CellPress

Trends in Plant Science

Spotlight

Microbiota-dependent and -independent autoimmunity in plants

Lijing Liu[1], Dev Patel[2], Olivia Cyburt[2], Daowen Wang[3,*], and Zheng Qing Fu[2,*]

In a recent study to identify arabidopsis (*Arabidopsis thaliana*) genes involved in maintaining normal leaf microbiota, Cheng *et al.* discovered *TIP GROWTH DEFECTIVE1* (*TIP1*)encoding an *S*-acyltransferase. The *tip1* mutant exhibits abnormal microbiota levels and phenotypes resembling autoimmune mutants. Further study revealed the existence of both microbiota-dependent and -independent autoimmunity in plants.

Over the past half-century, our understanding of the molecular interactions between plants and pathogens has increased significantly [1,2]. Plants rely on inherent physical defenses and inducible immune mechanisms to combat pathogen infections. The initial phase of these inducible defenses is known as pattern-triggered immunity (PTI). This is activated when plasma membrane-localized pattern recognition receptors detect conserved molecules in plant pathogens, known as pathogen-associated molecular patterns (PAMPs). To establish infections, successful pathogens deliver effectors into plant cells to inhibit PTI, leading to effector-triggered susceptibility. In response, plants have evolved intracellular proteins with nucleotide-binding sites and leucine-rich repeats (NBS-LRR or NLR) that can recognize pathogen effectors directly or sense the activities of their effectors indirectly, thereby initiating effector-triggered immunity (ETI) [3]. Recently, multiple studies have collectively shown that PTI and ETI enhance one another, resulting in a more robust and effective defense [4]. Activation of plant defense leads to elevated expression of defense genes and accumulation of salicylic acid (SA), a defense hormone. Overactivation of these immune responses in the absence of pathogen infection is known as plant autoimmunity and is often associated with dwarfism, leaf chlorosis, and necrosis [5].

Despite the omnipresence of nonpathogenic or commensal microorganisms around plants, our understanding of the intricate relationship between plant immunity and microbiota remains comparatively underexplored. Recently, a groundbreaking paper shed new light on this burgeoning area of research [6].

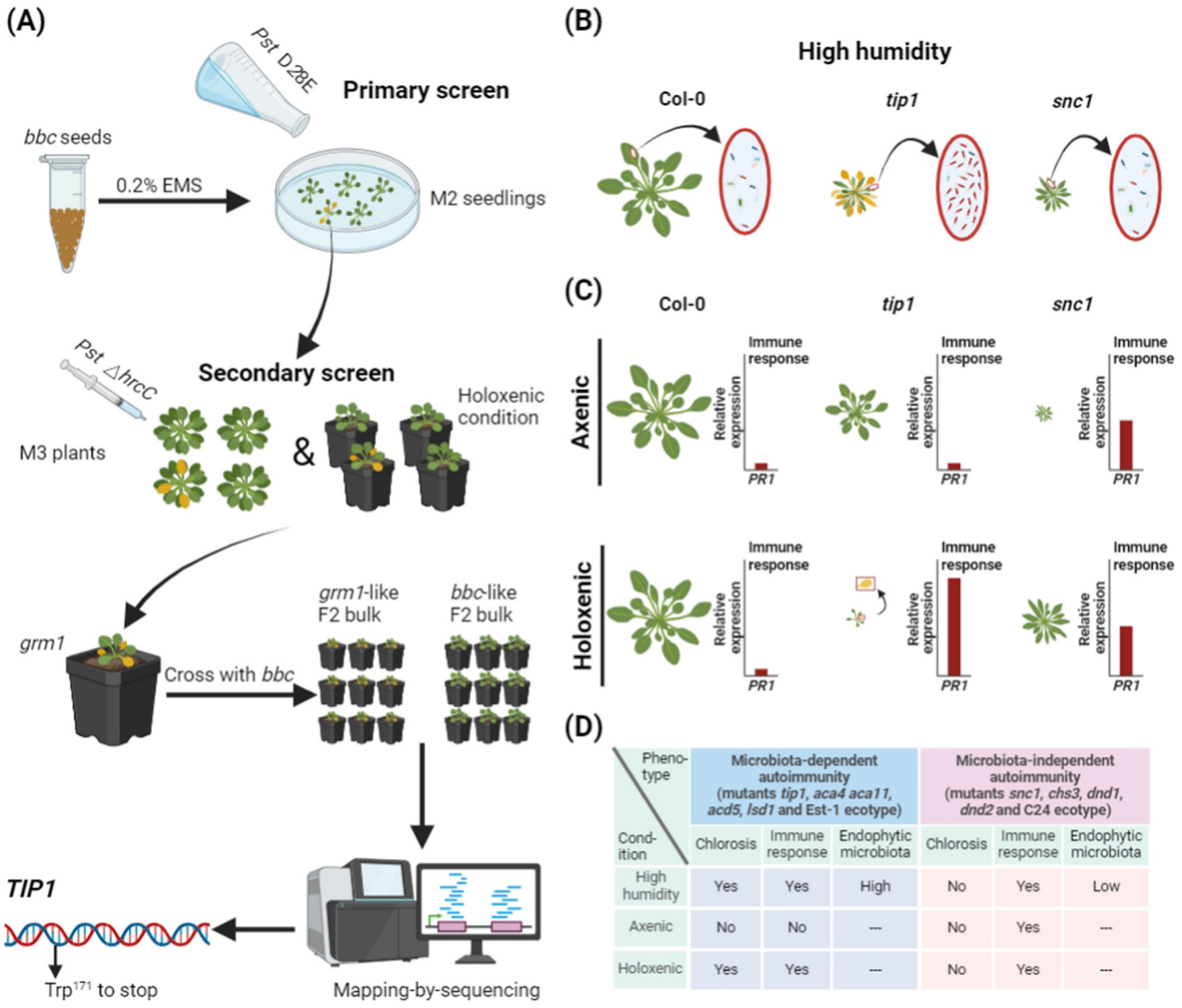
A previous investigation demonstrated that arabidopsis *bak1-5 bkk1-1 cerk1-2* (*bbc*) triple mutants, which lack functional pattern recognition co-receptor genes *BAK1*, *BKK1*, and *CERK1*, can still inhibit the excessive growth of nonpathogenic *Pseudomonas syringae* pv. tomato (*Pst*) DC3000 D28E mutants that are missing 28 effector genes and native leaf microbiota [7]. This indicates that other genes, which either function independently of, or in addition to, these co-receptors, have a role in plant interactions with nonpathogenic microbes.

Cheng *et al.* used a forward genetic screen in the arabidopsis *bbc* mutant background to pinpoint the arabidopsis genes essential for sustaining normal leaf microbiota (Figure 1A) [6]. They conducted comprehensive screening to identify mutants that exhibited disease-like symptoms when encounter nonpathogenic *Pst* D28E. Initially, they flood-inoculated 3-week-old M2 seedlings grown on plates with the nonpathogenic *Pst* D28E strain. Next, they syringe-infiltrated 4-week-old soil-grown M3 plants with the nonpathogenic *Pst* Δ*hrcC* strain, which lacks the type III secretion system needed to deliver effectors into plant cells, and monitored for disease-like symptoms. Last, they observed symptoms triggered by natural soil-derived microbiota under holoxenic conditions using a peat-based gnotobiotic system.

Through these thorough processes, they successfully identified ten *guardians of normal microbiota* (*grm*)mutants.Inresponse to exposure to nonpathogenic *Pst* strains and/or natural soil-derived microbiota, these mutants exhibited disease-like symptoms, including chlorosis and necrosis. To identify the causal mutation of the *grm1* mutant, a mapping-by-sequencing approach was utilized. The *grm1* mutant was backcrossed with the *bbc* mutant, generating an F2 population, which was then analyzed. The authors identified a G to A mutation on chromosome 5 of *TIP1* that is tightly associated with the *grm1* phenotypes. This G to A mutation occursatthesplicesiteofthe5′ end of the third intron, likely causing a disruption in the splicing process and resulting in a premature stop codon at the W171 position.

TIP1 encodes an S-acyltransferase and is essential for protecting leaves from abnormal microbiota levels and composition, particularly under conditions of high humidity (~95% relative humidity; RH). Cheng and colleagues then segregated the *tip1* mutation from the *bbc* triple mutations by crossing the *grm1* mutant with wild-type Col-0 plants and genotyping the resulting F2 population. Alongside the $tip1^{W171stop}$ mutant, Cheng and colleagues discovered two additional T-DNA knockout mutants of *TIP1*.They found that all three *tip1* mutants exhibit humidity-dependent dysbiosis symptoms, including leaf chlorosis and an overabundance of endophytic leaf microbiota.

To investigate the connection between microbiota and autoimmune responses, the researchers initially concentrated on the *tip1*



Phenotype / Condition	Microbiota-dependent autoimmunity (mutants *tip1, aca4 aca11, acd5, lsd1* and Est-1 ecotype)			Microbiota-independent autoimmunity (mutants *snc1, chs3, dnd1, dnd2* and C24 ecotype)		
	Chlorosis	Immune response	Endophytic microbiota	Chlorosis	Immune response	Endophytic microbiota
High humidity	Yes	Yes	High	No	Yes	Low
Axenic	No	No	---	No	Yes	---
Holoxenic	Yes	Yes	---	No	Yes	---

Figure 1. Discovery of *TIP GROWTH DEFECTIVE1* (*TIP1*) as a vital component for sustaining normal microbiota in arabidopsis (*Arabidopsis thaliana*) and characterization of microbiota-dependent and -independent autoimmunity. (A) A forward genetic screen was conducted to identify key components responsible for maintaining normal microbiota in arabidopsis, leading to the discovery of the *guardians of normal microbiota* (*grm*) mutant. *bak1-5 bkk1-1 cerk1-2* (*bbc*) mutant seeds were exposed to 0.2% ethyl methanesulfonate (EMS) to induce mutations, and M1 seeds were then harvested. Three-week-old M2 seedlings were grown on plates and later flood-inoculated with *Pseudomonas syringae pv. tomato* (*Pst*) D28E. Seedlings exhibiting disease-like symptoms were transplanted to soil for seed collection. A secondary screening was performed on 4-week-old M3 plants by observing disease-like symptoms on leaves infiltrated with *Pst ΔhrcC*. Furthermore, disease-like symptoms induced by microbiota in natural soil under holoxenic conditions were observed. Among the identified mutants, *grm1* was chosen for further investigation. The *grm1* mutant was backcrossed with the *bbc* mutant, and the F2 plants were categorized into *grm1*-like and *bbc*-like groups for sequencing. Analysis of the mapping-by-sequencing data revealed a G-to-A mutation at the *TIP1* splicing junction, resulting in a premature stop codon at Trp171. (B) Growth phenotypes of Col-0, *tip1*,and*snc1* under high humidity conditions. The population sizes and diversity of endophytic leaf microbiota in these plants were also assessed. (C) Development of Col-0, *tip1*, and *snc1* plants was examined in both sterile (axenic) and non-sterile (holoxenic) environments. The immune response in these plants, as represented by *PR1* expression levels, was determined. (D) Table outlining the phenotypes of microbiota-dependent autoimmunity and -independent autoimmunity across various conditions. The figure was created with the software BioRender (biorender.com).

mutant and the *snc1* mutant, the latter of which has a gain-of-function E552K mutation in the NLR protein SNC1, resulting in elevated levels of the SNC1^{E552K} protein [6]. Cheng and colleagues found that *tip1* leaves displayed pronounced chlorotic lesions following a 5-day exposure to high humidity. By contrast, *snc1* mutants showed no such symptoms under either normal or high

humidity conditions (Figure 1B). Remarkably, the endophytic bacterial load in *tip1* mutants surged over 1000-fold after 5 days of high humidity, while *snc1* mutants maintained bacterial levels comparable with those in Col-0 plants. Moreover, compared with Col-0, *tip1* mutants exhibited a notably diminished diversity of endophytic microbes, dominated largely by Gammaproteobacteria. Conversely, *snc1* mutants maintained a varied leaf endophytic microbial community akin to that of Col-0. Ultimately, even when cultivated on sterile agar plates, (i.e., without the presence of microbes), *snc1* plants still exhibited elevated expression of the immune-related marker genes *PR1* and *FRK1*.By contrast, the expression of *PR1* and *FRK1* in *tip1* mutant plants significantly diminished, nearing the minimal levels found in wild-type Col-0 plants.

Next, Cheng *et al.* examined arabidopsis Est-1 and C24 ecotypes with autoimmune phenotypes, togetherwithautoi mmune mutants including *tip1*, *acd5* with a ceramide kinase mutation, *lsd1* featuring a defective zinc-finger protein, NLR receptor gene mutants *snc1*, *chs3*, *defense no death 1* (*dnd1*), and *dnd2*, as well as the *aca4 aca11* double mutant, which has mutations in two vacuolar pumps (Figure 1C,D). Following exposure to high humidity for 5–7days,*tip1* (Figure 1C), *acd5, lsd1*, *aca4 aca11* mutants, and Est-1 ecotype displayed varying degrees of chlorosis and lesion, alongside with elevated levels of endophytic microbiota. By contrast, C24 ecotype, *dnd1*, *dnd2*, *snc1*,an d *chs3* mutants did not exhibit these phenotypes (Figure 1C). Using the peat-based gnotobiotic system, the authors demonstrated that the autoimmune phenotypes in *tip1, acd5, lsd1*, *aca4 aca11* mutants and the Est-1 ecotype were significantly influenced by the presence of microbiota, showing lesions, chlorosis, and heightened expression of the immune-related marker *PR1*. However, in the absence of microbiota, these mutants did not demonstrate autoimmune phenotypes. Interestingly, immune-related phenotypes arising from the C24 ecotype, *dnd1*, *dnd2*, *snc1*,and*chs3* mutants, did not require the presence of microbiota and may, in fact, have been somewhat mitigated by it. Consequently, these findings suggest that there are at least two distinct types of autoimmunity in plants: one that is microbiota dependent and another that is not (Figure 1D).

Lesion-mimic mutants can be caused by gain-of-function mutation in *NLR* genes, which result in improper activation or elevated levels of NLR proteins [8]. Since lesion-mimic mutant genes can be targets of pathogen effectors, guardees of NLR proteins, or decoys for pathogen effector targets and *TIP1* is not an *NLR* gene [9], it is worthwhile to determine whether TIP1 has one of the former three roles. Besides autoimmune mutants, arabidopsis Est-1 and C24 ecotypes both exhibit autoimmune phenotypes. Given that the Est-1 ecotype exhibits traits of TIP1-type microbiota-dependent autoimmunity, it would be intriguing to determine whether the autoimmunity observed in the Est-1 ecotype relies on the TIP1-dependent pathway [6].

The interplay between autoimmunity and microbiota in plants closely mirrors the relationship between autoimmunity and dysbiosis observed in animals. These parallels underline the sophisticated relationship between host immune systems and microbial communities across different biological realms. Studying this similarity between plant and animal autoimmunity modulation by microbiota has immediate implications in both zoology and botany, as well as medicine. Future studies should delve into understanding how TIP1 influences microbiota levels and composition. As an acyl-transferase, TIP1 is presumed to transfer an acyl group to a substrate protein. Future research should aim to identify the specific TIP1 substrate protein, which is crucial for preserving the normal microbiota in arabidopsis [6]. In addition, exploring how abnormal microbiota can initiate autoimmunity would be intriguing. For instance, *tip1* mutants exhibit increased levels of endophytic microbiota, albeit with markedly decreased microbial diversity, predominantly comprising Gammaproteobacteria under conditions of high humidity. Future research could investigate whether and how elevated levels of Gammaproteobacteria in *tip1* mutant plants are recognized by the immune system of the plant, subsequently activating immune responses.

Acknowledgments

This work is supported by a grant from the National Science Foundation (IOS-2207677) to Z.Q.F.

Declaration of interests

The authors declare no competing interests.

[1]The Key Laboratory of Plant Development and Environmental Adaptation Biology, Ministry of Education, School of Life Sciences, Shandong University, Qingdao 266237, China
[2]Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA
[3]State Key Laboratory of Wheat and Maize Crop Science, College of Agronomy and Center for Crop Genome Engineering, Henan Agricultural University, Longzi Lake Campus, Zhengzhou 450046, China

*Correspondence:
dwwang@henau.edu.cn (D. Wang) and
zfu@mailbox.sc.edu (Z.Q. Fu).

https://doi.org/10.1016/j.tplants.2024.12.003

Published by Elsevier Ltd.

References

1. Boyd, L.A. *et al.* (2013) Plant–pathogen interactions: disease resistance in modern agriculture. *Trends Genet.* 29, 233–240
2. Jones, J.D.G. *et al.* (2024) The plant immune system: from discovery to deployment. *Cell* 187, 2095–2116
3. Jones, J.D. and Dangl, J.L. (2006) The plant immune system. *Nature* 444, 323–329
4. Chang, M. *et al.* (2021) PTI and ETI: convergent pathways with diverse elicitors. *Trends Plant Sci.* 27, 113–115
5. Freh, M. *et al.* (2022) Plant autoimmunity-fresh insights into an old phenomenon. *Plant Physiol.* 188, 1419–1434
6. Cheng, Y.T. *et al.* (2024) Roles of microbiota in autoimmunity in *Arabidopsis* leaves. *Nat. Plants* 10, 1363–1376
7. Xin, X.F. *et al.* (2016) Bacteria establish an aqueous living space in plants crucial for virulence. *Nature* 539, 524–529
8. Chakraborty, J. *et al.* (2018) Autoimmunity in plants. *Planta* 248, 751–767
9. Rodriguez, E. *et al.* (2016) Making sense of plant autoimmunity and 'negative regulators'. *FEBS J.* 283, 1385–1391