Planta (2017) 245:237–253
DOI 10.1007/s00425-016-2628-x

REVIEW

Molecular functions of *Xanthomonas* type III effector AvrBsT and its plant interactors in cell death and defense signaling

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Received: 28 August 2016 / Accepted: 30 November 2016 / Published online: 7 December 2016

Abstract

Main conclusion ***Xanthomonas*** **effector AvrBsT interacts with plant defense proteins and triggers cell death and defense response. This review highlights our current understanding of the molecular functions of AvrBsT and its host interactor proteins.**

The AvrBsT protein is a member of a growing family of effector proteins in both plant and animal pathogens. *Xanthomonas* type III effector AvrBsT, a member of the YopJ/AvrRxv family, suppresses plant defense responses in susceptible hosts, but triggers cell death signaling leading to hypersensitive response (HR) and defense responses in resistant plants. AvrBsT interacts with host defense-related proteins to trigger the HR cell death and defense responses in plants. Here, we review and discuss recent progress in understanding the molecular functions of AvrBsT and its host interactor proteins in pepper (*Capsicum annuum*). Pepper arginine decarboxylase1 (CaADC1), pepper aldehyde dehydrogenase1 (CaALDH1), pepper heat shock protein 70a (CaHSP70a), pepper suppressor of the G2 allele of *skp1* (CaSGT1), pepper SNF1-related kinase1 (SnRK1), and *Arabidopsis* acetylated interacting protein1 (ACIP1) have been identified as AvrBsT interactors in pepper and *Arabidopsis*. Gene expression profiling, virus-induced gene silencing, and transient transgenic overexpression approaches have advanced the functional characterization of AvrBsT-interacting proteins in plants. AvrBsT is localized in the cytoplasm and forms protein–protein complexes with host interactors. All identified AvrBsT interactors regulate HR cell death and defense responses in plants. Notably, CaSGT1 physically binds to both AvrBsT and pepper receptor-like cytoplasmic kinase1 (CaPIK1) in the cytoplasm. During infection with *Xanthomonas campestris* pv. *vesicatoria* strain Ds1 (*avrBsT*), AvrBsT is phosphorylated by CaPIK1 and forms the active AvrBsT–CaSGT1–CaPIK1 complex, which ultimately triggers HR cell death and defense responses. Collectively, the AvrBsT interactor proteins are involved in plant cell death and immunity signaling.

Keywords AvrBsT interactors · Cell death · Plant defense · Defense-related genes · Hypersensitive response · *Xanthomonas* type III effector AvrBsT

Introduction

During their entire life cycle, plants are continually exposed to diverse microbial pathogens, including bacteria, fungi, and viruses. Adapted pathogens have evolved sophisticated invasion mechanisms to colonize and infect their hosts, and plants have developed complex defense mechanisms to obstruct pathogen attack. This interaction has been referred to as an "arms race" between pathogens and plants (Boller and He 2009). In many cases, plants successfully detect pathogen presence and/or infection via pattern recognition receptors (PRRs) localized on the plant cell surface, which perceive microbial- or pathogen-associated molecular patterns (MAMPS or PAMPs), such as bacterial flagellin, elongation factor Tu (EF-Tu),

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peptidoglycan (PGN), and lipopolysaccharides (Jones and Dangl 2006; Bohm et al. 2014; Zipfel 2014). Plant PRRs are typically leucine-rich repeat (LRR) kinases and lysine motif kinases that are broadly analogous to Toll-like receptors in animals (Dangl et al. 2013). The perception of PAMPs by PRRs at the plant cell surface initiates PAMP-triggered immunity (PTI), which usually suppresses pathogen infection in the plant (Chisholm et al. 2006). Downstream defense responses include the activation of mitogen-activated protein kinases (MAPKs) and calcium-dependent protein kinases (CDPKs), rapid generation of reactive oxygen species (ROS), enhanced expression of defense-related genes, and callose deposition at the cell wall (Boller and Felix 2009; Tena et al. 2011). The culmination of these PTI defense responses leads to the suppression of the ingress or growth of microbial plant pathogens (Macho and Zipfel 2015).

Adapted pathogens have evolved virulence strategies to overcome PTI and to cause disease on their respective host plants. Important virulence factors of a range of different pathogens are the so-called effector proteins which are produced by the pathogen to reprogram host cellular functions for its own benefit. In Gram-negative bacteria, these effectors are delivered into host cells by the type III secretion system (T3SS), which acts as a key pathogenicity factor for plant and animal hosts (He 1998; Ghosh 2004; Büttner and He 2009; Büttner and Bonas 2010). The T3SS is encoded by hypersensitive response and pathogenicity (*hrp*) genes that direct bacteria to trigger HR in non-host cells and cause disease in host cells (Lindgren 1997). Type III effectors may act on their plant host target proteins to interfere with PTI-mediated defense signaling cascades, ultimately promoting disease in susceptible host genotypes (Jones and Dangl 2006). However, effectors that enable pathogens to overcome PTI are directly or indirectly recognized by specific plant disease resistance (*R*) genes, which induce effector-triggered immunity (ETI) in resistant host genotypes (Jones and Dangl 2006). The recognized effectors are termed avirulence (Avr) proteins. Major specific R proteins include members of a polymorphic superfamily of intracellular nucleotide-binding leucine-rich repeat (NLR) receptors (Maekawa et al. 2011; Dangl et al. 2013). NLR recognition by specific pathogen effectors initiates ETI, which ultimately leads to robust resistant responses such as the hypersensitive response (HR). HR causes rapid plant cell death which drastically restricts pathogen growth at the initial infection site. During the last decades, many effector proteins from pathogenic fungi and bacteria, and their putative target proteins from host plants, have been identified and reviewed (Win et al. 2012; Giraldo and Valent 2013; Qi and Innes 2013; Macho and Zipfel 2015). However, further investigations of the molecular functions of pathogen effectors and their target proteins in host plants are required to better understand how pathogens cause disease and how plants express and orchestrate defense responses against pathogen attack.

Xanthomonas campestris pv. *vesicatoria* (*Xcv*) (Doidge) Dye causes a spot disease on foliage and fruit of pepper (*Capsicum annuum*) and tomato (*Solanum lycopersicum*) (Choi and Hwang 2015). This disease causes great fruit losses during warm and rainy seasons worldwide. *Xcv* translocates a cocktail of different type III effector proteins into the plant cell via the T3SS (Szczesny et al. 2010). *Xcv* type III effector proteins have been identified as AvrBsT, AvrBs1, AvrBs2, AvrBs3, and XopD (Escolar et al. 2001; Thieme et al. 2005; Ryan et al. 2011; Kim et al. 2013a; Choi and Hwang 2015). *Xcv* and its hosts are used as model pathosystems to study how Xcv effectors function in the context of virulence and avirulence. The *Xcv* type III effector protein AvrBsT elicits hypersensitive cell death in pepper and *Nicotiana benthamiana* leaves (Orth et al. 2000; Escolar et al. 2001; Kim et al. 2013b). The HR cell death response elicited by AvrBsT is similar to the resistance (*R*) gene-mediated defense response in plants (Eitas and Dangl 2010; Kim et al. 2010). However, the precise molecular mechanisms underlying AvrBsT recognition and cell death initiation have not been fully elucidated. The identification of host proteins that interact with AvrBsT will be required to define AvrBsT-triggered cell death and molecular defense mechanisms. Recent work demonstrated that *Xanthomonas* effectors and their targeted host proteins play significant roles in triggering or suppressing cell death and defense responses in plants (Boch et al. 2009; Kim et al. 2010; Szczesny et al. 2010; Kim et al. 2013a, b, 2014b; Cheong et al. 2014; Kim and Hwang 2015a, b). Here, we review and discuss recent progress in understanding the molecular functions of the *Xcv* type III effector AvrBsT and its host interactor proteins in plants.

Distribution, type III secretion, structure and biochemical function of *Xanthomonas* effector AvrBsT

The *avrBsT* gene encoding *Xanthomonas* effector AvrBsT was identified from *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) strains 75-3 and Bv5-4a which belong to the *Xcv* tomato group (Minsavage et al. 1990a, b; Jones et al. 1998; Kim et al. 2010). The *Xcv* tomato group is avirulent on pepper plants but virulent on tomato plants. *Xcv* strain 75-3 is avirulent on the near-isogenic pepper line Early Calwonder (ECW), but virulent on the tomato cultivar Walter. *Xcv* strain Bv5-4a triggers HR in all pepper cultivars tested (Hwang et al. 1995; Kim et al. 2010). The avirulence gene *avrBsT* induces a characteristic HR in all pepper lines (Minsavage et al. 1990b; Kim et al. 2010). The

Xcv avirulence genes *avrBs1*, *avrBs2*, and *avrBs3* specify disease resistance on pepper ECW-10R, ECW-20R, and ECW-30R lines carrying the corresponding resistance genes *Bs1*, *Bs2*, and *Bs3*, respectively (Minsavage et al. 1990b). The *avrBsT*, *avrBs1*, and *avrBs3* genes are located in the unique indigenous plasmids, whereas *avrBs2* is probably located on the chromosome (Bonas et al. 1989; Minsavage et al. 1990a, b). Notably, *avrBsT* is located on an indigenous plasmid of approximately 41 kb in *Xcv* strain 75-3 (Minsavage et al. 1990b). The spontaneous virulent *Xcv* strain 75-3 Δ*avrBsT*, which lacks the plasmid, successfully causes disease on the resistant pepper lines ECW-20R and ECW-30R.

AvrBsT is a type III effector of *Xcv*, which is secreted via T3SS and delivered into plant cells during *Xcv* infection (Escolar et al. 2001). Epitope-tagged AvrBsT proteins are detected in culture supernatants only in the presence of a functional type III secretion apparatus, suggesting that AvrBsT is secreted by the *Xcv* Hrp T3SS into the plant cell cytoplasm (Escolar et al. 2001). Expression of the *avrBsT* gene is constitutive and independent of the *hrp* gene regulators, *hrpG* and *hrpX*. In planta, recognition of AvrBsT occurs intracellularly during *Xcv* infection, when AvrBsT is translocated from *Xcv* into the plant cell. Targeting signals for type III secretion and translocation are proposed to be present in the N-terminal region of *Yersinia* effector proteins, which are translocated via T3SS into host cells during infection (Cornelis and Van Gijsegem 2000; Lloyd et al. 2001). A conserved amino acid motif consists of a proline (P) residue surrounded by basic amino acids. This conserved motif is present in the N-terminal region of all known *Xcv* effector proteins including AvrBsT, and it has been suggested to play a potential role in effector protein translocation into plant cells (Escolar et al. 2001).

DNA sequence analysis indicates that AvrBsT has a high level of sequence homology with the secreted virulence effector protein YopJ from the animal pathogenic bacteria *Yersinia* spp. (Ciesiolka et al. 1999). AvrBsT cloned from the *Xcv* strain Bv5-4a (Kim et al. 2010) differs in two amino acid residues (K90-H91; N90-D91) compared with the sequence of AvrBsT (accession no. AAD39255) from the *Xcv* strain 75-3 (Ciesiolka et al. 1999). *Xcv* AvrBsT is a member of the YopJ/AvrRxv family (Lewis et al. 2011). This family contains four known members in *Xanthomonas*: XopJ, AvrXvr4, AvrRxv, and AvrBsT (Büttner and Bonas 2010). Homologs of AvrBsT in animal pathogens are YopJ/YopP from *Yersinia* spp. (Galyov et al. 1994; Mills et al. 1997). AvrBsT contains a putative YopJ-like serine/threonine acetyltransferase domain (182 amino acids) in the central region (Fig. 1a, b). The conserved catalytic residues His (H154) and Cys (C222) are required for triggering cell death, AvrBsT-dependent acetyltransferase activity, and both auto- and *trans*-acetylation (Cheong et al. 2014). Auto-acetylation activity of AvrBsT is detected by incubating AvrBsT purified from *E. coli* with ^{14}C-acetyl-coenzyme A (acetyl-CoA) and inositol hexa*kis*phosphate (IP_6). IP_6 is a eukaryotic cofactor that promotes the acetyltransferase activity of YopJ family effectors (Mittal et al. 2010; Cheong et al. 2014). Wild-type GST-AvrBsT activity causes auto-acetylation of AvrBsT and *trans*-acetylation of GST-acetylated interacting protein 1 (ACIP1). By contrast, the catalytic residue mutants GST-AvrBsT (H154A) or GST-AvrBsT (C222A) do not exhibit either auto-acetylation or *trans*-acetylation activities (Cheong et al. 2014). The conserved residue Lys (K282) is indispensable for inducing cell death and the *trans*-acetylation activity of AvrBsT to its substrate. The conserved residues His (H154), Glu (E173), and Cys (C222) in the AvrBsT catalytic core are required for *Xcv*-mediated induction of localized cell death in *N. benthamiana* and pepper plants (Orth et al. 2000). Thus, the acetyltransferase activity of AvrBsT appears to be closely related to the cell death phenotype.

Molecular functions of *Xanthomonas* effector AvrBsT inside plant cells

Bacterial type III effector proteins have diverse functions and target multiple host cellular pathways, including gene expression, hormone signaling, proteasome-dependent protein degradation, and defense responses, to the benefit of bacterial pathogens (Speth et al. 2007; Block et al. 2008; Lewis et al. 2009; Üstün and Börnke 2014). The *Xcv* type III effector AvrBsT localizes in the cytoplasm and nucleus of host plant cells (Szczesny et al. 2010). AvrBsT triggers hypersensitive cell death in pepper, but suppresses defense responses in tomato (Kim et al. 2010). The mutant *Xcv* strain Bv5-4a Δ*avrBsT*, in which the biparental mating technique was used to delete *avrBsT* from the avirulent *Xcv* strain Bv5-4a (Lee et al. 2009), does not induce cell death, but causes a typical bacterial spot symptom to develop in pepper leaves. However, AvrBsT acts as a virulence factor in susceptible tomato plants to suppress early defense signaling. In tomato leaves, growth of *Xcv* strain Bv5-4a Δ*avrBsT* is dramatically reduced, and callose deposition and defense-marker gene expression are significantly disrupted, compared with those of the wild-type *Xcv* strain Bv5-4a (Kim et al. 2010). Introduction of AvrBsT into the virulent *Xcv* strain Ds1 renders the strain avirulent to pepper plants. Infection of pepper leaves with the *Xcv* strain Ds1 (*avrBsT*) expressing AvrBsT strongly induces defense signaling, including cell death, H_2O_2 production and accumulation, callose deposition, and defense-related gene expression.

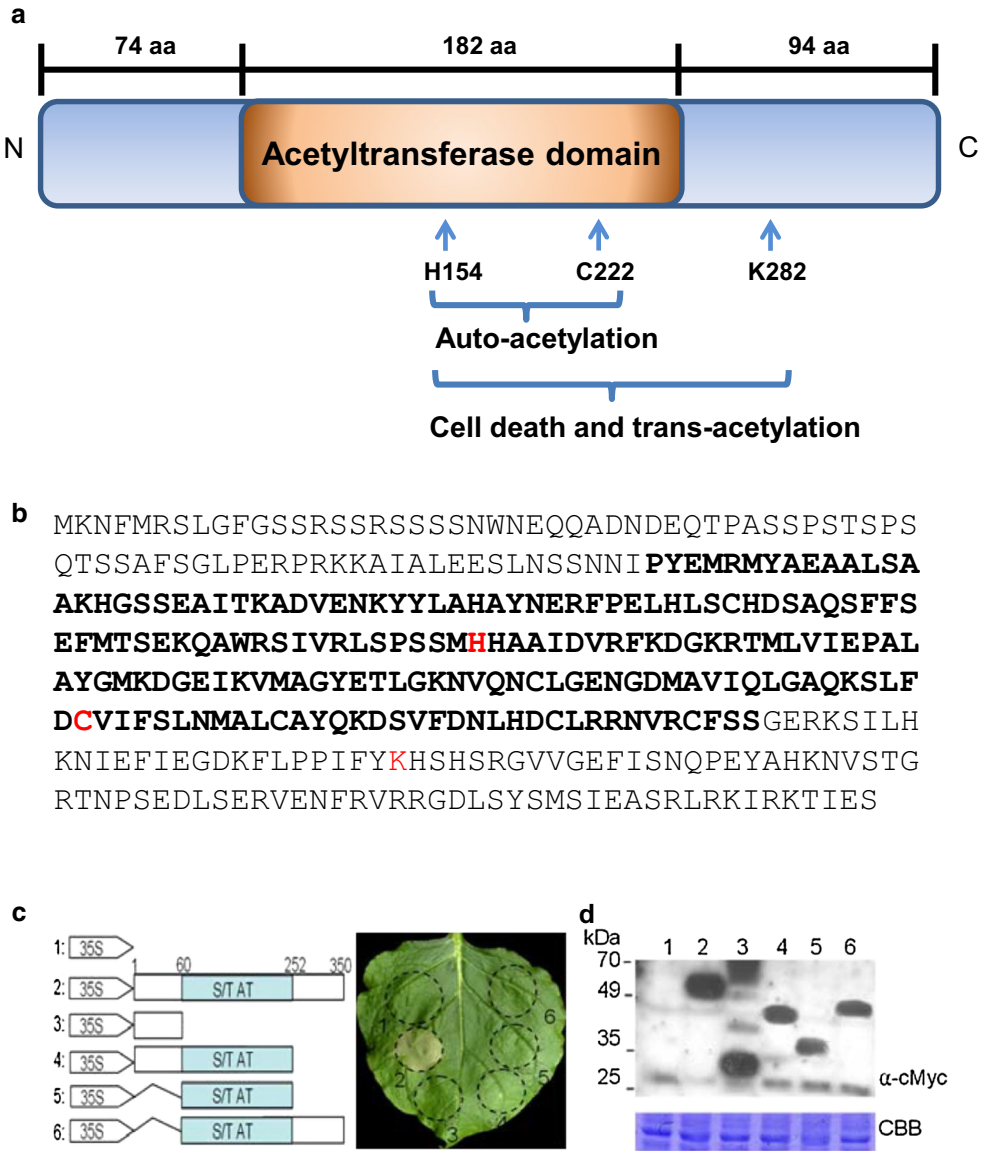

Fig. 1 Schematic of *Xanthomonas* effector AvrBsT. **a** AvrBsT possesses a putative acetyltransferase domain (182 amino acids) in the central region. The catalytic residues H154 and C222 are required to trigger cell death, AvrBsT-dependent catalytic activity, and both auto- and *trans*-acetylation. The conserved K282 is indispensable to induce cell death and *trans*-acetylation of its substrate. **b** Deduced amino acid sequence of AvrBsT (350 amino acids). *Bold letters* indicate the putative acetyltransferase domain. *Red letters* indicate the crucial residues for triggering AvrBsT-mediated cell death. **c** Transient expression of AvrBsT induces cell death in *N. benthamiana* leaves. *Agrobacterium* carrying the indicated AvrBsT deletion mutants (at a titer of $OD_{600} = 0.5$) were infiltrated into *N. benthamiana* leaves, which were photographed 48 h later (Kim et al. 2010). **d** Immunoblot analyses of AvrBsT deletion mutants. Proteins were extracted from *N. benthamiana* leaves infiltrated with *Agrobacterium* carrying the indicated constructs. AvrBsT was detected using the anti-cMyc antibody. The *numbers* above the immunoblot image represent the constructs indicated in **c** (Kim et al. 2010)

AvrBsT-mediated induction of cell death requires a functional catalytic domain (Orth et al. 2000; Kim et al. 2010). The central domain of AvrBsT, which is collinear with the YopJ serine/threonine acetyltransferase, is responsible for enzymatic activity but not for induction of cell death in plants. However, the N- and C-terminal regions are involved in the induction of cell death in *N. benthamiana* (Fig. 1c, d), which suggests that these domains may be essential for the binding of AvrBsT to host target protein(s) associated with cell death. The acetyltransferase domain of AvrBsT is also required to trigger cell death in *N. benthamiana* leaves as determined by *Agrobacterium*-mediated transient expression of *avrBsT* deletion mutants (Fig. 1c, d; Kim et al. 2010). The full-length AvrBsT protein distinctly induces AvrBsT-mediated cell death, but deletion mutants of the acetyltransferase domain, and N- and C-terminal regions do not induce any cell death response.

AvrBsT overexpression triggers both susceptible and resistant responses in *Arabidopsis* plants against the hemibiotrophic bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 and the obligate biotrophic oomycete pathogen *Hyaloperonospora arabidopsidis* (*Hpa*), respectively (Hwang et al. 2012). Overexpression of dexamethasone (DEX):*avrBsT* (DEX:*avrBsT*-OX) significantly induces disease-associated cell death and leads to higher bacterial growth in *Pst*-infected *Arabidopsis* plants. Overexpression of other bacterial effector proteins such as $HopF2_{pto}$ and AvrB in *Arabidopsis* plants disrupts PAMP-triggered host immunity (Shang et al. 2006; Cui et al. 2010; Wilton et al. 2010), which is consistent with the results of AvrBsT overexpression studies. *Arabidopsis* plants

overexpressing HopF2$_{pto}$ exhibit compromised AvrRpt2-mediated HR (Wilton et al. 2010). AvrB overexpression in *Arabidopsis* plants promotes the growth of nonpathogenic *P. syringae* by disrupting the functions of key defense components, such as RAR1, HSP90, MPK4, and RIN4 (Shang et al. 2006; Cui et al. 2010). By contrast, *DEX*:-*avrBsT*-OX *Arabidopsis* plants are less susceptible to *Hpa* infection, which is accompanied by HR and H_2O_2 accumulation (Hwang et al. 2012). Exogenous treatment of *Arabidopsis* cotyledons with purified GST-tagged AvrBsT proteins inhibits the growth and sporulation of *Hpa*. On the other hand, treatment with 10 μg mL^{-1} of GST-tagged AvrBsT induces a typical cell death phenotype on *Arabidopsis* seedlings, which suggests that AvrBsT functions to trigger sufficient basal defense responses to suppress *Hpa* infection.

Xanthomonas effector AvrBsT is proposed to suppress HR, which is elicited by the *Xcv* effector protein AvrBs1 in resistant pepper plants (Szczesny et al. 2010). *Xcv* strain 85-10 possesses the *avrBs1* gene and induces AvrBs1-specific HR in the pepper ECW-10R line (Ronald and Staskawicz 1988). Introduction of *avrBsT* into the *Xcv* strain 85-10 (*avrBsT*) significantly attenuates HR in resistant pepper plants (Szczesny et al. 2010). AvrBs1-specific HR depends on the conserved catalytic residues (H154, E173, and C222) in a putative acetyltransferase domain of AvrBsT and YopJ/AvrRxv family members (Fig. 1a, b; Orth et al. 2000). Ectopic expression of *avrBsT* (C222A) in *Xcv* strain 85-10 does not suppress the induction of AvrBs1-specific HR in resistant pepper plants (Szczesny et al. 2010). AvrBsT physically interacts with pepper SNF1-related kinase1 (SnRK1), a regulator of sugar metabolism involved in AvrBs1-triggered immunity (Szczesny et al. 2010).

AvrBsT possesses acetyltransferase activity and acetylates *Arabidopsis* acetylated interacting protein1 (ACIP1), which is required for both PTI and AvrBsT-triggered ETI during *Pst* infection (Cheong et al. 2014). AvrBsT *trans*-acetylation activity, but not auto-acetylation activity, triggers ETI in *Arabidopsis thaliana* Pi-0 leaves. Mutation of the conserved lysine (K) residue found in YopJ and YopJ-like effectors to arginine (R) in AvrBsT (K282R) (Tasset et al. 2010) does not affect AvrBsT auto-acetyltransferase activity in vitro, but it does inhibit AvrBsT *trans*-acetylation of ACIP1. The K282R mutation of AvrBsT attenuates AvrBsT-mediated activation of defense responses in *Arabidopsis* Pi-0 leaves, similar to that observed for the H154A mutation in the catalytic core (Cheong et al. 2014). Notably, *Pst* DC3000 expressing AvrBsT (K282R) does not elicit HR in *Arabidopsis* Pi-0 leaves, despite stable protein expression in leaves. These results suggest that the K282R mutation affects the AvrBsT *trans*-acetylation activity in vitro and defense-eliciting activity in planta.

Identification, in planta expression, and subcellular localization of AvrBsT-interacting plant proteins

Little is known about the corresponding plant molecular targets of *Xanthomonas* effector AvrBsT and their potential roles in HR cell death and plant defense responses. The host plant proteins that interact with *Xanthomonas* effector AvrBsT in yeast and in planta are differentially expressed in pepper plants after infection with avirulent pathogens and/or treatment with agents that induce defense responses (Kim et al. 2013b, 2014a, b). The yeast two-hybrid system (Fields and Song 1989) is used to isolate the molecular host components that physically interact with AvrBsT. A complementary DNA prey library is generated from pepper leaves undergoing HR by infection with the avirulent *Xcv* strain Bv5-4a expressing *avrBsT* (Jung and Hwang 2007; Kim et al. 2014b). A DNA-binding domain (BD) is fused with host interactors, and an activation domain (AD) is fused with AvrBsT to verify the interaction between AvrBsT and host interactors. Several AvrBsT-plant interactors have been identified from pepper and *Arabidopsis* cDNA libraries using *avrBsT* cDNA as bait, including pepper arginine decarboxylase1 (CaADC1) (Kim et al. 2013b), pepper aldehyde dehydrogenase1 (CaALDH1) (Kim and Hwang 2015a), pepper heat shock protein 70a (CaHSP70a) (Kim and Hwang 2015b), pepper suppressor of the G2 allele of *skp1* (CaSGT1) (Kim et al. 2014b), pepper SNF1-related kinase1 (SnRK1) (Szczesny et al. 2010), and *Arabidopsis* acetylated interacting protein1 (ACIP1) (Cheong et al. 2014) (Table 1). Bimolecular fluorescence complementation (BiFC) assays (Hu et al. 2002; Walter et al. 2004) and co-immunoprecipitation (Co-IP) assays (Phizicky and Fields 1995) provide in planta verification that AvrBsT physically interacts with its plant interactors.

Specific subcellular localizations and translocations of AvrBsT-interacting proteins are important for triggering downstream cell death-mediated defense signaling in plants. Specific plant disease resistance proteins detect specific pathogenic effectors, and subsequently act in the nucleus to trigger downstream signaling and defense pathways (Sheen and He 2007). For example, nuclear translocation of cytoplasmic fractions of *Arabidopsis* RPS4 (Wirthmueller et al. 2007) and tobacco N protein (Burch-Smith et al. 2007) is crucial for their activation, which ultimately leads to HR-mediated cell death. By contrast, potato Rx1 activates an antiviral mechanism in the cytoplasm but not in the nucleus (Slootweg et al. 2010). Full-

Table 1 Plant proteins that interact with the *Xanthomonas* effector AvrBsT

Interactor	Host	Accession number	Putative function	Localization	Reference
CaADC1	Pepper	DI336485	Arginine decarboxylase	Cytoplasm	Kim et al. (2013b)
CaALDH1	Pepper	KJ872510	Aldehyde dehydrogenase	Cytoplasm	Kim and Hwang (2015a)
CaHSP70a	Pepper	KJ619375	Heat shock protein 70	Cytoplasm	Kim and Hwang (2015b)
CaSGT1	Pepper	JN252483	Suppressor of the G2 allele of *skp1*	Cytoplasm Nucleus	Kim et al. (2014b)
SnRK1	Pepper	HQ176416	SNF1-related kinase	Cytoplasm	Szczesny et al. (2010)
ACIP1	*Arabidopsis*	AT3G09980	Unknown, but associated with microtubules	Microtubule	Cheong et al. (2014)

length pepper abscisic acid-responsive 1 (CaABR1) protein, which triggers cell death, specifically localizes to the nucleus in plants (Choi and Hwang 2011).

Pepper arginine decarboxylase1 (CaADC1)

The pepper CaADC1 interacts with AvrBsT in yeast and in planta (Kim et al. 2013b), and is involved in the biosynthesis of polyamines (PAs) (Walters 2003). PAs, including putrescine, spermidine, and spermine are positively charged secondary metabolites that are associated with plant resistance to microbial pathogens (Walters 2003; Jimenez-Bremont et al. 2014). BiFC and Co-IP analyses of *Agrobacterium*-mediated transient expression in *N. benthamiana* leaves verified the physical interaction of AvrBsT with CaADC1 in planta (Kim et al. 2013b). Arginine decarboxylase (ADC) activity requires pyridoxal phosphate binding at its lysine residue (Cohen et al. 1983). In the BiFC experiment, CaADC1 K154A, in which lysine (K) is replaced by alanine (A) at residue 154, does not bind to AvrBsT and does not enhance AvrBsT-triggered cell death response in *N. benthamiana* leaves (Kim et al. 2013b). The Co-IP assay indicates that AvrBsT forms a complex with CaADC1 in planta, but it does not form a complex with CaADC1 K154A. Pepper infection with avirulent *Xcv* strain Ds1 (*avrBsT*) or avirulent *Xcv* strain Bv5-4a, which possesses functional AvrBsT, rapidly and strongly induces the expression of pepper arginine decarboxylase1 (CaADC1) (Kim et al. 2013b). Exogenous treatment with defense-related hormones, such as salicylic acid (SA), methyl jasmonate (MeJA), and ethylene differentially induces *CaADC1* expression in pepper leaves. AvrBsT has been proposed to localize in the cytoplasm and nucleus (Szczesny et al. 2010). The CaADC1–AvrBsT complex is localized in the cytoplasm in *N. benthamiana* cells (Kim et al. 2013b).

Pepper aldehyde dehydrogenase1 (CaALDH1)

CaALDH1 also physically interacts with AvrBsT in yeast (Kim and Hwang 2015a). ALDHs catalyze aldehyde dehydrogenation via aldehyde oxidation to carboxylic acids. ALDHs are involved in plant growth, development, and stress responses (Kotchoni et al. 2006; Shin et al. 2009; Kim and Hwang 2015a). The in planta interactions of AvrBsT with CaALDH1 are confirmed by BiFC and Co-IP assays using an *Agrobacterium*-mediated transient expression system in *N. benthamiana* leaves (Kim and Hwang 2015a). AvrBsT-interacting CaALDH1 is expressed in pepper leaves inoculated with *Xcv* (Kim and Hwang 2015a). *CaALDH1* expression is specifically induced in pepper leaves by infection with avirulent *Xcv* strain Ds1 (*avrBsT*). *Agrobacterium*-mediated transient expression assays in *N. benthamiana* leaves indicate that CaALDH1:GFP fusion proteins are specifically localized in the cytoplasm, but not to mitochondria (Kim and Hwang 2015a). Human ALDHs are categorized as cytoplasmic ALDH1 and mitochondrial ALDH2, and are involved primarily in ethanol metabolism (Wang et al. 1998; Vasiliou et al. 1999). Family 1 ALDHs include the Class 1 ALDHs, which are localized in the cytoplasm. Family 2 ALDHs are classified as mitochondrial Class 2 ALDHs.

Pepper heat shock protein 70a (CaHSP70a)

Heat shock protein 70 (HSP70) is a ubiquitous essential protein chaperone and one of the most abundant and diverse heat stress proteins. HSP70 is involved in protein translocation, folding, synthesis, and macromolecular assemblies (Hartl and Hayer-Hartl 2002; Mayer and Bukau 2005). Pepper heat shock protein 70a (CaHSP70a) physically interacts with AvrBsT in yeast and in planta (Kim and Hwang 2015b). *Agrobacterium*-mediated transient expression assays and Co-IP assays in *N. benthamiana* showed that AvrBsT directly binds to CaHSP70a in planta (Kim and Hwang 2015b).

HSP70s are induced by environmental stresses and are required for plants to cope with heat (Feder and Hofmann 1999). *HSP70* expression is induced in *Arabidopsis* by diverse RNA viruses (Whitham et al. 2003). For example, *tomato yellow leaf curl virus* coat protein recruits host plant HSP70 during virus infection (Gorovits et al. 2013). HSP70s appear to be involved in regulating viral reproduction and protein folding and movement, which

ultimately promotes viral infection (Hafren et al. 2010). *CaHSP70* expression is induced in pepper leaves by heat stress and *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) infection (Guo et al. 2014; Kim and Hwang 2015b). Elevated temperatures promote *CaHSP70a* overexpression, which triggers cell death signaling pathways. CaHSP70a expression is strongly induced in pepper leaves by heat stress (37 °C), but it is not induced at a normal growth temperature (24 °C) (Fig. 2a; Kim and Hwang 2015b). Transient *CaHSP70a* overexpression under heat treatment (37 °C) drastically induces programed cell death in pepper leaves (Fig. 2b). The cellular heat stress response enhances expression of heat stress genes, including multigene families that encode molecular chaperones (Bukau et al. 2006; Nakamoto and Vigh 2007; Richter et al. 2010). *Xcv* infection of pepper leaves strongly induces *CaHSP70a* expression at the protein levels; however, CaHSP70a proteins are not detected in healthy and mock-treated leaves (Fig. 2c; Kim and Hwang 2015b). AvrBsT is required to promote *CaHSP70a* expression in pepper. The CaHSP70a protein level peaks 5 h after infection with avirulent *Xcv* strain Ds1 (*avrBsT*). Cytoplasmic localization of CaHSP70a positively regulates AvrBsT-triggered cell death caused by infection with *Xcv* strain Ds1 (*avrBsT*). CaHSP70a proteins are located primarily in the cytoplasmic region in pepper leaves infected with *Xcv* strains Ds1 and Ds1 (*avrBsT*) (Fig. 2d). Notably, CaHSP70a levels in pepper leaf cytoplasmic fractions are higher during infection with incompatible *Xcv* strain Ds1 (*avrBsT*) than during infection with *Xcv* strain Ds1. AvrBsT interacts with CaHSP70a in both the nucleus and the cytoplasm (Kim and Hwang 2015b). The AvrBsT–CaHSP70a complex promotes AvrBsT-triggered cell death in *N. benthamiana* leaves only when CaHSP70a is localized in the cytoplasm by attachment of the nuclear export signal (NES) (Slootweg et al. 2010; Choi et al. 2012). Transient *CaHSP70a* co-expression with *avrBsT* leads to cytoplasmic localization of the CaHSP70a–AvrBsT complex, and significantly enhances AvrBsT-triggered cell death in *N. benthamiana* (Fig. 3). CaHSP70a may physically interact with AvrBsT or unknown cell death-triggering host proteins in the cytoplasm.

Pepper suppressor of the G2 allele of *skp1* (CaSGT1)

Pepper suppressor of the G2 allele of *skp1* (CaSGT1) is identified as a host interactor of AvrBsT (Kim et al. 2014b). SGT1 is strongly conserved in eukaryotic organisms, and is involved in regulating the cAMP pathway, Skp1-Cullin-F-box (SCF)-mediated ubiquitination, and cell division (Kitagawa et al. 1999). The AvrBsT C222A mutant (Fig. 1a), which lacks acetyltransferase activity (Cheong et al. 2014), interacts with CaSGT1, indicating that the functional enzymatic activity of AvrBsT is not required for the formation of AvrBsT–CaSGT1 complexes. The pepper Bs2 resistance protein associates with SGT1 via its LRR domain (Leister et al. 2005). In *Arabidopsis*, SGT1 interacts with HSP90 and RAR1 (required for Mla 12 resistance1) and is essential for *R* gene-mediated disease resistance (Azevedo et al. 2002; Takahashi et al. 2003). CaSGT1 physically interacts with pepper pathogen-induced kinase1 (CaPIK1), a receptor-like cytoplasmic protein kinase, which is required for plant defense and cell death signaling (Kim and Hwang 2011; Kim et al. 2014b). SGT1 proteins contain three distinct domains: tetratricopeptide repeats (TPR), CHORD-containing protein and SGT1 (CS), and SGT1-specific domain (SGS) (Kadota et al. 2010). RAR1 and HSP90 are both required for the plant immune system, and bind to the CS domain of SGT1 (Azevedo et al. 2002; Takahashi et al. 2003). Domain deletion assays indicate that the central CS domain of CaSGT1 is required for binding to AvrBsT (Kim et al. 2014b). However, only the full-length CaSGT1 strongly interacts with CaPIK1 in the yeast two-hybrid system. BiFC and Co-IP assays in *N. benthamiana* leaves indicate that CaSGT1 forms a heterotrimeric complex with both AvrBsT and CaPIK1 in planta (Kim et al. 2014b).

In pepper, SGT1 is proposed to be essential for normal development, growth, and basal defense responses (Chung et al. 2006). *CaSGT1* expression is enhanced in pepper leaves by incompatible pathogen challenge and SA treatment. CaSGT1 forms a complex with AvrBsT and CaPIK1 in *N. benthamiana* leaves (Kim et al. 2014b). Both AvrBsT and SGT1 are localized in the cytoplasm and the nucleus (Noel et al. 2007; Szczesny et al. 2010); however, AvrBsT interacts with CaSGT1 in the cytoplasm (Kim et al. 2014b). The CaSGT1–CaSGT1 complex is present in the cytoplasm, and the CaSGT1–CaPIK1 complex is localized in both the cytoplasm and nucleus. Thus, the CaSGT1–CaSGT1–CaPIK1 complex may specifically form in the cytoplasm of plant cells. Yeast SGT1 physically binds to PLK1 (Polo-like kinase 1), which is essential for kinetochore-microtubule attachment in mitosis (Liu et al. 2012). Transient co-expression of AvrBsT, CaSGT1, and CaPIK1 results in cytoplasmic co-localization of both CaSGT1-CaPIK1 and AvrBsT–CaSGT1 complexes (Kim et al. 2014b).

Pepper SNF1-related kinase1 (SnRK1) and *Arabidopsis* acetylated interacting protein1 (ACIP1)

In pepper, AvrBsT targets a putative regulator of sugar metabolism, SnRK1, which is required for AvrBs1-induced immunity (Szczesny et al. 2010). The plant SnRK1 protein kinase is involved in sugar and abscisic acid (ABA) signaling pathways (Jossier et al. 2009). Yeast two-hybrid

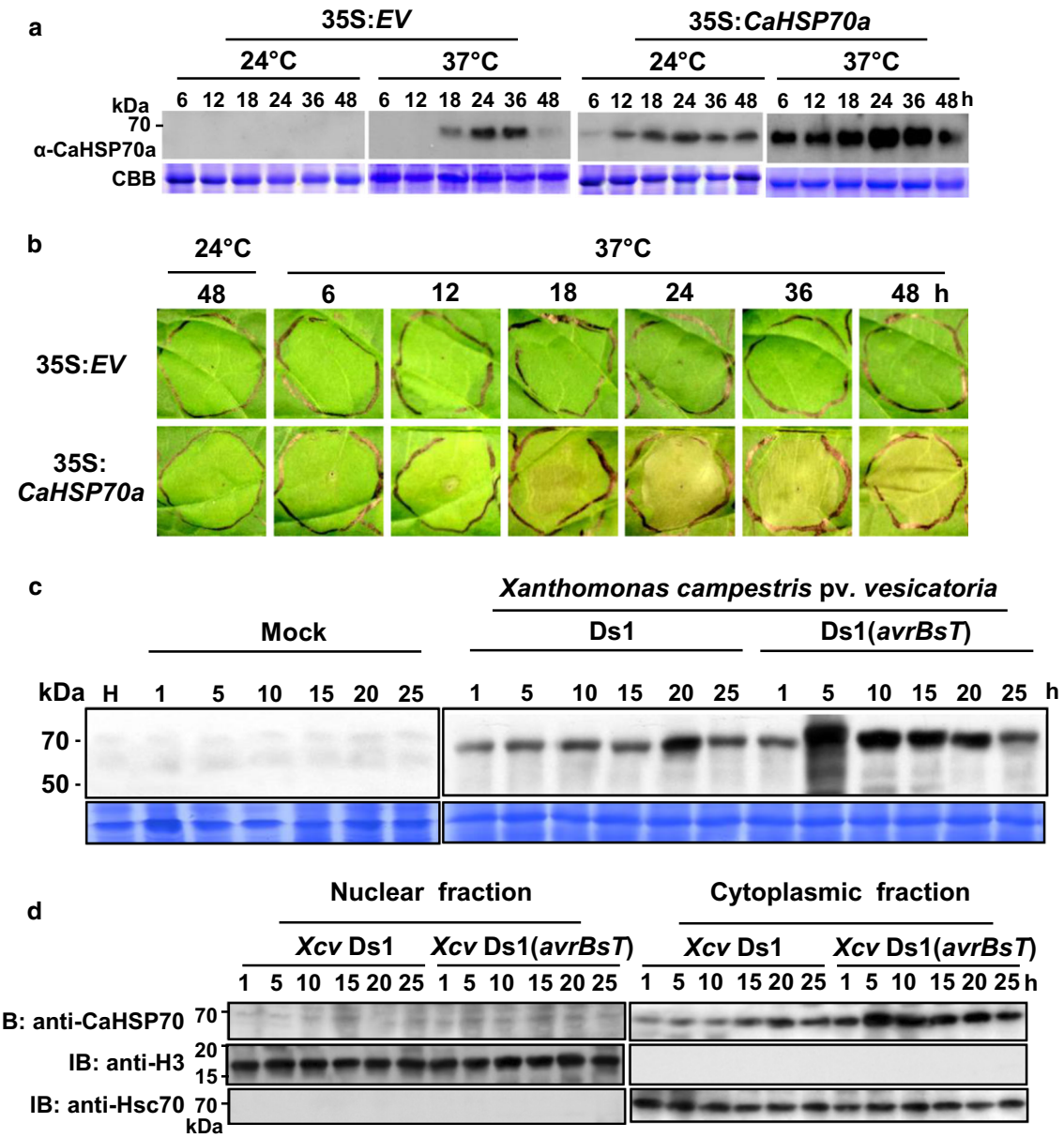

Fig. 2 Induction of *CaHSP70a* expression in pepper leaves by heat stress and *Xanthomonas campestris* pv. *vesicatoria* infection (Kim and Hwang 2015b). **a** Immunoblot analyses of *Agrobacterium*-mediated transient *CaHSP70a* expression in pepper leaves. Loaded proteins were checked by Coomassie brilliant blue (CBB) staining. **b** Cell death phenotypes triggered by transient *CaHSP70a* expression under heat stress. Pepper plants were kept at 37 °C for varying times, and then photographed 2 days after infiltration with *Agrobacterium* carrying an empty vector or a *CaHSP70a* construct ($OD_{600} = 0.5$). **c** Immunoblot analyses of *CaHSP70a* expression in leaves infected with virulent *Xcv* strain Ds1 or avirulent *Xcv* strain Ds1 (*avrBsT*) using anti-CaHSP70a antibody. Mock (10 mM $MgCl_2$) and *Xcv* strains (5×10^4 cfu mL^{-1}) were infiltrated into pepper leaves, and protein samples were recovered at the indicated times. **d** CaHSP70a protein levels in nuclear and cytoplasmic fractions from leaves infected with *Xcv* strain Ds1 or *Xcv* strain Ds1 (*avrBsT*). Anti-H3 and anti-Hsc70 antibodies were used to confirm nuclear histone 3 (H3) and cytoplasmic heat shock complex 70 (Hsc70) proteins, respectively

assays identified pepper SnRK1 as an AvrBsT interactor (Table 1; Szczesny et al. 2010). The interaction between AvrBsT and SnRK1 in planta has been verified by BiFC assays using *Agrobacterium*-mediated transient expression in *N. benthamiana* leaves. The *Arabidopsis* acetylated interacting protein1 (ACIP1) is an AvrBsT-interacting non-host protein that is proposed to preferentially bind AvrBsT in yeast (Table 1; Cheong et al. 2014). ACIP1 is a small, α-helical protein with 178 amino acids that is required for plant immunity. Glutathione *S*-transferase (GST) pull-down assays also reveal that AvrBsT interacts with *Arabidopsis* ACIP1 in vitro. However, physical interaction between AvrBsT and ACIP1 has not yet been verified in planta by any protein–protein interaction assays.

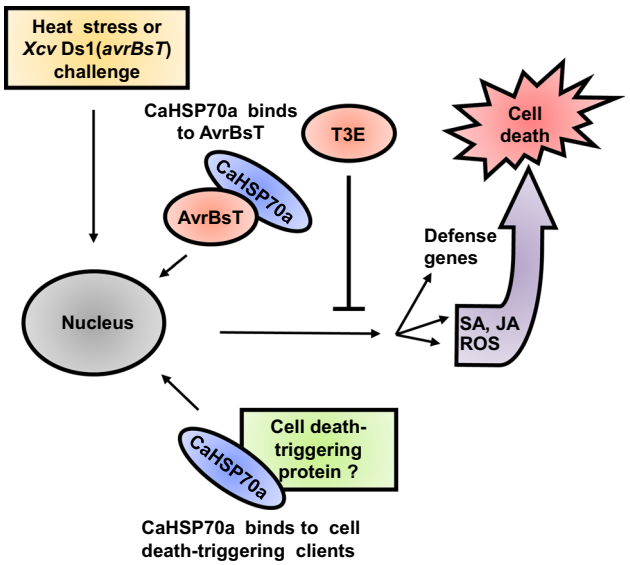

Fig. 3 Role of the CaHSP70a–AvrBsT complex in cell death and defense responses during heat stress or *Xanthomonas campestris* pv. *vesicatoria* strain Ds1 (*avrBsT*) challenge (Kim and Hwang 2015b). CaHSP70a is rapidly expressed in response to heat stress or *Xcv* strain Ds1 (*avrBsT*) challenge, and physically interacts with AvrBsT or unknown cytoplasmic host proteins that trigger cell death. Cell death signaling triggered by AvrBsT and/or host proteins induces salicylic acid (SA) and jasmonic acid (JA) accumulation, reactive oxygen species (ROS) burst, and defense-related gene expression, ultimately leading to cell death and defense responses. The defense responses may be interrupted by other type III effectors (T3E) from virulent *Xcv* strains

The AvrBsT-interacting protein SnRK1 is required for the induction of AvrBs1-specific HR in pepper (Szczesny et al. 2010). Virus-induced gene silencing shows that *SnRK1*-silenced plants exhibit a reduction in HR and reduced *SnRK1* transcript abundance. SnRK1 and AvrBsT interact in the plant cell cytoplasm (Szczesny et al. 2010). SnRK1:GFP and AvrBsT:GFP fusion proteins localize in both the cytoplasm and nucleus in *N. benthamiana*. BiFC assays also indicate that SnRK1 and AvrBsT are localized in the cytoplasm in planta. In *Arabidopsis*, AvrBsT acetylates ACIP1, a protein that associates with microtubules and is required for immunity (Cheong et al. 2014). As an AvrBsT-interacting protein, *Arabidopsis* ACIP1 is located to punctae on the cell cortex, and some of these punctae co-localize with cortical microtubules.

Molecular functions of AvrBsT-interacting proteins inside plant cells

Pepper arginine decarboxylase1 (CaADC1)

Pepper arginine decarboxylase1 interacts with AvrBsT and functions as a key defense and cell death regulator by modulating PA and γ-aminobutyric acid (GABA) metabolism (Kim et al. 2013b). Decarboxylation of arginine by ADC leads to the sequential production of PAs, such as putrescine, spermidine, and spermine (Fig. 4; Flores and Filner 1985). *Agrobacterium*-mediated transient *CaADC1* co-expression with *avrBsT* in *N. benthamiana* leaves specifically enhances AvrBsT-triggered cell death, which is accompanied by accumulation of PAs, nitric oxide (NO), and ROS bursts (Kim et al. 2013b). This enhancement may be dependent on the ADC activity of CaADC1. Transient expression of *CaADC1* in *N. benthamiana* leaves strongly induces PA accumulation. PA catabolism produces GABA, which can be further metabolized to glutamate or alanine (Fig. 4; Bouche and Fromm 2004). Transient *CaADC1* expression reduces the levels of arginine and alanine but not glutamate or GABA (Kim et al. 2013b), suggesting that *CaADC1* induction interferes with the accumulation of arginine and alanine. The nonprotein amino acid GABA may be involved in AvrBsT-triggered HR-like resistance against *Xcv* infection. GABA is a remarkably versatile signaling molecule, and is proposed to mediate communication between plants and microbial pathogens (Shelp et al. 2006). *CaADC1* expression induces an overaccumulation of PAs. Spermine could induce NO and ROS bursts, leading to cell death and defense responses (Fig. 4). During the resistance response of barley to powdery mildew (*Blumeria graminis* f.sp. *hordei*), high ornithine decarboxylase (ODC) or ADC activities result in higher PA levels (Cowley and Walters 2002). During *Pseudomonas*

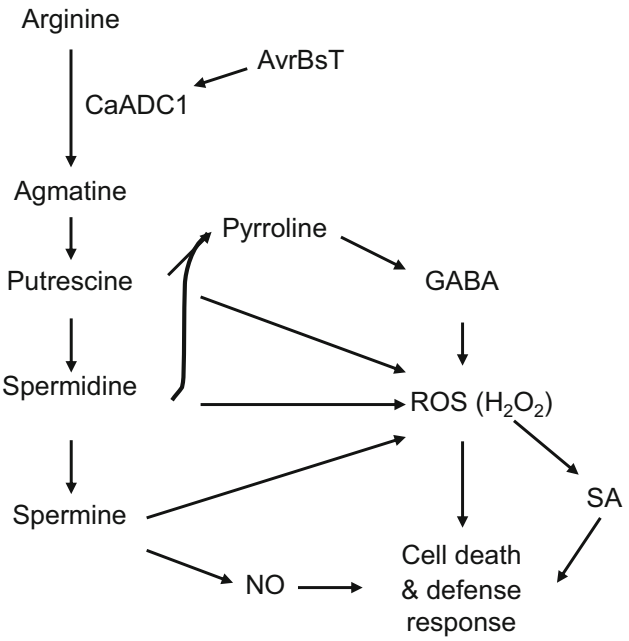

Fig. 4 Role of CaADC1 in cell death and defense response signaling mediated by polyamines (PAs) and γ-aminobutyric acid (GABA) in plants (Kim et al. 2013b)

viridiflava infection of tobacco (*Nicotiana tabacum*) plants, significantly higher spermine levels accumulate due to increased ADC activity (Marina et al. 2008). *CaADC1* silencing in pepper leaves compromises AvrBsT-triggered cell death, which is accompanied by reduced induction of H_2O_2, SA, and SA-dependent *CaPR1* and *CaPR10* defense genes during infection with avirulent *Xcv* strain Ds1 (*avrBsT*) (Kim et al. 2013b). *CaADC1*-silenced pepper leaves contain significantly lower levels of spermine and GABA. ADC functions as a rate-limiting factor that regulates PA levels in plants (Kasinathan and Wingler 2004; Alcazar et al. 2005). PA catabolism directly produces H_2O_2 (Bagni and Tassoni 2001). *CaADC1* expression confers HR cell death in response to AvrBsT, and this may be related in part to GABA. *CaADC1* silencing does not induce GABA accumulation during infection with avirulent *Xcv* strain Ds1 (*avrBsT*), and exogenous application of GABA significantly reduces the growth of avirulent *Xcv* strain Ds1 (*avrBsT*) in *CaADC1*-silenced pepper leaves. GABA may directly induce AvrBsT-triggered HR-like resistance that inhibits *Xcv* infection. There is evidence that GABA is linked to ROS generation and cell death in plants (Bouche et al. 2003). GABA accumulates in plants to counteract abiotic and biotic stresses (Choi et al. 2004; Lima et al. 2010).

Pepper aldehyde dehydrogenase1 (CaALDH1)

Aldehyde dehydrogenases (ALDHs) are involved in plant growth, development, and stress responses (Kotchoni et al. 2006; Shin et al. 2009). ALDHs also are predicted to play important roles in defense responses by detoxifying stress-generated aldehydes (Kirch et al. 2004). Cytoplasmic CaALDH1 interacts with AvrBsT and promotes AvrBsT-triggered cell death and defense responses in plants (Kim and Hwang 2015a). Heterologous transient co-expression of *CaALDH* with *avrBsT* significantly enhances AvrBsT-triggered cell death in *N. benthamiana* leaves. This cell death response depends on CaALDH1 activity. In pepper plants infected with avirulent *Xcv* (*avrBsT*), *CaALDH1* silencing attenuates ALDH activity and the ROS burst, and significantly reduces cell death and defense responses. *CaALDH1*-silenced pepper plants also exhibit significantly reduced expression of defense-related genes, such as *CaPR1* (Kim and Hwang 2000) and *CaPR10* (Choi et al. 2012). Transgenic *Arabidopsis* plants overexpressing *CaALDH1* exhibit enhanced defense responses to infection with *Pst* and *Hpa* (Kim and Hwang 2015a). *CaALDH1* overexpression in *Arabidopsis* suppresses the growth of *Pst* DC3000, *Pst* DC3000 (*avrRpm1*), and *Hpa* Noco2. *CaALDH1*-overexpressing plants accumulate significantly higher ROS levels during *Pst* infection than control plants, which ultimately enhances cell death. The ROS burst mediates cell-defense responses in pepper plants (Choi et al. 2007).

Pepper heat shock protein 70a (CaHSP70a)

Pepper heat shock protein 70a, which is targeted by *Xcv* type III effector AvrBsT, acts as a positive regulator of plant cell death and immunity signaling (Kim and Hwang 2015b). Transient *CaHSP70a* overexpression requires elevated temperatures to trigger cell death in pepper leaves. *CaHSP70a* expression induces hypersensitive cell death under heat stress, which is accompanied by strong induction of defense- and cell death-related genes. Heat shock proteins function as molecular chaperones, which are essential for the maintenance of protein homeostasis (Mayer et al. 2001). The cytoplasmic/nuclear heat shock cognate 70 (HSC70) chaperone, which is highly homologous to HSP70 (Tavaria et al. 1996), regulates *Arabidopsis* immune responses together with the suppressor of the G2 allele of *skp1* (SGT1) (Noel et al. 2007). In *N. benthamiana*, HSP70s are required for tabtoxinine-β-lactam-induced cell death (Ito et al. 2014). The Hopl1 effector protein from *P. syringae* pv. *maculicola* ES4326 directly binds *Arabidopsis* HSP70 to suppress plant immune responses (Jelenska et al. 2010). Cytoplasmic HSP70 and HSP90 are essential components of *Phytophthora infestans* INF1-mediated HR in *N. benthamiana* (Kanzaki et al. 2003). By contrast, *HSP70* expression reduces cell death triggered by SA in *N. tabacum* protoplasts (Cronjé et al. 2004). Overexpression of mitochondrial HSP70 suppresses heat- and H_2O_2-induced programmed cell death in rice (*Oryza sativa*) (Qi et al. 2011). The modular HSP70 structure consists of an N-terminal ATPase domain and a C-terminal peptide-binding domain that contains a β-sandwich subdomain with a peptide-binding cleft and an α-helical latch-like segment (Zhu et al. 1996; Hartl and Hayer-Hartl 2002). CaHSP70a contains these domains which are crucial for triggering cell death under heat stress conditions (Kim and Hwang 2015b). The actin-like ATPase- and peptide-binding domains may be responsible for the *CaHSP70a*-mediated cell death response under heat stress. In pepper leaves, transient expression of the 35S:*CaHSP70a* 195–648 deletion mutant induces a cell death response at 37 °C, similar to that induced by the full-length *CaHSP70a*. These domains may be involved in protein folding via substrate-binding cycles regulated by its ATPase activity (Hartl and Hayer-Hartl 2002). *CaHSP70a* expression contributes to both defense and ETI in pepper. The HR-like cell death response induced by *Xcv* strain Ds1 (*avrBsT*) is dramatically reduced in *CaHSP70a*-silenced pepper. *CaHSP70a* silencing in pepper enhances *Xcv* growth, but attenuates the ROS burst and cell death response during *Xcv* infection. The ROS burst closely parallels the temporal sequence of

plant cell death and defense signaling (Torres et al. 2006; Van Breusegem and Dat 2006). The levels of plant defense hormones such as SA and JA are significantly reduced in *CaHSP70a*-silenced pepper. These combined results demonstrate that *CaHSP70a* positively regulates downstream defense gene expression, SA and JA signaling, and ROS bursts, leading to the HR-like cell death response in pepper.

Pepper suppressor of the G2 allele of *skp1* (CaSGT1)

Pepper suppressor of the G2 allele of *skp1* (CaSGT1) interacts with both the *Xcv* type III effector AvrBsT and the pepper CaPIK1 to promote AvrBsT-triggered HR cell death associated with CaPIK1-mediated phosphorylation in plants (Kim et al. 2014b). SGT1 forms a chaperone complex with HSP90 to function as a sensor of the plant NLR immune receptor proteins (Shirasu 2009). Notably, SGT1 interacts with LRR domains and is essential for the maintenance of steady-state R protein levels (Leister et al. 2005; Azevedo et al. 2002). CaPIK1 is suggested to be crucial for the plant defense signaling and cell death responses (Kim and Hwang 2011). Protein kinases play key roles in signal transduction by phosphorylating target proteins involved in plant defense responses. Yeast SGT1 is phosphorylated by PLK1, a yeast SGT1-interacting protein (Liu et al. 2012). CaPIK1 specifically phosphorylates CaSGT1 and AvrBsT in vitro (Kim et al. 2014b). The kinase activity of CaPIK1 is blocked by AvrBsT. CaPIK1 autophosphorylation and phosphorylation of CaSGT1 decline in the presence of AvrBsT. However, the expression of both *CaSGT1* and *CaPIK1* promotes AvrBsT-triggered cell death in plants. CaSGT1 is essential for AvrBsT recognition and cell death, and for CaPIK1 recognition associated with phosphorylation. CaSGT1 forms a heterotrimeric complex with both AvrBsT and CaPIK1 primarily in the cytoplasm (Kim et al. 2014b). The binding of AvrBsT to CaSGT1 may induce a conformational change that alters the specific association between CaSGT1 and CaPIK1 in the cytoplasm (Shirasu 2009). AvrBsT specifically binds to the CS domain of CaSGT1, and blocks CaSGT1 phosphorylation by CaPIK1 and subsequent nuclear transport of the CaSGT1–CaPIK1 complex (Kim et al. 2014b). Liquid chromatography-tandem mass spectrometry of the proteolytic peptides of CaSGT1 identified residues Ser 98 and Ser 279 as the major CaPIK1-mediated phosphorylation sites. Phosphorylation of these sites is crucial for the activation of AvrBsT-triggered cell death in planta. CaPIK1 does not physically interact with AvrBsT in yeast cells; however, CaPIK1 phosphorylates AvrBsT in the presence of CaSGT1, and the phosphorylation of AvrBsT by CaPIK1 greatly increases in the presence of CaSGT1. Thus, AvrBsT may be phosphorylated by CaPIK1 only when CaSGT1 is present. AvrBsT reduces CaPIK1 autophosphorylation and CaSGT1 phosphorylation, and this inhibits nuclear localization of the CaSGT1–CaPIK1 complex. This suggests that recognition of AvrBsT by the CaSGT1–CaPIK1 complex is likely to occur in the cytoplasm. Addition of the nuclear localization signal (Bai et al. 2012) forces the nuclear localization of the CaSGT1–CaPIK1–AvrBsT complex and significantly suppresses cell death, which suggests that cytoplasmic localization and recognition of AvrBsT is required to trigger cell death and defense responses. *Agrobacterium*-mediated co-expression of *CaSGT1* and *CaPIK1* with *avrBsT* enhances AvrBsT-triggered cell death in *N. benthamiana*, which is dependent on CaPIK1 (Kim et al. 2014b). CaPIK1-mediated phosphorylation of AvrBsT enhances AvrBsT-triggered cell death. In pepper, virus-induced silencing of *CaSGT1* and/or *CaPIK1* compromises PTI, AvrBsT-triggered cell death, H_2O_2 production, defense gene induction and SA accumulation, leading to enhanced growth of bacterial pathogens in plants. These combined results indicate that CaSGT1 and CaPIK1 are required for basal resistance and AvrBsT-triggered cell death responses to *Xcv* infection in pepper.

Pepper SNF1-related kinase1 (SnRK1) and *Arabidopsis* acetylated interacting protein1 (ACIP1)

SnRK1 interacts with AvrBsT and is involved in AvrBsT-mediated suppression of AvrBs1-specific HR in pepper (Szczesny et al. 2010). SnRK1 does not interact directly with the *Xcv* effector AvrBs1, but is presumably indirectly involved in the recognition of AvrBs1 by the cognate *R* gene *Bs1*. AvrBsT suppresses AvrBs1-triggered HR in resistant pepper plants. *SnRK1* silencing strongly compromises AvrBs1-specific HR, which suggests that SnRK1 is required for the induction of AvrBs1-triggered plant immunity. Tomato SnRK1 may have a role in plant resistance to geminivirus infection (Shen et al. 2012). ACIP1 is an AvrBsT interactor from *Arabidopsis*, and is required for both PTI and AvrBsT-triggered ETI during *Pst* DC3000 infection (Cheong et al. 2014). Members of the ACIP family function as components of the defense signaling required for anti-bacterial immune responses. AvrBsT-dependent acetylation in planta alters the defense function of ACIP, which is linked to AvrBsT-dependent activation of ETI.

Regulation of plant cell death and defense responses by AvrBsT and AvrBsT interactors

The *Xanthomonas* effector AvrBsT and AvrBsT-interacting host proteins coordinately regulate HR cell death and defense responses in plants. CaADC1, CaALDH1,

CaHSP70a, CaSGT1, and SnRK1 are AvrBsT-interacting proteins identified in pepper (Table 1). A working model of the regulatory networks controlling AvrBst-mediated cell death and defense responses and AvrBsT-interacting and associated host proteins is presented in Fig. 5. AvrBsT is translocated into the plant cytoplasm via the *Xanthomonas* type III secretion apparatus, where it triggers HR-like cell death and defense responses in pepper leaves (Kim et al. 2010). In the presence of Ca^{2+}, the cytoplasmic CaCaM1 (Choi et al. 2009) translocates to the plant plasma membrane, where it physically binds to pepper mildew resistance locus O (CaMLO2) (Fig. 5; Kim and Hwang 2012, Kim et al. 2014a). The CaCaMLO2–CaCaM1 complex blocks AvrBsT-triggered Ca^{2+} influx and HR-like cell death and defense responses (Fig. 5). Pepper *CaMLO2* and *CaCaM1* genes are involved in disease-associated cell death and hypersensitive cell death, respectively (Kim et al. 2014a). However, infection with *Xcv* strain Bv5-4a harboring *avrBsT* triggers strong and early induction of *CaPR10* and *CaLRR1* in pepper leaves (Jung and Hwang 2007; Choi et al. 2012). In the presence of AvrBsT, CaPR10 physically interacts with CaLRR1 in the cytoplasm, which promotes CaPR10-mediated ROS burst, defense response gene expression, and cell death responses (Fig. 5; Choi et al. 2012).

CaADC1 plays a pivotal role in PA and GABA signaling in cell death and defense responses in plants (Fig. 4; Kim et al. 2013b). AvrBsT physically binds to CaADC1 in planta and presumably activates CaADC1. Activated CaADC1 catalyzes agmatine synthesis, which is further metabolized to putrescine (Fig. 4; Bagni and Tassoni 2001). Spermidine synthase adds aminopropyl groups to putrescine to produce spermidine (Bagni and Tassoni 2001; Walters 2003), which also generates H_2O_2 as a by-product. Subsequently, spermine synthase adds aminopropyl groups to spermidine to produce spermine (Bagni and Tassoni 2001), which also generates H_2O_2. Spermine can directly trigger the NO burst. PAs also are catabolized to pyrroline, which is further processed to form GABA (Flores and Filner 1985). GABA is proposed to contribute to defense responses by affecting the ROS burst during pathogen infection (Bouche et al. 2003). ROS are involved in SA signaling, which positively regulates cell death and defense responses in plants (Torres et al. 2006). These PA-mediated signaling processes suggest that CaADC1 functions as a key regulator of AvrBsT-triggered cell death and defense signaling to fine-tune PA and GABA metabolism in pepper plants (Figs. 4, 5).

CaALDH1 acts as a positive regulator of AvrBsT-triggered cell death and defense responses (Kim and Hwang

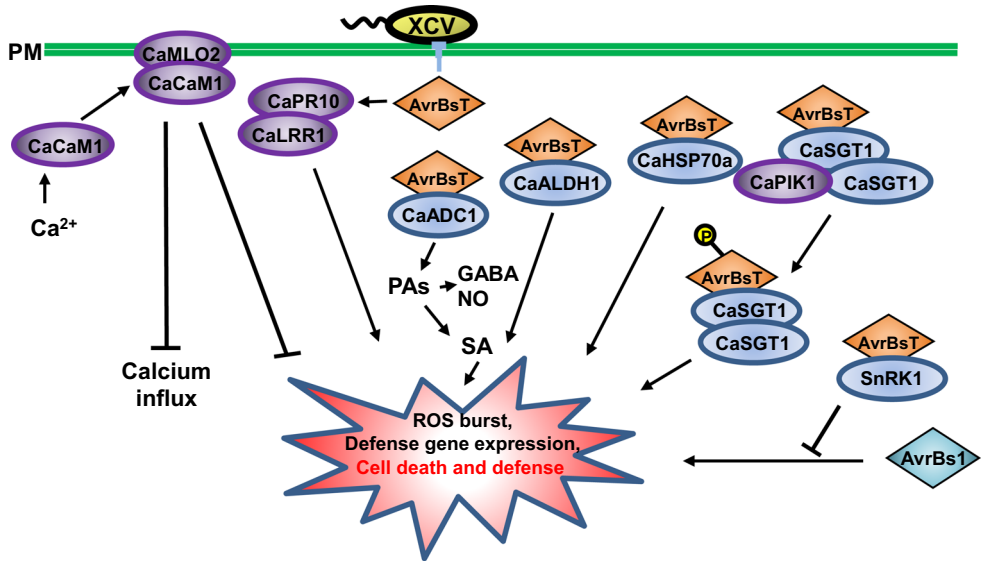

Fig. 5 Regulation of AvrBsT-triggered cell death and defense responses by AvrBsT-interacting and AvrBsT-associated proteins in plants. In the presence of Ca^{2+}, the cytoplasmic CaCaM1 translocates to the plasma membrane (PM) and physically binds to CaMLO2. The CaMLO2–CaCaM1 complex specifically suppresses AvrBsT-mediated Ca^{2+} influx, cell death, and defense responses. CaPR10 interacts with CaLRR1 and is strongly expressed in the presence of AvrBsT in the cytoplasm, which leads to the ROS burst, defense response gene expression, and cell death response. AvrBsT directly interacts with CaADC1 to regulate polyamine (PA)-associated cell death and defense response via γ-aminobutyric acid (GABA), nitric oxide (NO), and salicylic acid (SA) signaling pathways. CaALDH1 and CaHSP70a physically bind to AvrBsT and positively regulate AvrBsT-triggered cell death and defense responses. AvrBsT and CaPIK1 directly bind to CaSGT1. AvrBsT is subsequently phosphorylated by CaPIK1 and forms the active AvrBsT–SGT1–SGT1 complex, which promotes HR cell death and defense responses. By contrast, AvrBsT interacts with SnRK1 to specifically interrupt AvrBs1-triggered cell death. Proteins enclosed in blue and purple are AvrBsT-interacting and AvrBsT-associated proteins in plants, respectively

2015a). *CaALDH1*-mediated cell death depends on CaALDH1 activity. CaALDH1 interacts with AvrBsT in the plant cell cytoplasm and promotes AvrBsT-triggered cell death and defense responses (Fig. 5). Pepper *CaALDH1* expression is rapidly induced by *Xcv* (*avrBsT*) challenge (Kim and Hwang 2015a). The CaALDH1–AvrBsT complex promotes ALDH activity, which may trigger the ROS burst and induce some *PR* genes, such as *CaPR1* (Kim and Hwang 2000) and *CaPR10* (Choi et al. 2012). The cumulative effect of CaALDH1 activity enhances HR-like cell death and defense responses.

The CaHSP70a–AvrBsT complex mediates cell death and defense signaling in plants (Fig. 5; Kim et al. 2014b). Heat stress or *Xcv* (*avrBsT*) challenge triggers defense signaling and rapidly elevates *CaHSP70a* expression levels (Fig. 2). CaHSP70a binds to AvrBsT or to unknown host cell cytoplasmic client proteins that trigger cell death during infection with avirulent *Xcv* strain Ds1 (*avrBsT*) (Fig. 3). However, it remains unclear why AvrBsT targets some host proteins such as CaHSP70a. It is possible that CaHSP70a affects the folding and/or complex assembly of SA- or JA-dependent defense response proteins. AvrBsT may trigger cell death by altering CaHSP70a activity so that it facilitates the production or assembly of protein complexes that promote cell death. This cell death defense signaling is likely to induce SA and JA accumulation, ROS burst, and defense-related gene expression, which ultimately lead to HR-like cell death and defense responses (Figs. 4, 5).

Recognition of CaSGT1 by AvrBsT promotes hypersensitive cell death associated with CaPIK1-mediated phosphorylation (Kim et al. 2014b). Avirulent *Xcv* strain Ds1 (*avrBsT*) secretes the type III effector AvrBsT into host plant cells. CaSGT1 physically binds to both AvrBsT and CaPIK1, forming a transient AvrBsT–CaSGT1–CaSGT1–CaPIK1 complex in the cytoplasm. AvrBsT is subsequently phosphorylated by CaPIK1 to form the active AvrBsT–SGT1–SGT1 complex, possibly due to the negative regulation of CaSGT1 monomerization during *Xcv* strain Ds1 (*avrBsT*) infection, which further promotes HR cell death and defense responses (Fig. 5). CaPIK1 specifically phosphorylates CaSGT1 and AvrBsT (Kim et al. 2014b); however, CaPIK1 preferentially phosphorylates AvrBsT rather than CaSGT1, and then dissociates from the AvrBsT–CaSGT1–CaSGT1–CaPIK1 complex that may be transiently generated in the cytoplasm. Subsequently, the phosphorylated AvrBsT–CaSGT1–CaSGT1 complex appears to activate unknown host R proteins in the cytoplasm, ultimately leading to enhanced HR-like cell death in plants. Conversely, CaSGT1 may act as a scaffold for CaPIK1 and putative R proteins. The SGT1-HSP90 chaperone complex is involved in the maintenance of NLR-type R proteins (Shirasu 2009). SnRK1, a putative regulator of sugar metabolism, does not interact directly with the *Xanthomonas* effector AvrBs1, but is required for the induction of AvrBs1-induced immunity in plants (Szczesny et al. 2010). AvrBsT interacts indirectly with SnRK1 in planta and suppresses AvrBs1-mediated HR cell death and defense responses in resistant pepper plants (Fig. 5; Szczesny et al. 2010). Further studies are needed to determine whether AvrBsT modifies the formation of the predicted trimeric complex to which SnRK1 belongs, for example, by modifying the GAL83 homolog that interacts with SnRK1.

Conclusions and perspectives

Continued progress in understanding the molecular recognition systems of plants and pathogens has been facilitated by extensive studies of the interaction of pathogen effectors and host proteins using established biochemical techniques, such as the yeast two-hybrid system (Fields and Song 1989), BiFC assays (Hu et al. 2002; Walter et al. 2004), and Co-IP assays (Kim et al. 2013b). *Xanthomonas* type III effector proteins AvrBs1, AvrBs2, AvrBs3, and AvrBsT are secreted into the host cell and mediate ETI signaling in pepper (Choi and Hwang 2015). *Xanthomonas* effector AvrBsT suppresses plant defense responses in susceptible hosts, but triggers cell death signaling leading to HR and defense responses in resistant plants (Kim et al. 2010).

The R protein cognate of AvrBsT has not yet been identified in pepper. Extensive studies to identify the corresponding R protein cognate that directly interacts with AvrBsT to trigger defense responses in pepper have identified some of the AvrBsT-interacting plant proteins described in this review paper (Table 1). All identified AvrBsT interactors are involved in regulating HR-like cell death and defense responses in plants. *Agrobacterium*-mediated transient expression analyses and virus-induced gene silencing (Liu et al. 2002) have clearly demonstrated that AvrBsT-interacting plant proteins, such as CaADC1, CaALDH1, CaHSP70a, and CaSGT1 positively regulate HR-like cell death and defense responses against *Xcv* infection (Fig. 5).

The AvrBsT-interacting plant proteins may be useful targets for developing effective genetic markers for selecting elite lines, breeding resistant lines, and generating transgenic plant lines that are resistant to pathogen attack. AvrBsT is localized in the plant cytoplasm where it forms protein complexes with host interactors (Table 1). There may be other plant host components that control the expression of defense-related genes, ultimately leading to hypersensitive cell death and disease resistance responses. Thus, downstream host components involved in AvrBsT/interactor-mediated signaling cascades should be further

identified and characterized. Identification of a pepper NLR-type resistance protein corresponding for AvrBsT will provide new insights into novel AvrBsT-R protein interactions. The identified R protein could also be a valuable genetic resource for plant breeding and biotechnology of disease resistance.

Author contribution statement BKH designed the outline of the article. HWH and BKH wrote the manuscript. SWH composed the table and figures. BKH did the revisions of the manuscript. Both authors read and approved the manuscript.

Acknowledgements This work was supported by Grants from the National Research Foundation (NRF-2015R1A2A2A01004242, S. W. Han) and the Next-Generation BioGreen 21 Program (No. PJ011033012016, S. W. Han; No. PJ00802701, B. K. Hwang) of the Rural Development Administration, Republic of Korea.

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