Tomato defense mechanisms against herbivorous insects

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Abstract

The tomato plant is a vegetable crop of significant economical importance for the consumption of its edible berries and is considered a model plant within the Solanaceae. Cultivated tomato plants suffer from severe yield losses due to a variety of herbivorous pests. Insect herbivory has resulted in the evolution of a wide array of morphological and biochemical plant defense mechanisms. Plants respond to insect herbivory or wounding by expressing systemic signals that initiate different phytohormone signaling pathways. Systemin, an 18 amino acid peptide released from its 200 amino acid precursor prosystemin, coordinates the systemin-mediated signaling pathways in tomato which involves the activation of jasmonic acid and the release of volatile organic compounds. Tomato and its relatives also possess a wide range of glandular and non-glandular trichomes types. Glandular trichomes are specialized hairs which originate from the plants surface, capable of producing and releasing varying secondary metabolites. Many of the wild tomato species are more resistant to insect pests than cultivated tomatoes due to an enhanced production of resistance-related metabolites, such as acyl sugars, terpenes, and methyl ketones. Dissecting the systemin-mediated hormonal signaling pathways and trichome mediated defense responses in tomato will provide valuable information to improve future breeding strategies for new tomato varieties and to engineer tomato plants with enhanced insect resistance. Here, we review recent advances in molecular research on the role of systemic signaling and phytohormone pathways in the tomato defense response against insect herbivores, with a focus on systemin mediated signaling. Next, we highlight the role of different trichomes in tomato and its relatives and summarize the role of several important trichome-produced compounds in tomato.

Keywords: Tomato; systemin; plant-herbivore interactions; trichomes; pests

Layman's Summary

The tomato plant is a vegetable crop of huge economical importance. It is grown throughout the world for the consumption of its edible berries and is considered a model plant within the nightshade family, which consists among others of tomatoes, potatoes and eggplant. Cultivated tomato plants suffer from severe yield losses due to damage and diseases from many herbivorous pests. Insect herbivory has resulted in the evolution of a wide range of structural and biochemical plant defense mechanisms. Plants respond to insect herbivory or wounding by the expression of long-distance signals that initiate different plant hormone signaling pathways. Systemin, a small peptide released from a larger precursor called prosystemin, coordinates the defense signaling pathways in tomato which involves the activation of a specific plant hormone and the release of volatile organic compounds into the atmosphere. Tomato and its relatives also possess a wide range of trichomes types, of which some possess glands. These glandular trichomes are specialized hairs which originate from the plants surface, capable of producing and releasing different toxic compounds. Many of the wild tomato species are more resistant to insect pests than cultivated tomatoes due to their enhanced production of toxic compounds. Analyzing the systemin-mediated hormonal signaling pathways and trichome mediated defense responses in tomato will provide researchers with valuable information that can improve future breeding of new tomato varieties and can engineer tomato plants with increased resistance traits. In this review we show the recent advances in molecular research on the role of systemic signaling and phytohormone pathways in the tomato defense response against insect herbivores, with a focus on systemin-mediated signaling. Next, we explain the role of different trichomes in tomato and its relatives and summarize the role of several important toxic compounds in tomato.

1. Introduction

1.1. Cultivated tomato and its wild relatives

The cultivated tomato plant, *Solanum lycopersicum* L., is a vegetable crop of significant economical importance and is grown throughout the world for the consumption of its edible berries. Worldwide tomato production exceeds 180 million metric tons annually (FAO, 2020). Tomatoes and tomato-based foods provide a rich source of dietary fibers, minerals, carotenoids, and vitamin C (Beecher, 1998). *S. lycopersicum,* belongs to the diverse genus *Solanum* in the *Solonaceae* family and is part of sect. *Lycopersicon* (Mill.) Wettst, which furthermore comprises 12 closely related wild species: *S. arcanum, S. cheesmaniae, S. chilense, S. chmielewskii, S. corneliomulleri, S. galapagense, S. habrochaites, S. huaylasense, S. neorickii, S. pennellii, S. peruvianum* and *S. pimpinellifolium* (Table 1; Peralta et al., 2008). These species are all native to western South America, diversifying in desert-like conditions on the slopes of the Andes (Knapp & Peralta, 2016). *S. galapagense* and *S. cheesmaniae* are both endemic to the Galapagos Islands and form a closely related monophyletic group with *S. pimpinellifolium* and *S. lycopersicum* (Peralta et al., 2008; Rodriguez et al., 2009) (Table 1)*. S. pimpinellifolium* is native to areas of low elevation on Peru's Andean slopes and has been proposed as the closest wild relative of the cultivated tomato (Blanca et al., 2021). Colonizers of the New World brought the cultivated tomato to Europe in the 16th century. Although *S. lycopersicum* originated in South America, debate about the geographical origin of tomato domestication has been ongoing for several centuries (Jenkins, 1948). Recent studies proposed a two-step process for tomato domestication (Blanca et al., 2015; Razifard et al., 2020). According to that model, *S. lycopersicum* var. *cerasiforme* (Alef.) D.M. Spooner et al., a wild small-fruited *S. lycopersicum* variety, diverged from *S. pimpinellifolium* and was first domesticated in northern Peru and then moved to Mesoamerica, where it was further domesticated into the big-fruited *S. lycopersicum* var. *lycopersicum* (Razifard et al., 2020). However, Blanca et al. (2021) suggests

that *S. lycopersicum* var. *cerasiforme*, after diverging from *S. pimpinellifolium*, spread from the Peruvian desert to Mesoamerica, unrelated to human activity. Humans then took the wild *S. lycopersicum* var. *cerasiforme* from Mexico to South America, where it was first domesticated, and brought it back to Mesoamerica, where it was further domesticated into the big-fruited *S. lycopersicum* var. *lycopersicum* (Blanca et al., 2021).

Table 1: Members of sect. *Lycopersicon* and their geographical distribution. Adapted from Peralta et al. (2008).

Group	Species	Geographical distribution
Lycopersicon	*S. lycopersicum* L.	Globally cultivated
	S. pimpinellifolium L.	Southwestern Ecuador to northern Chile
	S. cheesmaniae (L. Riley) Fosberg	Galápagos Islands
	S. galapagense S.C. Darwin and Peralta	Galápagos Islands
Neolycopersicon	*S. pennellii* Correll	Western slopes from Peru to Chile
Eriopersicon	*S. habrochaites* S. Knapp and D. M. Spooner	Andean mountains of Ecuador and Peru
	S. huaylasense Peralta	The Santa Valley (Callejón de Huaylas) in Peru
	S. corneliomulleri J. F. Macbr.	Western slopes of southern Peru
	S. peruvianum L.	Central Peru to northern Chile
	*S. chilense (*Dunal) Reiche	Coastal Chile and southern Peru
Acanum	*S. arcanum* Peralta	Northern Peru
	S. chmielewskii (C. M. Rick et al.), D. M. Spooner et al.	Southern Peru and northern Bolivia
	S. neorickii D. M. Spooner et al.	Southern Ecuador to southern Peru

The domestication events of many vegetable crops drastically changed a wide range of phenotypical traits distinguishing them from their wild ancestors, often referred to as the domestication syndrome (Chen et al., 2015). Although cultivated tomato accessions show great morphological diversity, they represent a narrow genetic base caused by strong genetic

bottlenecks introduced during early domestication in Mesoamerica and the following modern breeding programs in Europe (Bai & Lindhout, 2007). Exchange of genetic information between cultivated tomatoes and wild germplasm most likely did not take place until ca. 1940 (Bai & Lindhout, 2007). The domesticated tomato has been selected for a wide variety of desirable characteristics. Early tomato domestication transformed the smaller wild tomatoes into the larger present-day varieties, brought on by massive changes in fruit size and fruit shape (Bai & Lindhout, 2007; Paran & van der Knaap, 2007). Furthermore, tomatoes were selected for other traits such as growth habit (apical dominance), leaf morphology, flavor (soluble solids), texture and color (Liu et al., 2020; Paran & van der Knaap, 2007). From the 1970s till early 2000s, tomato breeding went through roughly four phases: breeding for yield, shelf-life, taste, and nutritional quality (Bai & Lindhout, 2007). The current tomato breeding goals aim at breeding for increased resistance to biotic and abiotic stresses, as the use of fungicides and pesticides is severely restricted and global climates are changing (Zeist et al., 2019). The reduced genetic variation acquired from tomato domestication most likely reduced their ability to withstand biotic and abiotic stresses. Crop domestication has long been hypothesized to result in reduced plant resistance to insect herbivores (Chen et al., 2015; Whitehead et al., 2017). Next to genetic bottlenecks, humans may have selected for traits improving plant quality for herbivory insects, by increasing nutritional content. Furthermore, humans may have selected directly against defense traits, such as distasteful or anti-nutritive secondary metabolites (Whitehead et al., 2017).

Cultivated tomato plants suffer from severe yield losses due to a variety of herbivorous pests, such as the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aphididae), the aphids *Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae), the thrips *Frankliniella schultzei* (Trybom) (Thysanoptera: Thripidae), the tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), the American serpentine leafminer *Liriomyza*

trifolii (Burgess) (Diptera: Agromyzidae), the corn earworm *Helicoverpa zea* (Boddie) and cotton bollworm *H. armigera* (Hübner) (Lepidoptera: Noctuidade), the tomato fruit bearer moth *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae), the caterpillar and the spider mites *Tetranychus urticae* (Koch) and *T. evansi* (Baker and Pritchard) (Acari: Tetranychidae) (Zeist et al., 2019). Many of the wild tomato species are more resistant to these pests than cultivated tomatoes, often due to an increased production of resistance-related metabolites. *S. pennellii, S. habrochaites, S. galapagense, S. peruvianum, S. pimpinellifolium, S. cheesmaniae* and *S. chmielewskii* have a rich genetic background and are an important source of diversity for the development of resistant tomato varieties through genetic introgression of important resistance traits (Stout et al., 2018).

Plants and herbivorous insects have co-evolved for millions of years. Insect herbivory has resulted in the evolution of a wide array of morphological and biochemical plant defense mechanisms, capable of restricting insect pests (War et al., 2018). Plant defense-traits are usually divided into constitutive or induced defense mechanisms. Constitutive defenses represent preformed mechanical or anatomical defenses, such a trichomes, thorns, and sclerophylly, which offer continuous protection by interfering with arthropod mobility (War et al., 2012). Upon insect attack, pants may induce several defense mechanisms that may involve the production of toxic secondary metabolites (War et al., 2012), the increase of trichome densities on newly expanding leaves (Boughton et al., 2005) or the emission of volatiles (Aljbory & Chen, 2018). Tomato and its relatives have evolved a wide range of trichomes types, glandular (capable of secondary metabolite synthesis) and non-glandular, each with different roles in plant resistance (Glas et al., 2012). Most defense-related traits affect pests or pathogens directly, however some act indirectly through the attraction of natural predators (Aljbory & Chen, 2018). From the moment an insect herbivore starts feeding on a plant, several intracellular signals and pathways are initiated. Plants can recognize insect herbivory through

the feeding strategies of different feeding guilds, insect ovipositing, and oral secretions (Bonaventure, 2012). Insects have evolved a variety of mouthparts used for chewing, pierce-sucking, and phloem-sucking of several plant tissues to obtain the necessary nutrients to complete their development (Bonaventure, 2012). In response to insect herbivory or wounding, systemic signals are expressed, followed by the initiation of different phytohormone signaling pathways. The phytohormones jasmonic acid (JA) and salicylic acid (SA) play important roles in these signaling pathways and are co-regulated by other phytohormones, such as ethylene (ET) and abscisic acid (ABA) (Pieterse et al., 2009; Vos et al., 2013). JA-dependent defenses are mostly involved in plant responses to insect herbivores, whereas SA-dependent defenses are primarily induced by biotrophic pathogens. However, SA accumulation has also been reported by the attack of phloem-sucking insects and spider mites, resulting in the expression of SA-inducible genes (Weinblum et al., 2021). SA- and JA-signaling pathways can affect each other's signaling antagonistically (Pieterse et al., 2001, 2009; Vos et al., 2013). This crosstalk between signaling pathways allows plants to regulate multiple resistance mechanisms thereby prioritizing the activation of defense pathways against specific pests (Vos et al., 2013).

This review attempts to provide a comprehensive overview and discuss recent advances in molecular research on defense mechanisms against herbivorous insects of tomato species in sect. *Lycopersicon*. First, it describes the role of systemic signals and phytohormone pathways in the tomato defense response against insect herbivores, with a particular focus on systemin mediated signaling of the jasmonate pathway. Next, it describes the role of different trichomes in the tomato defense and summarizes the role of several important trichome-produced compounds in tomato.

2.   Role of phytohormonal and signaling pathways in the tomato defense response

2.1.   Systemic wound signaling in tomato

In response to herbivory or wounding, plants express systemic signals that induce the synthesis of defensive proteinase inhibitors (PIs), which interfere with the digestive proteinases of insects (Ryan, 1990). This systemic response has been well-studied using tomato as a model system, by investigating the plants local and distal defense processes after injury. Pearce et al. (1991) first discovered an 18-amino acid proline-rich peptide, called Systemin (Sys), in the extracts of wounded tomato leaves which was found to trigger systemic defenses upon wounding or herbivory. Systemin-like homologs were quickly identified in several other species, such as potato (*S. tuberosum* L.) and bell pepper (*Capsicum annuum* L.) and black nightshade (*S. nigrum* L.) (Constabel et al., 1998; Pearce & Ryan, 2003). In addition, systemic hydroxyproline-rich glycopeptides were isolated from tomato, potato, and tobacco (*Nicotiana tabacum* L.) plants (Bhattacharya et al., 2013; Pearce, 2011; Pearce et al., 2001; Pearce & Ryan, 2003). These hydroxyproline-rich systemins (HypSys) belong to the same family of peptides as Sys and are also strong activators of PI expression. HypSys peptides are structurally unrelated to Sys but took its name from their systemin-like function (Zhang et al., 2020). The upregulation of JA-production to induce a strong systemic response has been shown to be co-operatively regulated by prosystemin (ProSys) and HypSys in the tomato defense response (Narváez-Vásquez et al., 2007).

Pearce et al. (1991) reported that Sys induced PIs both locally in damaged leaves and distally in undamaged leaves. Sys stimulates distal defense responses in tomato by amplifying a systemic signal through the JA pathway (for a detailed review, see Zhang et al., 2020). Sys is cleaved from the C-terminal region of its 200-amino acid precursor proprotein, ProSys (McGurl et al., 1992). The overexpression of the ProSys gene generated a systemic signal, activating the wound response pathway, constitutively inducing PIs, and increasing resistance to herbivorous

insects, such as potato aphid (*M. euphorbiae*), African cotton leafworm (*Spodoptera littoralis* Boisduval) and tobacco hornworm (*Manduca sexta* L.) and phytopathogenic fungi including *Botrytis cinerea* Pers. and *Alternaria solani* Sorauer (Coppola et al., 2015; McGurl et al., 1994; Orozco-Cardenas et al., 1993). Conversely, the transformation of tomato plants with an antisense ProSys gene, driven by a *35S-CaMV* promotor, resulted in impaired JA signaling (Stenzel et al., 2003), impaired PI accumulation in response to wounding and decreased resistance to *M. sexta* larvae (Orozco-Cardenas et al., 1993). These results substantiated Sys as the mobile defense signal mediating a long-distance systemic response in tomato (Figure 1). Li et al. (2002) used grafting experiments with mutant tomato plants that blocked JA biosynthesis, JA perception or Sys signaling and reported that Sys was not the mobile defense signal. However, a recent study by Mucha et al. (2019) used capillary electrophoresis and found that Sys peptide injected into mature tomato foliage was transported throughout the whole plant.

ProSys is synthesized upon wounding or insect attack, followed by the proteolytic release of Sys from residues 179-196. Subsequently, Sys is translocated through the petiole phloem into distal undamaged leaves and plant tissues (Mucha et al., 2019), where it interacts with leucine-rich repeat receptor-like kinases, Sys receptor 1 (SYR1) and Sys receptor 2 (SYR2) (Wang et al., 2018). Sys perception, by primarily SYR1, at the surface leads to the accumulation of reactive oxygen species and is coupled with influxes of Ca^{2+} and H^+ and effluxes of K^+ in the cell (Ryan, 2000; Wang et al., 2018; Zhang et al., 2020). These changes are followed by cell membrane depolarization and the rapid activation of mitogen-activated protein kinases (MAPKs) (Haj Ahmad et al., 2019). It has been suggested that MAPKs coupled with Phospholipase A2 (PLA2) could be responsible for the release of linolenic acid (LA) from membrane phospholipids (Narváez-Vásquez et al., 1999). LA is released into the cytoplasm, followed by the formation of JA and its derivatives methyl jasmonate (MeJA) and the bioactive

jasmonoyl isoleucine (JA-Ile), ultimately leading to the biosynthesis of PIs (Narváez-Vásquez et al., 1999; Zhang et al., 2020).

However, recent studies have demonstrated the intrinsically disordered structure of the ProSys proprotein and suggested it could have a substantially larger role in defense responses that previously thought (Buonanno et al., 2018; Zhang et al., 2020). Intrinsically disorder proteins (IDPs) are a group of large proteins lacking a well-defined structure, allowing them to assume a wide range of configuration states, suggesting that they can bind with several other partners (Buonanno et al., 2018; Uversky, 2013). A recent study by Molisso et al. (2022) produced transgenic tomato plants expressing a truncated ProSys gene (ProSys$_{(1-178)}$, devoid of the Sys coding region). Both the transgenic plants and plants treated with the recombinant protein demonstrated expression of defense-related genes and resistance to *S. littoralis* and *B. cinerea*. In transgenic plants, the authors reported down-regulation of genes involved in the JA-, SA-, ET- and gibberellin-pathways, which they attributed to the absence of the Sys protein. However, the authors demonstrate that many genes involved in physical barriers, related to cell-wall reinforcement and callose synthesis are up-regulated, independent from phytohormone-regulated pathways (Molisso et al., 2022). Furthermore, ProSys$_{(1-178)}$ seems involved in the activation of defense pathways associated with the production of oligogalacturonides (OGs) (Molisso et al., 2022). Besides its function in Sys release, ProSys may have its own biological functions, and could be involved in the activation of multiple defense pathways (Figure 1; Molisso et al., 2022; Zhang et al., 2020).

Tomato plants respond to mechanical wounding, herbivorous insects, or pathogen infections by releasing volatile organic compounds (VOCs) (Bezerra et al., 2021). Tomato plants overexpressing ProSys have been shown to produce different VOC profiles than wild-type plants, providing a higher level of indirect attractiveness to natural insect enemies (Corrado et al., 2007; Degenhardt et al., 2010). Coppola et al. (2017) proposed that plant-to-plant

communication through volatile compounds is controlled by Sys-dependent pathways. Sys treatment and ProSys overexpression changed the expression pattern of plant defense genes in surrounding tomato plants, improving their resistance to insect pests (Coppola et al., 2017). The authors suggest that unchallenged neighboring plants are primed by the activation of a Sys-mediated systemic response in attacked tomato plants (Figure 1). However, they note that many of the upregulated genes in primed plants are not directly involved in insect deterrence, unlike Sys-mediated defense within the attacked plant (Coppola et al., 2017).
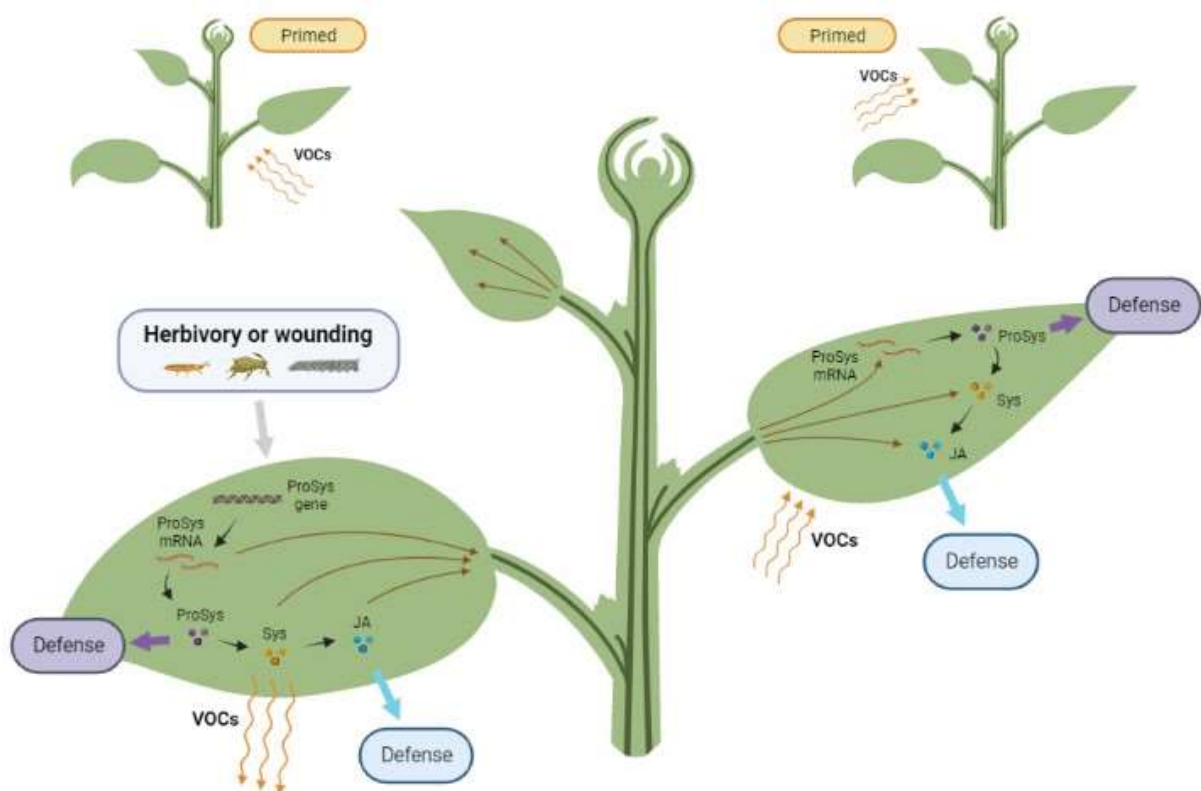

Figure 1: Schematic model of the Sys-mediated defense response in tomato. Upon herbivory or wounding (on the left leaf), the *ProSys* gene is expressed, resulting in the translation of *ProSys* mRNA into ProSys proteins in the damage leaf. ProSys activates defense genes related to physical barriers and OG production, independently from phytohormone pathways (Molisso et al., 2022). Sys is released from the ProSys protein, inducing JA biosynthesis and local JA-mediated defenses. Either *ProSys* mRNA or ProSys can move through the phloem into distal tissues (right leaf) for release of additional systemin or activation of defense-related genes (Jacinto et al., 1997; Narváez-Vásquez & Ryan, 2004). Sys and JA

have both been reported as phloem-mobile signals (Mucha et al., 2019; Zhang et al., 2020). In the distal (right) leaf, JA-mediated defenses are initiated by either mobile JA or Sys. The activation of this Sys-mediated systemic response releases VOCs that prime unchallenged neighboring plants and distal leaves, increasing their pest resistance (Coppola et al., 2017). Adapted from Zhang et al. (2020). Created in Biorender.com

2.2. JA- and SA-mediated defense in tomato

Hormonal control by the two primary phytohormones, JA and SA, has been extensively investigated in *Arabidopsis thaliana* (L.) Heynh. (hereafter Arabidopsis) and tomato. JA is a naturally occurring phytohormone involved in regulating germination, growth, development, and fruit ripening. As previously mentioned, wounding, insect herbivory, and even some necrotrophic pathogens activate the octadecanoid pathway, triggering JA-dependent defenses that activate the expression of defense genes involved in the accumulation of PIs, polyphenol oxidases (PPOs), and secondary metabolites (Sun et al., 2011). Activation of the octadecanoid pathway increases the endogenous levels of members of the jasmonate family, including JA, MeJA, JA-Ile, and their metabolic precursor, 12-oxo-phytodienoic acid (OPDA) (Sun et al., 2011). JA and its derivatives are derived from LA upon its release into the cytoplasm (Zhang et al., 2020). It was recently demonstrated that acetic acid could enhance tomato resistance against cotton leafworm (*Spodoptera litura* Fabricius) by pre-treatment of tomato seedlings. Acetic acid pre-treatment resulted in increased transcription of defense genes and JA accumulation after *S. litura* attack (Chen et al., 2019). However, Zhang et al. (2020) reported a possible negative correlation between defense priming and constitutive defenses in tomato.

SA- and JA-signaling pathways can affect each other's signaling antagonistically (Pieterse et al., 2001, 2009; Vos et al., 2013). This crosstalk between signaling pathways allows plants to regulate multiple resistance mechanisms by prioritizing the activation of defense pathways against specific pests (Vos et al., 2013). SA-dependent defenses are primarily induced

by biotrophic pathogens but are also upon attack by phloem-sucking insects and spider mites, resulting in the expression of SA-inducible genes (Weinblum et al., 2021). Insect oviposition has also been found to induce SA-related genes (Bruessow et al., 2010). For example, egg-derived elicitors from the butterfly, *Pieris brassicae* L., inhibited the expression of JA-mediated defense genes in Arabidopsis (Bruessow et al., 2010). In addition, Colorado potato beetles (*Leptinotarsa decemlineata* Say) suppress JA-dependent defenses in tomato by using bacteria in their oral secretions and thereby increasing larval growth (Chung et al., 2013). The activation of SA through insect oviposition can lead to a reduction in JA signaling, subsequently rendering plants more susceptible to herbivorous insects (Koornneef & Pieterse, 2008). Furthermore, some herbivores are capable of directly suppressing JA or SA responses. For example, the mites *T. urticae* and *T. evansi* can suppress both JA and SA simultaneously in tomato (Alba et al., 2015; Sarmento et al., 2011). Suppression occurred downstream of JA and SA accumulation (Alba et al., 2015). These examples indicate that insect herbivores can circumvent plant defenses by exploiting their elicitors or effectors to increase survival (Kant et al., 2015).

Besides defense response regulation, it has become clear that JA is also involved in trichome formation and plays an important role in their development. JA-deficient tomato plants were reported to have less glandular trichomes (Yan et al., 2013). In addition, MeJA treatment has shown to increase trichome densities on newly developing tomato leaves (Boughton et al., 2005). Furthermore, it has been shown that spraying plants with JA induces the production of many secondary metabolites such as, terpenoids, acyl sugars and alkaloids, that can provide resistance to many insect pests (Glas et al., 2012).

3. Trichomes as resistance mechanisms in sect. Lycopersicon

3.1. Plant trichomes

The plants outer surface acts as a protective barrier against many different pathogens and insect herbivores. Hair-like epidermal structures, called trichomes, originate from the outer epidermal

cell tissues from plants and can mitigate the negative effects opposed by biotic and abiotic stresses (Wagner et al., 2004; Zhang et al., 2020). Trichomes have been shown to reduce pest and pathogen attack, oviposition, protection against freezing and drought, reduce heat loss, and reduce heat load from excessive light (Bar & Shtein, 2019; Wagner et al., 2004). Trichomes range in many different sizes and forms, from unicellular to multicellular, peltate, branched or unbranched and can be found on nearly all plant parts (Wagner et al., 2004). Furthermore, they are often morphologically classified as glandular or non-glandular (Bar & Shtein, 2019; Glas et al., 2012). Non-glandular trichomes are often unicellular, inhibiting the movement and oviposition of insects, whereas glandular trichomes are most often multicellular and can synthesize, store, and secrete many classes of secondary metabolites in apical cells (Schuurink & Tissier, 2020). The specialized metabolites produced in trichomes provide great opportunities and have received a lot of attention as natural pesticides, and even as food additives or pharmaceuticals. Trichomes are seen as a great target for the genomic engineering of plant-pest interactions by providing increased protection to herbivorous insects in many commercially important crops.

3.2.   Tomato trichomes and diversity in wild species

Trichomes are broadly distributed and have been extensively studied in several different species, such as Arabidopsis, *Artemisia annua* (sweet wormwood), *Ocimum basilicum* (sweet basil), *Gossypium hirsutum* (cotton), tobacco, and tomato (Bar & Shtein, 2019; Chalvin et al., 2020; Glas et al., 2012). Trichome density in different species is thought to be influenced by their surrounding environmental conditions as protection to biotic or abiotic threats. *Solanum* spp. contain very diverse trichome types, as previously reviewed by Bar & Shtein (2019) and Glas et al. (2012). They are known to present up to eight different trichome types on their outer surface, which have been described using a Roman numeral system, from I to VIII (Fig. 2). These are divided in glandular (I, IV, VI, and VII) and non-glandular trichomes (II, III, V, and

VIII). Glandular trichomes differ in morphology and can be peltate or capitate. Peltate trichomes (types VI and VII) consist of a short uni- or bicellular stalk with a multicellular head of several secretory cells, whereas capitate trichomes (types I and IV) are often larger, consisting of a multicellular stalk with a small unicellular secretory head (Feng et al., 2021).
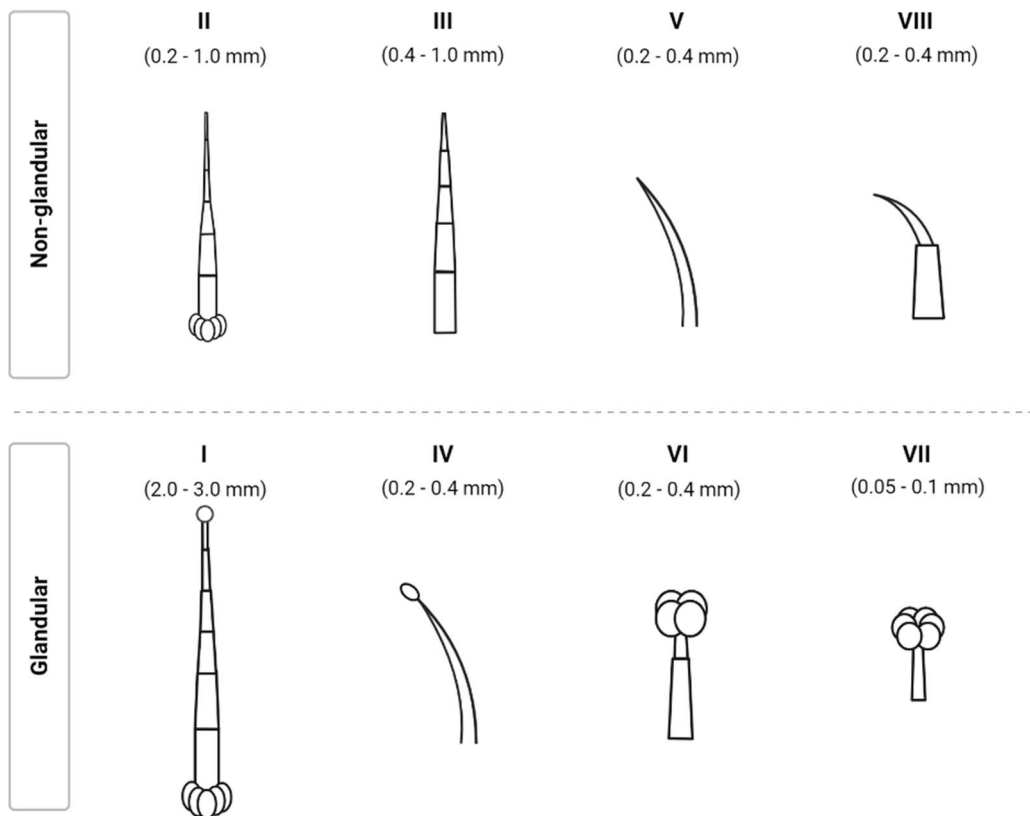

Figure 2: Different trichome types in Solanum sect. Lycopersicon as described by Channarayappa et al. (1992) and Glas et al. (2012). Created in Biorender.com

Type I trichomes consist of a globular multicellular base with a stalk of six to 10 cells and a small glandular cell in the tip. Type II trichomes are like type I trichomes, with a multicellular glandular base, but shorter and a non-glandular tip. Type III trichomes consist of four to eight cells, with a flat unicellular base, and no terminal gland. Type IV and V trichomes are very alike in height and build, with unicellular flat bases, but type IV trichomes contain a small glandular cell in the tip like that of type I. Type VI trichomes are short, glandular trichomes, consisting of a two-celled stalk, with a flat base, and a four-celled glandular head

(Glas et al., 2012). The secretory heads of type VI trichomes are structurally different between some members of sect. *Lycopersicon* (Bergau et al., 2015). The authors used a variety of cell imaging techniques to distinguish the clearly distinguishable contour of the four secretory cells in cultivated tomato. However, in *S. habrochaites* the glandular head looks round and the cells can not be distinguished from the outside. Also, the type VI trichomes of *S. habrochaites* contained larger intercellular cavities for storage (Bergau et al., 2015). Type VII trichomes are very small, with a four to eight-celled glandular head. Last, type VIII trichomes consist of a large basal cell with a thinner, often slanted tip.

3.3.   Glandular trichomes of sect. *Lycopersicon* in herbivore defense

The study of trichomes in *Solanum* spp., especially the defensive function of glandular trichomes and their specialized metabolites, is of tremendous relevance for crop protection against herbivorous pests. The cultivated tomato possesses the trichome types I, III, IV, V, VI, VII, and VIII, but type IV trichomes have only been found on cotyledons and juvenile leaves, besides several developed mutants (Vendemiatti et al., 2017). Many of the glandular trichomes differ in cellular buildup of the stalk and secretory cells, but also in the excretion of different defensive chemicals (Bergau et al., 2015; Glas et al., 2012). Trichome presence and density differs between species of sect. *Lycopersicon*, but also between cultivars, and it could even depend on tissue type or environmental conditions (Kortbeek et al., 2021).

Insect and mite resistance has been suggested to be based on specific plant compounds present in glandular trichomes (Glas et al., 2012). The main classes of secondary chemicals that are produced in glandular trichomes include terpenoids, phenylpropenes and flavonoids, methyl ketones, acyl sugars, and defensive proteins (see Glas et al., 2012 for an extensive review on trichome produced metabolites in Solanaceae). Many of these secondary metabolites have been found in trace amounts or have been lost in the domesticated tomato, making them more susceptible to pests (Besser et al., 2009). Acyl sugars are primarily produced in the secretory

cells of type I and IV trichomes whereas type VI trichomes are mainly involved in terpenoid, flavonoid and methyl ketone secretion (Balcke et al., 2017; Bergau et al., 2015; Fan et al., 2019). *S. cheesmaniae* and S. *galapagense* are very closely related but differ strongly in the presence of type IV trichomes (Lucatti et al., 2013). These are present on *S. galapagense* but absent on *S. cheesmaniae* leaves (Lucatti et al., 2013). Multiple studies have performed Quantitative Trait Locus (QTL) mapping of F2 populations derived from a *S. lycopersicum* x *S. galapagense* cross (Firdaus et al., 2013; Vosman et al., 2019). The authors identified Wf-1, a major QTL that regulates the formation of glandular type IV trichomes, governing resistance to different whitefly species (Firdaus et al., 2013; Vosman et al., 2019). In addition to *S. galapagense*, both *S. pennellii* and *S. pimpinellifolium* also contain high type IV trichome densities that are associated with the secretion of acyl sugars (Firdaus et al., 2013). The nature of type VII trichome secretion is not clearly established, although in *S. habrochaites* it has been proposed that they are less involved in the synthesis of previously mentioned secondary metabolites and are more involved in PI or alkaloid synthesis (McDowell et al., 2011). A recent study by Xu et al. (2018) has shown that SlMYC1 plays a role in type VI glandular trichome formation and is involved in the regulation of several terpene synthases.

4.   Role of glandular trichome-produced compounds

4.1.   Terpenes

Terpenoids represent a large class of diverse metabolites with roles in plants, such as photosynthesis, protein glycosylation, electron transport, membrane structure and function and in phytohormonal regulation (Pichersky & Raguso, 2018; Tholl, 2015). A few notable examples are carotenoids, chlorophylls, quinones, sterols, and the phytohormones abscisic acid, strigolactones, gibberellins and brassinosteroids (Pichersky & Raguso, 2018; Zi et al., 2014). However, the majority of terpenoids are volatile in nature and play roles involved biotic and abiotic stresses, such as attracting pollinators, defense against pests, and in attracting beneficial

soil micro-organisms (Pichersky & Raguso, 2018). Several extensive reviews on terpenoids and terpenoid biochemistry have been published in the last decade (see e.g., Hemmerlin et al., 2012; Pichersky & Raguso, 2018; Tholl, 2015; Zi et al., 2014). In short, all terpenoids originate from the five carbon (C_5) "building blocks" isomers isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), which can be synthesized by two alternative pathways, the mevalonic acid (MVA) and the methylerythritol phosphate (MEP) pathway (Hemmerlin et al., 2012). Both pathways operate independently from each other, with the MVA pathway in the cytosol and the MEP pathway in plastids (Hemmerlin et al., 2012). However, there are indications of metabolic cross talk between them (Tholl, 2015). IPP and DMAPP serve as substrates for elongation and are utilized by prenyltransferases to form larger prenyl diphosphate intermediates, namely C_{10} geranyl diphosphate (GPP), C_{15} farnesyl diphosphate (FPP), C_{20} geranylgeranyl diphosphate (GGPP). Through cyclization of these linear precursors, C_{10} monoterpenes, C_{15} sesquiterpenes, and C_{20} diterpenes are synthesized, whereas through condensation C_{10} irregular monoterpenes, C_{30} triterpenes, and C_{40} tetraterpenes are formed (Hemmerlin et al., 2012).

Terpenoids are major components of herbivore-induced plant volatiles (HIPVs), attracting predators and parasitoids to plants under attack from herbivores. For example, the mite *T. urticae* induced indirect defenses in tomato by the biosynthesis of monoterpenes and diterpenes within one day and a delayed increase in volatile terpenoid emission after 4 days (Kant et al., 2004). In addition, terpenoids have also been reported as toxic or repellent, at higher concentrations, and can play roles in direct defenses against pests (de Oliveira et al., 2020). The presence of sesquiterpenes in trichomes of some wild tomato species were found to be important for resistance to several insect pests, such as Colorado potato beetles (Carter et al., 1989), beet armyworms (*S. exigua* Hübner) (Eigenbrode et al., 1994), spider mites (de Oliveira et al., 2018; Maluf et al., 2001), and whiteflies (Bleeker et al., 2011). In many of these examples, the presence of sesquiterpene 7-epi-zingiberene, produced by type VI trichomes, was found to be

frequently responsible for toxicity and repellence (Bleeker et al., 2011, 2012; de Oliveira et al., 2018). Recently, two more toxic derivatives from 7-epi-zingiberene were described, 9-hydroxy-zingiberene and 9-hydroxy-10,11-epoxyzingiberene, with repellent effects to whiteflies and spider mites (Dawood & Snyder, 2020; Zabel et al., 2021). Zabel et al. (2021) reported a gene, encoding a cytochrome P450 oxygenase, that is responsible for the oxidation of 7-epi-zingiberene into its derivatives. Wang et al. (2020) reported that 7-epi-zingiberene did not have a major affect on phloem-sucking potato aphids. However, the authors described two distinct groups of sesquiterpenes, β-caryophyllene/α-humulene and α-santalene/α-bergamotene/β-bergamotene, present in larger quantities in *S. habrochaites,* that did affect potato aphid feeding and performance.

4.2.   Methyl Ketones

Methyl ketones are a widely distributed class of fatty-acid derived compounds highly effective in protecting plants against pests (Williams et al., 1980). Methyl ketones synthesized in plants have an odd number of carbons, C_7 to C_{15}. These include, C_7 2-heptanone, C_9 2-nonanone, C_{11} 2-undecanone, C_{13} 2-tridecanone and C_{15} 2-pentadecanone (Fridman et al., 2005). Methyl ketone biosynthesis has been well described in *S. habrochaites* and consists of two steps (Yu et al., 2010). During fatty acid biosynthesis in chloroplasts, a 3-ketoacyl-acyl carrier protein intermediate is hydrolyzed, resulting in 3-ketoacids. This step is catalyzed by a methyl ketone synthase 2 (MKS2) enzyme. Catalyzed by another methyl ketone synthase enzyme, MSK1, these 3-ketoacids are then decarboxylated into methyl ketones (Yu et al., 2010). Methyl ketones, primarily 2-tridecanone and 2-undecanone have been linked to resistance in *S. habrochaites* against several insect pests, such as spider mites (Chatzivasileiadis & Sabelis, 1997; Farrar & Kennedy, 1987), corn earworm (Farrar & Kennedy, 1987), tobacco hornworm and, cotton aphid (*Aphis gossypii* Glover) (Williams et al., 1980).

4.3.   Acyl sugars

Sugar esters, also called acyl sugars, are nonvolatile metabolites which are produced, stored, and exuded from several glandular trichomes, providing broad-spectrum insect resistance in many tomato species (Vosman et al., 2018). Acyl sugars comprise of a glucose or sucrose base with 3 to 4 esterified methyl-branched fatty acid side chains which vary in length and branching patterns (see Fan et al., 2019 for a review on acyl sugar biosynthesis and diversity). Trichome types I, IV and even VI have been reported to exudate acyl sugars, however they are mostly exudated from trichome type IV. In *S. pennellii,* acyl sugars can represent up to 20% of the leaf dry weight (Fobes et al., 1985), where they are capable of immobilizing or suffocating insects by their emulsifying and surfactant nature which allow them to easily stick to insect cuticles (Wagner et al., 2004). In addition, acyl sugars may also have toxic properties to insects (Luu et al., 2017). Glandular trichomes density and acyl sugars content was shown to correlate with whitefly and spider mite resistance (Alba et al., 2009). It has been indicated that acyl sugar mediated resistance depends on the type of sugar backbone and acyl chain composition (Fan et al., 2019; Leckie et al., 2016). Acyl sucroses and acyl sugars enriched in 3-methylbutanoic (i-C5) fatty acids were best capable of suppressing whitefly oviposition (Leckie et al., 2016). Ben-Mahmoud et al. (2018) reported the suppression of western flower thrips (*F. occidentalis* Pergande) oviposition by acyl sugars. The authors reported the involvement of i-C5, i-C9 and i-C11 acyl sugar fatty acids responsible for reduced oviposition.

5.   Conclusions and perspectives

The development of new tomato cultivars has been conducted for several decades. The cultivated tomato has been selected for a wide variety of desirable characteristics such as, changes in fruit size, fruit shape, growth habit (apical dominance), leaf morphology, flavor (soluble solids), texture and color (Bai & Lindhout, 2007; Liu et al., 2020; Paran & van der Knaap, 2007). The current tomato breeding goals aim at breeding for increased resistance to

biotic and abiotic stresses, as the use of fungicides and pesticides is severely restricted and global climates are changing (Zeist et al., 2019). Many wild tomato species are more resistant to insect pests than cultivated tomatoes. They posses a rich genetic background and are an important source of diversity for the development of resistant tomato varieties through genetic transfer of important resistance traits (Stout et al., 2018). Glandular trichomes and their trichome-produced compounds are an important line of defense and can be exploited for increased resistance against herbivorous insects and pathogens in cultivated tomato varieties (Glas et al., 2012).

The availability of genomic data has allowed researchers and breeders to discover various genes involved in the biosynthesis pathways of trichome-produced compounds (Zeist et al., 2019). Respective biosynthetic genes have been identified in *S. pennellii, S. habrochaites,* and *S. galapagense* (Leckie et al., 2012; Therezan et al., 2021; Zeist et al., 2019). For example, it has recently been shown that *S. habrochaites* trichome morphology could be mimicked in cultivated tomato by transferring plastid-derived sesquiterpene synthesis to type-VI trichomes of cultivated tomato (Therezan et al., 2021). Furthermore, the broad-spectrum insect resistance of *S. galapagense* holds great promises for transfer into cultivated tomato. The Wf1 QTL region previously identified in *S. galapagense* contains several structural and transcription factor genes that may be responsible for changes in insect resistance and metabolite synthesis between *S. galapagense* and cultivated tomato (Vosman et al., 2018). Leckie et al. (2012) reported the introgression of QTL regions from *S. pnnellii* in cultivated tomato, which increased acyl sugar content and increased whitefly resistance. The extensive potential of glandular trichomes and trichome-produced compounds has not remotely been exploited due to the complex and multilayered nature of plant secondary metabolism. The outcomes of such manipulations are not easily predictable. However, through breeding or genetic engineering researchers can

acquire a stronger grip on how to obtain the desired levels of chemical compounds in insect resistant tomato varieties.

Insect herbivory triggers systemic signals that are perceived by receptors on the plasma membrane, such as the SYR1 in the systemin-mediated defense pathway in tomato (Wang et al., 2018). It has become increasingly clear that protein translocation on the plasma membrane is involved in the organization of signaling compounds during membrane signaling in cells (Zhang et al., 2020). A recent study by Toyota et al. (2018) reported that glutamate triggers a long-distance calcium-based signaling pathway that activated defense responses in undamaged plant parts of Arabidopsis. This raises the question that systemically propagated electric potentials could also participate in Sys-mediated defense responses in tomato plants. Future research into systemin, electrical potentials and ProSys-dependent pathways will contribute to our understanding of systemic defense regulation in plants.

6.   References

Alba, J. M., Montserrat, M., & Fernández-Muñoz, R. (2009). Resistance to the two-spotted spider mite (Tetranychus urticae) by acylsucroses of wild tomato (Solanum pimpinellifolium) trichomes studied in a recombinant inbred line population. *Experimental and Applied Acarology*, *47*, 35–47. https://doi.org/10.1007/s10493-008-9192-4

Alba, J. M., Schimmel, B. C. J., Glas, J. J., Ataide, L. M. S., Pappas, M. L., Villarroel, C. A., Schuurink, R. C., Sabelis, M. W., & Kant, M. R. (2015). Spider mites suppress tomato defenses downstream of jasmonate and salicylate independently of hormonal crosstalk. *New Phytologist*, *205*, 828–840. https://doi.org/10.1111/nph.13075

Aljbory, Z., & Chen, M.-S. (2018). Indirect plant defense against insect herbivores. *Insect Science*, *25*, 2–23. https://doi.org/10.1111/1744-7917.12436

Bai, Y., & Lindhout, P. (2007). Domestication and breeding of tomatoes: What have we gained and what can we gain in the future? *Annals of Botany, 100*, 1085–1094. https://doi.org/10.1093/aob/mcm150

Balcke, G. U., Bennewitz, S., Bergau, N., Athmer, B., Henning, A., Majovsky, P., Jiménez-Gómez, J. M., Hoehenwarter, W., & Tissier, A. (2017). Multi-omics of tomato glandular trichomes reveals distinct features of central carbon metabolism supporting high productivity of specialized metabolites. *Plant Cell, 29*, 960–983. https://doi.org/10.1105/tpc.17.00060

Bar, M., & Shtein, I. (2019). Plant trichomes and biomechanics of defense in various systems with Solanaceae as a model. *Botany, 97*, 651–660. https://mc06.manuscriptcentral.com/botany-pubsBotany

Beecher, G. R. (1998). Nutrient content of tomatoes and tomato products. *Experimental Biology and Medicine, 218*, 98–100. https://doi.org/10.3181/00379727-218-44282a

Ben-Mahmoud, S., Smeda, J. R., Chappell, T. M., Stafford-Banks, C., Kaplinsky, C. H., Anderson, T., Mutschler, M. A., Kennedy, G. G., & Ullman, D. E. (2018). Acylsugar amount and fatty acid profile differentially suppress oviposition by western flower thrips, frankliniella occidentalis, on tomato and interspecific hybrid flowers. *PLoS ONE, 13*. https://doi.org/10.1371/journal.pone.0201583

Bergau, N., Bennewitz, S., Syrowatka, F., Hause, G., & Tissier, A. (2015). The development of type VI glandular trichomes in the cultivated tomato Solanum lycopersicum and a related wild species S. habrochaites. *BMC Plant Biology, 15*. https://doi.org/10.1186/s12870-015-0678-z

Besser, K., Harper, A., Welsby, N., Schauvinhold, I., Slocombe, S., Li, Y., Dixon, R. A., & Broun, P. (2009). Divergent regulation of terpenoid metabolism in the trichomes of wild

and cultivated tomato species. *Plant Physiology*, *149*, 499–514. https://doi.org/10.1104/pp.108.126276

Bezerra, R. H. S., Sousa-Souto, L., Santana, A. E. G., & Ambrogi, B. G. (2021). Indirect plant defenses: volatile organic compounds and extrafloral nectar. In *Arthropod-Plant Interactions* (Vol. 15, pp. 467–489). Springer Science and Business Media B.V. https://doi.org/10.1007/s11829-021-09837-1

Bhattacharya, R., Koramutla, M. krishna, Negi, M., Pearce, G., & Ryan, C. A. (2013). Hydroxyproline-rich glycopeptide signals in potato elicit signalling associated with defense against insects and pathogens. *Plant Science*, *207*, 88–97. https://doi.org/10.1016/j.plantsci.2013.03.002

Blanca, J., Montero-Pau, J., Sauvage, C., Bauchet, G., Illa, E., Díez, M. J., Francis, D., Causse, M., van der Knaap, E., & Cañizares, J. (2015). Genomic variation in tomato, from wild ancestors to contemporary breeding accessions. *BMC Genomics*, *16*. https://doi.org/10.1186/s12864-015-1444-1

Blanca, J., Sanchez-Matarredona, D., Ziarsolo, P., Montero-Pau, J., van der Knaap, E., José Díez, M., & Cañizares, J. (2021). Haplotype analyses reveal novel insights into tomato history and domestication including long-distance migrations and latitudinal adaptations. *Horticulture Research*. https://doi.org/10.1101/2021.06.18.448912

Bleeker, P. M., Diergaarde, P. J., Ament, K., Schütz, S., Johne, B., Dijkink, J., Hiemstra, H., de Gelder, R., de Both, M. T. J., Sabelis, M. W., Haring, M. A., & Schuurink, R. C. (2011). Tomato-produced 7-epizingiberene and R-curcumene act as repellents to whiteflies. *Phytochemistry*, *72*, 68–73. https://doi.org/10.1016/j.phytochem.2010.10.014

Bleeker, P. M., Mirabella, R., Diergaarde, P. J., VanDoorn, A., Tissier, A., Kant, M. R., Prins, M., de Vos, M., Haring, M. A., & Schuurink, R. C. (2012). Improved herbivore resistance in cultivated tomato with the sesquiterpene biosynthetic pathway from a wild relative.

Proceedings of the National Academy of Sciences, *109*, 20124–20129. https://doi.org/10.1073/pnas.1208756109

Bonaventure, G. (2012). Perception of insect feeding by plants. *Plant Biology*, *14*, 872–880. https://doi.org/10.1111/j.1438-8677.2012.00650.x

Boughton, A. J., Hoover, K., & Felton, G. W. (2005). Methyl jasmonate application induces increased densities of glandular trichomes on tomato, Lycopersicon esculentum. *Journal of Chemical Ecology*, *31*, 2211–2216. https://doi.org/10.1007/s10886-005-6228-7

Bruessow, F., Gouhier-Darimont, C., Buchala, A., Metraux, J. P., & Reymond, P. (2010). Insect eggs suppress plant defence against chewing herbivores. *Plant Journal*, *62*, 876–885. https://doi.org/10.1111/j.1365-313X.2010.04200.x

Buonanno, M., Coppola, M., di Lelio, I., Molisso, D., Leone, M., Pennacchio, F., Langella, E., Rao, R., & Monti, S. M. (2018). Prosystemin, a prohormone that modulates plant defense barriers, is an intrinsically disordered protein. *Protein Science*, *27*, 620–632. https://doi.org/10.1002/pro.3348

Carter, C. D., Sacalis, J. N., & Gianfagna, T. J. (1989). Zingiberene and resistance to Colorado potato beetle in Lycopersicon hirsutum f. hirsutum. *Journal of Agricultural and Food Chemistry*, *37*, 206–210.

Chalvin, C., Drevensek, S., Dron, M., Bendahmane, A., & Boualem, A. (2020). Genetic control of glandular trichome development. In *Trends in Plant Science* (Vol. 25, pp. 477–487). Elsevier Ltd. https://doi.org/10.1016/j.tplants.2019.12.025

Channarayappa, C., Shivashankar, G., Muniyappa, V., & Frist, R. H. (1992). Resistance of Lycopersicon species to Bemisia tabaci, a tomato leaf curl virus vector. *Canadian Journal of Botany*, *70*, 2184–2192.

Chatzivasileiadis, E. A., & Sabelis, M. W. (1997). Toxicity of methyl ketones from tomato trichomes to Tetranychus urticae Koch. *Experimental and Applied Acarology*, *21*, 473–484. https://doi.org/10.1023/a:1018436113892

Chen, D., Shao, M., Sun, S., Liu, T., Zhang, H., Qin, N., Zeng, R., & Song, Y. (2019). Enhancement of jasmonate-mediated antiherbivore defense responses in tomato by acetic acid, a potent inducer for plant protection. *Frontiers in Plant Science*, *10*. https://doi.org/10.3389/fpls.2019.00764

Chen, Y. H., Gols, R., & Benrey, B. (2015). Crop domestication and its impact on naturally selected trophic interactions. *Annual Review of Entomology*, *60*, 35–58. https://doi.org/10.1146/annurev-ento-010814-020601

Chung, S. H., Rosa, C., Hoover, K., Luthe, D. S., & Felton, G. W. (2013). Colorado potato beetle manipulates plant defenses in local and systemic leaves. *Plant Signaling and Behavior*, *8*. https://doi.org/10.4161/psb.27592

Constabel, C. P., Yip, L., & Ryan, C. A. (1998). Prosystemin from potato, black nightshade, and bell pepper: primary structure and biological activity of predicted systemin polypeptides. *Plant Molecular Biology*, *36*, 55.

Coppola, M., Cascone, P., Madonna, V., di Lelio, I., Esposito, F., Avitabile, C., Romanelli, A., Guerrieri, E., Vitiello, A., Pennacchio, F., Rao, R., & Corrado, G. (2017). Plant-to-plant communication triggered by systemin primes anti-herbivore resistance in tomato. *Scientific Reports*, *7*. https://doi.org/10.1038/s41598-017-15481-8

Coppola, M., Corrado, G., Coppola, V., Cascone, P., Martinelli, R., Digilio, M. C., Pennacchio, F., & Rao, R. (2015). Prosystemin overexpression in tomato enhances resistance to different biotic stresses by activating genes of multiple signaling pathways. *Plant Molecular Biology Reporter*, *33*, 1270–1285. https://doi.org/10.1007/s11105-014-0834-x

Corrado, G., Sasso, R., Pasquariello, M., Iodice, L., Carretta, A., Cascone, P., Ariati, L., Digilio, M. C., Guerrieri, E., & Rao, R. (2007). Systemin regulates both systemic and volatile signaling in tomato plants. *Journal of Chemical Ecology*, *33*, 669–681. https://doi.org/10.1007/s10886-007-9254-9

Dawood, M. H., & Snyder, J. C. (2020). The alcohol and epoxy alcohol of zingiberene, produced in trichomes of wild tomato, are more repellent to spider mites than zingiberene. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.00035

de Oliveira, J. R. F., de Resende, J. T. V., Filho, R. B. de L., Roberto, S. R., da Silva, P. R., Rech, C., & Nardi, C. (2020). Tomato breeding for sustainable crop systems: high levels of zingiberene providing resistance to multiple arthropods. *Horticulturae*, *6*, 1–14. https://doi.org/10.3390/horticulturae6020034

de Oliveira, J. R. F., de Resende, J. T. V., Maluf, W. R., Lucini, T., de Lima Filho, R. B., de Lima, I. P., & Nardi, C. (2018). Trichomes and allelochemicals in tomato genotypes have antagonistic effects upon behavior and biology of tetranychus urticae. *Frontiers in Plant Science*, *9*. https://doi.org/10.3389/fpls.2018.01132

Degenhardt, D. C., Refi-Hind, S., Stratmann, J. W., & Lincoln, D. E. (2010). Systemin and jasmonic acid regulate constitutive and herbivore-induced systemic volatile emissions in tomato, Solanum lycopersicum. *Phytochemistry*, *71*, 2024–2037. https://doi.org/10.1016/j.phytochem.2010.09.010

Eigenbrode, S. D., Trumble, J. T., Millar, J. G., & White, K. (1994). Topical toxicity of tomato sesquiterpenes to the beet armyworm and the role of these compounds in resistance derived from an accession of Lycoperiscon hirsutum f. typicum. *Journal of Agricultural and Food Chemistry*, *42*, 807–810. https://doi.org/10.1021/jf9802979

Fan, P., Leong, B. J., & Last, R. L. (2019). Tip of the trichome: evolution of acylsugar metabolic diversity in Solanaceae. *Current Opinion in Plant Biology*, *49*, 8–16. https://doi.org/10.1016/j.pbi.2019.03.005

FAO. (2020). *FAOSTAT Statistical Database*. Food and Agriculture Organization of the United Nations. https://www.fao.org/faostat/en/#data/QCL

Farrar, R. R., & Kennedy, G. G. (1987). 2-Undecanone, a constituent of the glandular trichomes of Lycopersicon hirsutum f. glabratum: effects on Heliothis zea and Manduca sexta growth and survival. *Entomologia Experimentalis et Applicata*, *43*, 17–23. https://doi.org/10.1111/j.1570-7458.1987.tb02196.x

Feng, Z., Bartholomew, E. S., Liu, Z., Cui, Y., Dong, Y., Li, S., Wu, H., Ren, H., & Liu, X. (2021). Glandular trichomes: new focus on horticultural crops. *Horticulture Research*, *8*. https://doi.org/10.1038/s41438-021-00592-1

Firdaus, S., van Heusden, A. W., Hidayati, N., Supena, E. D. J., Mumm, R., de Vos, R. C. H., Visser, R. G. F., & Vosman, B. (2013). Identification and QTL mapping of whitefly resistance components in Solanum galapagense. *Theoretical and Applied Genetics*, *126*, 1487–1501. https://doi.org/10.1007/s00122-013-2067-z

Fobes, J. F., Mudd, J. B., & Marsden, M. P. F. (1985). Epicuticular lipid accumulation on the leaves of Lycopersicon pennellii (Corr.) D'Arcy and Lycopersicon esculentum Mill. *Plant Physiol*, *77*, 567–570.

Fridman, E., Wang, J., Iijima, Y., Froehlich, J. E., Gang, D. R., Ohlrogge, J., & Pichersky, E. (2005). Metabolic, genomic, and biochemical analyses of glandular trichomes from the wild tomato species Lycopersicon hirsutum identify a key enzyme in the biosynthesis of methylketones. *Plant Cell*, *17*, 1252–1267. https://doi.org/10.1105/tpc.104.029736

Glas, J. J., Schimmel, B. C. J., Alba, J. M., Escobar-Bravo, R., Schuurink, R. C., & Kant, M. R. (2012). Plant glandular trichomes as targets for breeding or engineering of resistance to

herbivores. *International Journal of Molecular Sciences*, *13*, 17077–17103. https://doi.org/10.3390/ijms131217077

Haj Ahmad, F., Wu, X. N., Stintzi, A., Schaller, A., & Schulze, W. X. (2019). The systemin signaling cascade as derived from time course analyses of the systemin-responsive phosphoproteome. *Molecular and Cellular Proteomics*, *18*, 1526–1542. https://doi.org/10.1074/mcp.RA119.001367

Hemmerlin, A., Harwood, J. L., & Bach, T. J. (2012). A raison d'être for two distinct pathways in the early steps of plant isoprenoid biosynthesis? *Progress in Lipid Research*, *51*, 95–148. https://doi.org/10.1016/j.plipres.2011.12.001

Jacinto, T., McGurl, B., Franceschi, V., Delano-Freier, J., & Ryan, C. A. (1997). Tomato prosystemin promoter confers wound-inducible, vascular bundle-specific expression of the β-glucuronidase gene in transgenic tomato plants. *Planta*, *203*, 406–412.

Jenkins, J. A. (1948). The origin of the cultivated tomato. *Economic Botany*, *2*, 379–392.

Kant, M. R., Ament, K., Sabelis, M. W., Haring, M. A., & Schuurink, R. C. (2004). Differential timing of spider mite-induced direct and indirect defenses in tomato plants. *Plant Physiology*, *135*, 483–495. https://doi.org/10.1104/pp.103.038315

Kant, M. R., Jonckheere, W., Knegt, B., Lemos, F., Liu, J., Schimmel, B. C. J., Villarroel, C. A., Ataide, L. M. S., Dermauw, W., Glas, J. J., Egas, M., Janssen, A., van Leeuwen, T., Schuurink, R. C., Sabelis, M. W., & Alba, J. M. (2015). Mechanisms and ecological consequences of plant defence induction and suppression in herbivore communities. *Annals of Botany*, *115*, 1015–1051. https://doi.org/10.1093/aob/mcv054

Knapp, S., & Peralta, I. E. (2016). The tomato (Solanum lycopersicum L., Solanaceae) and its botanical relatives. In *The Tomato Genome*. http://tgrc.ucdavis.edu/key.html

Koornneef, A., & Pieterse, C. M. J. (2008). Cross talk in defense signaling. *Plant Physiology*, *146*, 839–844. https://doi.org/10.1104/pp.107.112029

Kortbeek, R. W. J., Galland, M. D., Muras, A., van der Kloet, F. M., André, B., Heilijgers, M., van Hijum, S. A. F. T., Haring, M. A., Schuurink, R. C., & Bleeker, P. M. (2021). Natural variation in wild tomato trichomes; selecting metabolites that contribute to insect resistance using a random forest approach. *BMC Plant Biology*, *21*. https://doi.org/10.1186/s12870-021-03070-x

Leckie, B. M., D'Ambrosio, D. A., Chappell, T. M., Halitschke, R., de Jong, D. M., Kessler, A., Kennedy, G. G., & Mutschler, M. A. (2016). Differential and synergistic functionality of acylsugars in suppressing oviposition by insect herbivores. *PLoS ONE*, *11*. https://doi.org/10.1371/journal.pone.0153345

Leckie, B. M., de Jong, D. M., & Mutschler, M. A. (2012). Quantitative trait loci increasing acylsugars in tomato breeding lines and their impacts on silverleaf whiteflies. *Molecular Breeding*, *30*, 1621–1634. https://doi.org/10.1007/s11032-012-9746-3

Li, L., Li, C., Lee, G. I., Howe, G. A., & Ryan, C. A. (2002). Distinct roles for jasmonate synthesis and action in the systemic wound response of tomato. *PNAS*, *99*, 6416–6421. https://www.pnas.org

Liu, D., Yang, L., Zhang, J., Zhu, G., Lü, H., Lü, Y., Wang, Y., Cao, X., Sun, T., Huang, S., & Wu, Y. (2020). Domestication and breeding changed tomato fruit transcriptome. *Journal of Integrative Agriculture*, *19*, 120–132. https://doi.org/10.1016/S2095-3119(19)62824-8

Lucatti, A. F., van Heusden, A. W., de Vos, R. C. H., Visser, R. G. F., & Vosman, B. (2013). Differences in insect resistance between tomato species endemic to the Galapagos Islands. *BMC Evolutionary Biology*, *13*. https://doi.org/10.1186/1471-2148-13-175

Luu, V. T., Weinhold, A., Ullah, C., Dressel, S., Schoettner, M., Gase, K., Gaquerel, E., Xu, S., & Baldwin, I. T. (2017). O-Acyl sugars protect a wild tobacco from both native fungal pathogens and a specialist herbivore. *Plant Physiology*, *174*, 370–386. https://doi.org/10.1104/pp.16.01904

Maluf, W. R., Campos, G. A., & das Graças Cardoso, M. (2001). Relationships between trichome types and spider mite (Tetranychus evansi) repellence in tomatoes with respect to foliar zingiberene contents. *Euphytica*, *121*, 73–80.

McDowell, E. T., Kapteyn, J., Schmidt, A., Li, C., Kang, J. H., Descour, A., Shi, F., Larson, M., Schilmiller, A., An, L., Daniel Jones, A., Pichersky, E., Soderlund, C. A., & Gang, D. R. (2011). Comparative functional genomic analysis of solanum glandular trichome types. *Plant Physiology*, *155*, 524–539. https://doi.org/10.1104/pp.110.167114

McGurl, B., Orozco-Cardenas, M., Pearce, G., & Ryan, C. A. (1994). Overexpression of the prosystemin gene in transgenic tomato plants generates a systemic signal that constitutively induces proteinase inhibitor synthesis. *Proceedings of the National Academy of Sciences*, *91*, 9799–9802. https://doi.org/10.1073/pnas.91.21.9799

McGurl, B., Pearce, G., Orozco-Cardenas, M., & Ryan, C. A. (1992). Structure, Expression, and Antisense Inhibition of the Systemin Precursor Gene. In *New Series* (Vol. 255, Issue 5051).

Molisso, D., Coppola, M., Buonanno, M., di Lelio, I., Monti, S. M., Melchiorre, C., Amoresano, A., Corrado, G., Delano-Frier, J. P., Becchimanzi, A., Pennacchio, F., & Rao, R. (2022). Tomato Prosystemin Is Much More than a Simple Systemin Precursor. *Biology*, *11*(1). https://doi.org/10.3390/biology11010124

Mucha, P., Ruczynski, J., Dobkowski, M., Backtrog, E., & Rekowski, P. (2019). Capillary electrophoresis study of systemin peptides spreading in tomato plant. *Electrophoresis*, *40*, 336–342. https://doi.org/10.1002/elps.201800206

Narváez-Vásquez, J., Florin-Christensen, J., & Ryan, C. A. (1999). Positional specificity of a phospholipase A activity induced by wounding, systemin, and oligosaccharide elicitors in tomato leaves. *The Plant Cell*, *11*, 2249–2260. www.plantcell.org

Narváez-Vásquez, J., Orozco-Cárdenas, M. L., & Ryan, C. A. (2007). Systemic wound signaling in tomato leaves is cooperatively regulated by systemin and hydroxyproline-rich glycopeptide signals. *Plant Molecular Biology*, *65*, 711–718. https://doi.org/10.1007/s11103-007-9242-z

Narváez-Vásquez, J., & Ryan, C. A. (2004). The cellular localization of prosystemin: A functional role for phloem parenchyma in systemic wound signaling. *Planta*, *218*, 360–369. https://doi.org/10.1007/s00425-003-1115-3

Orozco-Cardenas, M., Mcgurl, B., & Ryant, C. A. (1993). Expression of an antisense prosystemin gene in tomato plants reduces resistance toward Manduca sexta larvae. *Proceedings of the National Academy of Sciences*, *90*, 8273–8276.

Paran, I., & van der Knaap, E. (2007). Genetic and molecular regulation of fruit and plant domestication traits in tomato and pepper. *Journal of Experimental Botany*, *58*, 3841–3852. https://doi.org/10.1093/jxb/erm257

Pearce, G. (2011). Systemin, hydroxyproline-rich systemin and the induction of protease inhibitors. *Current Protein & Peptide Science*, *12*, 399–408. https://doi.org/10.2174/138920311796391106

Pearce, G., Moura, D. S., Stratmann, J., & Ryan, C. A. (2001). Production of multiple planthormones from a single polyprotein precursor. *Nature*, *411*, 817–820.

Pearce, G., & Ryan, C. A. (2003). Systemic signaling in tomato plants for defense against herbivores: isolation and characterization of three novel defense-signaling glycopeptide hormones coded in a single precursor gene. *Journal of Biological Chemistry*, *278*, 30044–30050. https://doi.org/10.1074/jbc.M304159200

Pearce, G., Strydom, D., Johnson, S., & Ryan, C. A. (1991). A polypeptide from tomato leaves induces wound-inducible proteinase inhibitor proteins. *New Series*, *253*, 895–898.

Peralta, I. E., Spooner, D. M., & Knapp, S. (2008). Taxonomy of Wild Tomatoes and Their Relatives (Solanum sect. Lycopersicoides, sect. Juglandifolia, sect. Lycopersicon; Solanaceae). In *Monographs* (Vol. 84, pp. 1–186).

Pichersky, E., & Raguso, R. A. (2018). Why do plants produce so many terpenoid compounds? *New Phytologist*, *220*, 692–702. https://doi.org/10.1111/nph.14178

Pieterse, C. M. J., Leon-Reyes, A., van der Ent, S., & van Wees, S. C. M. (2009). Networking by small-molecule hormones in plant immunity. *Nature Chemical Biology*, *5*, 308–316. https://doi.org/10.1038/nchembio.164

Pieterse, C. M. J., Ton, J., & van Loon, L. C. (2001). Cross-talk between plant defence signalling pathways: boost or burden? *AgBiotechNet*, *3*. http://www.bio.uu.nl/~fytopath

Razifard, H., Ramos, A., della Valle, A. L., Bodary, C., Goetz, E., Manser, E. J., Li, X., Zhang, L., Visa, S., Tieman, D., van der Knaap, E., & Caicedo, A. L. (2020). Genomic evidence for complex domestication history of the cultivated tomato in Latin America. *Molecular Biology and Evolution*, *37*, 1118–1132. https://doi.org/10.1093/molbev/msz297

Rodriguez, F., Wu, F., Ané, C., Tanksley, S., & Spooner, D. M. (2009). Do potatoes and tomatoes have a single evolutionary history, and what proportion of the genome supports this history? *BMC Evolutionary Biology*, *9*. https://doi.org/10.1186/1471-2148-9-191

Ryan, C. A. (1990). Protease inhibitors in plants: genes for improving defenses against insects and pathogens. *Annual Review of Phytopathology*, *28*, 425–474. www.annualreviews.org

Ryan, C. A. (2000). The systemin signaling pathway: differential activation of plant defensive genes. *Biochimica et Biophysica Acta*, *1477*, 112–121. www.elsevier.com/locate/bba

Sarmento, R. A., Lemos, F., Bleeker, P. M., Schuurink, R. C., Pallini, A., Oliveira, M. G. A., Lima, E. R., Kant, M., Sabelis, M. W., & Janssen, A. (2011). A herbivore that manipulates plant defence. *Ecology Letters*, *14*, 229–236. https://doi.org/10.1111/j.1461-0248.2010.01575.x

Schuurink, R., & Tissier, A. (2020). Glandular trichomes: micro-organs with model status? *New Phytologist*, *225*, 2251–2266. https://doi.org/10.1111/nph.16283

Stenzel, I., Hause, B., Maucher, H., Pitzschke, A., Miersch, O., Ziegler, J., Ryan, C. A., & Wasternack, C. (2003). Allene oxide cyclase dependence of the wound response and vascular bundle-specific generation of jasmonates in tomato – amplification in wound signalling. *Plant*, *33*, 577–589.

Stout, M. J., Kurabchew, H., & Leite, G. L. D. (2018). Host-plant resistance in tomato. In *Sustainable Management of Arthropod Pests of Tomato* (pp. 217–236). Elsevier Inc. https://doi.org/10.1016/B978-0-12-802441-6.00009-7

Sun, J.-Q., Jiang, H.-L., & Li, C.-Y. (2011). Systemin/Jasmonate-mediated systemic defense signaling in tomato. *Molecular Plant*, *4*, 607–615. https://doi.org/10.1093/mp/ssr008

Therezan, R., Kortbeek, R., Vendemiatti, E., Legarrea, S., de Alencar, S. M., Schuurink, R. C., Bleeker, P., & Peres, L. E. P. (2021). Introgression of the sesquiterpene biosynthesis from Solanum habrochaites to cultivated tomato offers insights into trichome morphology and arthropod resistance. *Planta*, *254*. https://doi.org/10.1007/s00425-021-03651-y

Tholl, D. (2015). Biosynthesis and biological functions of terpenoids in plants. In J. Schrader & J. Bohlmann (Eds.), *Biotechnology of Isoprenoids. Advances in Biochemical Engineering/Biotechnology* (Vol. 148, pp. 63–106). Springer, Cham. https://doi.org/10.1007/10_2014_295

Toyota, M., Spencer, D., Sawai-Toyota, S., Jiaqi, W., Zhang, T., Koo, A. J., Howe, G. A., & Gilroy, S. (2018). Glutamate triggers long-distance, calcium-based plant defense signaling. *Botany*, *361*, 1112–1115. https://www.science.org

Uversky, V. N. (2013). Intrinsic disorder-based protein interactions and their modulators. *Current Pharmaceutical Design*, *19*, 4191–4213.

Vendemiatti, E., Zsögön, A., Silva, G. F. F. e., de Jesus, F. A., Cutri, L., Figueiredo, C. R. F., Tanaka, F. A. O., Nogueira, F. T. S., & Peres, L. E. P. (2017). Loss of type-IV glandular trichomes is a heterochronic trait in tomato and can be reverted by promoting juvenility. *Plant Science*, *259*, 35–47. https://doi.org/10.1016/j.plantsci.2017.03.006

Vos, I. A., Pieterse, C. M. J., & van Wees, S. C. M. (2013). Costs and benefits of hormone-regulated plant defences. *Plant Pathology*, *62*, 43–55. https://doi.org/10.1111/ppa.12105

Vosman, B., Kashaninia, A., van't Westende, W., Meijer-Dekens, F., van Eekelen, H., Visser, R. G. F., de Vos, R. C. H., & Voorrips, R. E. (2019). QTL mapping of insect resistance components of Solanum galapagense. *Theoretical and Applied Genetics*, *132*, 531–541. https://doi.org/10.1007/s00122-018-3239-7

Vosman, B., van't Westende, W. P. C., Henken, B., van Eekelen, H. D. L. M., de Vos, R. C. H., & Voorrips, R. E. (2018). Broad spectrum insect resistance and metabolites in close relatives of the cultivated tomato. *Euphytica*, *214*. https://doi.org/10.1007/s10681-018-2124-4

Wagner, G. J., Wang, E., & Shepherd, R. W. (2004). New approaches for studying and exploiting an old protuberance, the plant trichome. *Annals of Botany*, *93*, 3–11. https://doi.org/10.1093/aob/mch011

Wang, F., Park, Y.-L., & Gutensohn, M. (2020). Glandular trichome-derived sesquiterpenes of wild tomato accessions (Solanum habrochaites) affect aphid performance and feeding behavior. *Phytochemistry*, *180*. https://doi.org/10.1016/j.phytochem.2020.112532

Wang, L., Einig, E., Almeida-Trapp, M., Albert, M., Fliegmann, J., Mithöfer, A., Kalbacher, H., & Felix, G. (2018). The systemin receptor SYR1 enhances resistance of tomato against herbivorous insects. *Nature Plants*, *4*(3), 152–156. https://doi.org/10.1038/s41477-018-0106-0

War, A. R., Paulraj, M. G., Ahmad, T., Buhroo, A. A., Hussain, B., Ignacimuthu, S., & Sharma, H. C. (2012). Mechanisms of plant defense against insect herbivores. *Plant Signaling and Behavior*, *7*. https://doi.org/10.4161/psb.21663

War, A. R., Taggar, G. K., Hussain, B., Taggar, M. S., Nair, R. M., & Sharma, H. C. (2018). Plant defence against herbivory and insect adaptations. *AoB PLANTS*, *10*. https://doi.org/10.1093/aobpla/ply037

Weinblum, N., Cna'ani, A., Yaakov, B., Sadeh, A., Avraham, L., Opatovsky, I., & Tzin, V. (2021). Tomato cultivars resistant or susceptible to spider mites differ in their biosynthesis and metabolic profile of the monoterpenoid pathway. *Frontiers in Plant Science*, *12*. https://doi.org/10.3389/fpls.2021.630155

Whitehead, S. R., Turcotte, M. M., & Poveda, K. (2017). Domestication impacts on plant-herbivore interactions: A meta-analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1712). https://doi.org/10.1098/rstb.2016.0034

Williams, W. G., Kennedy, G. G., Yamamoto, R. T., Thacker, J. D., & Bordner, J. (1980). 2-Tridecanone: a naturally occurring insecticide from the wild tomato Lycopersicon hirsutum f. glabratum. *Science*, *207*, 888–889. https://doi.org/10.1126/science.207.4433.888

Xu, J., van Herwijnen, Z. O., Dräger, D. B., Sui, C., Haring, M. A., & Schuurink, R. C. (2018). SlMYC1 regulates type VI glandular trichome formation and terpene biosynthesis in tomato glandular cells. *Plant Cell*, *30*, 2988–3005. https://doi.org/10.1105/tpc.18.00571

Yan, L., Zhai, Q., Wei, J., Li, S., Wang, B., Huang, T., Du, M., Sun, J., Kang, L., Li, C. B., & Li, C. (2013). Role of tomato lipoxygenase D in wound-induced jasmonate biosynthesis and plant immunity to insect herbivores. *PLoS Genetics*, *9*. https://doi.org/10.1371/journal.pgen.1003964

Yu, G., Nguyen, T. T. H., Guo, Y., Schauvinhold, I., Auldridge, M. E., Bhuiyan, N., Ben-Israel, I., Iijima, Y., Fridman, E., Noel, J. P., & Pichersky, E. (2010). Enzymatic functions of wild tomato methylketone synthases 1 and 2. *Plant Physiology*, *154*, 67–77. https://doi.org/10.1104/pp.110.157073

Zabel, S., Brandt, W., Porzel, A., Athmer, B., Bennewitz, S., Schäfer, P., Kortbeek, R., Bleeker, P., & Tissier, A. (2021). A single cytochrome P450 oxidase from Solanum habrochaites sequentially oxidizes 7-epi-zingiberene to derivatives toxic to whiteflies and various microorganisms. *Plant Journal*, *105*, 1309–1325. https://doi.org/10.1111/tpj.15113

Zeist, A. R., da Silva, A. A., Maluf, W. R., de Resende, J. T. v., Gabriel, A., Guerra, E. P., & Zanin, D. S. (2019). Tomato Breeding for Insect-Pest Resistance. In *Recent Advances in Tomato Breeding and Production*. IntechOpen. https://doi.org/10.5772/intechopen.75978

Zhang, H., Zhang, H., & Lin, J. (2020). Systemin-mediated long-distance systemic defense responses. *New Phytologist*, *226*(6), 1573–1582. https://doi.org/10.1111/nph.16495

Zhang, P. J., Zhao, C., Ye, Z. H., & Yu, X. P. (2020). Trade-off between defense priming by herbivore-induced plant volatiles and constitutive defense in tomato. *Pest Management Science*, *76*, 1893–1901. https://doi.org/10.1002/ps.5720

Zhang, Y., Song, H., Wang, X., Zhou, X., Zhang, K., Chen, X., Liu, J., Han, J., & Wang, A. (2020). The roles of different types of trichomes in tomato resistance to cold, drought, whiteflies, and botrytis. *Agronomy*, *10*. https://doi.org/10.3390/agronomy10030411

Zi, J., Mafu, S., & Peters, R. J. (2014). To gibberellins and beyond! Surveying the evolution of (Di)terpenoid metabolism. *Annual Review of Plant Biology*, *65*, 259–286. https://doi.org/10.1146/annurev-arplant-050213-035705