REVIEW ARTICLE

Biotic Stress Related Functions of Hydroxycinnamic Acid Amide in Plants

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Abstract The study of hydroxycinnamic acid amides (HCAAs) which is a group of secondary metabolites in plants have been an interesting research subject and become of greater importance at present. Several plant amides have shown important role in plant-pathogen interaction and also in different biotic and abiotic stresses. This review paper aims to give a thorough understanding on the emerging functions of HCAA accumulation in plants related to pathogen infections. In addition, this paper discusses the current biochemical mechanisms on the formation of various classes of HCAAs in relation to plant immunity against pathogens. HCAAs contribute to several developmental processes as well as both biotic and abiotic stress responses which remains unclear up to date and there is a need to further investigate it from different plant species of various tissues or organs and cell cultures.

Keywords Biotic stress, Cell wall, Hydroxycinnamic acid amides, Phenylpropanoid pathway

Introduction

Hydroxycinnamic acid amides (HCAAs) are diversely distributed group of plant secondary metabolites and considered one of the major classes of phenylpropanoid metabolites found in nature (Herrmann et al. 1989). HCAAs have been associated with growth and developmental processes including, flower development, senescence, sexual differentiation, plant defense, tuberization, cell division, cyto-morphogenesis cell wall cross-linking, and stress responses (Tamagnone et al. 1998; Bouchereau et al. 1999; Facchini et al. 2002; Luo et al. 2009;

Bassard et al. 2010; Yogendra et al. 2014). Hydroxycinnamate conjugates play a vital role in the plant's defense responses against biotic and abiotic stresses. Plant defense responses involve activation of the phenylpropanoid pathway leading to the synthesis of low-molecular-mass natural chemicals characteristic of many cellular regulatory processes in plant physiology known as secondary metabolites. These compounds can provide protection against environmental changes and biotic or abiotic stresses (Dixon 2001; Jahangir et al. 2009). Evidence has emerged during the past decades demonstrating the importance of products from the phenylpropanoid pathway in plant defense response against pathogens (Dixon et al. 2002; Jahangir et al. 2009).

HCAA have important functions in plant adaptation to stress owing to their antioxidant properties (Bouchereau et al. 1999). It has been proposed that it also have some other functions in resistance to cold stress or viruses, floral induction and reproduction (Martin-Tanguy 1997). HCAA are important for the control of intracellular polyamine concentrations (Alcázar et al. 2006; Kuznetsov et al. 2006; Alcázar et al. 2010) and free polyamines have been observed to decrease during senescence (Ten Chen et al. 1991; Hurng et al. 1993). However, specific role of many HCAAs in plant biology is still not fully conceptualized (Jin et al. 2003; Walters 2003).

The study of HCAAs which is a group of secondary metabolites have been an interesting research subject for past few years and even become of greater importance at present. Several plant amides have shown important role in plant-pathogen interaction and also in different biotic and abiotic stresses has been cited by many present studies. This review paper aims to give a thorough understanding of the physiology and functions of HCAA accumulation in plants related to defense responses.

Anti-oxidant/anti-inflammatory Property

It has been investigated that some HCAA possess antioxidant

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property. Peptide conjugates of hydroxycinnamic acids such as ferulic acid, caffeic acid, and sinapic acid showed a synergistic antioxidative activity and could be a valuable subject of study as an active ingredient for cosmetics and preservatives for food (Kwak et al. 2009). In addition, *trans N*-feruloylnoradrenaline was found to have a very outstanding antioxidant activity in the defense response of tomato plants against bacterial infection (López-Gresa et al. 2011). HCAAs like coumaroyldopamine and coumaroyltyramine have been reported as novel induced HCAAs upon colonization of resistant tomato plants by *P. syringae* and were shown to have both antioxidant activities (Zacares et al. 2007). Serotonin derivative synthesized by serotonin N-hydroxycinnamoyl transferase (SHT) in safflower seeds was also identified to have strong antioxidant activity and other therapeutic properties making them possible to have high potential in treatment and prophylaxis, as cosmetic ingredients, and as major components of functional foods or feeds that have health-improving effects (Kang et al. 2009). Aside from its antioxidant property, cinnamic acids, p-coumaric acid and ferulic acid, p-dicoumaroylputrescine and diferuloylputrescine isolated from corn bran were found potential source of natural anti-inflammatory agents as well (Kim et al. 2012).

Pathogen Infection

Hydroxycinnamate conjugates play a vital role in the plant's defense responses against biotic- and abiotic-stresses. Plant defense responses involve activation of the phenylpropanoid pathway leading to the synthesis of low-molecular-mass natural chemicals characteristic of many cellular regulatory processes in plant physiology known as secondary metabolites. These compounds can provide protection against environmental changes and biotic or abiotic stresses (Dixon 2001; Jahangir et al. 2009). Evidence has emerged during the past decades demonstrating the importance of products from the phenylpropanoid pathway in plant defense response against pathogens (Dixon et al. 2002; Jahangir et al. 2009). In past few years, detailed investigations on the activities of metabolites in plant tissues have been conducted especially on plant pathogen interactions such as tobacco/tobacco mosaic virus (Choi et al. 2006), saskatoons/*Entomosporium mespili* (Wolski et al. 2010), tomato plants to pathogens (Bednarek et al. 2004; Zacares et al. 2007) and A*rabidopsis/ Pseudomonas syringae* (Hagemeier et al. 2001; Tan et al. 2004).

A nontarget metabolomics based on liquid chromatography and high-resolution mass spectrometry (LC HRMS) was conducted and performed for identification of resistance-related metabolites including phenylpropanoid pathway and HCAA against pathogen stress (Dixon et al. 2002; Bollina et al. 2010; Gunnaiah et al. 2012). HCAAs including feruloylputrescine, p-

coumaroyltyramine, N-feruloyltyramine, 4-coumaroyl-3-hydroxyagmatine, feruloylagmatine, 4-coumaroylagmatine, terrestriamide, and feruloylserotonin are known to be accumulated upon pathogen infection (Yogendra et al. 2014). In plants, phenylpropanoids can function as inducible physical barriers and also as chemical antagonists for the invading pathogens (Dixon et al. 2002).

Several candidate genes in the phenylpropanoid pathway were induced against plant pathogens, including 4-coumarate: CoA ligase (Fritzemeier et al. 1987; Cuypers et al. 1988), tyrosine decarboxylase, and phenylalanine ammonia-lyase in potato cell suspension culture treated with *P. infestans* (Schmidt et al. 1998), tyramine hydroxycinnamoyl transferase; in potato against *P. infestans* (Schmidt et al. 1999) and agmatine coumaryl transferase (ACT) against *Alternaria brassicicola* in Arabidopsis (Muroi et al. 2009). However, the production and role of biochemicals associated with these genes are unclear. Nontargeted metabolomic approach can help in the detection of an array of biochemicals that can be correlated to biotic stress resistance, which are governed by monogenes or polygenes (Kushalappa et al. 2013). A growing number of plant-specific acyl-CoA dependent acyltransferases, the so-called BAHD superfamily enzymes such as *Clarkia breweri* benzyl alcohol *O*acetyltransferase (*BEAT*); *Gentiana triflora* anthocyanin *O*hydroxycinnamoyltransferase (*AHCT*); *Dianthus caryophyllus* anthranilate *N* hydroxycinnamoyl/benzoyl transferase (*HCBT*); and *Catharanthus roseus* deacetylvindoline 4-O-acetyltransferase (*DAT*) were identified and characterized through genetic and biochemical studies (St-Pierre et al. 1998; D'Auria 2006).

Hydroxycinnamoyl coumaroyl and feruloyl putrescine accumulate in rust-infected wheat (*Triticum aestivum*) (Samborski et al. 1970) and agmatine was found in powdery mildew infected barley (*Hordeum vulgare*) (Smith et al. 1978). The presence of an accompanying minor complex of cross-linked dimers containing both feruloyltyramines and feruloyloctopamines and the characterization of cross-linked hydroxycinnamic acid amides were found to be associated with wound healing in potato (*Solanum tuberosum*) tubers (King et al. 2005). Chemical structure, accumulation kinetics, and functional significance of diverse compounds from the phenylpropanoid metabolism in tomato and cucumber infected with different pathogens were determined in previous studies (Fayos et al. 2006; Zacares et al. 2007; Bellés et al. 2008; López-Gresa et al. 2011). Challenging Rutgers tomato with *P. syringae* pv. *tomato DC3000* rapidly induced the expression of the hydroxycinnamoyl-CoA:tyramine hydroxycinnamoyltransferase (THT) gene, coding for the key enzyme responsible for the synthesis of hydroxycinnamoyl amides. Notably, and in agreement with this increase, a substantial accumulation of hydroxycinnamoyl amides of dopamine with positive antibacterial and antioxidative effects was

detected (Zacares et al. 2007).

HCAA metabolites produced in the phenylpropanoid pathway were detected in early response to *Fusarium* attack (Torras-Claveria et al. 2012). A fast accumulation of phytoalexin hydroxycinnamic acid amides (HCAA) of octopamine (*cis/trans N-p*-coumaroyloctopamine and *cis/trans N*-feruloyloctopamine) and noradrenaline (*cis/trans N-p*-coumaroylnoradrenaline and *cis/trans N*-feruloylnoradrenline) was detected after *Pseudomonas syringae* pv. *tomato DC3000* infiltration. (López-Gresa et al. 2011). *N-(E)* feruloyloctopamine and feruloyltyramine (FTA), *p*-coumaroyloctopamine (COA) and *N-(E)*-feruloyl-4'-*O*-methyloctopamine accumulate in potato cell suspensions during the first few hours of the *Pseudomonas syringae* or *Ralstonia solanacearum* or with pathogen-related elicitors interaction and were sensitive to oxidative stress (Baker et al. 2008). The kinetics of individual extracellular phenolic compounds and the concurrent oxidative stress during the first few hours of a plant–bacterial interaction were examined and found out that caffeoylputrescine and feruloylputrescine accumulate in the extracellular environment of *Nicotiana tabacum* suspension cells during the first few hours of the interaction between plant cells and *Pseudomonas syringae* (Baker et al. 2005). Fig. 2 shows different HCAA accumulation upon various pathogen infections.

HCAAs accumulate in incompatible interactions between plants and a variety of pathogens and have function in plant Hypersensitive Response upon pathogen infection. Free polyamine spermine has role in the hypersensitive response of barley to powdery mildew and particularly in tobacco to TMV (Walters 2003). Inoculation of tomato plants (*Solanum lycopersicum cv. Rutgers*) with *Pseudomonas syringae* pv. *tomato DC3000* led to the production of a hypersensitive-like response in this pathovar of tomato. Accumulation of hydroxycinnamic acid amides (HCAA) of tyramine (p-coumaroyltyramine and feruloyltyramine) and dopamine (p-coumaroyldopamine and feruloyldopamine) was detected after bacterial infection (Zacares et al. 2007). Transcriptional network and metabolome analysis indicate that the strong accumulation of HCAAs constitute a key class of metabolites which regulated the expression of WRKY transcription factors and genes are associated are involved in the HR activity in tomato (Etalo et al. 2013). Transgenic tomato plants overexpressing tyramine N hydroxycinnamoyltransferase exhibit elevated hydroxycinnamic acid amide levels such as coumaroyltyramine, feruloyltyramine, octopamine, and noradrenaline which leads to enhanced resistance to *Pseudomonas syringae*. HCAA accumulation was found to induce increase in salicylic acid levels as well as pathogenesis-related gene. With this, HCAA is believed to play an important function in the tomato defense system against *P. syringae* infection (Campos et al. 2014). *Torenia hybrida cv. Summerwave Blue* was created to produce Arabidopsis agmatine coumaroyltrans-

ferase (AtACT). These transgenic plants accumulated *p*-coumaroylagmatine which was found responsible to make plant resistant to the necrotrophic fungus, *Botrytis cinerea* (Muroi et al. 2012). Silencing of polyphenol oxidase (PPO) led to changes in the metabolism of phenolic compounds and its derivatives such as coumaric acid and catechin as well as in the gene expression of phenylpropanoid pathway. Most of the metabolic changes are related to PPO in the metabolism of tyrosine and in the hydroxycoumarin esculentin biosynthesis. Exogenous application of tyramine which is derived from tyrosine elicits cell death in walnut and several other plant species. With this, it was suggested that PPO plays an important role in secondary metabolism and acts as an indirect regulator of cell death in walnut (Araji et al. 2014). In potato (*Solanum tuberosum*), tomato, and tobacco (*Nicotiana tabacum*), THTs play a pivotal regulatory role in the biosynthesis of HCAAs by catalyzing the transfer of hydroxycinnamic acids from the respective CoA esters to tyramine, dopamine, octamine, and various other amines (Guillet et al. 2005; Kang et al. 2006). Phe and Tyr, in combination with various amines, are the primary precursors for the induced HCAA biosynthesis during the defense of plants against pathogens and wounding (Facchini et al. 2002; Von Roepenack-Lahaye et al. 2003; Walters 2003; López-Gresa et al. 2011). Similarly, endogenous THT and TYDC activities were wound-induced. The rate of wound-induced HCAA accumulation was highest in transgenic plants with elevated THT and TYDC activities showing that both enzymes
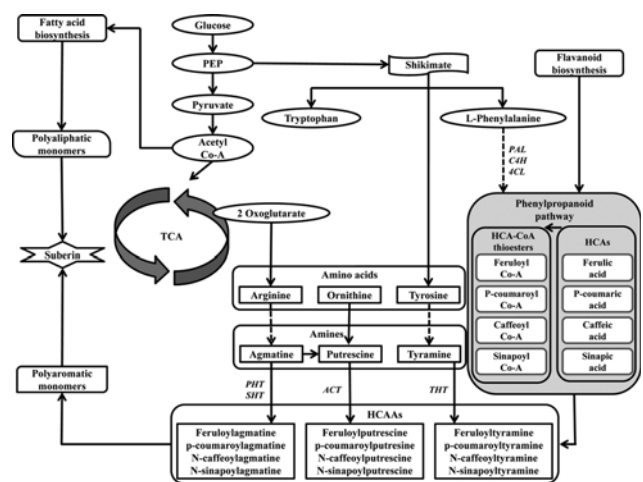

Fig. 1. Metabolic pathway in potato upon *Phytophthora infestans* infection. The resistance- related (RR) metabolites were detected in potato-inoculated with *P. infestans*. Part of the phenylpropanoid pathway showed resistance-related metabolites and their catalyzing enzymes involved in their biosynthesis. HCAAs: hydroxycinnamic acid amides, PAL: phenyl alanine ammonia lyase, C4H: cinnamate 4-hydroxylase, 4-CL: 4-coumarate: CoA ligase, ACT: agmatine coumaryl transferase, TyDC: tyrosine decarboxylase, THT: tyramine hydroxycinnamoyl transferase, SHT: spermidine hydroxycinnamoyl transferase, and PHT: putrescence hydroxycinnamoyl transferase.

exert control over the flux of intermediates involved in HCAAT biosynthesis under some conditions (Hagel et al. 2005).

A nontargeted metabolomics approach was used to identify pathogenesis-related (RR) metabolites in a resistant genotype and found out that high fold increase in phenylpropanoid pathway related compounds such as flavonoid, fatty acid, and alkaloid chemical groups. The most important phenylpropanoids identified are shown in Fig. 1. Hydroxycinnamic acid amides such as feruloylputrescine, p coumaroyltyramine, N-feruloyltyramine, 4-coumaroyl-3-hydroxyagmatine, feruloyla-gmatine, 4-coumaroylagmatine, terrestriamide, and feruloylserotonin are accumulated. HCAAs are the polyaromatic domain of suberin that is known to be associated with cell wall reinforcement. There was an upregulation of 4-coumarate: CoA ligase (4-CL), tyrosine decarboxylase (TyDC), and tyramine hydroxycinnamoyl transferase (THT) in the pathogen-inoculated resistant genotype than in susceptible (Yogendra et al. 2014).

Ethylene has been shown to be required for the induction of N-hydroxycinnamoyl-coenzyme A:tyramine N-hydroxy-cinnamoyl transferase (THT) gene expression and the

Table 1. Various HCAA accumulation in plants related to pathogen infection

HCAA	Plant	Findings	Author, Date
Ferouloyloctopamine Coumaroyloctopamine	Eggplant	HCAA are released into soil as allepathic agents	Yoshihara, 1978
Coumaroylputrecine		Antiviral activity against TMV	Martin et al, 1978
Cinnamic acid amides	Potato	HCAAs accumulate in response to Phytophthora in festans	Clarke 1982
Tyramine, octopamine	Potato	Bind rapidly to cell walls following exposure of tuber s to Phytophthora infestans	Clark 1982
Feruloyltyramine	Onion	Accumulate in roots colonized by mycorrhizal fungi	Grandmaison et al. 19 93
Suberin HCAA	Potato	Wounding	Bernards, 1994
Avenanthramides	Oat	Crown rust fungus Puccinia coronate accumulate H CAA	Miyagawa et al. 1995
Coumaroylhydroxyagmatine	Oat	Suppress Erysiphe graminis, the powdery mildew fu ngus, to cause infection	Carver et al. 1996
Feruloyltyramine, Feruloyloctapamine	Potato	Constitutes the polyaromatic domain of potato suberi n	Negrel et al. 1996/ Le wis, 1998
Coumaroylputrescine, coumaroy lagmatine	Barley	Methyljasmonate induce HCAA	Lee et al. 1997
Coumaroylagmatine, coumaroyl putrescine	Barley	Accumulate in roots as a putative component of a transient defense response in mycorrhiza format ion	Peipp et al. 1997
4-hydroxy-benzaldehyde, 4-hydr oxy-benzoate, N-4-coumaroyl-N f eruloyl-tyramine	Potato	Induced by Phytophthora infestans infection	Schmidt et al, 1997
Feruloyltyramine, coumaroyltyra mine	Tomato	Wounding or chitosan treatment increase HCAA ind ependent of jasmonic acid or	Pearce et al. 1998
Hydroxycinnamoyltyramines	Potato	Amides accumulate in elicitor-treated cell cultures	Schmidt et al. 1998
Tyramine, feruloyl tyramine	Tobacco	Inoculated with TMV	Negrel1992
Hydroxycinnamoyl esters	Parsley	Found in cell walls of elicitor-treated cell cultures	Franke et al. 1998
Coumaroyltyramine, feruloyltyra mine, hydroxylbenzaldehyde, hy droxyl-benzoate	Potato	Increased upon treatment with an elicitor from Phyto phthora infestans increased the incorporation of into the cell wall	Schmidt et al. 1998
Coumaroylagmatine	Barley	Induced when infected with Erysiphe graminis f. sp. hordei	von Röpenack et al., 1 998
Feruloylmethoxytyramine, ferulo yltyramine,coumaroyltyramine, c oumaroylglucose, hydroxylhydroxyphenyl ethylferu late	Onion	FMT, CT and FT has no antifungal activity against B otrytis allii and it just prevent fungal degradation of t he cell wall	McLusky et al. 1999
Coumaroylagmantine, Coumaroy lhydroxy agmantine	Winter wheat	HCAA protect plant against snow mold under snow cover	Shigeki et al, 2002
Hydroxycinnamoylagmantine/ ho rdatines	Barley	Powdery mildew fungus	Burhenne et al, 2003
Hydroxyanthranilte hydroxinamo yl transfersae and caffeoyl-CoA 3 -0-methyltransferase	Oat	Crown rust fungus Puccinia coronata	Yang et al , 2004
Feruloyl-L-phenylalanine t-butyl ester, sinapoyl-L-phenylalanine t -butyl ester		Has antioxidant activity	Spasova, 2005
Spermidine	Arabidopsis	Expressed in anther tapetum cells in the early stage s of flower development	Grienenberger et al. 2 009
Noradrenaline, octopamine	Tomato	Infected with Pseudomonas	Gresa et al, 2011
Coumaroylagmantine	Torenia	Accumulated after Botrytis cinerea	Muroi, 2012
Polyphenol oxidase isoenzymes	Dandelion	induced by B. cinerea and P. syringae pv. tomato	Richter, 2012
Coumaroyltyramine, feruloyltyra mine, octopamine, and noradren aline	Tomato	Accumulated in response to Pseudomonas syringae pv. tomato infection	Campos et al, 2014
Polyphenol oxidase (PPO)	Walnut	plays a fundamental role in secondary metabolism a nd acts as an indirect regulator of cell death	Araji et al. 2014

accumulation of HCAAs during the defense of tomato against the bacterial pathogen *Pseudomonas syringae* (López-Gresa et al. 2011). HCAA also accumulated in transgenic *NahG* tomato plants overexpressing a bacterial salicylic hydroxylase but treatment of plants with the ethylene biosynthesis inhibitor aminoethoxyvinilglycine, led to a reduction in the accumulation of THT transcripts and HCAA suggesting that that pathogen-induced induction of ethylene is essential for HCAA synthesis, whereas salicylic acid is not required for this response (Zacares et al. 2007). HCAAs were induced by ethylene gene, while the mutant failed to induce in Arabidopsis against the necrotrophic pathogen *Botrytis cinerea* (Lloyd et al. 2011). Table 1 shows the list of related studies on the accumulation of HCAAs in different plants upon pathogen infection. The study on HCAA induction in relation to pathogenesis started way back in 1978 and is continually investigated up to date. More HCAA compounds were discovered and more functions of HCAAs were identified aside from its role in plant defense system.

Cell Wall Fortification

The HCAAs constitute the polyaromatic domain of suberin, which is a complex, intractable biopolymer deposited apoplastically between the primary cell wall and plasmalemma (Graça 2010). These polymers increase the cell wall thickness, limiting the spread of pathogen and also act as antifungal, antimicrobial, and antibacterial compounds. The effectiveness of HCAAs as a plant defense response requires the deposition of amide conjugates in the cell wall as a means to reduce the ability of fungal pathogens to penetrate and infect cells. HCAA biosynthesis appears to occur in the cytosol based on the apparent lack of targeting signals and the solubility of key enzymes such as TYDC and THT in the pathway. Accumulation of HCAAs in the cell wall ensures a durable barrier against pathogens by reducing the cell wall

Fig. 2. Different HCAAs accumulated upon various pathogen infections.

digestibility by hydrolytic enzymes of the pathogen and/or by directly inhibiting further proliferation of the pathogen (Grandmaison et al. 1993; Facchini 1998; von Röpenack et al. 1998; Yu et al. 2009).

Previous studies have demonstrated the synthesis and integration of HCAAs into cell walls as an initial response of potato tubers to fungal attack (Clarke 1982) and four feruloyl amides: N-transferuloyloctopamine, N-cis-feruloyloctopamine, N-transferuloyltyramine, and N-cis-feruloyltyramine have been identified in potato tuber against scab caused by Streptomyces scabies (King et al. 2005). Furthermore, the incorporation of hydroxycinnamate conjugates into the cell wall is a major factor increasing the wall's biodegradability (Liu 2010). The resulting fortified and less permeable cell wall ensures exclusion of the pathogen from accessing plant nutrients and water.

HCAAs, such as coumaroylputrescine, feruloylputrescine, cinnamoyltyramine, cis-p-coumaroylagmatine, feruloylagmatine, coumaroyl serotonin, caffeoylserotonin, and feruloylserotonin, have also been proved to increase cell wall thickness, limiting the *F. graminearum* movement, especially in rachis of wheat near isogenic lines (NILs) with QTL-Fhb1 (Gunnaiah et al. 2012). Likewise, HCAAs derived from tyramine: p-coumaroyltyramine and feruloyltyramine and from dopamine: p coumaroyldopamine and feruloyldopamine (Zacares et al. 2007), noradrenaline (cis/trans N pcoumaroylnoradrenaline, and cis/trans N-feruloylnoradrenline), and octopamine (cis/trans N-p-coumaroyloctopamine and cis/trans N-feruloylo-ctopamine), which act as antibacterial and antioxidant compounds, were reported in tomato infected with the bacterial pathogen *Pseudomonas syringae* (López-Gresa et al. 2011). Feruloyltyramine and 4-coumaroyltyramine participate in the defense of plants against pathogens through their extracellular peroxidative polymerization, which is thought to reduce cell wall digestibility (Hagel et al. 2005). Similarly, feruloyl-3-methoxytyramine, feruloyltyramine, and coumaroyltyramine were detected in the cell walls of epidermal onion cells at the site of *Botrytis allii* penetration (McLusky et al. 1999).

Various methanol soluble granules accumulated on the inner face of the cell wall outside the plasma membrane and it includes HCAA components. With this, it is possible that the HCAA synthesis in the cytosol is delivered to different vesicles and then transported to the plasma membrane allowing HCAA to be deposited into the cell wall. A recombinant GST from *Opium poppy* was reported to bind HCAAs and hydroxycinnamoyl-CoA thioesters in a the same manner which has been reported for GSTs from maize and petunia (*Petunia hybrida*), which are clearly involved in the transport of anthocyanins to the vacuole suggesting that glutathione *S*-transferases (GSTs) play important role as amide carrier proteins (Edwards et al. 2000; Mueller et al. 2000, Yu and Facchini 2000). Fig. 3 shows the localization
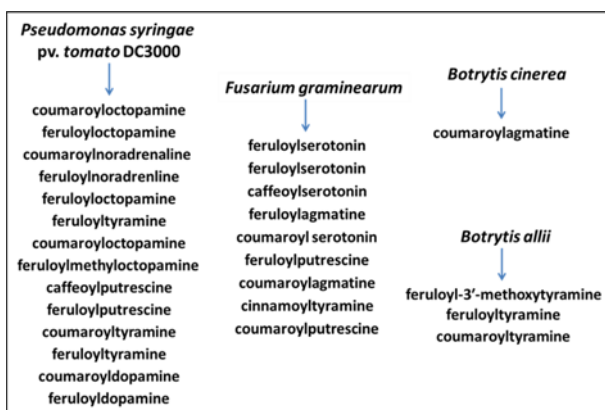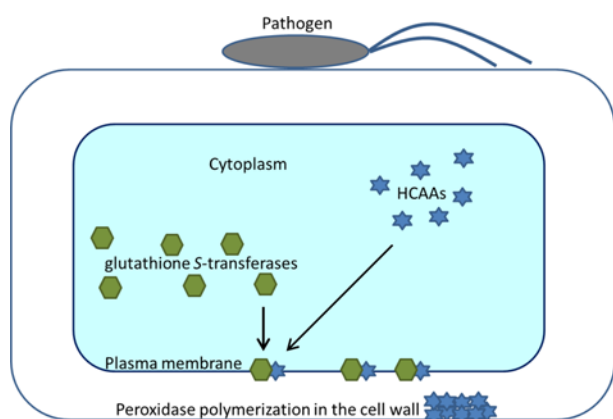
Fig. 3. HCAA biosynthesis occurs in the cytosol. Upon pathogen infection, glutathione S-transferases (GSTs) possibly play important role as amide carrier proteins of HCAA translocation to the plasma membrane. Peroxidative polymerization is necessary for the cross-linking of HCAAs into the cell wall which creates a durable barrier against pathogens by reducing the cell wall digestibility by hydrolytic enzymes of the pathogen or by directly inhibiting further proliferation of the pathogen.

of HCAA biosynthesis and its translocation upon pathogen infection in order to create a thicker cell wall protecting plants from invading pathogens. Accordingly, it is said that peroxidase activity is necessary for the cross-linking of HCAAs into the cell wall (Iiyama et al. 1994, Negrel et al. 1996). Peroxidase activity was associated with onion cell walls which leads to accumulation of auto fluorescent phenolics reported in histochemical localization test. Localized changes in pH and calcium levels regulated the rapid synthesis and enzyme secretion involved in the increase of peroxidase activity (McLusky et al. 1999).

Serotonin and its HCAAs, p-coumaroylserotonin and feruloylserotonin, were also accumulated in *Bipolaris oryzae* infected leaves of rice (Ishihara et al. 2008). Aside from cell wall fortification, HCAAs in their free form have additional functions since there is massive production of HCAA exceeding the rate of incorporation into the cell wall thus indicating that the host plant is mounting a barrier against invading pathogens. In wheat, a confocal microscopy study revealed cell wall thickening in rachis following *Fusarium graminearum* inoculation, which prevented further spread of pathogen within spike (Gunnaiah et al. 2012). In spite of numerous studies suggesting the importance of HCAA and cell wall components, more research is needed to further elucidate the role of amides in cell wall fortification and plant-pathogen interaction.

Future Perspectives

Further studies on HCAA must be conducted since these secondary metabolites are interconnected with various aspects of plant mechanisms against stress responses. HCAAs play important roles in the plants and therefore should be given more emphasis in further investigations. We are still searching continuously and fully elucidating the molecular/biochemical/physiological and holistic role of HCAAs in relation to biotic stress responses. Pathogenesis research can be done to improve defense responses of common crops through genetic manipulation of specific HCAAs. Genetic engineering of amide metabolism in the future will lead to better understand the biochemical and molecular activities of HCAAs in relation to plant defense systems. In addition, the contribution of HCAAs in many developmental processes and biotic and abiotic stress responses remains unclear and there is still a need to further investigate it from different plant species various tissues / organs and cell cultures.

Author's Contributions

DMM and MGK wrote the manuscript with input from other authors. WYK and SYL edited the paper, gave support and conceptual advice. All authors discussed the contents and approved the manuscript and agreed on the contents of the paper and post no conflicting interest.

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