Agricultural Science & Technology, 2013, 14(12): 1698–1701, 1718

Advances on the Molecular Mechanism of the Interaction between *Ralstonia solanacearum* and Hosts

Dousheng WU, Gaofei JIANG, Yanmei YU, Ying LIU, Wei DING*

College of Plant Protection, Southwest University, Chongqing 400716, China

Abstract *Ralstonia solanacearum* is an important model phytopathogenic bacterium that causes bacterial wilt disease on many plant species and leads to serious economic losses. The interactions between *R. solanacearum* and host plants have become a model system for the study of plants and pathogens interactions. This paper reviews the advances on the molecular mechanisms between *R. solanacearum* and hosts interaction including the formation of plant innate immunity, the suppression of plant innate immunity by this pathogen and the activation of effector–triggered immunity. Furthermore, we made a prospect on how to utilize the interaction mechanism between *R. solanacearum* and hosts to control the disease.
Key words *Ralstonia solanacearum*; Host plants; Interaction; Plant immunity

Bacterial wilt caused by *Ralstonia solanacearum* is one of the most important plant diseases worldwide, both in terms of field losses and economic losses. *R. solanacearum* is a gram-negative soil-borne phytopathogenic bacterium with a wide host range of more than 250 plant species including tomato, potato and tobacco[1]. This bacterium is considered to be a very species complex with broad genetic diversity. Based on the scientific and economic importance, *R. solanacearum* was ranked the second most important plant pathogenic bacteria in molecular plant pathology research[2]. In recent years, bacterial wilt spreads gradually from low altitude to high altitude with climate change and new hosts of *R. solanacearum* strains are reported continuously[3–4], which show a serious threat to agricultural production.

All the remarkable and extraordinary features of bacterial wilt are due to the long term co-evolution and interaction between *R. solanacearum* and its hosts. Faced with the attack of a variety of pathogenic microorganisms in the environment, host plants evolved the innate immunity. Pattern-recognition receptors (PRRs) located on the plant cell surface could perceive pathogen-associated molecular patterns (PAMPs) of pathogens and activate the immune signals in plants, resulting in PAMPs-triggered immunity (PTI) that can resist the invasion of most pathogens[5]. In order to infect plants successfully, pathogens like *R. solanacearum* evolved effector proteins to suppress PTI or enhance the virulence. These results in effector-triggered susceptibility (ETS). Seldom hosts in order to avoid the suppression of PTI, they evolved resistance proteins which can specifically recognize the corresponding effectors, resulting in effector-triggered immunity (ETI) that makes hosts resistant to a specific pathogen. The co-evolution in the dynamic interaction between *R. solanacearum* and hosts leads to some hosts susceptible while others resistant to this special pathogen.

The molecular mechanism of *R. solanacearum* and hosts interaction was reviewed in this paper, which aims to provide the basis for further study of molecular mechanism of interaction, and at the same time, to provide some new ideas for the control of this disease.

The Formation of PTI

Host plants are surrounded by a large number of potentially pathogenic microorganisms during different developmental processes. These potentially pathogenic microorganisms evolved various strategies to attack plants as well as formed some conservational PAMPs such as bacterial flagellin, elongation factor Tu (EF-Tu), lipopolysaccharide, peptidoglycan and cold shock proteins. PAMPs are similar of its kind and have very few variations. Host plants evolved PRRs in the long process of struggling with pathogens in order to achieve self-protection. PRRs can either directly or indirectly recognize PAMPs, which leads to the activation of the complex defense response signaling pathway and triggers PTI[6–7].

Flagellin, the major component of flagella, is one of the PAMPs that have been well studied. A synthetic 22-amino-acid peptide (flg22) which is highly conserved in gram-negative bacteria existed in the N terminal of the flagellin[5, 8]. In *R. solanacearum*, flg22 as an extracellular PAMP can be recognized by various plant species. The receptor like protein kinase FLS2 in *Arabidopsis thaliana* can specifically recognize the flg22[9]. And FLS2 homologous proteins also existed in tobacco and tomato[10–11]. The direct interaction between FLS2 and flg22 will

Supported by Fundamental Research Funds for the Central Universities (110201202002).
* Corresponding author. E-mail: dwing818@163.com
Received: November 10, 2013 Accepted: December 9, 2013

lead to the activation of plant resistance signals. EF-Tu induces similar defense responses to flg22[12]. A leucine rich repeat (LRR) kinase named EFR was identified to specifically recognize EF-Tu in *A. thaliana*. PRRs including FLS2 and EFR have a high degree of specialization. That means recognition happens only when hosts have a specific PRR that corresponds to a specific PAMP in pathogens. Up to present, several PAMPs have been identified in pathogenic bacteria, but less is known about their targets in plants. Therefore, identifying more PRRs and understanding the interaction mechanism will be the focus of PTI research in the future.

R. solanacearum Secrets Effectors to Achieve Successfully Infection

Host plants successfully resist the invasion of most pathogenic microorganisms including *R. solanac-earum* after PTI. In order to successfully infect some hosts again, *R. solanacearum* evolved a special secretion system, termed type III secretion system (T3SS), to inject effector proteins directly into host cells. The effectors secreted by T3SS can either mimic the function of key proteins in hosts or interfere with certain proteasome pathways, causing plant diseases. T3SS of *R. solanacearum* was encoded by the hypersensitive response and pathogenicity (*hrp*) gene cluster and regulated by both PrhA and Phc transcriptional regulators[13]. And T3SS delivers a large set of approximately 75 type III effectors, which is much more than the average effectors (30–40) of phytopathogens, into host cells. The function of 23 effectors have been revealed in some host plants and some effectors are considered to have diversity and specificity in host selection[14–17].

GALA protein family and AWR protein family are two important effector protein families in *R. solanacearum*[18-20]. There are seven GALA members in model strain GMI1000 [20–21], while only six members exist in strain Molk2 and UW55 [19]. GALA proteins are highly conserved in *R. solanacearum* and contain both a LRR and F-box domain. These proteins interact with a subset of 19 different *Arabidopsis* Skp1-like proteins, forming ubiquitin ligase complex which controls specific protein ubiquitinylation[20]. A seven GALA effector genes deleted strain was completely avirulent on *Arabidopsis* and less virulent on tomato. Furthermore, GALA7 is a host-specificity factor which is required for pathogenicity on *Medicago truncatula* plants[19–20]. AWR protein family is a recently described effector protein family in *R. solanacearum*. Five members are included in this family and all of them except for awr1 exist in all *R. solanacearum* strains[22]. A *R. solanacearum* strain in which all of the five *AWR* effector genes have been deleted severely impaired its capacity to multiply in natural host plants. Further studies have shown that AWR2 is mainly contributed to pathogen virulence[18]. Interestingly, AWR4 and AWR5 displayed the opposite phenotype. AWR4 restricted the growth of *R. solanacearum* in *Arabidopsis* and tomato and AWR5 inhibited the growth of this bacterium in *Arabidopsis* and eggplant. Therefore, AWR effector genes exhibit some functional redundancy[18]. Except for the two effector protein families described above, there are some other protein families such as HLK and SKWP in *R. solanacearum*[23]. The HLK family contains three members. Deletion of all HLK genes significantly reduced the virulence on tomato, but the mutant was as virulent as the wild type on eggplant and tobacco[24].

Many single effectors were contained in *R. solanacearum*, most of which can promote growth in natural hosts. AvrPphF, PopP2, AWR1, AWR2 and Rip34 facilitate bacteria growth in tomato; SKWP4, Rsp0842, AvrPphF, Rip3, Rip23, Rip39, Rip55 and Rip64 facilitate bacteria growth in eggplant; AvrPphF, PopP2, Rsp0842 and Rip34 facilitate bacteria growth in bean[14–16]. Rsc0411 enhanced bacterial motility, biofilm formation and root attachment, so as to promote the host pathogenicity and increased membrane permeability. And likewise, this effector protein has a strain-specific role in T3SS activity of *R. solanacearum*[25].

Formation of ETI

Effectors evolved by bacteria were initially used for suppression of PTI, causing successful infection. Under the stress of effector proteins, host plants evolved resistance proteins, which could specifically recognize effector proteins in pathogens, for successful survival[5]. The direct recognition between resistance proteins and effector proteins results in ETI, making hosts become resistant once again. Effectors that can cause ETI were called avirulence proteins.

PopP2, a member of the YopJ/AvrRxv family of type III effector proteins, is one of the avirulence proteins that have been described in detail in *R. solanacearum*. PopP2 specifically interacts with RRS1-R protein which contains the Toll/Interleukin1 receptor-nucleotide binding site leu-rich repeat (NBS-LRR) domains in resistant Nd-1 *A. thaliana* ecotype[26–28]. This direct interaction was localized in the nucleus and severely impaired the colonization ability of *R. solanacearum* in hosts. In addition, RD19, the Arabidopsis Cys protease, was identified as a PopP2-interacting protein and induced by *R. solanacearum* infection. During this process, RD19 was specifically relocalized to the plant nucleus, which is very similar to PopP2 and RRS1-R interaction. RD19 is required for activation of the RRS1-R mediated resistance response by forming a nucleus complex with PopP2[29]. Further study has shown that PopP2 have acetyltransferase activity which leads to its autoacetylation on a particular lysine residue. The autoacetylation of PopP2 is required for resistance mediated by PopP2 and RRS1-R interaction[30]. This specific and direct interaction between the two proteins results in the formation of ETI, which makes seldom host containing the resistance proteins being resistant. It is a new stage of plant-microbe interaction when ETI works.

AvrA is another important avirulence protein in *R. solanacearum*. It can induce hypersensitive response (HR) on tobacco, leading to the bacterium losing the ability to infect[31–33]. PopP1, which also belongs to the YopJ/AvrRxv family, can induce species specific HR on *Petunia*[15]. The *popp*1 gene mutant strain became

pathogenic to *Petunia* that is resistant to the wild-type strain. And a recent study found that PopP1 together with AvrA induced HR on host tobacco. Deletion of both *popp*1 and *avrA* made the strain GMI1000 pathogenic on tobacco[32–33]. Although PopP1 and AvrA were identified as avirulence proteins and could induce HR on specific hosts, the target and the interaction mechanism of the two proteins remain unclear. Other effectors such as AWR5 also can induce the formation of ETI on special hosts[18].

Outlook

Plant-microbe interaction is a hot topic of plant pathology in recent years. *R. solanacearum* is regarded as the model phytopathogenic bacterium. In the long process of co-evolution, *R. solanacearum* formed its own unique infection mechanism; meanwhile, host plants formed the unique defense mechanism. The bacterial wilt disease occurs, because in the process of *R. solanacearum* and hosts interaction, the defense mechanism formed by host plants is insufficient to resist the invasion of the pathogen. Therefore, to gain insight into the molecular mechanism of *R. solanacearum* and hosts interaction, explicating the shortage of the host plant defense mechanism and the redundancy of *R. solanacearum* infection mechanism, will provide new train of thought for the control of this destructive disease. To date, a lot of progresses have been made on the interaction mechanism between *R. solanacearum* and hosts. There are still many things need deep thinking for further comprehensive investigating the interaction mechanism between *R. solanacearum* and hosts.

The function and target of type III effector proteins need for further study

Type III effector proteins play a vital role in plant-microbe interaction. They either prominently enhanced the pathogenicity of pathogens or successfully suppressed the PTI of hosts. Thus, it will have significant meaning to clarify the accurate function of each effector protein in *R. solanacearum*. Three methods were used for the study of each individual effector contributes to bacterial fitness in planta[14, 16, 18]. The first is to measure the growth of effector gene deleted mutant strains inside natural host such as tomato and eggplant. The second is to measure the growth of *Pseudomonas syrinage* which heterologously expresses *R. solanacearum* effector genes in *Arabidopsis*. The third is to use competitive index assays between co-inoculated wild-type and mutant strains, which was considered to be a very sensitive method. After knowing the contribution of each individual effector protein in planta, we need to further investigate the target in hosts. According to the gene for gene hypothesis, a virulence gene in pathogens corresponds to a susceptible gene in hosts, and an avirulence gene in pathogens corresponds to a resistant gene in hosts. Identifying more susceptible genes or resistant genes in plants is an extremely complicated work. Identification of susceptible genes and resistant genes will be conducive in breeding for resistance plants[34–35].

Can we activate PTI of host plants?

PTI is the most basic immune responses in plants. Activation of PTI may lead to hosts exhibiting resistance to a class of pathogenic microorganisms which have the conservative PAMPs. The direct recognition between PAMPs and PRRs is the beginning of the activation of plant immune response. Theoretically, two methods can be used for humans to activate the PTI of host plants. One is to synthesize PAMPs based on the structure of the existing PAMPs or to extract PAMPs directly from pathogens. PTI of plants is induced when treated with these PAMPs exogenously. The other is to clone the *PRR* genes that have been well studied from resistant plants and then turn them into the conventional hosts, resulting in resistance occurs.

Can we reduce the virulence of *R. solanacearum* by inhibiting the T3SS?

T3SS is a decisive virulence factor in *R. solanacearum* as well as in many other plant pathogenic or animal pathogenic pathogens. Without T3SS, many bacteria are no longer pathogenic on natural hosts, making the T3SS an attractive target for the control of diseases. Studies have shown that T3SS can be specifically inhibited by chemical compounds or small molecules in animal pathogenic bacteria[36–38]. Salicylidene acylhydrazides inhibited the T3SS of *Yersinia pseudotuberculosis* and reduced its motility[36]. 2-imino-5-arylidene thiazolidinone, a small molecule, was identified to block secretion and virulence functions of a large variety of animal bacterial pathogens[39]. T3SS in phytopathogenic bacteria can be inhibited too[40]. Compounds belong to salicylidene acylhydrazides inhibited T3SS gene expression in *Erwinia amylovora* under *hrp*-inducing conditions[41]. The plant phenolic compounds acted as T3SS inhibitors in plant pathogens *E. amylovora* and *Dickeya dadantii*[40, 42]. All of the above indicate that T3SS inhibitors do exist in pathogens that have this special secretion system. Are there T3SS inhibitors in *R. solanacearum*? If any, how T3SS is inhibited? These will be very interesting and valuable questions to be answered.

Can we utilize the existing resistance gene for breeding transgenic plants?

According to ETI, when a small number of host plants evolved a resistance gene that can specifically recognize the avirulence gene in *R. solanacearum*, host resistance occurs. For example, *RRS*1-*R*, a well-studied resistance gene in *A. thaliana* ecotype Nd-1, specifically interacts with *PopP*2 in *R. solanacearum*. Under this interaction, the strain GMI1000 loses its pathogenicity on *A. thaliana*. Now if we clone the *RRS*1-*R* gene from *A. thaliana* and transfer it successfully into susceptible hosts such as tomato and pepper, the transgenic plant will be resistant to GMI1000. And therefore, the utilization of resistance genes could make a lot of plants become resistance to *R. solanacearum*.

References

[1] HAYWARD AC. Biology and epidemiology of bacterial wilt caused by *Pseudomonas Solanacearum*[J]. Annu Rev of Phytopathol, 1991, 29: 65–87.

[2] MANSFIELD J, GENIN S, MAGORI S, *et al*. Top 10 plant pathogenic bacteria in molecular plant pathology[J]. Mol Plant Pathol, 2012, 13(6): 614–629.

[3] SHE XM, HE ZF, LUO FF, *et al*. First report of bacterial wilt caused by *Ralstonia solanacearum* on Ageratum conyzoides in China[J]. Plant Dis, 2013, 97(3): 418.

[4] PEETERS N, GUIDOT A, VAILLEAU F, *et al*. *Ralstonia solanacearum*, a widespread bacterial plant pathogen in the post-genomic era[J]. Mol Plant Pathol, 2013, 14(7): 651–662.

[5] JONES JD, DANGL JL. The plant immune system [J]. Nature, 2006, 444 (7117): 323–329.

[6] SCHWESSINGER B, ZIPFEL C. News from the frontline: recent insights into PAMP-triggered immunity in plants [J]. Curr Opin Plant Biol, 2008, 11(4): 389–395.

[7] KEMMERLING B, POSTEL S. Plant systems for recognition of pathogen-associated molecular patterns[J]. Semin Cell Dev Biol, 2009, 20(9): 1025–1031.

[8] CHISHLOM ST, COAKER G, DAY B, *et al*. Host-microbe interactions: shaping the evolution of the plant immune response[J]. Cell, 2006, 124(4): 803–814.

[9] DUNNING FM, SUN W, JANSEN KL, *et al*. Identification and mutational analysis of *Arabidopsis* FLS2 leucine-rich repeat domain residues that contribute to flagellin perception [J]. Plant Cell, 2007, 19 (10): 3297–3313.

[10] ROBATZEK S, BITTEL P, CHINCHILLA D, *et al*. Molecular identification and characterization of the tomato flagellin receptor LeFLS2, an orthologue of *Arabidopsis* FLS2 exhibiting characteristically different perception specificities[J]. Plant Mol Biol, 2007, 64 (5): 539–547.

[11] HANN DR, RATHJEN JP. Early events in the pathogenicity of *Pseudomonas syringae* on *Nicotiana benthamiana*[J]. Plant J, 2007, 49(4): 607–618.

[12] KUNZE G, ZIPFEL C, ROBATZEK S, *et al*. The N terminus of bacterial elongation factor Tu elicits innate immunity in *Arabidopsis* plants[J]. Plant Cell, 2004, 16(12): 3496–3507.

[13] GENIN S, BOUCHER C. Lessons learned from the genome analysis of *Ralstonia sonalacearum*[J]. Annual Review of Phytopathology, 2004, 42: 107–134.

[14] COLL NS, VALLS M. Current knowledge on the *Ralstonia solanacearum*-type III secretion system[J]. Microb Biotechnol, 2013, 6(6): 614–620.

[15] LAIVE M, SHILLINGTON E, EGUILUZ C, *et al*. PopP1, a new member of the YopJ/AvrRxv family of type III effector proteins, acts as a host-specificity factor and modulates aggressiveness of *Ralstonia solanacearum* [J]. Mol Plant Microbe Interact, 2002, 15(10): 1058–1068.

[16] MACHO AP, GUIDOT A, BARBERIS P, *et al*. A competitive index assay identifies several *Ralstonia solanacearum* type III effector mutant strains with reduced fitness in host plants[J]. Mol Plant Microbe Interact, 2010, 23 (9): 1197–1205.

[17] MACHO AP, RUIZ-ALBERT J, TORNERO P, *et al*. Identification of new type III effectors and analysis of the plant response by competitive index[J]. Mol Plant Pathol, 2009, 10(1): 69–80.

[18] SOLE M, POPA C, MITH O, *et al*. The awr gene family encodes a novel class of *Ralstonia solanacearum* type III effectors displaying virulence and avirulence activities [J]. Mol Plant Microbe Interact, 2012, 25(7): 941–953.

[19] REMIGI P, ANISIMOVA M, GUIDOT A, *et al*. Functional diversification of the GALA type III effector family contributes to *Ralstonia solanacearum*adaptation on different plant hosts[J]. New Phytol, 2011, 192(4): 976–987.

[20] ANGOT A, PEETERS N, LECHNER E, *et al*. *Ralstonia solanacearum*requires F-box-like domain-containing type III effectors to promote disease on several host plants [J]. Proc Natl Acad Sci USA, 2006, 103 (39): 14620 – 14625.

[21] ALLEN C, GABRIEL DW, SCHELL M, *et al*. Identification of open reading frames unique to a select agent: *Ralstonia solanacearum* race 3 biovar 2 [J]. Mol Plant Microbe Interact, 2006, 19(1): 69–79.

[22] GUIDOT A, PRIOR P, SCHOENFELD J, *et al*. Genomic structure and phylogeny of the plant pathogen *Ralstonia solanacearum* inferred from gene distribution analysis[J]. J Bacteriol, 2007, 189(2): 377–387.

[23] POUEYMIRO M, GENIN S. Secreted proteins from *Ralstonia solanacearum*: a hundred tricks to kill a plant [J]. Curr Opin Microbiol, 2009, 12(1): 44–52.

[24] CHEN L, SHIROTA M, ZHANG Y, *et al*. Involvement of HLK effectors in *Ralstonia solanacearum*disease development in tomato[J]. J Gen Plant Pathol, 2013, DOI 10.1007/s10327 – 013–0490–2.

[25] LIN YM, YANG WC, CHENG YS, *et al*. *Ralstonia solanacearum* RSc0411 (lptC) is a determinant for full virulence and has a strain-specific novel function in the T3SS activity[J]. Microbiol, 2013, 159: 1136–1148.

[26] DENLANDES L, PILEUR F, LIAUBET Laurence, *et al*. Genetic characterization of RRS1, a recessive locus in *Arabidopsis thaliana* that confers resistance to the bacterial soilborne pathogen Ralstonia solanacearum [J]. Mol Plant Microbe Interact, 1998, 11(7): 659–667.

[27] DENLANDES L, OLIVIER J, THEULIERES F, *et al*. Resistance to *Ralstonia solanacearum* in *Arabidopsis* thaliana is conferred by the recessive *RRS*1-*R* gene, a member of a novel family of resistance genes [J]. Proc Natl Acad Sci U S A, 2002, 99 (4): 2404–2409.

[28] DENLANDES L, OLIVIER J, PEETERS N, *et al*. Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus [J]. Proc Natl Acad Sci U S A, 2003, 100(13): 8024–8029.

[29] BERNOUX M, TIMMERS T, JAUNEAU A, *et al*. RD19, an *Arabidopsis* cysteine protease required for RRS1-R-mediated resistance, is relocalized to the nucleus by the *Ralstonia solanacearum*PopP2 effector[J]. Plant Cell, 2008, 20(8): 2252–2264.

[30] TASSET C, BERNOUX M, JAUNEAU A, *et al*. Autoacetylation of the *Ralstonia solanacearum*effector PopP2 targets a lysine residue essential for RRS1-R-mediated immunity in *Arabidopsis*[J]. PLoS Pathog, 2010, 6(11): 1001202.

[31] DENNY TP, CARNEY BF. A cloned avirulence gene from *Pseudomonas solanacearum* determines incompatibility on *Nicotiana tabacum* at the host species level[J]. J Bacteriol, 1990, 172 (9): 4836–4843.

[32] ROBERTSON AE, WECHTER WP, DENNY TP, *et al*. Relationship between avirulence gene (*avrA*) diversity in *Ralstonia solanacearum* and bacterial wilt incidence[J]. Mol Plant Microbe Interact, 2004, 17(12): 1376–1384.

[33] POUEYMIRO M, CUNNAC S, BARBERIS P, *et al*. Two type III secretion system effectors from *Ralstonia solanacearum* GMI1000 determine host-range specificity on tobacco [J]. Mol Plant Microbe Interact, 2009, 22 (5): 538–550.

[34] GAWEHNS F, CORNELISSEN BJ, TAKKEN FL. The potential of effector-target genes in breeding for plant innate immunity [J]. Microb Biotechnol, 2013, 6(3): 223–229.

[35] DESLANDES L, RIVAS S. Catch me if you can: bacterial effectors and plant targets [J]. Trends Plant Sci, 2012, 17 (11): 644–655.

[36] DUNCAN MC, LININGTON RG, AUERBUCH V. Chemical inhibitors of the type three secretion system: disarming bacterial pathogens[J]. Antimicrob Agents Chemother, 2012, 56(11): 5433–5441.

[37] TSOU LK, DOSSA PD, HANG HC. Small molecules aimed at type III secretion systems to inhibit bacterial virulence[J]. Med Chem Commum, 2013, 4(1): 68.

[38] MAY AE, KHOLSLA C. Discovery and mechanism of type III secretion sys-

(Continued on page 1718)

nous hormones can cause a physiological toxicity[16].

The change in each of POD, PPO and IAAO has displayed a certain trend during the rooting process of *S. microphylla* cuttings, and the change is noticeable after the IBA treatment, but some changes are hard to be explained reasonably. The changes in the three types of oxidative enzymes are partly consistent with, and partly different from those discovered in previous studies. POD activity in our study was contrary to that in Chinese herbaceous peony[13] or *Aquilaria sinensis*[14], which may be due to different plant species and different types of exogenous hormones. Thus, changes in activities of the three types of oxidative enzymes are closely related but also vary during the rooting process in different plant species, and what the correlation is between the changes of three enzymes, and which substance plays a leading role in the occurrence of adventitious roots require further exploration.

References

[1] ZHANG TL (张天麟). Garden 1600 tree species (园林树木 1600 种)[M]. Beijing: China Architecture & Building Press (北京: 中国建筑工业出版社), 2012: 461–462.

[2] YU SX(余树勋). *Syriga microphylla*(丁香花) [M]. Shanghai: Shanghai Science and Technology Press (上海: 上海科学技术出版社), 2000.

[3] REN L(任俐), LIU XD(刘小东), LI YW(李耀文). Study on influence of three plant hormone on *Syringa oblata* cottage (三种植物激素对紫丁香扦插的影响)[J]. Journal of Harbin University of Commerce(哈尔滨商业大学学报), 2006, 22 (2): 36–39.

[4] WU SJ, ZHOU XH. Study on the cutting rooting of *Syriga microphylla* with tender branches [J]. Agricultural Science & Technology, 2010, 11 (11/12): 37–38, 81.

[5] LI XF(李晓峰), LI YX(李艳星), GUO WL (郭伟略). Influence of different hormone treatments on *Syzygium aromaticum* Cuttage (不同激素处理对丁香扦插生根的影响)[J]. Journal of Chinese Urban Forestry (中国城市林业), 2012, 10(4): 55–56.

[6] LI GC(李广春), SUN LP(孙丽萍). Study on lilac cuttage seedling experiment (紫丁香扦插育苗技术试验研究)[J]. Shandong Forestry Science and Technology (山东林业科技), 2006, 38(3): 42–45.

[7] MU HX (牟洪香). Study on rapid micropropagation technique of triploid clones of populus tomentosa (三倍体毛白杨优良无性系微体快速繁殖技术研究)[D]. Jinan: Shandong Agricultural University (济南: 山东农业大学), 2003.

[8] HAO JJ(郝建军), KANG ZL(康宗利), YU Y (于洋). Plant Physiology experiment technique (植物生理学实验技术)[M]. Beijing: Chemical Industry Press (北京: 化学工业出版社), 2007: 62–64.

[9] LI HX (李焕秀), WANG QC (王乔春), LI CX(李春秀). Primary study on pear bud and stem tip PPO activity and total phenol content (梨芽和茎尖多酚氧化酶活性和总酚含量的初步研究)[J]. Journal of Sichuan Agricultural University (四川农业大学学报), 1994, 2(2): 218–222.

[10] ZHANG ZL(张志良), QU WJ(瞿伟菁). The experimental guide for Plant Physiology (植物生理学实验指导)[M]. Beijing: Higher Education Press(北京: 高等教育出版社), 2003: 123–124, 188–189.

[11] WU MJ (吴明江), YU P (于萍). The physiological role of plant peroxidase (植物过氧化物酶的生理作用)[J]. Journal of Biology (生物学杂志), 1994(6): 14–16.

[12] GEBHARDT K. Activation of indole–3–acetic acid oxidase from horseradish and prunus by phenols and hydrogen peroxide (H_2O_2)[J]. Plant Growth Regulation,1982, 1(2): 73–84.

[13] HAISSIF BE. Metabolic processes in adventitious rooting of cutting [M]// JACKSON MB. New Root Formation in Plant and Cuttings. Lancaster: Martinus Nijhoff, 1986: 141–189.

[14] HU HJ(扈红军), CAO BH(曹帮华), YIN WL(尹伟伦), *et al*. Changes in the relevant oxidases during cutting rooting of Corylus heterophylla and the cutting propagation technique (榛子嫩枝扦插生根相关氧化酶活性变化及繁殖技术)[J]. Scientia Silvae Sinicae(林业科学), 2008, 44(6): 60–65.

[15] FU XL (付喜玲), GUO XF (郭先锋), KANG XF(康晓飞), *et al*. Effects of IBA on stem cutting and activity of related enzymes during rooting of Paeonia lactiflora Pall.(IBA 对芍药扦插生根的影响及生根过程中相关酶活性的变化)[J]. Acta Horticulturae Sinica (园艺学报), 2009, 36 (6): 849–854.

[16] ZHANG YC(张玉臣). Cutting propagation techniques and rooting mechanism of Aquilaria Sinensis (Lour.) Gilg (白木香扦插繁殖技术及生根机理研究)[D]. Beijing: Chinese Academy of Forestry (北京: 中国林业科学研究院), 2010.

[17] SONG JY(宋金耀), HE WL(何文林), LI SB (李松波), *et al*. Analysis of physiological and biochemical characteristics related to cutting and rooting of chimera Inpopulus tomentosa carr.(毛白杨嵌合体扦插生根相关理化特性分析)[J]. Scientia Silvae Sinicae (林业科学), 2001, 37(5): 64–67.

[18] BITTIN A, EXCESS N. Application inhibits rooting: The influence of nutrient supply to azalea other plant on the rooting of cuttings [J]. Gartner-borse and Gartenwelt, 1990, 90(6): 232–255.

[19] SONG LH(宋丽红), CAO BH(曹帮华). Studies on activities of indoleacetic acid oxidase, polyphenol oxidase and peroxidase in cuttings of *Broussonetia papyrifera* during rooting process(光叶楮扦插生根的吲哚乙酸氧化酶、多酚氧化酶、过氧化物酶活性变化研究)[J]. Journal of Wuhan Botanical Research (武汉植物学研究), 2005, 23 (4): 347–350.

[20] ZENG DX (曾端香), YIN WL (尹伟伦), WANG YH(王玉华), *et al*. Propagation with etiolated softwood cuttings of five dwarf cultivars of Chinese tree peony (5 个矮生牡丹品种黄化嫩枝扦插技术研究[J]. Acta Horticulturae Sinica(园艺学报), 2005, 32(4): 725–728.

Responsible editor: Fujuan QI **Responsible proofreader: Xiaoyan WU**

(Continued from page 1701)

tem inhibitors[J]. Israel J Chem, 2013, 53(8): 577–587.

[39] FELISE HB, NGUYEN HV, PFUETZNER RA, *et al*. An inhibitor of gram-negative bacterial virulence protein secretion [J]. Cell Host Microbe, 2008, 4 (4): 325–336.

[40] KHOKHANI D, ZHANG C, LI Y, *et al*. Discovery of plant phenolic compounds that act as type three secretion system inhibitors or inducers of fire blight pathogen *Erwinia amylovora*[J]. Appl Environ Microbiol, 2013, DOI: 10.1128/AEM.00845–13

[41] YANG F, KORBAN SS, PUSEY PL, *et al*. Small-molecule inhibitors suppress the expression of both type III secretion and amylovoran biosynthesis genes in *Erwinia amylovora* [J]. Mol Plant Pathol, 2013, DOI: 10.1111/mpp.12064

[42] LI Y, PENG Q, SELIMI D, *et al*. The plant phenolic compound p-coumaric acid represses gene expression in the *Dickeya dadantii* type III secretion system[J]. Appl Environ Microbiol, 2009, 75(5): 1223–1228.

Responsible editor: Qingqing YIN **Responsible proofreader: Xiaoyan WU**