fig. R18* and *Fig. R19,* respectively). Considering the hierarchy structures of GO systems, we used the ReviGO tool to collectively visualise the enriched GO terms for each DEG cluster (Supplementary *Fig. S1* and *Fig. S2;* Supek *et al*., 2011).

Fig. R17. **(A) Dendrogram graph showing global relationships of samples**. Six aphid RNA samples were used in this study (three maintained on MM plants and three in ABL10-4 plants). **(B) Hierarchical clustering graph of DEGs found between ABL10-4–MM samples**. Heatmap shows the significant differentially expressed *Macrosiphum euphorbiae* genes when are exposed to Moneymaker and ABL10-4 tomato plants. Each row represents a gene and column represents different aphid samples (3 per condition).



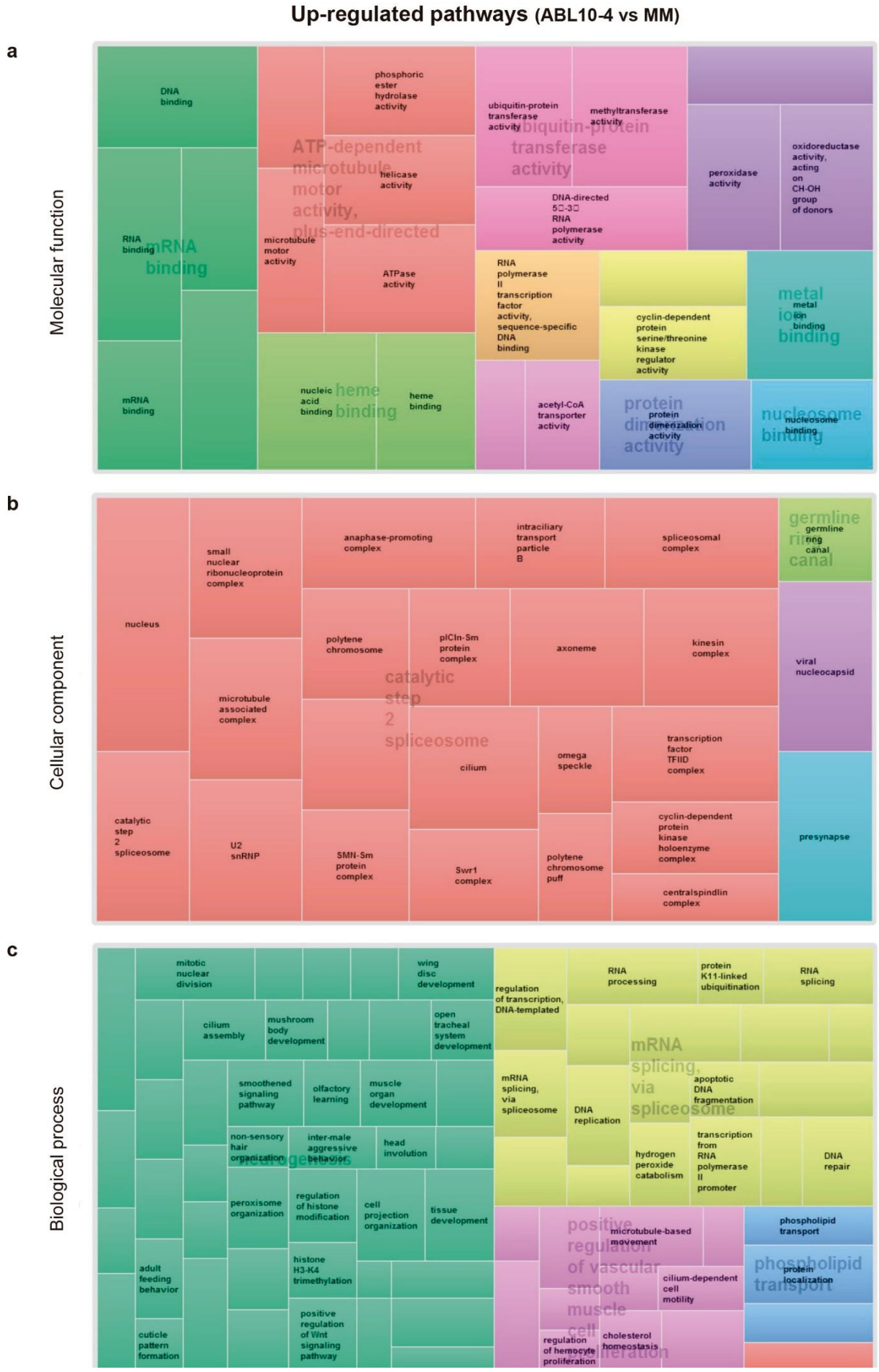


Fig. R18. **Up-regulated pathways in aphids reared on ABL10-4 comparing to MM.** Illustration of superclusters of overrepresented GO-terms visualised in semantic similarity-based treemap views from REVIGO program, for (a) molecular functions (b) cellular component and (c) biological process. Rectangles in the treemaps are size-adjusted to reflect the corrected *P-value* (i.e. larger rectangles represent the most significant GO-terms). Each rectangle in the treemap view has a single cluster for representation. These representatives are further joined together to build superclusters that are related terms and displayed in different colours.

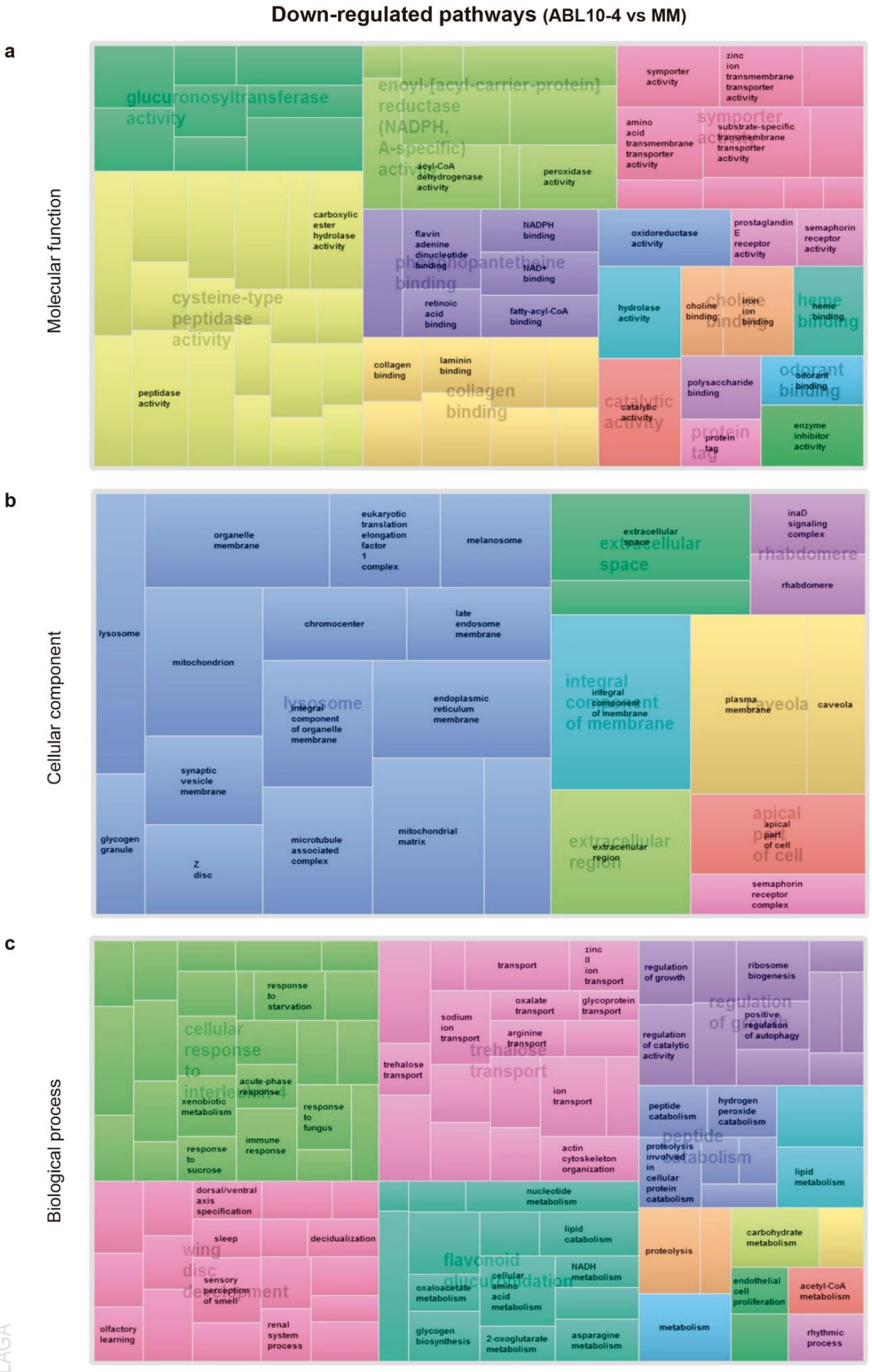


Fig. R19. **Down-regulated pathways in aphids reared on ABL10-4 comparing to MM.** Illustration of superclusters of overrepresented GO-terms visualised in semantic similarity-based treemap views from REVIGO program, for (a) molecular functions (b) cellular component and (c) biological process. Rectangles in the treemaps are size-adjusted to reflect the corrected *P-value* (i.e., larger rectangles represent the most significant GO-terms). Each rectangle in the treemap view has a single cluster for representation. These representatives are further joined together to build superclusters that are related terms and displayed in different colours.

Among the 1,159 genes that showed repression, the most enriched categories were those involved in the immune system, the xenobiotic metabolism and oxidative stress responses (e.g., *Cytochromes P450 family*, *peroxidase*), glycogen and fatty acid biosynthesis (e.g., *Glycogenin*, *desaturase 1*), carbohydrate and lipid metabolism (e.g., *Fatty acid synthase 1*, *phospholipase A2*), regulation of growth and rhythmic process (*Clock*, *vrille*). In addition, genes involved in transmembrane transport also showed a negative regulation on aphids reared on the ABL10-4 genotype (e.g., *ABC transporter B*, *D* and *G family members* and UDP glycosyltransferase *Dorothy*, among others).

Among the induced genes in DEG, it is worth noting those related to epidermal formation, such as larval cuticle protein and ecdysteroid biosynthesis (*shade* and *disembodied*, among others). Genes related to adult feeding behaviour, DNA repair and wing development were also up-regulated in ABL10-4 aphids.

D.3 Identification and molecular characterization of biomarkers in *M. euphorbiae*

Then, it was made a systemic search of the biomarkers in the transcriptome that could be useful to detect the main physiological changes on the insect. A total of 14 genes from *M. euphorbiae* involved in critical pathways for the survival of the aphid were *de novo* identify and characterized. The search rendered different sequences with open reading frames (ORFs) for their corresponding proteins. Those sequences were identified, analysed and deposited in the National Centre for Biotechnology Information (NCBI) database, GenBank. A table summary of all de novo characterised genes with their corresponding ORF and protein length as well as their database accession number is shown in the *Table R1.*

***Table R1*. Summary of the de novo characterised *Macrosiphum euphorbiae'* genes.** Involved pathway, gene name, ORF lengths of the de novo characterised *M. euphorbiae* genes as well as their corresponding database accession number.

Pathway	Gene	Accession number	ORF length (bp)
Cell stress	*Hsp70*	MT157251	640
Ecdysone	*EcR*	MT157253	539
	FTZ-F1	MT157250	712
	E74	MT157254	286
	HR38	MT157256	474
	HR4	MT157255	510
Energy metabolism	*GAPDH*	MT157252	332
Detoxification & oxidative stress	*cyp4g15*	MT105339	1701
	cyp6a13-like	MT105340	1122
	cyp6k1-like	MT105341	299
	cyp4c1	MT105342	630
	Cu-Zn SOD-like	MT105343	465
	GST	MT105344	629
	GPx	MT105345	454

D.4 Characterisation of gene sequences encoding stress-response, ecdysone, detoxification and biotransformation-related genes

Genes related to the principal pathways involved in the survival of the aphid, were identified: (i) ecdysone synthesis and response pathway: *phm, ERR, EcR, E74, ftz-f1, HR38, HR4*, (ii) cell stress response: *hsp70, hsp17, hsp10*, (iii) energy metabolism: *GAPDH*, (iv) detoxification and oxidative stress: *cyp4g15, cyp6a13-like, cyp6k1-like, cyp4c1, Cu-Zn SOD-like, GST and GPx*.

A systematic search in the de novo transcriptome of *M. euphorbiae*. rendered sequences with open reading frames (ORFs) for some proteins. Six sequences with complete ORF (*EcR*, *FTZ-F1*, *Hsp70*, *GAPDH*, *cyp4g15* and *Cu–Zn SOD-like*) and eight with incomplete ORFs (*E74*, *HR38*, *HR4*, *cyp6a13-like, cyp6k1-like, cyp4c1, GST* and *GPx*) were obtained. *Fig. R20* shows the relevant domains of each ORF. The accession numbers, the lengths of ORFs, and the closest match in the database in BLAST tool (NCBI) with another specie are presented in the Supplementary *Table S5* and *Table S6*).

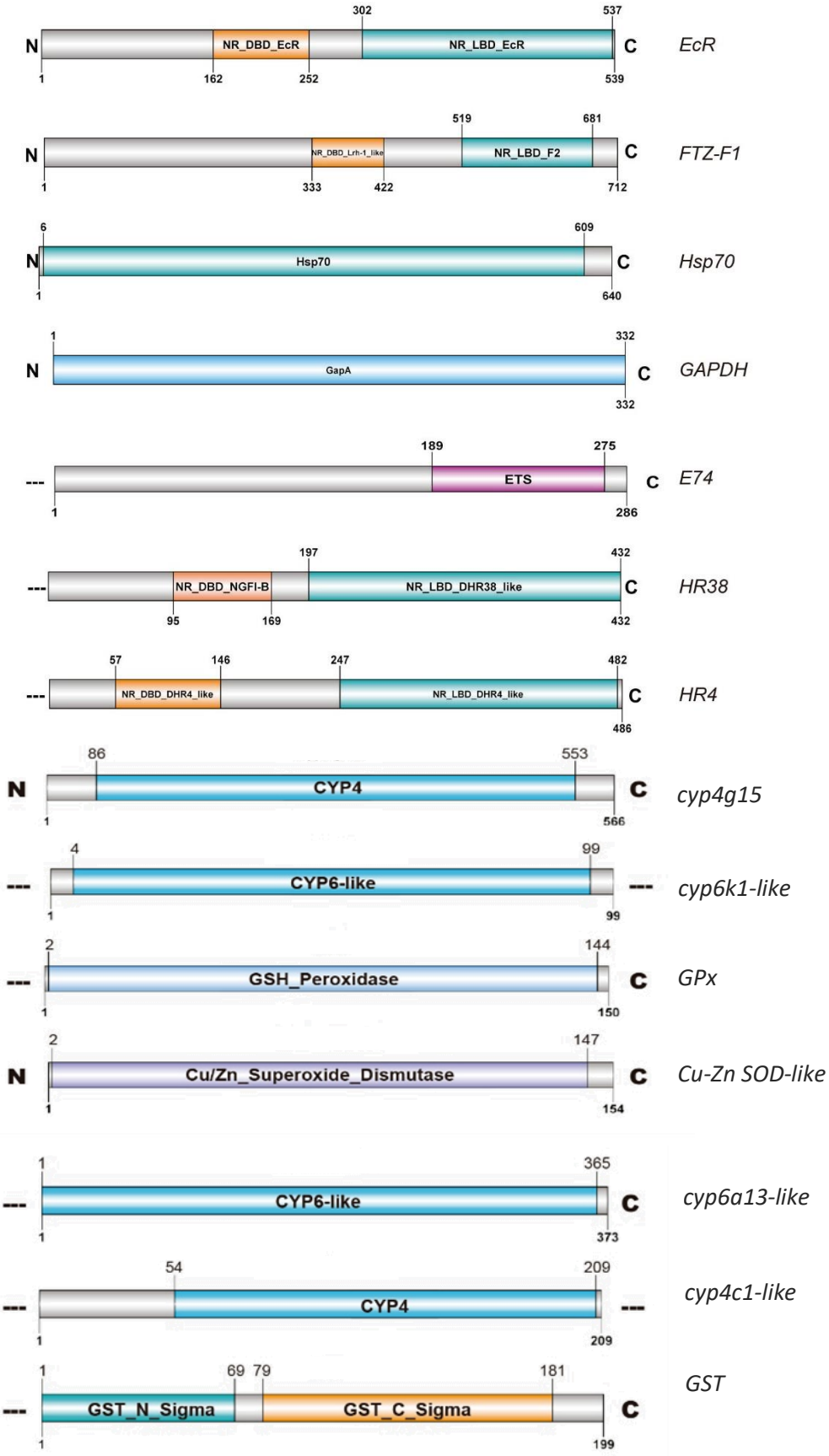


Fig. R20. **Characterization of stress-response ecdysone-related, detoxification and oxidative stress proteins proteins identified in *de novo* transcriptome of *Macrosiphum euphorbiae*.** Diagram of the protein of *M. euphorbiae* identified as putative mRNAs and their conserved domains. Diagram designed with DOG V.2 software.

D.4.1 Ecdysone-related proteins

- EcR:

The complete ORF of EcR was coded by a DNA of 1620 bp and it had a length of 539 aa in length. The protein had a DNA-binding domain and a ligand-binding domain. These two domains are Ecdysone receptor characteristic and it shared 99% identity to the ecdysone receptor protein from *Acyrthosiphon pisum* and *Myzus persicae*.

- FTZ-F1

The complete ORF of FTZ-F1 was 712 aa in length. It had a DNA-binding domain of Lrh-1 like and a ligand-binding domain both domains of nuclear receptor family. The protein also showed high identity to the nuclear hormone receptor FTZ-F1 from *A. pisum* and *M. persicae* (99% and 98%, respectively).

- E74

An incomplete ORF of E74 was identified, with 286 aa in length.

- HR38

The incomplete ORF of HR38 covered a region of 474 aa of the C-terminal. It had a DNA-binding domain of the orphan nuclear receptor and a ligand-binding domain of DHR38-like proteins. The protein showed (98%) identity with HR38 protein from *A. pisum* and *M persicae*.

- HR4

An incomplete ORF sequence found in the transcriptome was a 1535 bp DNA and coded an ORF of 510 aa that shared 99% identity to hormone receptor 4 from *A. pisum* and *M. persicae*.

D.4.2 Cell stress-related protein

- Hsp70

The Hsp70 ORF was 640 aa in length. It has a characteristic Hsp70 domain and protein shares 97% identity to the heat shock protein 70 from *A. pisum* and *M. persicae*.

D.4.3 Energy metabolism-related protein

- GAPDH

The complete ORF of GAPDH was coded by a DNA of 999 bp and it had a length of 332 aa in length, sharing 99% identity to corresponding protein from *A. pisum* and *Aphis gossypii*.

D.4.4 Detoxification and oxidative stress-related proteins

Among the detoxification and oxidative stress genes, four of the genes were identified and characterized due to the presence of a Cyp450 domain and some of the most conserved motifs for CYP proteins: heme-binding region (FXXGXRXCXG), meander motif (PXXFXPXXF), K-helix region (EXXR) and C-helix region (WXXXR) (*Fig. R20*; *Supplementary Table S7*). The ORF was complete in one of these sequences (cyp4g15), while the other three were incomplete.

- Cyp4g15

A DNA of 1,701 bp coded the complete ORF; it had a length of 566 aa and it shared 99% and 98% identity with cyp4g15 of *Acyrthosiphon pisum* and *Myzus persicae*, respectively. This amino acid sequence contained the C-helix region conserved motif (WRAHR; residues 141-145), the K-helix sequence motif (ETLR, residues 421-424), the meander motif region (PNPEVFNPDNF; residues 472-482) and, the heme-binding sequence motif (FSAGPRSCVG; residues 498-507).

- Cyp6a13

The first incomplete sequence was a 1,122 bp DNA with an ORF of 373 aa that contained the heme-binding domain (FGDGPRHCIG), the K-helix motif (ETHR) and the meander motif region (PETFDPERF). It shared 97% and 95% identity to cyp6a13 from *Acyrthosiphon pisum* and *Myzus persicae*, respectively.

- Cyp450

The incomplete ORF of Cyp450 was 299 bp in length, and the protein had 99 aa and contained the K-helix motif (ETER) and the meander motif region (PLRFDPERF). The highest identity (91%) was with the cyp6k1 of *Acyrthosiphon pisum*.

- Cyp4

The last cytochrome identified belonged to the Cyp4 family, and the size of the DNA was 630 bp. It coded for a 209 aa protein and contained the C-helix motif (WQTR) and the meander motif region (PLRFDPERF). It showed 98% and 92% of identity with cyp4c1 from *Acyrthosiphon pisum* and *Myzus persicae*, respectively.

Different proteins were identified concerning biotransformation processes.

- Cu/Zn-SOD

First, the complete ORF (465 bp) of a superoxide dismutase was identified coding a protein of 154 aa. This protein contained the two Cu/Zn-SOD family signature sequences: GMHIHQFGDNT (residues 45–55) and GNAGARPACGVI (residues 139–150) and shared 63% identity with Cu–Zn SOD of *Macconellicocus hirsutus*.

- GST

The incomplete ORF of GST covered a region of 199 aa; it was 629 bp in length, and it shared 76% identity to GST Sigma-like from *Aedes aegypti*.

The GSH binding site (G-site) on the domain GST_N_Sigma (residues 1–69) and the substrate binding pocket (H-site) on the domain GST_C_Sigma (residues 79–181) were found in this amino acid sequence. Both regions contained all the residues that compose these conserved features.

- GPx

The last incomplete sequence was a 454 bp DNA coding an ORF of 150 aa. It shared 53% identity to glutathione peroxidase from *Melanaphis sacchari* and contained the three highly conserved motifs of GPx: GKVVLVVNTASKCG (GPx signature 1), ILAFPCNQF (GPx signature 2) and WNFEKF (conserved active site motif).

The rest of the genes, not present in the de novo transcriptome, were identified using primers from other insect species (*Table M2*), and their identity was verified by sequencing and BLAST comparison.

D.5 Gene expression analysis of sequences encoding for proteins involved in stress-response, ecdysone and energy metabolism pathways

Analyses of the transcriptional activity of the previous identified targets were carried out in viviparous females exposed to MM and ABL10-4 plants, respectively. In terms of endocrine processes, different changes on the expression profile of ecdysone related genes were observed. Surprisingly, the gene coding for Phantom, a cytochrome P450 enzyme essential for ecdysone biosynthesis, was induced in ABL 10-4, suggesting that ecdysone production increased in aphids exposed to ABL 10-4 (*Fig. R21* (A)). By comparison to aphids on MM, genes coding for the ecdysone and estrogen-related receptors (*EcR and ERR*) (*Fig. R21*(C), (D)) were also up-regulated in aphids on ABL 10-4 exudates, with a mean increase in transcription of 1.4- and 2.3-fold, respectively, relative to MM. Although this induction was not significant for EcR, a concomitant tendency was detected downstream to the ecdysone response pathway. Significant (*Fig. R21* (D)–(G)) up-regulations were detected in all, early and late ecdysone-responsive genes (*E74, ftz-f1, HR38, HR4*) selected for this study.

The transcription of genes known to be involved in the cell stress response (*hsp70, hsp17, hsp10*) showed similar patterns for those observed in hormone genes. In the ABL 10-4 treatment, the three *hsp* genes analyzed (*Fig. R21* (H)–(J)) significantly increased their transcriptional activity. The *hsp70* gene was significantly overexpressed in individuals exposed to ABL 10-4 plants, up to 3.7-fold. (*Fig. R21* (H)). Genes coding for small HSPs (*hsp17* and *hsp10*) were also induced (up to 2.3- and 2.4-fold, respectively) in ABL 10-4 (*Fig. R21* (I), (J)).

Finally, dramatic effect was observed on the transcriptional activity of the GAPDH gene (*Fig. R21* (K)), leading to a significant reduction in ABL 10-4 group comparing to MM, with values of about 85% below the control.

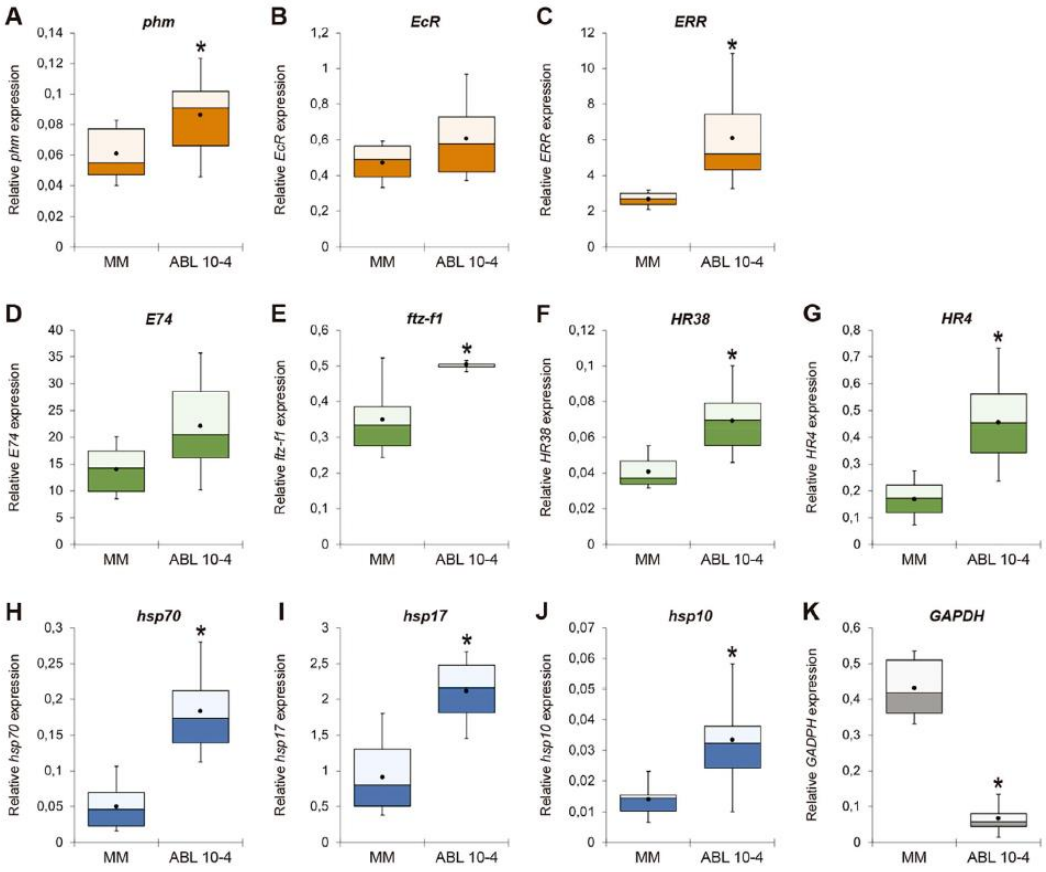


***Fig. R21*. Changes in expression of genes related to hormonal pathways, stress cell response and energy metabolism in *Macrosiphum euphorbiae* adult females exposed to MM and ABL 10-4 line tomato plants.** Box-and-whisker plots represent the expression patterns of the studied genes measured by real-time qPCR: (A) phm; (B) EcR; (C) ERR; (D) E74; (E) ftz-f1; (F) HR38; (G) HR4; (H) hsp70; (I) hsp17; (J) hsp10; and (K) GAPDH. For each experimental condition, three independent experiments were performed, and RNA was extracted from groups of 25 aphids. Box and whiskers represent the 25–75 percentile and the minimum/maximum measured values, respectively; the mean is represented by a dot, whereas the horizontal line separating the lower (dark) and the upper (light) area represents the median. Asterisks indicate significant differences $P \leq 0.05$ (*) between MM and ABL 10-4 aphids.

D.6 Gene expression analysis of gene sequences encoding detoxification and biotransformation proteins

In terms of detoxification processes, different changes in expression profile were observed depending on the gene. Compared to MM aphids, significant repression of *cyp4g15*, *cyp6k1-like* and *cyp4c1* in ABL10-4 (up to 5.6-fold, 2.7-fold and 1,9-fold respectively) were observed in individuals reared on ABL10-4 (*P*=0,001, *P*=0,011 and *P*=0,019, respectively). In contrast, a significant induction of *cyp6a13-like* expression (a ean of 5.7-fold; *P*=0,008) was detected in these aphids (*Fig. R22*).

Gene expression of *GPx*, *GST* and *Cu-Zn SOD-like*, involved in biotransformation processes, showed a similar tendency to that observed for *cyp6a13-like*. The overexpression of *GST* and *Cu-Zn SOD-like* were significant (*P*=0,015 and *P*=0,017, respectively), while *GPx* P-value was in the limit of significance (*P*=0,0503) in aphids after exposure to the ABL10-4 plants in comparison to the MM condition (*Fig. R22*).

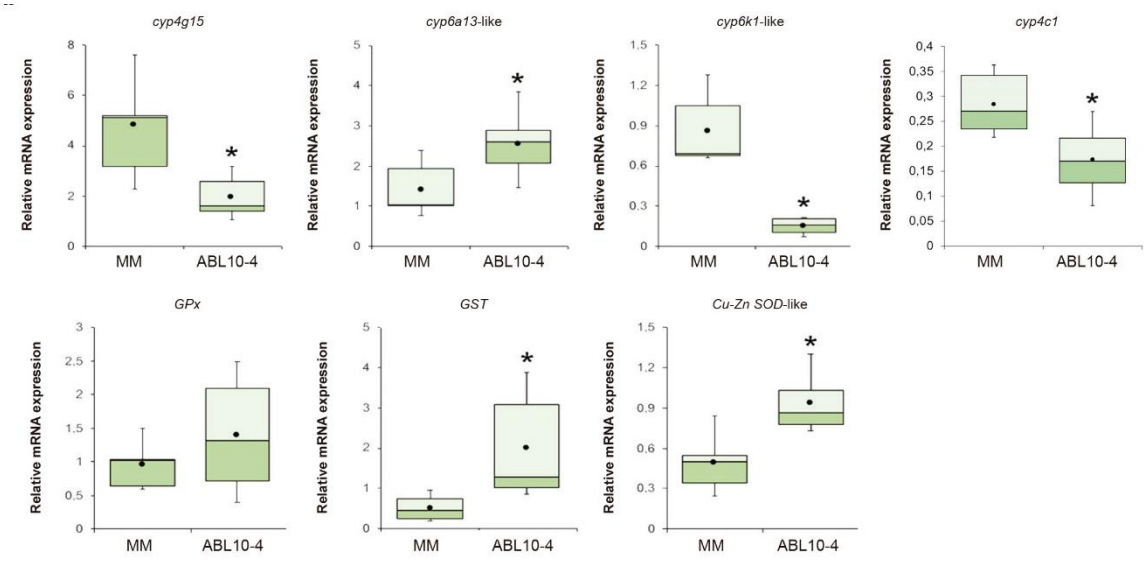


Fig. R22. **Transcriptional activity of Cyp4g15, Cyp6a13-like, Cyp6k1-like, Cyp4c1, GPx, GST, Cu-Zn SOD-like in *Macrosiphum euphorbiae*.** Box and whisker plots represent the expression patterns of Cyp4g15, Cyp6a13-like, Cyp6k1-like, Cyp4c1, GPx, GST, Cu-Zn SOD-like measured by real-time RT-qPCR. Box and whiskers represent the 25-75 percentile and the minimum/maximum measured values; mean is represented by a dot; horizontal line separating the lower (dark) and the upper (light) area represents the median. (*) Asterisks indicate significant differences between MM and ABL10-4 aphids, *P*<0,05**.**

DISCUSSION

V. DISCUSSION

Addressing not only the extent of the interaction between tomato plants and the aphid *M. euphorbiae*, but also the mechanisms operating the defence response, is a complex exercise that requires multidisciplinary approaches to obtain a complete overview. In this study, this general objective has been achieved by breaking it down into more specific objectives: 1) Evaluate the impact of the domestication on the tolerance and resistance of tomato plants against *M. euphorbiae*; 2) Determine the effect of glandular trichomes type IV and their acylsucroses production on *M. euphorbiae*'s behaviour; 3) Identify the signallying hormonal pathways underlying the induced defence response; 4) Analyze the effect of glandular trichome type IV secretions on the physiology of *M. euphorbiae* by means of a transcriptomic study.

The intricate web of interactions between plants and insects has played a crucial role in shaping the evolutionary history of both groups (Ehrlich & Raven, 1964; Hembry *et al.*, 2014). This PhD sed light in the interaction among one of the most important crops on a global scale and its frequent pest, tomato and the aphid *M. euphorbiae.* As detailed on the specific objectives, this work combines ecological and molecular perspectives to evaluate, on one hand, the plant defence response and impact on the insect behaviour, and on the other, mechanisms of the insect to cope with the defence. Consequently, covering the two sides of the interaction.

A Domestication impact on tolerance and resistance against *M. euphorbiae*

Plants, as sessile organisms that continuously face biotic stresses, have evolved different mechanisms to deal with insects. Against herbivores they deploy a mixed pattern of defence by allocating the available resources to two different defence strategies: resistance, that reduces the damage by impairing the herbivore's performance, and tolerance which buffers the negative impact of the pest (Núñez-Farfán *et al.*, 2007). Although several hypothesis has been proposed, it is still not clear how the mixed pattern of defence allocation works and how tolerance and resistance characters map onto phylogeny.

For centuries humans have imposed strong selection on domesticated crops, resulting in drastically altered crop phenotypes compared with wild ancestors. It has been hypothesized that in the process of domestication the strong selection for traits of agronomic importance have encompassed the loss of many other traits such as resistance to diverse herbivores. And therefore, cultivated varieties tend to be more susceptible compared with their wild relatives (Chen *et al*., 2015). In this situation, ecological theory recognizes the occurrence of *trade-offs*, whereby plant investment in resistance goes at the cost of other important life-history traits, such as the tolerance to pests and diseases. This work started by comparing 23 different genotypes of *S. lycopersicum* that represent different stages in the domestication history, categorized as: wild relatives, early domesticated genotypes, local and modern varieties. Although important differences were found either in both plant resistance and tolerance to pests depending on tomato provenance, the partial resistances detected were not phylogenetically structured and, hence, were not related to different genetic lineages in the putative domestication process.

A.1 Phylogenetic relationships

The 23 tomato varieties showed a very low genetic differentiation, supporting previous results for tomato that used other molecular markers like simple sequence repeats (SSR) and sequence related amplified polymorphism (SRAP) (Ruiz *et al*., 2005c; Mazzucato *et al*., 2008). Tomato, *S. lycopersicum*, is divided into two main groups of varieties: the cultivated *S. lycopersicum* var. *lycopersicum*, and the weedy *S. lycopersicum* var. *cerasiforme* (early domesticated tomatoes), while *S. pimpinellifolium* is thought to be the most closely related wild species (Víquez-Zamora *et al*., 2013). The domestication process of tomato is still not completely understood but both genome comparison and biogeographical studies suggest that *S. pimpinellifolium* and *S. lycopersicum* var. *cerasiforme* played an important role in the early stages of domestication. Recent studies additionally show that some *cerasiforme* accessions are genetically close to the cultivated *S. lycopersicum* group while others are an admixture between cultivated tomatoes and *S. pimpinellifolium* (Ranc *et al*., 2008, Blanca *et al*., 2012). The phylogenetic reconstruction based on GBS supported this statement, as one of the early domesticated tomatoes included in our study, Mex-89 (NR0275) an accession from

Mexico, was clearly integrated in the *S. lycopersicum* clade whereas the other, PE-55 (NR0407) from Peru, was ancestral to the whole group together with *S. pimpinellifolium* genotype also from Peru. These two accessions also responded differently to pests.

Tomato is an excellent example of a crop whose wild ancestor, *S. pimpinellifolium*, has little resemblance to modern varieties. This analysis with different genotypes showed substantial variation among closely related wild tomato species, early domesticated varieties (*cerasiforme*) and traditional local landraces. In these selected genotypes, there was no evidence found for any phylogenetic signal in the responses to the two different herbivores, aphids and caterpillars. In terms of resistance, this study shows that intraspecific variation can be equal or larger than interspecific variation. These results highlight important aspects on the effects of artificial selection on resistance to herbivory during the early domestication of tomato. As seen in the glasshouse comparisons, resistance to herbivores is rather labile within the selected genotypes. For example, the differences observed in resistance between the genotypes of *S. pimpinellifolium* or the two varieties of *cerasiforme* were as contrasting as the differences observed between *S. pimpinellifolium* and modern varieties. A second aspect drawn by the results of this work is that there is little evidence for big limitations in the resistance to functionally contrasting herbivore pests. This means that being resistant to one herbivore species seems to be independent from being (or not) resistant to a different herbivore. For tomato, as for many other species, it is generally assumed that chewing and sap-sucking insects trigger different hormonal pathways in planta to induce plant defence, that is jasmonate and salicylic pathways respectively (Mewis *et al*., 2005). Accordingly, in phytopathological studies it is often assumed that resistance to a certain pest goes at the cost of resistance to a different pest. However, we could not find such pattern among the compared genotypes, which showed independent responses to the two herbivores, from genotypes with a high general resistance (e.g., *S. habrochaites*) to genotypes highly susceptible to the three different pests. Contrary to what occurs in other groups, phylogenetic comparisons at larger evolutionary scales within *Solanum* do not reveal clear patterns on the evolution of defence syndromes, that is closely related genotypes that show similar suits of resistance traits (Futuyma & Agrawal, 2009; Losos, 2011). The lack of genetic correlation between resistance traits in our experimental set-

up goes in line with previous findings in wild tomatoes (e.g., Thaler & Karban, 1997; Koricheva *et al*., 2004), but also extends the observed pattern to later stages of the domestication of tomato (see also Carrillo *et al*., 2019).

A.2 Resistance and tolerance trade-offs

In modern agriculture lots of efforts have been conducted to develop engineered plants that express toxic compounds or optimized plant defence routes to reduce pest damage (Douglas, 2017). However, in a crop protection context it is impotant to differenciate between tolerance and resistance strategies in order to match the most effective defensive tratits to the risk that a certain pest pose. On this basis, a solid understanding of the mechanisms underpinning defensive traits is necessary.

Resistance to herbivores depends on physical and chemical defensive traits that either impair or reduce feeding, oviposition and development of larvae and/or adults. Tomato plants have glandular trichomes as the first line of defence against herbivores. Epidermal hairs present on aerial parts that can secrete sticky and volatile substances (Kennedy, 2013) with a deterrent and repellent activity (Bennett & Wallsgrove, 1994). Although they are present on many vascular plants, trichomes in *Solanum* species have been studied in detail, where four types of glandular trichomes (I, IV, VI, VII) and four nonglandular (II, III, V, VIII) have been described (Glas *et al*., 2012). Different wild tomato relatives such as *S. habrochaites*, *S. lycopersicum* and *S. pennellii* show a wide array of glandular and nonglandular trichomes (i.e., I, III, IV, V, VI and VII) (Reeves, 1977; Lemke & Mutschler, 1984; Dai *et al*., 2010). Glandular trichomes and the associated allelochemicals for example, acylsugars, sesquiterpenes, and methylketones, are present in tomato wild relatives (i.e., *S. habrochaites*, *S. pimpinellifolium*) and many studies confirm the role of trichomes in the resistance of tomato against different insect pests (Maluf *et al*., 2010; Mutschler *et al*., 1996; Freitas *et al*., 2002; Muigai *et al*., 2003; Bleeker *et al*., 2009). Interestingly, most domesticated tomatoes lack glandular trichomes and their excreted metabolites, making them susceptible to the very same pests (Besser *et al*., 2009; McDowell *et al*., 2011). From the species included in this study, two genotypes have glandular trichomes, that is *S. habrochaites* and *S. pimpinellifolium*. Interestingly, *S. habrochaites* (showing type IV and type VI) had the lowest number of

aphids in the presented glasshouse experiment. The poor performance of aphids can be attributed here to the deterrent effect of trichome exudates and the high concentration of terpenes on the leaf surface. For *S. pimpinellifolium*, two different genotypes were used. Genotype LA1589 that has glandular trichomes type VI while TO-937 displays type IV and a susbstantial production of acylsucroses (Escobar-Bravo, 2013). This latter genotype (TO-937) is resistant to the two-spotted spider mite *Tetranychus urticae* and the *whitefly B. tabaci* (Fernández-Muñoz *et al*., 2000; Alba *et al*., 2006; Rodríguez-López *et al*., 2011). Genetic and biochemical studies have shown that this resistance is mainly associated with type IV glandular leaf trichomes (Fernández-Muñoz *et al*., 2003; Alba *et al*., 2009). Also, a recent study with a different introgressed line shows that the resistance of tomato plants against *Tuta absoluta* is mediated by glandular trichomes type IV (de Resende *et al*., 2022). These results showed a greater resistance of TO-937, the cultivar with type IV trichomes, to aphids compared with LA1589, cultivar without type IV, which further confirms that excretions produced by type IV glandular trichomes are also involved in tomato defence against aphids (Goffreda *et al*., 1988, 1989, 1990). The importance of glandular trichomes type IV in the resistance against *M. euphorbiae* is further corroborated by the near-isogenic introgressed line ABL10-4 which was generated from the initial cross *S. lycopersicum* cv Moneymaker x *S. pimpinellifolium*. This crossing resulted in a tomato line with a Moneymaker (MM) genetic background but producing type IV glandular trichomes. From the results of this work is very remarkable that the number of aphids in the introgressed line was not only the lowest among the cultivar and local varieties but also even lower than in the wild parental line. Furthermore, the mechanisms of partial resistance seem to be linked to glandular trichomes since we did not detect any other mechanisms of resistance to the aphid *Macrosiphum euphorbiae* in the accessions without trichomes.

Focusing on the tolerance, these assays show that the wild and early domesticated varieties have a greater tolerance against the caterpillars compared to locals and cultivars. A reduction in trichome density and trichome-borne metabolites has been previously shown to render plants more attractive to chewing herbivores (Bosch *et al*., 2014). However, in this study differences in the weight of caterpillars after feeding on plants of different types were not found. Still, local and cultivars experienced greater

reductions in biomass due to caterpillars. While no effects were found according to the degree of domestication when treated with aphids.

The use of tolerant genotypes as a complement to pest management can become very successful to pest control. Environmental agencies and industry have traditionally promoted practices among farmers of planting susceptible (refuge) crop lines combined with resistant lines that secrete a high dose of toxic compounds. Although this practice can reduce the evolution of pest resistance (Gould, 2003), farmers have been reticent to give any portion to the susceptible genotypes due to the field losses. However, tolerant genotypes could be used as refuge lines, reducing yield losses but without enhancing pest resistance through a coevolutionary response on the herbivores (Garrido-Espinosa & Foroni, 2006).

In this first part of the study, where it was compared the variation and possible trade-offs in resistance and tolerance to three different herbivore pests of tomato using 23 varieties with different domestication status, substantial variation among the genotypes was detected and no evidence for phylogenetic conservatism among resistance traits was found. Data from these experiments also indicated that there was no evidence for similar or contrasting resistance to functionally different herbivore pests and do not put forward any significant *trade-off* in resistance to herbivores which probably reveal a pleiotropic origin in the appearance and loss of resistance to insect pests driven by the selection for other agronomic characters. These findings complement previous work comparing defensive syndromes in wild tomato relatives and underpin complex eco-evolutionary and breeding feedbacks during the early stages of domestication in *S. lycopersicum*. The combination, in this experiment, of different pests revealed that wild and early domesticated cultivars showed more tolerance to pests than modern-bred varieties and highlighted the necessity of focusing on tolerance mechanisms to understand the process of domestication in tomato and in develop new breeding strategies.

Traditional landraces are of special interest to farmers, consumers, chefs and the general public due to their adaptation to low-input crop systems (Krishna *et al*. 2010) and the lack of taste of commercial tomatoes (Fernqvist and Hunter 2012; Fernqvist and Ekelund 2013). Interestingly, here it is important to highlight the great value of these

varieties also as a reservoir of partial resistance to pest that can be implemented in the future to ensure food production in a sustainable way.

Furthermore, it has been described that resistance can decrease the incidence of attack of other genotypes with different allocation patterns in their defence strategies (Barbosa *et al*., 2009 y Garrido *et al*., 2016). Consequently, wild, early-domesticated and the introgressed-line ABL 10-4, as resistant varieties, become very relevant to design current practice to control pest resistance.

Moreover, focusing on the tomato aphid, the density of *M. euphorbiae* decreased in early-domesticated cultivars (Mex-89), some wild genotypes (PI-134419) and the introgressed line ABL 10-4 (cultivar); in concordance with previous studies that also point a reduction of the herbivore density in resistant genotypes (Underwood &Rausher, 2000; Thaler *et al*., 2001; Garrido *et al*., 2016). This points to a lower amount of damage which, in turn, would mean a decrease in the incidence of this aphid in nearby genotypes with different distribution strategies and which would indirectly benefit from this resistance.

Macrosiphum euphorbiae currently seems unable to overcome glandular trichome defences, not even by modulating basic physiological profiles related to survival and secondary metabolite detoxification. Thus, this defence is consolidated as a particularly relevant defence trait for future breeding and pest management strategies. Nevertheless, the possibility of local adaptation by *M. euphorbiae* to these defences should not be excluded. A plant trait that deters feeding, is likely to impose a strong selection on the arthropod to overcome plant resistance (Janzen, 1980). In this evolutional scenario that would lead to an increase of the aphid population, tolerance genotypes would be favoured. When resistance is unable to eliminate herbivory, at low levels of damage, allocation to tolerance is favoured. Because both genotypes pay an equivalent cost of defence but the one biased towards tolerance will obtain higher fitness benefits. This positive demographic effect of tolerance will have a limit when herbivory has risen up to a point where tolerant genotypes are no longer able to compensate any increasing level of damage (reviewed in Garrido *et al*., 2016). A cyclical dynamic where neither maximum allocation to resistance nor that to tolerance would

be evolutionary stable as we have seen in the different tomato genotypes studied. Which taken into account may help to improve current management practices.

B Role of glandular trichomes type IV on the resistance of tomato plants against *M. euphorbiae*

Revealed the importance of the glandular trichomes type IV in the specific resistance against *M. euphorbiae*, the next step was to study this interaction in more detail. In order to do so, MM and ABL 10-4 lines were compared, since differences between them could be ascribed only to the presence of glandular trichomes type IV. It is interesting how previous studies with spider mites (Rakha *et al*., 2017), whiteflies (Lucatti *et al*., 2013), and thrips (Mirnezhad *et al*., 2010) pointed that not only the presence of glandular trichomes on tomato surface but also reach certain amount of acylsucroses is required to develop fully resistance in wild and cultivated tomatoes. Having this in mind, the comparisons began with a morphological study of the leaf surface of MM and ABL 10-4 genotypes and with a quatification of the amount of acylsugars. After that, to complete the study of the role of glandular trichomes type IV in the defence response, some choice and population dynamic assays using both genotypes were carried out

Host selection by aphids is not a random process and a variety of sensorial mechanisms are used to locate suitable host plants. An optimal host choice by insects is vital for survival, feeding, breeding, and progeny development. Chemicals present on the leaf, provide critical information and influence insect choices (Pickett, *et al*., 1992). The no-choice bioassays, that mock a situation where all plants offered to *M. euphorbiae* are of the same genotype, showed that aphids were attracted to MM but not to ABL 10-4. Aphids tended to remain on the experimental arena when both leaflets offered were of the later genotype. In other species like whiteflies, settling and feeding behavior has also been altered when type IV glandular trichomes are present (Rodríguez-López *et al*., 2012). Moreover, Goffreda *et al*., (1988) showed that glandular exudates of *S. pennellii* trichomes deter aphid feeding on *M. euphorbiae*. Although later studies on aphid settling have also shown a direct relationship between glandular trichomes and deterrence, the underlying mechanism remains unclear. Acylsucroses, one of the primary metabolites produced by glandular trichomes type IV, have been related with

increased mortality on diverse herbivores due to poisoning, entrapment and insects' chemical labeling that increase their recognition by predators. (Glas *et al*., 2012; Weinhold & Baldwin, 2011). This study confirms that the high densities of trichomes type IV in ABL 10-4 plants were accompanied by the accumulation of acylsugars as previously described by Escobar-Bravo (2013) and, consequently, *M. euphorbiae*'s repellence can be attributed to these compounds. Taken together, the selection trials evidence a clear preference of *M. euphorbiae* for MM leaflets and a clear deterrent activity of ABL 10-4 explained by the secretions of type IV glandular trichomes. These results linked the presence of type IV glandular trichomes with an avoidance behavior in *M. euphorbiae*, as seen previously in others phytophagous pests. i.e., spider mites (Alba *et al*., 2009) and whiteflies (Escobar-Bravo *et al*., 2016). Under free-choice conditions, when aphids were exposed to both genotypes at the same time, aphids remained more frequently on the arena. A lack of choice that can be explained due to the methodology used. Assays using leaf-discs or leaflets detached from plants may present different volatile profiles than whole plants. Different studies with several herbivorous insect species have described that a single volatile component or complex background odors are able to mask attractive ques (Schöder & Hiker, 2008). Also, the damage caused by handling plants may affect the plant physiology and these subtle differences may influence the results. Nevertheless, whenever aphids made a choice, a clear preference for MM leaflets was observed.

To determine the resistance of tomato plants to *M. euphorbiae*, aphids' growth was evaluated on the two genetic lines. While aphid population on MM plants followed an exponential growth as expected, those on ABL 10-4 plants barely survived. These findings, together with those of the choice experiments, showed not only that aphids prefer not to settle on ABL 10-4 plants if they have the choice, but when no other host is available, population growth collapses, unable to multiply on plants with type IV glandular trichomes. The chain of events that results in reproductive impairment and lack of population growth is difficult to establish but we consider insect starvation as the main underlying mechanism, exacerbated by the toxic effect of trichome exudates. In this way, previous studies using whiteflies show a severe detrimental effect of type IV glandular trichomes on probing and feeding (Rodríguez-López *et al*.,2011; 2012).

Moreover, the relationship between type IV trichome secretions and the observed effects needs further experimental consideration since it is possible that other metabolites may be important as well. The production of acylsucroses is not only restricted to type IV trichomes, e.g., type-I are also producers of acylsugars and terpenoids. What can be deducted from this work is that, in this particular case, trichomes type I were uniformly low on the abaxial and adaxial leaf surfaces in comparison with glandular trichomes type IV, that seem to be the responsible for the most production of acylsucroses, very similar to what Rakha and colleagues described (2017). However, a detailed analysis of the different types of acylsucroses produced was not performed and therefore, further analysis on the trichome's acylsucroses production is required to: (i) pinpoint and further characterize the response of aphids to different trichomes exudates; and (ii) to determine whether the observed effect should be attributed to single or to a blend of compounds.

Taken all together, the results from these trichome type IV resistance studies with MM and ABL 10-4 lines confirmed that this specific trait and its exudates (i.e., mainly acylsucroses) play a key role in the defence of tomato plants against the aphid *M. euphorbiae*. The bioassays developed in this part of the study proved the impact of type IV glandular trichomes in terms of deterrence and impairment of population build-up in *M. euphorbiae.*

C Signallying mechanisms underlying the induced defence response of tomato plants against *M. euphorbiae*

C.1 Priming effect on the latter defence response

The underlying mechanisms operating this defence response are unknown. Generalized plant responses to aphid feeding are mediated by phytohormonal signalling that promote the expression of genes involved in herbivore deterrence and activation of inducible defences (Howe & Jander, 2008). After that, plants can switch to a primed state of enhanced defence from where they can respond faster and stronger when a subsequent biotic stress appears (Conrath, 2015). The plant hormones JA and SA are known to play major roles in the activation of induced defences (Gimenez-Ibanez & Solano, 2013; Pieterse *et al.*, 2012). However, their effect on aphid-plant interactions

still remains controversial. Even though it is generally assumed that aphid feeding mostly induces the SA pathway, the activation of this pathway has been shown to confer inconsistent defence against aphids. In this sense, several studies have also reported that aphids trigger JA-mediated defence response while in other cases, it has been observed a negative impact of JA induction on aphids feeding (Ellis *et al*., 2002; Gao *et al*., 2007, Brunissen *et al*., 2010).

Although it is well known that prior herbivory can modify plant responses to subsequent herbivores, especially when exposed to different feeding guilds, little is known about how earlier herbivory can modify plant responses mediated by glandular trichomes to later arriving herbivores. In this sense, it has only been described that the application of JA to tomato plants induced the production of acylsucroses by type IV glandular trichomes (Escobar-Bravo *et al*. 2016). Therefore, to unravel further implications of a priming response on the trichome-mediated responses, plants were challenging with herbivores with contrasting feeding strategies in order to trigger the different hormonal cascades and then, the effect of the different pathways on a subsequent *M. euphorbiae* attack either on MM and ABL 10-4 plants, was eventually addressed. The work with these isogenic lines allowed to distinguish the effects of a known resistance trait (i.e., the type IV trichomes) from the responses mediated by hormonal pathways and at the same time, indirectly evaluate the effect of different pathways on *M. euphorbiae*. Numerous studies have investigated how prior herbivory affects the performance of subsequent herbivores, particularly using contrasting feeding guilds with different outcomes in interaction (e.g., positive, neutral, or negative for the second herbivorous insect) (Soler *et al*. 2012; Schweiger *et al*. 2014, Rodríguez-Saona *et al*. 2010; Eisenring *et al*. 2018; Li *et al*. 2018, Kroes *et al*. 2016). Results showed that ABL 10-4 plants, with type IV glandular trichomes, were overall more resistant to *M. euphorbiae* than MM, as previously seen in the first part of this dissertation and in the same line with previous studies using other herbivorous insects (Escobar-Bravo *et al*., 2016; Rodríguez-López *et al*., 2020). However, resistance differed depending on the type of herbivory previously faced. In this sense, plants previously infested with *S. littoralis* were more resistant than control plants, indicating that a first attack by the chewing herbivore, triggering the JA pathway resulted in an enhanced resistance against *M. euphorbiae*. Regardless of the

presence of type IV glandular trichomes and acylsucrose production. Leaf-chewing herbivores inducing JA on the plant have been shown to reduce the performance of aphids on radish, milkweed, and tomato (Agrawal, 1998; Ali & Agrawal, 2014). These results also point that those plants previously infested with *M. euphorbiae*, were more susceptible to a second attack of the aphid. Similar studies in other plant families and using other aphid clones have shown a lot of variability in the outcome of the interaction (Züst & Agrawal, 2016). However, works focused on aphid choices at small scales indicated a preference and enhanced feeding on pre-infested leaves and a positive effect on nymph survival (Dugravot *et al*., 2007). In this sense, it has been hypothesized that specialized aphids seem to have gained the ability to manipulate plant defences to their benefit using the plant's own hormonal crosstalk, a line of thought in accordance with our observations (Züst & Agrawal, 2016; Ali and Agrawal, 2014; Moreira *et al*., 2018).

Many inducible defences are expressed rapidly (i.e., within hours) in leaves of herbivore-challenged plants. In tomato plants, wounding causes a systemic reprogramming of leaf cells that results in the synthesis of different defence-related proteins (Ryan *et al*. 2000). The JA-responsive *proteinase inhibitor II* (*PI-II*) gene, is a well-characterized marker of induced resistance to insects (Howe and Ryan, 1999; Li *et al*., 2003) that inhibits the activity of digestive enzymes in the gut of the herbivore (Ryan, 1990). Transcriptional activation of proteinase inhibitor genes in response to wounding and systemin depends on the action of JA (Ryan, 2000). For this reason, the expression profile of *PI-II* was evaluated after 48h, when its maximum expression level is reached (Kawazu *et al.,* 2012; Sanmartin *et al*., 2020), in order to verify that the priming stimulus worked. Real-time qPCR analysis showed a considerable overexpression of *PI-II* not only in plants sprayed with JA, as expected, but also in those primed with the caterpillar *S. littoralis;* indicating that this herbivore effectively triggered the JA signaling pathway. Previous studies have revealed that the application of MeJA in the ABL 10-4 plants increase the density of the glandular trichomes type-IV and their associated production of metabolites (Escobar-Bravo *et al*., 2016). Here it can be noticed a similar increase of the *PI-II* levels in both genotypes, ABL 10-4 and MM, treated with a JA precursor solution and previously induced with the caterpillar. The lack of significant differences on the expression of *PI-II*

after infestation with *M. euphorbiae* can be attributed to the fact that pierce sucking herbivores and specialized aphids in particular, activate the antagonist SA pathway (de Ilarduya *et al*. 2003; Züst & Agrawal, 2016). As a matter of fact, de Ilarduya and colleages (2003) suggested that both JA and SA signaling pathways are activated by *M. euphorbiae* and *M. persicae* feeding in tomato plants. However, the methodologies of their works and the present one differ since they evaluated the transcripts accumulation by RNA blot analyses and not qPCR.

C.2 Role of the principal hormonal pathways on the defence response

Considering the complexity of plant-aphid interactions and the unequivocal role of hormonal pathways in plant defence to aphid herbivory, the following step of this work was to address the role of JA and SA in the resistance to *M. euphorbiae* in tomato plants under experimental conditions.

The JA signaling pathway is involved extensively in plant resistance against herbivore insects by regulating the production of secondary metabolites and defensive proteins that are harmful or deterrent for insect herbivores. The relevance of jasmonic acid in defence responses has been concluded in different ways: 1) by the fact that exogenous application of JA or MeJA induces these defence responses (Boughton *et al*., 2005; Escobar-Bravo *et al*. 2016; Chen *et al*., 2018); 2) the occurrence of increased level of endogenous JA after wounding is correlated with the induction of defence responses (Howe, 2004; Kesser & Baldwin, 2002); 3) the inhibition of the JA production pathway also inhibits the induction of the defence responses (Li *et al*., 2002). For these reasons, and in order to avoid additional effects from the glandular trichomes that could mask the underlying defence pathway, aphid's performance was firstly evaluated on mutants impaired in the JA pathway. Results showed that the number of aphids on *spr2* plants was significantly higher than on the wild-type tomato CM; *spr2* has no ability to synthesize JAs (Li *et al.*, 2003) and therefore, these plants are unable to interact with SA or analogous pathways. Which points to a critical role of JA on the defence of tomato plants against the aphid *M. euphorbiae*. Something that contrasts with the study of Avila and colleagues (2012) which results are completely the opposite, suggesting that the population growth of *M. euphorbiae* was significantly lower on the *spr2* mutant than on

wild-type tomato plants (cv Castlemart). They point to a failure to the population growth due to the mutation on the JA pathway, either on the synthesis or perception, that not only disagree with this work, but also, with the fact that these mutations improve host suitability for other herbivores (Li *et al*., 2003, 2004, 2005). In addition, Avila and colleagues proposed, like other previous studies, that SA can contribute to plant defences against aphids in tomato genotypes (Li *et al*., 2006; Avila *et al*., 2012). Which differs again with the present data that shows a reduction in the population of *M. euphorbiae* in transgenic plants *NahG* (where SA accumulation is inhibited due to degradation). Actually, density intervals clearly reflect that there was no data overlapping between both groups and that the susceptibility of *NahG* plants was plain. With all these last results, the hypothesis of an antagonism between the JA and SA pathways, where these phloem-feeding insects limit the induction of JA-dependent defences by inducing SA (Zhu-Salzman *et al*., 2004; de Vos *et al*., 2005) makes sense. Although it is difficult to pinpoint with the information available the exact reasons for the discrepancies among the studies compared, differences may be ascribed to the aphid densities used in the experimental set-ups, genotype variation or the used aphid clones, as it has been shown that variation in bacterial symbionts may strongly determine the outcome of plant aphid interactions. In any case, the results present on this study together with previous observations, indicate that the role of hormonal pathways may be strongly unique.

Taken all together, the results from the study of the signallying mechanisms underlying the defence, suggest that the role of JA in the regulation of *M. eurphorbiae* and tomato should be reconsidered. They also show that the early herbivory of aphids or caterpillars could modulate the later resistance of tomato plants against the aphid *M. euphorbiae*. Being the susceptibility augmented when the plants were previously challenged with aphids and being more resistant if the previous herbivory was due to caterpillars. At the same time, these results further support the breeding tomato varieties of tomato varieties with higher densities of glandular trichomes and higher levels of acylsugars, for pest control as these genotypes show consistently very strong responses regardless of the induced defensive pathways.

D Physiological adaptations of *M. euphorbiae* to overcome trichome type IV defences

Hitherto, the scope of this work has been focused on plant defences, the effect of glandular trichomes type IV on tomato resistance and aphid's performance. However, little is known on the other side of the interaction, on the mechanisms evolved by insects to overcome these defences. Consequently, in order to complete this study, an analysis to study the aphid physiology when exposed to glandular trichomes and their secretions was set out.

Understanding the responses of insect herbivores to plant chemical defences is pivotal for the management of crops and pests. Although different strategies used by specialist aphids to deal with certain metabolites have been documented, such as avoidance of uptake by the gut, active elimination from the body cavity, degradation by detoxifying enzymes after uptake, and/or development of insensitive target sites for plant toxins (Castañeda *et al*., 2009; Ramsey *et al*., 2010; Shavit *et al*., 2018); insects' physiological mechanisms of adaptation to plant defences are not entirely understood. Genomic, transcriptomic and metabolomics analyses of model organisms constitute the traditional tools to identify novel target genes that can be involved in the adaptation response. However, there is little or no genomic information available for most agricultural pests, and prior to this work, none related with *M. euphorbiae*. In this context, using RNA-seq, a cost-effective and powerful tool for obtaining many functional genes in non-model organisms, a *de novo* transcriptome was built from females from four populations of *M. euphorbiae*. This transcriptome became the first resource for downstream applications, and it was used to detect potential biomarkers related to the response to plant defences. The use of MM and ABL 10-4 lines, allowed to infer the effect of glandular trichomes type IV and their associated exudates on the aphids, shedding light on the adaptation mechanisms that the insect undergo when interacting with these tomato natural defences.

This transcriptome comprises ca. 699 million reads, assembled into 240,067 contigs with an average size of 563 bp and N50 of 771 and with 29128 contigs longer than 1,000 bp. From the total number of contigs, 189,229 unigenes were identified distributed in X GO

terms. The number of annotated contigs were 87,781 and 32,601 of them were unique annotations. The number of genes successfully annotated (87,781) in this de novo *M. euphorbiae* reference transcriptome was higher than expected, compared to the total number of genes found in other studies with aphids with whole transcriptome sequences available, such as *M. euphorbiae* with 20,254 annotated contigs (Teixeira *et al*., 2018), *Myzus persicae* with 33,543 annotated transcripts (Ji *et al*., 2016), *Sitobion avenae* salivary glands with 10,776 annotated unigenes (Zhang *et al*., 2017), 27,112 annotated transcripts in the de novo transcriptome of the mustard aphid *Lipaphis erysimi* (Chongtham *et al*., 2019) and *P. solenopsis* with 38,725 (Arya *et al*., 2018).

To explore the potential effect of natural defences through the exposure to glandular trichomes, enrichment and DEG analyses comparing aphids reared on MM and ABL10-4 were performed, leading to 658 up-regulated unigenes and 1,159 down-regulated unigenes in aphids reared on ABL10-4. The most abundant GO Terms for *Biological Processes* among the up-regulated and down-regulated transcripts (see also Supplementary *Fig. S1* and *Fig. S2* respectively) give the major biological processes attributed to plant defences. The selected enriched pathways that were critical in aphid survival and development were compared: 'oxidation-reduction', 'transport', 'carbohydrate and lipid metabolism', 'development' and 'oxidative stress response and detoxification'. Additionally, the expression of conserved biomarkers that are fundamental for arthropod survival were also analysed: cell stress response (*hsp70*, *hsp17* and *hsp10*), primary metabolism (*GAPDH*), and developmental pathways (*Phantom*, *P450 enzyme*, *EcR*, *ERR*, *E74*, *ftz-f1*, *HR38* and *HR4*) when exposed to glandular trichomes type IV, that helped to characterize aphids' responses to trichomes as observed in the laboratory and green house experiments.

D.1 Cell stress response

When insects are exposed to chemical stresses that can imbalance their physiological parameters with the dangerous effects, a wide variety of responses are activated to tolerate this distrubances and survive. The most representative is the rapid increase in the synthesis of a set of conserved polypeptides collectively referred to as heat shock proteins (HSPs) (Feder & Hofmann, 1999; Zhao & Jones, 2012). Under normal conditions,

these HSPs facilitate the folding and assembly of newly synthesised proteins, while under conditions of environmental stress, they protect target proteins and repair denatured proteins (Gupta *et al*., 2010). Real-time qPCR analysis showed an active stress response when the aphid was on ABL 10-4 plants, with all the genes evaluated, *hsp70*, *hsp17*, and *hsp10*, overexpressed. In model insects, different pollutants and mixtures with heavy metals activate the inducible form of the *hsp70* gene as a response to mitigate cell stress (Zhao & Jones, 2012; Yoshimi *et al*., 2002; Planelló *et al*., 2008). However, this is the first study conducted with aphids and Hsp genes, a novel work which results also demonstrate that the blend of secretions and volatiles secreted by type IV trichomes entails a considerable stressor to *M. euphorbiae*. Despite this, it should be considered the possible effect of other exudates with a different origin present on ABL 10-4 surface.

D.2 Energy metabolism

The energy metabolism can be a sensor of the optimal functioning of the organism. GAPDH is an enzyme involved in energy production through glycolysis and also in multiple cellular processes such as apoptosis, oxidative stress, DNA repair or metabolic and physiologic pathways (Tristan, 2011). Observations of the GAPDH gene's significant repression on aphids growing on ABL 10-4 tomato line showed the possible toxic interaction between ABL 10-4 trichome's secretions and the energy metabolism of *M. euphorbiae*. This gene has a crucial role in the energetic metabolism that suggests an important energetic impairment of *M. euphorbiae* in this genotype. The almost total absence of GAPDH activity in aphids from ABL 10-4 (up to 84% below control), is consistent with two not mutually exclusive hypotheses: first, aphids on ABL 10-4 reduce their metabolism as an adaptive response to plant defences effects; second, aphids experience an energetic impairment due to their prevention fromfeeding on ABL 10-4. Although GAPDH is one of the top 10 reference genes most frequently used in real-time qPCR quantification (Lü *et al*., 2018), results in this work certify a variable expression under certain conditions. Since other studies also reflected its instability (Bustin, 2010; Herrero *et al*., 2017), the use of this gene as an internal housekeeping should be evaluated carefully.

D.3 Oxydation-reduction

Oxidation-reduction was one of the most abundant biological processes identified among the down-regulated transcripts in ABL10-4 aphids compared to MM aphids. Transcripts involved in oxidation-reduction were related to proteins involved in a diverse range of pathways: metabolism of insect hormones (CYP18a1 and CYP305a1); breakdown of synthetic insecticides (CYP6a14); growth and development (genes coding inosine-5'-monophosphate dehydrogenase, glucose dehydrogenase and chorion peroxidase, respectively), response to oxidative stress (sulfiredoxin gene-srx), and immune response (peroxidasin (pxn), prophenoloxidase 2 (PPO2)).

D.4 Transport

Genes involved in transport also showed a negative regulation on aphids reared on the ABL10-4 genotype. 60 down-regulated transcripts were identified related to oxalate, trehalose, glycoproteins and ions transport. Moreover, 37 genes involved in transmembrane transport, including insecticides (e.g., ABC transporter B, D and G family members and multidrug resistance-associated proteins), and sugar transmembrane transport (UDP glycosyltransferase Dorothy and other glucuronosyltransferase genes) were affected.

D.5 Carbohydrate and lipid metabolism

Carbohydrate metabolism was also an enriched down-regulated pathway in ABL10-4 with respect to MM. Similarly, transcripts associated with chitinase (*idgf*), fructose-1,6-bisphosphatase (*fbp)*, fructose-2,6-bisphosphatase (*pfrx*), 1,5-anhydro-D-fructose reductase (*akr1e2*), and fructosebisphosphate aldolase (*ald1*) gene expression were also down-regulated in ABL10-4 compared to MM, among other interesting genes (*Table D1*). These transcripts, which code for enzymes related to glycolysis and gluconeogenesis, are expected to be induced in feeding aphids (Chongtham *et al*., 2019; Sun & Li, 2021).

Other transcripts related to starch and sucrose metabolism -trehalase (*treh*), glycogenin-1 (*gyg1*), and glycogen synthase (*glyS*)- were also repressed in ABL10-4 aphids. A significant down-regulation of chitinase and fructose, 1,6-bisphosphatase genes has

been described in *Liphapis erysimi* under starvation (Chongtham *et al*., 2019). Gene expression analyses indicate that glycogen storage, gluconeogenesis and glycolytic processes took place normally in MM aphids, while in ABL10-4 were affected, underlying an inhibition or impairment in feeding due to the presence of type IV trichomes and their secretions.

In the same direction, the observed shifts in the expression of genes related to lipid metabolism point at aphid starvation on ABL10-4. In this sense, relevant genes of fatty acids biosynthesis, elongation and degradation pathways -acetyl-CoA carboxylase (*acc*), fatty acid synthase (*fasn*), acetyl-CoA acetyltransferase (*acat2*) genes were significantly repressed in aphids reared on ABL10-4 compared to MM.

In ABL10-4 aphids, genes related to the glycerophospholipid metabolism were down-regulated included *gpdh* (glycerol-3-phosphate dehydrogenase), *cisY1* and *cysY2* (citrate synthase 1 and 2, respectively), together with a decrease in the expression of aldehyde dehydrogenase (*aldh*), aldo–keto reductase *(akr1)*, diacylglycerol o-acyltransferase 2 (*dgat2*) and pancreatic triacylglycerol lipase (*pnlip*), which are involved in the glycerolipid metabolism. Furthermore, *ppap2a* (phosphatidic acid phosphatase 2A), involved in the synthesis of triacylglycerols (TAGs), was also down-regulated in ABL10-4 aphids. In insects, TAGs are stored in specialised lipid droplets, which are relevant for passive storage and also actively participate in fat and energy metabolism (Arrese & Soulages, 2010). Results also show concomitant repression of lipid storage droplet protein 1 (*LSD-1*) gene transcriptional activity, which underlies how the lipolytic machinery is compromised in the aphid. Lipids are known to play critical roles in energy homeostasis, membrane structure, and signalling. Triglycerides, along with glycogen and protein granules, are the major component of the lipid droplets and occupy most of the intracellular space in the fat body of insects (Dean *et al*., 1985). The level of reserves accumulated in the fat body modulates several essential aspects of the insect's life, such as the rate of insect growth, the timing of metamorphosis, and egg development (Mirth & Riddiford, 2007). The observed results at the transcriptomic level on carbohydrate, energy and lipid metabolism genes show the inhibition of the corresponding metabolic pathways and, consequently, point at the metabolic basis for the impairment observed

in feeding, growth, oviposition in aphids reared on ABL10-4 plants as hypothesized before.

D.6 Development

Hormonal control is essential for the coordination and regulation of many aspects of the developmental process of insects and under many circumstances can be related with the energy metabolism. Insects' endocrine system is one of the best studied, and the ecdysone genomic response has been largely characterised in model organisms such as *Drosophila spp*. or *Chironomus spp*. Moreover, the endocrine system is an important link between the target cell and the environment, and environmental factors are critical players in their regulation. For this reason, the ecdysone pathway's alterations were examined, a cascade of signals whose activation promotes developmental progression, molt and metamorphosis on insects (Henrich, 2005). A clear activation of the whole route on *M. euphorbiae* individuals reared on ABL 10-4 plants was detected. The relative expression of *ERR* (estrogen-related receptor) was significantly higher on aphids grown on ABL 10-4 plants. Furthermore, aphids exposed to ABL 10-4 plants showed a higher expression of the ecdysone receptor gene (*EcR*) compared to those in MM. When *EcR* gets activated it triggers early response genes but also late response ones. In this study *phm* gen (*cyp306a1*) was upregulated on aphids from ABL 10-4 plants, suggesting that ABL-10-4 secretions to modulate the synthesis levels of the ecdysone in aphids reared on this genotype. Data also showed the overexpression of the early response gene *E74* and a concomitant activation of genes at the end of the ecdysone related pathway: *HR4*, *HR38* and *ftz-f1* genes were all significantly upregulated on aphids from ABL 10-4 plants when compared with those from MM. All these data suggest that the upregulation of ecdysteroids receptors are related to higher levels of ecdysone in ABL 10-4 aphids. These final analyses reveal that, despite the major energetic impairment (*GAPDH* repression) because of the exposition to glandular trichomes type-IV and its associated secretions, *M. euphorbiae* individuals try to reproduce as the last survival option for the population (ecdysone pathway active). Which would be in concordance with the assertion of Powell and colleagues (2006) that the wingless adults invest more resources in reproduction.

Interestingly, the repression observed in genes related to carbohydrate, lipid and energy metabolism is accompanied by the up-regulation of developmental genes. Homeobox protein genes (*prospero (pros)*, *fork head domain-containing protein FD4 (fd96Ca)*, *dscam2*) were significantly induced in ABL10-4 aphids. Wing forms can be an induced adaptive response of aphids to environmental changes, with starvation being a well-known condition for inducing wing development in aphids (Chongtham *et al*., 2019, Hardie, 1986; Weisser & Braendle, 2001; Müller *et al.*, 2001). Wingless females feeding on deteriorating plant sources promoted the production of winged offspring in *Aphis craccivora* and *A. pisum* (Johnson, 1966; Harrewijn,1976; Sutherland, 1969ab). Wing development was an enriched (up-regulated) biological process in aphids exposed to glandular trichomes. Genes involved in wing development, some of them identified by Brisson *et al*.73 in *A. pisum* (i.e., *engrailed (en)*, *hedgehog (hh)*, *wnt-2*, *protocadherin-like wing polarity protein (stan)* and *cubitus interruptus (ci)*), were also induced in ABL10-4 reared aphids.

D.7 Oxidative stress response and detoxification

The effect of type IV glandular trichomes goes beyond changes in the energy and lipids metabolism and developmental changes (induction of winged morphs). However, it also implies changes in the capacity of aphids to deal with oxidative and toxic stress, which underlies the toxic character of the blend of secondary metabolites (mainly acylsucroses but also other metabolites) produced by glandular trichomes and affecting the aphids at a different level. The accumulation of ROS in the cells can involve potential toxicity since they can damage a variety of essential macromolecules such as nucleic acids, lipids and proteins. The evolution of the antioxidant defence system allows organisms to prevent oxidative damage caused by toxic substances and to maintain body homeostasis (Ighodaro & Akinloye, 2018). Detoxification enzymes are critical components of this system, and their genes are expected to respond to toxic chemicals exposure.

Response to oxidative stress was also down-regulated on aphids fed ABL10-4. Aphids reared in MM showed higher levels of oxidative stress-related transcripts, while these were compromised in ABL10-4 aphids. Some saliva genes protect aphids against reactive oxygen species (ROS) produced by plants after insect attack. Among them, catalases

(CATs) and peroxidases (Pxd) convert H_2O_2 into water and oxygen (DeJong *et al*., 2007), and glutathione S-transferases (GSTs) belongs to the Phase II biotransformation process and detoxifies secondary oxidation products generated from ROS (Hayes & McLellan, 1999). Activity levels of these enzymes in insects are believed to be crucial factors in determining their resistance to a broad spectrum of toxic chemicals (Després & Gallet, 2007). Comparisons of activities of genes coding for antioxidant enzymes in aphids reared on ABL10-4 and MM shed some light on how aphids respond to oxidative stress. Genes coding glucose dehydrogenase (*gld*), glucose oxidase (*gox*) and s-galactosidase (*glb*), associated with aphid Saliva (Carolan *et al*., 2011), were significantly repressed in ABL10-4 aphids. Trehalases, responsible for the hydrolysis of trehalose to glucose under stress conditions (Rao *et al*., 2013), are also associated with environmental stress, and were downregulated in ABL10-4 aphids. Peroxidase acts as an antioxidant enzyme, so peroxidase detected in aphids may protect aphids from oxidative stress caused by plant metabolites, detoxifying exposed organisms and playing an essential role in suppressing ROS production and ROS-induced plant defence responses (Carolan *et al*., 2011; Vandermoten *et al*., 2014). All these changes suggest that the capacity to respond to oxidative stress is compromised in aphids exposed to glandular trichomes. The lower gene transcriptional activities found in ABL10-4 aphids suggest that these responses pathways do not activate, maybe as a consequence of starvation. It has been described that plant genotype had a significant effect on aphid's choice. Data of no-choice assay performed under the same conditions described the preference of aphid choice for MM (70%) comparing to that of aphid choosing an ABL 10–4 plant (30%) (Besser *et al*., 2009). A clear effect of plant genotype on the selection rate was also observed under free-choice conditions, when aphids could choose either MM or ABL 10–4. Since aphids seem not to feed on ABL10-4 plants as regularly as on MM plants, they might not induce the secretion of detoxification proteins located in saliva to response to oxidative stress provoked by plants.

Detoxification systems are one of the different physiological pathways that may be affected by toxicants. To date, information about the molecular effects of natural plants defences and sensitive targets of exposure to these secretions in aphids is scarce. Cytochrome P450 monooxygenases (P450s, CYP3 and CYP4 clade) constitute essential

metabolic systems since they are involved in the oxidative detoxification of plant secondary metabolites and synthetic insecticides (Feyereisen, 2005). Our findings show a differential response of cytochrome P450 genes to the secretions of ABL10-4 plants. The decrease of some cytochromes (*cyp4g15*, *cyp4c1* and *cyp6k1-like*) could be caused by oxidative stress. The up-regulation of the gene coding for cytochrome *cyp6a13-like* could also be involved in the biotransformation response of aphids exposed to the trichome secretions of ABL10-4 plants. The enhanced transcriptional activities of genes coding cytochrome P450 monooxygenases conferring insecticide resistance have been well described in insects such as *N. lugens* (Zhang *et al*., 2016), *B. tabaci* (Karunker *et al*., 2008; 2009) and *M. persicae* (Puinean *et al*., 2010). However, to date, no data about detoxification genes and their role in insect response to plant defences have been reported in *M. euphorbiae*. Recently, it has been described that the suppression of some CYP genes increased the sensitivity of resistant *A. gossypii* to pesticide (Wu *et al*., 2018). In this sense, the repression of the expression of most of the CYP genes analysed in *M. euphorbiae* exposed to ABL10-4 could be related to the higher sensitivity of individuals to this plant tomato genotype and the significant changes observed at the physiological/behaviour level.

Regarding GST, it is an abundant antioxidant involved in the detoxification of many xenobiotics. GST catalyses the transformation of electrophilic compounds to less toxic substances, by conjugating them to GSH (Monteiro *et al*., 2010). The increased expression of genes encoding glutathione-S-transferase (*GST*), superoxide dismutase (*SOD*), and glutathione peroxidase (*GPx*) might be due to antioxidation defence and detoxification mechanisms. GPx is important in decomposing peroxide and protecting the integrity of the structure and function of the membrane by eliminating the harmful metabolite peroxide and interrupting the chain reaction of lipid peroxidation (Ighodaro & Akinloye,2018). SOD helps to facilitate the transformation of superoxide anion radicals to hydrogen peroxide (H2O2). The increased SOD activity in the *M. euphorbiae* might be a compensation mechanism against secondary metabolite intoxication from trichomes. Moreover, some aphid effectors have been identified having a GST and SOD activities and whilst GSTs have previously been mainly associated with resistance to insecticides, the findings described here underpin the role of GSTs, SOD and GPx in manipulating

plant defences when delivered into the plant (Kettles & Kaloshian, 2016; MacWilliams *et al*., 2020).

All these results underly the key adaptations and metabolic trade-offs in aphids exposed to glandular trichomes, revealing central processes and mechanisms to understand the adaptation of aphids to plants. The transcriptome generated represents a valuable genomic resource for screening potential gene targets in *M. euphorbiae.* Fourteen novel genes related to detoxification mechanisms and oxidative stress were de novo identified and showed differential expression in *M. euphorbiae*, proving to be effective potential biomarkers at the molecular level to better understand the physiological response of aphids to trichome secretions and plant defences. Furthermore, the observed differences in the transcriptome between the aphids reared in the two tomato lines give insights into the mechanisms responsible for the resistance mediated by glandular trichomes in tomato. Starvation together with the effect of glandular trichomes (and acylsucroses) results in the repression of genes related to carbohydrate and lipid metabolism, genes involved in response to oxidative stress genes, the induction of developmental genes responsible for the appearance of winged morphs, together with important effects in detoxification pathways. Our results demonstrate the detrimental effect of glandular trichomes (type IV) on the aphid and put forward their mode of action. Here we show that conserved biomarkers (fundamental for arthropod survival) are affected by glandular trichomes and their exudates and consequently other functional groups (e.g., pollinators or natural enemies of insect pests) could also experience similar responses when exposed to these defensive structures. The implications of our study go therefore beyond plant-aphid interactions but have consequences in our understanding of plant defences and the functional and ecological basis of sustainable pest management.

E Future perspectives

The experimental assays and genetic studies carried out in this dissertation with the selected tomato lines and the aphid *M. euphorbiae* contribute to a better understanding not only of the mechanisms of plant resistance modulated by glandular trichomes but also have implications in the implementation of future management strategies of insect pests.

The mechanistic complexities of insect-plant interactions also encompass the interaction with the natural enemies of the herbivores, parasitoids and predators. Consequently, in order to complete the study of the tritrophic interaction, it would be also necessary to asses the effect of type IV glandular trichomes on the natural enemies of the aphids. It has been described that nonglandular trichomes may impede the searching behaviour of parasitoids and predators while glandular trichomes may reduce predator mobility due to the entrapment of the sticky substances secreted (reviewed in Kennedy, 2003), nevertheless, the potential toxicity of glandular trichomes type IV' secretions on natural enemies has not been described. Previous studies with other pests like mites show contradictory results in terms of the negative or positive impact of the glandular trichomes on their predators (Loughner *et al*., 2010; van Houten *et al.,* 2013) which is a crucial knowledge fot the biological control of the pest. In this sense, if new strategies based on biological control are projected to be developed regarding tomato plants and *M. euphorbiae* it is esentiall to understand how the glandular trichomes type IV and their secretions affect the natural enemies of the aphid.

Following the idea of developing new management strategies that are more sustainable, it would be also adequate to focus on the effect of the JA in the increase of the trichome type IV density and their production of acylsucoses, which posses a clearly detrimental impact on the aphid *M. euphorbiae*. From this perspective, any mechanism to promote the activation of this route on the plant, via natural or artificial means (e.g application of synthetic elicitors of the response such as MeJA) would lead to feasible alternatives to increase pest resistance in tomato.

Since this works presents a turning point over the role of the JA in the in the defence of tomato plants against aphids, further experiments could be conducted to support what

has been raised. The transgenic tomato (*Solanum lycopersicum*) plants that overexpress the Prosystemin gene (35S::PS) constitutively accumulates high levels of PIs throughout the entire plant (McGurl *et al.*, 1994) and exhibit an enhanced resistance to caterpillar herbivory (Chen *et al*., 2005). However, no studies have been conducted with aphids and therefore, becomes a suitable candidate to work with in further studies.

Glandular trichomes (type IV) are also occurring in other members of the Solanaceae (Fan *et al*., 2019) and therefore, the mode of action on glandular trichomes described in this article are potentially operating in other (related) plant species (Cucurbitaceae, Pteridaceae, Lamiaceae, Solanaceae, Asteraceae and Cannabaceae among others; Tissier, 2012). The mechanisms by which the exposure to glandular trichomes affect the physiology of the insects are crucial to unravel the complexities of plant-insect interactions. Given the prevalence some of the investigated molecular biomarkers in insects in general, our results provide relevant mechanisms to understand the effect of trichomes not only on herbivorous insects but also on other trophic levels. Furthermore, this *de novo* transcriptome provides a valuable resource for future molecular analysis and assemblies of closely related species.

Additionally, this work leaves some open questions regarding the specific content of glandular trichomes and their role on the plant defence response, which can be addressed in future research. It has been showed that other trichomes present in wild species, like type VI in *S. habrochaites*, proven to be very resistant against different pests (Kennedy, 2003), seems to have also a very detrimental effect on *M. euphorbiae*. Therefore, a complete screening for potential toxicity of glandular trichome type IV secretions would promote the identification of chemical compunds of interest, that in turn, could also be evaluated on the aphid physiology (using the described biomarkers). The LC-MS is a technique that would allow to identify and measure the different metabolites present on leave samples, for example from glandular trichomes type IV (to complete this study) and also glandular trichomes type VI, since these two has been described as the predominant trichomes associated with negative effect on pests (Kennedy, 2003).

In relation to the behaviour of *M. euphorbiae*, some EPG (Electrical Penetration Graph) analysis would unequivocally indicate if the aphid is feeding from the phloem and

therefore, support or refute the the hyphotesis we propose that the starvation is due to glandular trichomes type IV secretions. EPG involves making the organism a part of an electrical circuit, and measuring changes in voltage that result during feeding (Walker & Backus, 2000).

Finally, to validate the results from the selection assays, some choice experiment could be conducted in an olfactomer, so entire plants can be used, and therefore, problems derived from using a leaflet cut ore derived from the odor mix from both genotypes would not appear.

This study shows the principal role of the glandular trichome type IV in the defence response of tomato plants against the aphid *M. euphorbiae*. Which may place this natural defence as the target for future breeding strategies that look for an enhanced resistance of tomato plants against the potato aphid.

Table D1. **Main biological functions associated with relevant differentially expressed (DE) genes.** Summary of results of differential expression profile in aphids reared ABL10-4 and MM tomato plants. Table shows relevant DE genes from enriched pathways in aphids reared on ABL10-4 compared to those reared on MM.

Differential expression profile in aphids (ABL10-4 vs MM)			
Pathway		Gene	Expression
Carbohydrate metabolism	Glycolysis / gluconeogenesis	*idgf* *fbp* *pfrx* *akr1e2* *ald1*	↓
	Starch / sucrose metabolism	*treh* *gyg1* *glyS*	↓
Lipid metabolism		*acc* *fasn* *acat2* *lsd-1*	↓
Glycerolipid metabolism		*gpdh* *cisy1/cys2* *aldh* *akr1* *dgat2* *pnlip*	↓
Hormone metabolism	Ecdysteroid biosynthesis	*cyp18a1* *cyp305a1* *shd* *dib*	↑
Immune system		*pxn* *PPO2*	↓
Rhythmic process		*clock* *vri*	↓
Detoxification / oxidative stress		*cyp6a14* *cyp4g15* *cyp6k1-like* *cyp4c1* *srx*	↓
		cyp6a13-like *Cu-Zn SOD-like* *GST* *GPx*	↑
Growth / development		*pxt* *gld* *pros* *fd96Ca* *dscam2*	↑
Wing development		*en* *hh* *wnt-2* *stan* *ci*	↑
Transport	Sugar transport	*ugt36A1/Dot* tre12 tret1	↓
	ABC transporter / multidrug resistance	L259 mdr1A ABCB6 ABDG23	↓

CONCLUSIONS

VI. CONCLUSIONS

1. The defence response of tomato plants against aphids and caterpillars vary among the compared genotypes but this was not phylogenetically structured, nor related with their degree of domestication. Nevertheless, overall, wild and early domesticated cultivars of *S. lycopersicum* show more resistance to aphids and a greater tolerance to caterpillars than modern-bred varieties.

2. Both the presence of glandular trichome IV in tomato accessions and their principal secretions, acylsucroses, mediate the resistance in tomato plants against the potato aphid *M. euphorbiae* by repelling the insect and impairing its population built up.

3. The previous attack of *Spodoptera littoralis* on tomato plants elicits a defence response mediated by JA, resulting in increased resistance against *M. euphorbiae* during subsequent attacks. Furthermore, the improved multiplication of the aphid observed in genotypes with impaired JA pathways prompts a reassessment of the presumed role of JA in regulating the interaction between this aphid and tomato plants.

4. The resistance mediated by type IV trichomes against aphids is effective regardless of the type of previous infestation.

5. The transcriptome of *M. euphorbiae,* generated for the first time, represents a valuable genomic resource to identify potential molecular biomarkers involved in the main regulatory mechanisms of the aphid's physiology in its interaction with plants. Which facilitates further research on basic aphid biology and the development of new techniques for biological control.

6. *M. euphorbiae* exposure to glandular trichome type-IV seccretions has revealed a unique and distinct gene expression profile resulting from changes in detoxification mechanisms, cell stress response, ecdysone pathway and energy metabolism. Fourteen novel genes were identified as strong biomarkers for these principal routes affected in the tomato-aphid interaction.

7. Starvation, in combination with the toxic effects of acylsucrose secretions of glandular trichomes type IV lead to a stress-related response in *M. euphorbiae* responsible of the collapse of the population in ABL 10-4 plants. Which proves that the role of glandular trichomes in plant defence goes beyond the physical impairment of insect activity.

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SUPPLEMENTARY MATERIAL

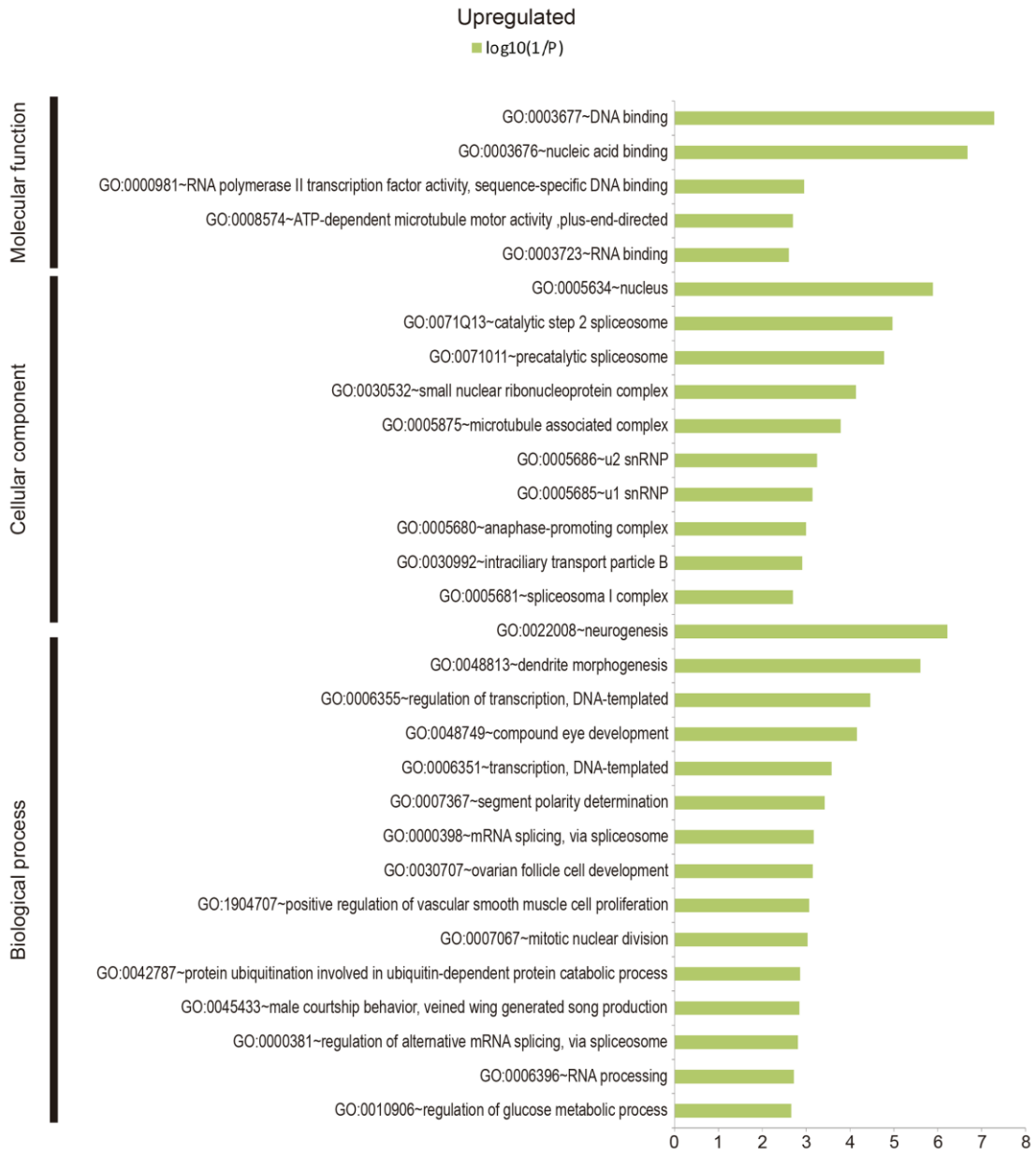

Fig. S1. **Enriched GO Terms in up-regulated genes (ABL10-4 aphids vs MM aphids).** GO enrichment analysis obtained with differentially up-regulated genes (DEGs). The results were summarized in three major categories, including biological process, cellular component, and molecular function. Graphics represent the significantly enriched (FDR 5%) GO terms obtained from up-regulated DEGs in ABL 10-4 aphids when compared to MM aphids. P values are represented as log10(1/P) to facilitate visualization.

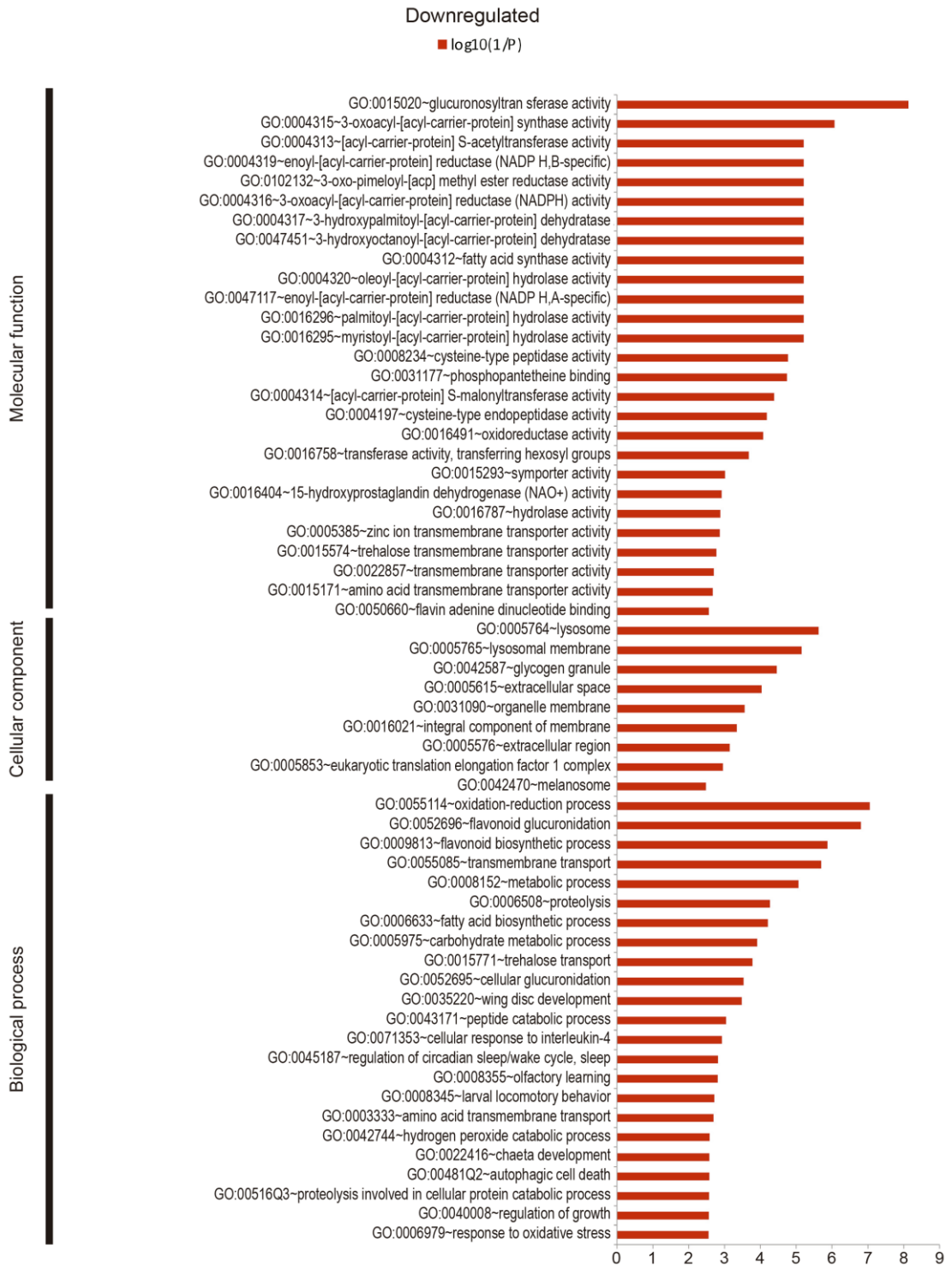

Fig. S2. **Enriched GO Terms in down-regulated genes (ABL10-4 aphids vs MM aphids).** GO enrichment analysis obtained with differentially down-regulated genes (DEGs). The results were summarized in three major categories, including biological process, cellular component, and molecular function. Graphics represent the significantly enriched (FDR 5%) GO terms obtained from down-regulated DEGs, in ABL 10-4 aphids when compared to MM aphids. *P-values* are represented as log10(1/P) to facilitate visualization.

Table S1. **Descriptors of the *Solanum sp* accessions used in this study**. Common name, country and locality of origin, type of plant growth and inflorescences (Infloresc.), fruit characteristics, pericarp colour and seed weight (of 1000 seeds) are included. Classification of accessions according to their degree of domestication is also included; from less domesticated to more domesticated: wild, early-domesticated, local, cultivar. Abbreviations: *S. habro*, *S. habrochaites*; *S. pim, S. pimpinellifolium*; *S. lyc cera*, *S. lycopersicum* var. cerasiforme; *S. lyc, S. lycopersicum*; Early-dom, early-domesticated; Indet, interdeterminate; Deter, determinate.

Accession no.	Species	Common name	Type	Country	Growth	Fruit width [mm]	Fruit length [mm]	Fruit weight [g]	Shape	Transversal section	Pericarp colour
NR0136	*S. habro*	PI134418	Wild	Ecuador	Indet			8			Green
NR0937	*S. pim*	TO 93715	Wild	Peru	Indet	13.3	12.7	1.5	Lobulate-irregular	Round	Red
NR1021	*S. pim*	LA1589	Wild								
NR0275	*S. lyc cera*	Mex 89	Early-dom	Mexico	Indet	18	17.5	4.4	Slightly flattened	Round	Red
NR0407	*S. lyc cera*	PE-55	Early-dom								
NR0006	*S. lyc*	Kalohi	Local	Nigeria	Deter	68.8	56.3	170	Slightly flattened	Round	Red
NR0025	*S. lyc*	Melillero	Local	Spain	Indet	72.8	63.4	156	Slightly flattened	Round- Angular	Red
NR0027	*S. lyc*	Muchamiel	Local								
NR0030	*S. lyc*	Valenciano	Local	Spain	Indet	71	73.5	162	Round	Round- Angular	Red
NR0044	*S. lyc*	Flor Baladre	Local	Spain	Indet	65.1	57.2	151.3	Slightly flattened	Round	Pinkish
NR0063	*S. lyc*	Marmande	Local	France	Indet	95.8	61.5	364.3	Flattened	Irregular	Red
NR0071	*S. lyc*	San Marzano	Local	Italy	Indet			59			
NR0166	*S. lyc*	Moruno	Local	Spain	Indet			166			
NR0432	*S. lyc*	Quicena	Local	Spain	Indet	89.5	56.9	280.4	Flattened	Round	Red
NR0504	*S. lyc*	Cazorla	Local	Spain	Indet	107.2	79.9	613.6	Round-lobulate	Irregular	Red
NR0561	*S. lyc*	H. de Toro	Local	Spain	Indet	93.8	70.4	303.8	Slightly flattened	Lobulate	Red
NR0612	*S. lyc*	De Penjar	Local	Spain	Indet	46	52	37	Round-flattened	Lobulate	Orange
NR0711	*S. lyc*	Pontevedra	Local	Spain							
NR1080	*S. lyc*	Periana	Local								
ABL104	*S. lyc*	ABL 10-4	Cultivar								
NR0080	*S. lyc*	Moneymaker	Cultivar	Spain (?)	Indet	63	51	126	Round	Round	Red
NR0705	*S. lyc*	Edkawi	Cultivar	Egipt	Indet	94.2	59.7	244.3	Flattened	Irregular	Red
NR0816	*S. lyc*	Monita	Cultivar	France	Indet	60	50	107	Round	Round	Red

Table S2. **Fixed effects of the Bayesian analyses of generalized linear multilevel models.** Fixed effects of the Bayesian analyses of generalized linear multilevel models relating biomass with the interaction between treatment and type of tomato, the number of aphids and caterpillar's weight gain. Estimates are presented with 95% higher posterior density interval (95% HPD).

		POSTERIOR MEAN	L-95% CI	U-95% CI	PMCMC
BIOMASS	(Intercept)	1.26	0.845	1.793	**0.004**
	Aphid	-0.239	-0.39	-0.067	**0.006**
	Caterpillar	-0.6	-0.984	-0.263	**0.002**
	Early-domesticated	0.105	-0.435	0.535	0.63
	Local	0.265	-0.096	0.63	0.146
	Cultivar	0.318	-0.128	0.78	0.162
	Aphid: Early-domesticated	0.104	-0.134	0.362	0.392
	Caterpillar: Early-domesticated	-0.396	-0.988	0.143	0.168
	Aphid:Local	0.046	-0.15	0.223	0.604
	Caterpillar:Local	-0.586	-0.97	-0.192	**0.008**
	Aphid:Cultivar	-0.01	-0.254	0.218	0.926
	Caterpillar:Cultivar	-0.632	-1.107	-0.121	**0.016**
APHIDS	(Intercept)	-0.773	-1.563	-0.028	0.048
	Early-domesticated	0.343	-0.92	1.443	0.52
	Local	0.904	0.16	1.72	**0.022**
	Cultivar	0.689	-0.402	1.528	0.164
	ln_Biom	0.052	-0.221	0.361	0.738
CATERPILLARS	(Intercept)	-0.687	-1.217	-0.14	0.02
	Early-domesticated	1.255	0.522	2.029	**<0.001**
	Local	0.493	-0.108	1.002	0.082
	Cultivar	0.186	-0.606	0.816	0.586

***Table S3*. Descriptive values for plant biomass under different herbivore treatments, and herbivores performance.** Plant biomass under different herbivore treatments, number of aphids (N Aphids) in the plants and caterpillars' weight gained (*Spodoptera* weight gain) in trial. Values are given as mean and standard error. N, number of plants treated. S. lyc, *Solanum lycopersicum*; S habro, *S. habrochaites (glabatum);* S. pim, *S. pimpinellifolium*; S. lyc cer, *S. lycopersicum var cerasiforme*.

		Control		Spodoptera				Aphids		
Accession	**Common name**	**N**	**Biomass**	**N**	**Survival**	**Biomass**	**Spodoptera weight gain**	**N**	**Biomass**	**N Aphids**
ABL104	ABL 10-4	15	3.708±0.371	5	0.33	1.034±0.845	0.147±0.072	15	3.129±0.298	32.857±5.848
NR0006	Kalohi	15	3.901±0.355	5	0.33	0.858±0.835	0.239±0.086	15	3.304±0.381	81.800±16.117
NR0025	Melillero	15	4.624±0.363	4	0.27	0.308±0.278	0.121±0.038	15	3.264±0.219	83.767±16.294
NR0027	Muchamiel	15	4.514±0.461	4	0.27	0.035±0.022	0.182±0.034	15	4.097±0.462	46.786±6.038
NR0030	Valenciano	15	2.129±0.290		0.00			15	1.482±0.188	93.200±15.432
NR0044	Flor de Baladre	15	3.818±0.239	9	0.60	0.772±0.361	0.223±0.028	15	3.087±0.178	67.600±8.974
NR0063	Marmande	15	4.065±0.314	6	0.40	0.445±0.180	0.180±0.039	15	2.905±0.298	62.600±9.607
NR0071	San Marzano	15	4.141±0.393	9	0.60	0.117±0.047	0.111±0.016	15	3.274±0.190	75.933±11.032
NR0080	Moneymaker	14	4.077±0.420	7	0.47	1.264±0.772	0.167±0.034	15	3.235±0.398	74.786±10.850
NR0136	PI134418	15	1.437±0.121	2	0.13			15	0.929±0.116	5.714±1.758
NR0166	Moruno	15	4.471±0.329	10	0.67	0.543±0.268	0.303±0.100	15	4.293±0.281	95.467±17.031
NR0275	Mex 89	15	2.328±0.285	2	0.13			15	2.125±0.251	28.400±5.319
NR0407	PE-55	15	3.981±0.370	11	0.73	0.846±0.487	0.424±0.129	13	3.143±0.338	64.571±10.676
NR0432	Rosa de Huesca	15	5.228±0.311	4	0.27	0.253±0.187	0.185±0.049	15	3.607±0.439	72.067±12.197
NR0504	Cazorla	15	3.881±0.349	5	0.33	1.432±0.736	0.309±0.075	16	3.013±0.340	77.067±12.028
ANL101	ANL101 - Huevo Toro	15	3.997±0.372	7	0.47	0.646±0.422	0.204±0.099	15	3.659±0.417	70.643±13.404
NR0612	De Penjar	15	3.718±0.358	7	0.47	0.841±0.539	0.244±0.063	14	2.959±0.132	52.692±9.271
NR0705	Edkawi	15	4.746±0.711	9	0.60	0.762±0.398	0.192±0.042	15	2.555±0.465	66.833±9.463
NR0711	Pontevedra	14	3.001±0.287	4	0.27	0.370±0.133	0.422±0.014	15	2.035±0.226	97.857±14.889
NR0816	Monita	15	4.286±0.467	11	0.87	0.125±0.073	0.221±0.027	15	2.423±0.264	86.533±14.926
NR0937	TO 93715	15	4.589±0.350	13	0.80	1.593±0.452	0.195±0.026	15	3.951±0.286	68.600±12.164
NR1021	LA1589	15	2.815±0.510	12	0.27	1.058±0.281	0.168±0.039	15	1.512±0.258	77.929±8.268

Table S4 **Summary of *M. euphorbiae* transcriptome assembly**.

Sample	Merge	
	All transcript contigs	Only longest isoform per 'gene'
Total trinity 'genes'	189,229	189,229
Total trinity transcripts	240,067	189,229
Percent GC	40.28	40.99
N90	244	235
N80	308	284
N70	405	356
N60	550	464
N50	771	635
N40	1,100	912
N30	1,593	1,357
N20	2,247	2,059
N10	3,227	3,105
Maximum contig length	15,848	15,848
Minimum contig length	201	201
Average contig length	563.74	513.03
Total assembled bases	135,334,529	97,079,564

Table S5 **. Species, accession number, length and identity of amino acid sequences used in BLAST analysis.**

	Specie	Accession number	ORF length (aa)	Identity (%)
EcR	*Macrosiphum euphorbiae*	MT157253	539	-
	Acyrthosiphon pisum	NP_001152832.1	539	99%
	Myzus persicae	XP_022175072.1	540	99%
FTZ-F1	*Macrosiphum euphorbiae*	MT157250	712	-
	Acyrthosiphon pisum	XP_001947027.2	712	99%
	Myzus persicae	XP_022165907.1	712	98%
Hsp70	*Macrosiphum euphorbiae*	MT157251	640	-
	Acyrthosiphon pisum	XP_001945786.2	637	97%
	Myzus persicae	XP_022167905.1	640	97%
GAPDH	*Macrosiphum euphorbiae*	MT157252	332	-
	Acyrthosiphon pisum	NP_001280403.1	332	99%
	Myzus persicae	XP_022171133.1	332	99%
E74	*Macrosiphum euphorbiae*	MT157254	286	-
	Acyrthosiphon pisum	XP_029347438.1	597	96%
	Myzus persicae	XP_022170040.1	574	97%
HR38	*Macrosiphum euphorbiae*	MT157256	474	-
	Acyrthosiphon pisum	XP_029345431.1	751	98%
	Myzus persicae	XP_022165760.1	753	98%
HR4	*Macrosiphum euphorbiae*	MT157255	510	-
	Acyrthosiphon pisum	XP_008182814.1	946	99%
	Myzus persicae	XP_022179977.1	944	99%

***Table S6 De novo* characterised *M. euphorbiae* genes related to biotransformation and detoxification.** Gene name, accession number, ORF and protein lengths of the *de novo* characterised *M. euphorbiae* genes as well as % of identity to closest species on databases.

Gene	Accession number	ORF length	Protein length	% identity
cyp4g15	MT105339	1701 bp	566 aa	99% (*Acyrthosiphon pisum*)
cyp6a13-like	MT105340	1122 bp	373 aa (Incomplete)	97% (*Acyrthosiphon pisum*)
cyp6k1-like	MT105341	299 bp	99 aa (Incomplete)	91% (*Acyrthosiphon pisum*)
cyp4c1	MT105342	630 bp	209 aa (Incomplete)	98% (*Acyrthosiphon pisum*)
Cu-Zn SOD-like	MT105343	465 bp	154 aa	63% (*Macconellicocus hirsutus*)
GST	MT105344	629 bp	199 aa (Incomplete)	76% (*Aedes aegypti*)
GPx	MT105345	454 bp	150 aa (Incomplete)	*53%* (*Melanaphis sacchari*)

Table S7. **List of differentially expressed CYP transcripts detected by RNA-SEq between aphids exposed to ABL 10-4 and MM tomato plants**.

RNA-seq (ABL10-4 vs MM)			
Contig ID	**Gene identification**	**Foldchange (Fc)**	**P-value**
c234766_g1_i1	*Cyp4c1*	-9,0422372	0,00034929
c254058_g1_i1	*Cyp4g15*	-7,3997128	0,00405308
c300320_g1_i3	*Cyp6a13-like*	2,90562582	0,0301665
c307953_g1_i2	*Cyp6k1-like*	-12,456248	0,00014581

RESUMEN EXTENDIDO EN ESPAÑOL

ANNEX 2 – RESUMEN EXTENDIDO EN ESPAÑOL

Efecto de los tricomas glandulares tipo IV en la resistencia del tomate frente al pulgón *Macrosiphum euphorbiae*

La agricultura es imprescindible en una sociedad en la que se espera que el crecimiento exponencial y los cambios de dieta que experimenta la población aumenten la demanda de alimentos en los próximos años. Actualmente, una de las mayores amenazas para la producción agrícola son las plagas de insectos. Éstos provocan grandes pérdidas en los principales cultivos para el consumo humano a través del daño directo que infligen en las plantas, pero también de forma indirecta al transmitir diversas enfermedades de las que son portadores. Tradicionalmente, e incluso a día de hoy en muchas partes del mundo, el control de plagas en cultivos se ha basado en el uso de pesticidas químicos, lo que ha supuesto la aparición de importantes problemas, por un lado, de resistencia en los herbívoros y, por otro, de salud pública. Acrecentando de este modo la preocupación en la sociedad y la aparición de políticas que limitan su uso. En este contexto, uno de los grandes retos de la agricultura del siglo XXI es controlar el impacto de las plagas sobre los cultivos, pero desarrollando métodos de producción más eficientes y sostenibles. Para lograr esto, es fundamental contar con un sólido conocimiento de la interacción planta insecto y de los mecanismos de defensa que operan.

Las plantas, al ser organismos sésiles, sufren constantemente ataques de diversas plagas y patógenos. Sin embargo, han ido evolucionando diferentes mecanismos que les permiten reconocer los distintos estreses bióticos a los que se enfrentan y activar a su vez vías complejas de señalización (generalmente mediadas por hormonas) que originan respuestas adaptativas precisas frente a estas amenazas. Aquí, cobran especial relevancia las defensas inducidas, que son aquellas que aparecen en respuesta a un ataque y que están reguladas principalmente por las fitohormonas ácido jasmónico (JA) y ácido salicílico (SA). Está descrito que, dependiendo del modo de alimentación del herbívoro y del grado de daño tisular que causa, la vía de señalización y genes que se activan tras el ataque, serán una u otra y así la resistencia inducida. Generalmente se asume que los herbívoros masticadores, como las orugas, conducen a una inducción de

vías defensivas reguladas principalmente por JA, mientras que la señalización de SA participa principalmente en la defensa contra herbívoros perforadores-chupadores que se alimentan del floema y causan menos daño en la hoja, como los pulgones y las moscas blancas. En concreto, la ruta de respuesta a daño mecánico mediada por jasmonatos en plantas de tomate ha sido especialmente estudiada por su importancia en la defensa frente a herbívoros que infligen distintos tipos de daño tisular. De forma muy resumida, la síntesis de JA activa la expresión de un subconjunto de genes diana entre los que destacan los inhibidores de proteinasas defensivas (*PIN*s) que bloquean las enzimas digestivas de los insectos.

Para evitar movilizar recursos a una defensa que podría no necesitarse, las plantas cuentan con estrategias que permiten ajustar su grado de resistencia en función de las necesidades. En este sentido, cuando son atacadas, alcanzan un estado de defensa mejorado que se denomina *priming* y desde el cual pueden responder con una activación más rápida y más potente de sus defensas frente a ataques posteriores. Como consecuencia, estas defensas inducidas pueden afectar, tanto a otros herbívoros que están alimentándose en la misma planta, como a aquellos que llegan después. Diversos estudios han demostrado que los ataques secuenciales de herbívoros funcionalmente distintos (*p. ej*. succionadores y masticadores) desencadenan diferentes vías de señalización que pueden dar lugar a una interacción entre ellas. Esto supone que los primeros atacantes pueden determinar la magnitud y el signo de los efectos sobre los siguientes. Sin embargo, este tipo de interacciones no están bien caracterizadas y se requieren más estudios.

Actualmente, uno de los modelos científicos más interesantes para los estudios planta-insecto es el tomate. Por un lado, debido a su relevancia agrícola, al tratarse de uno de los cultivos más importantes a nivel mundial y, por otro, gracias a su fácil manejo y características específicas que han permitido un desarrollo importante en la biotecnología, biología molecular y mejora genética de este cultivo, favoreciendo los distintos tipos de estudios que pueden llevarse a cabo.

El tomate durante años se ha seleccionado de forma artificial primando aquellas características beneficiosas para el consumo humano. Sin embargo, este proceso de selección ha conllevado una pérdida involuntaria genes y rasgos defensivos contra

plagas y enfermedades. Por este motivo, los rasgos de defensa presentes en tomates silvestres se han convertido en diana de números estudios y técnicas que permiten mejorar la protección de cultivos susceptibles. De entre estos rasgos imprescindibles para la defensa frente a herbívoros destacan los tricomas glandulares. Se trata de pelos epidérmicos que aparecen en la superficie del tomate con glándulas capaces de producir y segregar compuestos con efecto nocivo para los insectos. De los siete tipos descritos en tomate (I-VII), el IV y el VI son los que principalmente se relacionan con efectos negativos en plagas y, por tanto, son más relevantes para estudios de defensa en plantas.

Los pulgones son una de las plagas comunes del tomate, y en concreto, *M. euphorbiae* es un pulgón bien adaptado a las solanáceas que no sólo causa daño directo en la planta, sino que además es vector de numerosas enfermedades virales. Para mantenerse alimentándose del floema, los pulgones necesitan superar la batería de respuestas de defensa de las plantas que se activan. Algunas estrategias como evadir las primeras respuestas tratando de pasar inadvertidos o reprogramar los mecanismos de señalización, han sido descritos. Sin embargo, el conocimiento sobre los cambios fisiológicos que sufre el insecto cuando está expuesto a las defensas vegetales sigue siendo limitado. La exposición a agentes biocidas como pueden ser los metabolitos secundarios de las plantas, desencadena respuestas biológicas en los insectos que resultan en alteraciones en los perfiles transcriptómicos. Estos cambios en la expresión de genes juegan un papel relevante en su adaptación fisiológica al medio vegetal y, en última instancia, su supervivencia. Sin embargo, hasta la fecha, la información disponible sobre biomarcadores moleculares en insectos fitófagos expuestos a metabolitos secundarios de plantas hospedadoras es limitada. Además, la falta de un transcriptoma descrito para el pulgón *M. euphorbiae* dificulta el cribado de otras posibles dianas génicas implicadas en rutas críticas para la supervivencia del insecto. Estas alteraciones no sólo implicarían su papel clave en la adaptación fisiológica de *M. euphorbiae* a las plantas de tomate, sino que también proporcionarían información sobre los efectos más sutiles de las defensas vegetales sobre el organismo del pulgón.

El trabajo de esta tesis se centra en el estudio de la interacción entre el tomate *Solanum lycopersicum*, uno de los cultivos más importantes a escala mundial y una de sus plagas

más *frecuentes*, el pulgón *Macrosiphum euphorbiae*, a través del estudio del papel que desempeñan los tricomas glandulares tipo IV. Este objetivo principal se subdivide en los siguientes objetivos específicos: (1) Evaluar el impacto de la domesticación en la resistencia y la tolerancia del tomate frente a herbívoros con modos de alimentación diferentes. (2) Determinar el efecto de los tricomas glandulares tipo IV y su producción de acilsacarosas en el comportamiento de *M. euphorbiae*. (3) Identificar las rutas de señalización implicadas en el mecanismo de defensa inducido contra *M. euphorbiae*. (4) Analizar los efectos de las secreciones de los tricomas glandulares tipo IV en *M. euphorbiae* mediante un estudio transcriptional.

De acuerdo a los objetivos planteados, los resultados del trabajo quedan agrupados en cuatro secciones que se corresponden con cada uno de los objetivos planteados.

En relación al primer objetivo, una hipótesis recurrente que explica la elevada susceptibilidad de muchos cultivos a plagas y enfermedades es que, en el proceso de domesticación, los cultivos han perdido genes y rasgos defensivos contra plagas y enfermedades. La teoría ecológica predice procesos de compensación (o *trade-offs*) por los que la resistencia y la tolerancia frente a herbívoros pueden ir uno a costa del otro. Para evaluar experimentalmente si la resistencia y la tolerancia frente a diferentes herbívoros estaba estructurada filogenéticamente o variaba según el grado de domesticación, se seleccionaron 23 genotipos de tomate (*S. lycopersicum*) que fueron agrupados según su grado de domesticación en: variedades silvestres, variedades primitivas (de domesticación temprana), variedades locales tradicionales y cultivos. Los diferentes genotipos fueron expuestos a pulgones *M. euphorbiae* (herbívoro succionador) y larvas de *Spodoptera littoralis* (herbívoro masticador), y mediante genotipado por secuenciación se reconstruyeron las relaciones filogenéticas de los genotipos de tomate usados. Los resultados mostraron que, en términos de resistencia, las diferencias observadas entre los genotipos no estaban relacionadas con el grado de domesticación ni con el parentesco genético, pudiendo ser la variación entre especies igual o mayor que la que se encuentra dentro de una misma especie. Lo que pone de manifiesto una base genética compleja de la resistencia e indica que los rasgos de resistencia aparecieron en diferentes etapas y en linajes genéticos no relacionados. Aun así, las variedades silvestres y las primitivas mostraron una mayor resistencia a los

pulgones que los cultivos actuales. Por otro lado, con respecto a la tolerancia, las variedades silvestres y primitivas presentaron una mayor tolerancia frente a las orugas en comparación con las autóctonas y los cultivos. En la comparación global entre genotipos se observó que aquellos con tricomas glandulares tipo IV parecían ser más resistentes a *M. euphorbiae.* Aunque se sabe que los tricomas glandulares son esenciales en la defensa de las plantas contra las plagas, no se conoce completamente su mecanismo de acción. Por este motivo, en el segundo objetivo, se abordó en mayor profundidad el efecto de los tricomas glandulares tipo IV sobre la actividad del pulgón.

Para ello, se realizaron comparaciones entre dos líneas isogénicas de tomate, es decir, con un fondo genético compartido, y que sólo difieren en la presencia de tricomas glandulares de tipo IV. De manera que las diferencias encontradas sólo podían ser vinculadas a la presencia de esta defensa inducida. Estas líneas seleccionadas fueron la variedad comercial *S. lycopersicum* cv. 'Moneymaker' (MM) y una línea introgresada de *S. pimpinellifolium* con tricomas tipo IV (ABL 10-4).

El estudio morfológico de la superficie foliar mostró que mientras el tricoma predominante en MM era un tricoma no glandular, en la superficie de ABL 10-4 predominaban los tricomas glandulares tipo IV. Presencia que además estaba relacionada con un aumento muy significativo en la producción de acilsacarosas, lo que señala a los tricomas glandulares tipo IV como los principales productores de estos compuestos. Asimismo, se observó que la presencia de esta defensa inducida afectaba a la selección de hospedador por parte de los pulgones y también a la proliferación de los mismos. Ensayos de preferencia pusieron de manifiesto que los pulgones eran atraídos por MM, el genotipo comercial sin tricomas glandulares tipo IV, y repelidos por ABL 10-4 (con tricomas glandulares IV). Lo que, a su vez, en bioensayos de crecimiento poblacional, se correspondía con la incapacidad de *M. euphorbiae* para desarrollarse en plantas ABL 10-4. En conjunto, estos resultados indicaron, no sólo que los pulgones prefieren no asentarse en plantas con tricomas glandulares tipo IV, sino que cuando no hay otro huésped disponible el crecimiento de la población colapsa, incapaz de multiplicarse en presencia de esta defensa. Aunque es difícil establecer la cadena de acontecimientos que provoca el deterioro reproductivo y la falta de crecimiento de la

población, parece ser que la inanición de los insectos es el principal mecanismo subyacente, exacerbado por el efecto tóxico de los exudados de los tricomas.

A continuación, y como tercer objetivo, se estudiaron los mecanismos de señalización subyacentes a la respuesta inducida de defensa. En primer lugar, se exploró, en este sistema de estudio, el proceso por el que las plantas ajustan su respuesta a factores estresantes en función de una exposición previa. Es decir, cómo el fenómeno de *priming* puede modular la resistencia del tomate mediada por los tricomas glandulares, tipo IV, frente a *M. euphorbiae*. Los ensayos realizados mostraron que una infestación previa de larvas de *Spodoptera littoralis* aumentaba la resistencia a *M. euphorbiae*. En cambio, aquellas plantas infestadas previamente con *M. euphorbiae* eran más susceptibles a un segundo ataque de este mismo pulgón. Además, quedó de manifiesto que los tricomas glandulares tipo IV son eficaces contra los pulgones independientemente del tipo de infestación previa, en línea con los anteriores resultados. Por otro lado, se evaluó la resistencia en genotipos con capacidades limitadas en la señalización hormonal mediada por jasmonato y por el salicílico (*spr2* y *NahG*). Los resultados mostraron que en *spr2*, el genotipo incapaz de sintetizar JA (y por tanto sin niveles basales de esta fitohormona), fue más susceptible que su *wild-type* (genotipo control). Lo que enfatizaba la importancia del JA en la defensa del tomate frente a pulgón y opuesto a lo que se había descrito previamente. Asimismo, el tomate transgénico *NahG*, sin capacidad para acumular SA, mostró una mayor susceptibilidad comparado con MM. Lo que de nuevo reforzaba la hipótesis de la interacción negativa de las rutas del JA y SA descrita anteriormente y la relevancia del JA en la defensa de plantas de tomate frente a pulgón.

En el último bloque de trabajo, y para abordar el cuarto objetivo planteado, se estudió el efecto de las secreciones de los tricomas glandulares tipo IV del tomate sobre *M. euphorbiae* mediante un estudio transcriptómico. En primer lugar, se produjo el transcriptoma *de novo* de *M. euphorbiae* como herramienta imprescindible para poder detectar biomarcadores relacionados con la respuesta del pulgón a las defensas del tomate. Después, se analizaron los posibles efectos de la exposición del pulgón a las secreciones de los tricomas glandulares mediante análisis de enriquecimiento y de expresión genética diferencial (DEG) entre pulgones criados en MM (sin tricomas glandulares IV) y en ABL 10-4 (con tricomas glandulares IV). Lo que originó por primera

vez un perfil de expresión génica único y distinto correspondiente a la exposición del pulgón a los tricomas glandulares de tipo IV y a sus acilsacarosas. Se observó de esta manera una respuesta activa de estrés celular cuando el pulgón estaba en plantas ABL 10-4 (con todos los genes evaluados, *hsp70*, *hsp17* y *hsp10*, sobreexpresados). Una familia de genes que en condiciones de estrés se encarga de proteger proteínas diana y reparar otras desnaturalizadas, mitigando el estrés celular. Además, se observó que el metabolismo energético, un sensor del correcto funcionamiento del organismo, a través de la enzima GAPDH (implicada en la producción de energía mediante la glucólisis), era reprimido en aquellos pulgones que se encontraban en ABL 10-4. Lo que pone de manifiesto un daño energético considerable en este genotipo, que, a su vez, podría denotar un efecto tóxico de las secreciones de los tricomas. Asimismo, los procesos de oxidación reducción, de transporte y el metabolismo de carbohidratos y lípidos, se vieron también fuertemente reprimidos en los pulgones expuestos a los tricomas. Además de los genes implicados en la detoxificación de compuestos químicos cuya expresión era mucho menor también en los individuos crecidos en ABL 10-4. Por su parte, aquellos genes relacionados con el desarrollo hormonal estaban sobreexpresados. Todos estos resultados dan pistas de las principales adaptaciones y compensaciones metabólicas de los pulgones expuestos a los tricomas glandulares, revelando procesos y mecanismos cruciales que permiten comprender la adaptación de los pulgones a las plantas. Las diferencias observadas en el transcriptoma entre los pulgones criados en las dos líneas de tomate permiten comprender mejor los mecanismos responsables de la resistencia mediada por los tricomas glandulares. La inanición junto con el efecto de los tricomas glandulares (y acilsucrosas) genera la represión de genes implicados en la respuesta a estrés oxidativo, detoxificación y de aquellos relacionados con el metabolismo energético, de carbohidratos y lípidos. Además de provocar la inducción de genes de desarrollo responsables de la aparición de morfos alados. Estos resultados demuestran el efecto perjudicial de los tricomas glandulares (tipo IV) sobre el pulgón y plantean su modo de acción. El presente trabajo pone de manifiesto que biomarcadores conservados en artrópodos (fundamentales para su supervivencia) se ven afectados por los tricomas glandulares y sus secreciones y, en consecuencia, otros grupos funcionales (por ejemplo, polinizadores o enemigos naturales de las plagas de insectos) también podrían experimentar respuestas similares

cuando se exponen a estas estructuras defensivas. Las implicaciones de este estudio van, por tanto, más allá de las interacciones planta-pulgón, dado que tienen consecuencias para la comprensión de las defensas de las plantas, así como en la base funcional y ecológica de la gestión sostenible de plagas.

En resumen, los resultados de esta tesis doctoral proporcionan información que conduce a una mejor comprensión de las relaciones planta-insecto, y en particular de la interacción entre *S. lycopersicum* y *M. euphorbiae* y permite desarrollar estrategias de control más sostenibles que pueden ser transferidas a otros cultivos y modelos. En concreto, este estudio muestra el papel principal del tricoma glandular tipo IV en la respuesta de defensa de las plantas de tomate frente al pulgón *M. euphorbiae*. Lo que puede situar a esta defensa natural como objetivo de futuras estrategias de mejora genética que busquen una mayor resistencia de las plantas de tomate frente a diferentes plagas.

PEER-REVIEWED PUBLICATIONS

Research

New Phytologist

Complex patterns in tolerance and resistance to pests and diseases underpin the domestication of tomato

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Key words: domestication syndrome, herbivory, phylogenetic signal, plant defences, plant diseases, *Solanum lycopersicum*.

Summary

- A frequent hypothesis explaining the high susceptibility of many crops to pests and diseases is that, in the process of domestication, crops have lost defensive genes and traits against pests and diseases. Ecological theory predicts trade-offs whereby resistance and tolerance go at the cost of each other. We used wild relatives, early domesticated varieties, traditional local landraces and cultivars of tomato (*Solanum lycopersicum*) to test whether resistance and tolerance trade-offs were phylogenetically structured or varied according to degree of domestication.
- We exposed tomato genotypes to the aphid *Macrosiphum euphorbiae*, the cotton leafworm *Spodoptera littoralis*, the root knot nematode *Meloidogyne incognita* and two common insect-transmitted plant viruses, and reconstructed their phylogenetic relationships using Genotyping-by-Sequencing.
- We found differences in the performance and effect of pest and diseases but such differences were not related with domestication degree nor genetic relatedness, which probably underlie a complex genetic basis for resistance and indicate that resistance traits appeared at different stages and in unrelated genetic lineages. Still, wild and early domesticated accessions showed greater resistance to aphids and tolerance to caterpillars, nematodes and diseases than modern cultivars.
- Our findings help to understand how domestication affects plant pest interactions and underline the importance of tolerance in crop breeding.

Introduction

Domestication is characterised by the strong artificial selection of a wide range of morphological and physiological traits that results in phenotypes that differ considerably from their wild ancestor (Hammer, 1984; Doebley *et al.*, 2006). A frequent hypothesis explaining the high susceptibility of many crops to pests and diseases is that, in the process of domestication, crops have lost defensive genes and traits against pests and diseases (Khush, 2001; Whitehead *et al.*, 2017). Artificial selection has prioritised, for obvious reasons, plant genotypes that show low toxicity or low concentrations of secondary metabolites detrimental for human consumption (Benrey *et al.*, 1998; Wittkop *et al.*, 2009); and fruit traits for differences in colour, shape or taste or increased nutritional aspects (Newell-McGloughlin, 2008; Delgado-Baquerizo *et al.*, 2016). At the same time, intensification of agricultural practices and the use of pesticides have also conditioned plant–pest interactions (Macfadyen & Bohan 2010; Milla *et al.*, 2015). Most of these actions are thought to have rendered varieties with low defences against pests and diseases.

Phytopathological theory predicts two not mutually exclusive scenarios to understand plant–disease interactions: resistance and tolerance (Rosenthal & Kotanen, 1994; Krimmel & Pearse, 2016; McNickle & Evans, 2018). Resistance is defined as a reduction in the multiplication of the pest/disease (Strauss & Agrawal, 1999) and the mechanisms involve mainly defensive strategies (e.g. mechanical and chemical defence), or strategies that let plants to go unnoticed by enemies (Agrawal, 2011; Johnson, 2011). Tolerance reflects the degree to which plants suffer losses of performance upon pest/pathogen infestation/infection, that is a perfectly tolerant plant would achieve equal fitness when damaged or undamaged (Strauss & Agrawal, 1999; Little *et al.*, 2010). The interplay between these two phenomena is still incompletely understood. After Fineblum & Rausher (1995)

Research Article

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Characterization of the detrimental effects of type IV glandular trichomes on the aphid *Macrosiphum euphorbiae* in tomato

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Abstract

BACKGROUND: Glandular trichomes are essential in plants' defence against pests however, the mechanisms of action are not completely understood. While there is considerable evidence of feeding and movement impairment by trichomes, the effect on other traits is less clear. We combined laboratory and greenhouse experiments with molecular analysis to understand how glandular trichomes affect the behavior, population growth, and the expression of biomarkers involved in detoxification, primary metabolism, and developmental pathways of the aphid *Macrosiphum euphorbiae*. We used two isogenic tomato lines that differ in the presence of type IV glandular trichomes and production of acylsucroses; i.e.,*Solanum lycopersicum* cv. 'Moneymaker' and an introgressed line from *Solanum pimpinellifolium* (with trichomes type IV).

RESULTS: Type IV glandular trichomes affected host selection and aphid proliferation with aphids avoiding, and showing impaired multiplication on the genotype with trichomes. The exposure to type IV glandular trichomes resulted in the overexpression of detoxication markers (i.e., *Hsp70*, *Hsp17*, *Hsp10*); the repression of the energetic metabolism (*GAPDH*), and the activation of the ecdysone pathway; all these, underlying the key adaptations and metabolic trade-offs in aphids exposed to glandular trichomes.

CONCLUSION: Our results demonstrate the detrimental effect of glandular trichomes (type IV) on the aphid and put forward their mode of action. Given the prevalence of glandular trichomes in wild and cultivated Solanaceae; and of the investigated molecular biomarkers in insects in general, our results provide relevant mechanisms to understand the effect of trichomes not only on herbivorous insects but also on other trophic levels.

Supporting information may be found in the online version of this article.

Keywords: acylsucroses; glandular trichomes; molecular biomarkers; physiological responses; *Solanum pimpinellifolium*; *Solanum lycopersicum*

1 INTRODUCTION

Insect herbivory is a critical component in the coevolution of plants and insects.[1] To deal with phytophagous insects, plants show different defensive strategies (e.g., morphological and chemical adaptations) that impair insect movement, feeding and reproduction.[2] Herbivorous insects have developed morphological, behavioral and metabolic adaptations that enable them to deal (temporarily or permanently) with plant defenses.[3] Glandular trichomes, i.e., epidermal structures widely conserved across the plant kingdom,[4] play a key role in the defense of plants against herbivorous insects by producing allelochemicals that potentially affect insect pests behavior and life-cycle.[5] To date, the mechanisms by which glandular trichomes affect herbivore insects are not completely understood. While there is considerable evidence

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Transcriptome analysis of aphids exposed to glandular trichomes in tomato reveals stress and starvation related responses

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Understanding the responses of insect herbivores to plant chemical defences is pivotal for the management of crops and pests. However, the mechanisms of interaction are not entirely understood. In this study, we compared the whole transcriptome gene expression of the aphid *Macrosiphum euphorbiae* grown on two different varieties of tomato that differ in their inducible chemical defences. We used two isogenic lines of tomato with a shared genetic background that only differ in the presence of type IV glandular trichomes and their associated acylsucrose excretions. This works also reports a de novo transcriptome of the aphid *M. euphorbiae*. Subsequently, we identified a unique and distinct gene expression profile for the first time corresponding to aphid´s exposure to type IV glandular trichomes and acylsugars. The analysis of the aphid transcriptome shows that tomato glandular trichomes and their associated secretions are highly efficient in triggering stress-related responses in the aphid, and demonstrating that their role in plant defence goes beyond the physical impediment of herbivore activity. Some of the differentially expressed genes were associated with carbohydrate, lipid and xenobiotic metabolisms, immune system, oxidative stress response and hormone biosynthesis pathways. Also, the observed responses are compatible with a starvation syndrome. The transcriptome analysis puts forward a wide range of genes involved in the synthesis and regulation of detoxification enzymes that reveal important underlying mechanisms in the interaction of the aphid with its host plant and provides a valuable genomic resource for future study of biological processes at the molecular level using this aphid.

Although insect herbivores potentially have an abundance of plant species available for feeding, herbivory is often limited by the defence mechanisms that plants have evolved to level off insect attacks[1]. The co-evolution of phytophagous insects and their host plants has shaped the evolutionary history of both groups[2,3]. Despite the importance of this 'arms race', our knowledge of the mechanisms operating is still limited, and while plant defences and their effects on herbivores are well documented, much less is known on the mechanisms evolved by insects to overcome these defences[4,5].

Aphids are important pests on virtually all crops and are specialised herbivores that feed on the phloem of vascular plants[6]. They can greatly reduce crop yield by removing large quantities of sugar-rich sap from their hosts and by transmitting detrimental plant viruses and pathogens[7,8]. Compared to chewing insects, aphid feeding causes little structural harm to a plant. Negative impacts of sustained aphid feeding often arise from the rapid clonal reproduction of aphids and subsequent depletion of the plant's resources. Moreover, aphids can modulate and suppress the phytohormonal and defensive response[9,10].

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Plant-Insect Interactions

More than trichomes and acylsugars: the role of jasmonic acid as mediator of aphid resistance in tomato

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ABSTRACT

This study investigates the impact of priming on the resistance of tomato plants to the potato aphid *Macrosiphum euphorbiae*, focusing on the role of glandular trichomes. Glandular trichomes are specific hairs that provide protection to tomato plants against herbivorous insects. The experimental priming conducted in this study revealed that prior infestation by *Spodoptera littoralis* caterpillars increased the plant's resistance against *M. euphorbiae*, pointing at the jasmonic acid (JA) signaling pathway in regulating this plant-aphid interaction. Glandular trichomes type IV were effective against aphids regardless of the previous infestation. Using JA-deficient tomato (*spr2*), we observed that *M. euphorbiae* multiplication increased, while the number of aphids on salicylic -deficient *NahG* plants was lower than in the wildtype Moneymaker. These findings emphasize the crucial role of the JA signaling pathway in tomato plant resistance to aphids and the importance of glandular trichomes to enhance plant defences against pests.

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Solanum lycopersicum; potato aphid; hormonal pathways; plant defences; glandular trichomes; cross-talk; *Nahg*; *Spr2*

Introduction

Plants often suffer from attack by multiple herbivorous insects, either simultaneously or sequentially that may reduce the survival and fitness. To cope with the diversity of insect herbivory, plants have evolved different defense mechanisms to perceive and respond appropriately to specific attackers (Erb and Reymond 2019). While some defences are only induced in response to an herbivore attack other defence mechanisms are expressed constitutively (War et al. 2012; Fürstenberg-Hägg et al. 2013). Inducible defenses are activated when the surveillance system of the plant recognizes elicitors contained in insect oral secretions or signals released by injured cells which are followed by rapid activation of sophisticated plant signaling pathways (Nguyen et al. 2016). Moreover, induced defenses are not only triggered in the insect-attacked leaves but can also occur systemically i.e. in distal areas from the wounded parts facilitating rapid and effective responses to incoming herbivore attacks (Conrath et al. 2015; Erb and Reymond 2019). Plants expressing induced defences often exhibit a faster and stronger activation of specific defense responses after they have been previously challenged by an insect herbivore or a pathogen. This capacity for augmented defense expression is called priming, which is an adaptive strategy that improves the defensive capacity of plants. Induced plant defenses can have an impact on other herbivores that feed on the same plant simultaneously or subsequently (Agrawal 2014; Stam et al. 2014). Phytohormones, such as Jasmonic acid (JA) and salicylic acid (SA), play a crucial role in the activation of these induced plant defenses (Pieterse et al. 2012; Steinbrenner et al. 2020). The activation of these pathways by herbivores is often herbivore-specific, and their induction can be partly predicted by the feeding mode of the herbivore.

To fine-tune defense mechanisms in plants, the SA- and JA-responsive signaling pathways are interdependent and act through a complex network of regulatory interactions. One of the best characterized interactions in defense-related signaling is the crosstalk between the JA and SA response pathways. The crosstalk hypothesis predicts interference between plant signaling pathways when initial and subsequent attackers induce defenses associated with different pathways (Pieterse et al. 2012; Eisenring et al. 2018; Erb and Reymond 2019). Nonetheless, synergistic interactions have been described as well. For instance, a meta-analysis on plant-mediated effects of initial attackers on the performance of subsequent attackers indicated that JA-inducing herbivores resulted in a reduction in the performance of both JA- and SA- inducing herbivores (Moreira et al. 2018).

It is generally assumed that leaf-chewing herbivores, such as caterpillars, lead to an induction of defensive pathways mainly regulated by JA, whereas SA signaling primarily participates in defense against piercing-sucking herbivores that feed on the phloem, such as aphids and whiteflies (Morkunas et al. 2011; Züst and Agrawal 2016; Steinbrenner et al. 2020). However, the impact of SA defense pathway on resistance to

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