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Exploring Plant Proteinase Inhibitors

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Abstract Proteinase Inhibitors (PIs) are small, natural antagonists of proteinases and present in all life forms. PIs are widely present in plants and often found in storage organs. They are known to be inducible in plants by injuries, such as insect damage. PIs have enormous diversity of function through regulation of target proteinases. Various plant sources have been explored for isolating PIs and broad-spectrum of biological activities have been elucidated. A range of strategies have been attempted to improve effectiveness of proteinase inhibitors as antimetabolites towards insects, bacteria and fungi. Much emphasis is yet to be given to address the health benefits of the PIs and implementing it in the most available forms throughout.

Keywords Serine proteinase inhibitor; Trypsin inhibitor; Insect pest; Cloning

Plants are continuously exposed to insect pest and pathogen attacks during their life cycle. Like animals, plants cannot move away from an endangering environment nor do they possess a characteristic immune system. Each plant species or cultivars have developed diverse defense mechanism, which includes both specific and general defense responses. Herbivorous insects, mites and nematodes are major contributors to yield loss either directly through consumption of plant biomass or indirectly as vectors/facilitators of pathogen infection. Consequently, introgression of insect-pest resistance into crops has become one of the major priorities for plant breeders. Resistant cultivars reduce dependence on insecticides and need for crop rotation, provide economic benefits without compromising the environmental stewardship. Studies on host plant resistance reveal that plants have evolved natural defense strategies against pests, including the production of compounds that contribute directly or indirectly, protection against herbivore invasion. The best-known plant substances supposedly involved in defense mechanisms against phytophagous insects are ribosome-inactivating proteins (RIPs), protease inhibitors (PIs), amylase inhibitors (áAl-I) and lectins. Insects have developed several strategies to overcome plant defense barriers, allowing them to feed, grow and reproduce on their host plants. However, insects possess a powerful assemblage of enzymes that constitute their defense against chemical toxicant (Ryan, 1990; Smith and Boyko, 2006).The major digestive proteases present in herbivorous insect gut are serine, cysteine, and aspartyl proteases. Inhibitors of these proteinases have been identified in plants that inhibit gut proteolytic activity, and adversely affect the growth and development of insect pests. Studies reveal that PIs are induced under various stress conditions such as insect attack, mechanical wounding, pathogen attack and exposure to UV. Role of PIs in plants as natural defense has greatly stimulated thinking towards enhancing the plant defense against insect pests through genetic engineering. The strategy to improve plant defenses against insect pests is being tried using serine proteinase inhibitors. Evolutionarily during plant–insect interactions, insects have adapted to the PIs of their host plants. Therefore, non-host plants were also evaluated and subsequently used for transferring these traits into commercial cultivars.

PIs have broad-spectrum of biological activity, which includes suppression of pathogenic nematodes (Williamson and Hussey, 1996), inhibition of spore germination and mycelium growth (Dunaevskii et al., 1997) and hampering the growth of pathogenic fungi (Joshi et al., 1998; Bhattacharjee and Prasad, 2005). In addition, PIs also contribute to better and enhanced nutritional quality of grains, as they are rich in cysteine and lysine (Ryan, 1989, Bhattacharjee et al., 2006). PIs are specific for each of four mechanistic classes of proteolytic enzymes, classified as serine, cysteine, aspartic and metallo-protease based on the active amino acid in their reaction center. In general, PIs are competitive inhibitors, bind to the active site of the enzyme. For instance serine PIs bind to serine proteases, which include trypsin, chymotrypsin, elastase, subtilisin and thrombin. Serpins (serine protease inhibitors or classified inhibitor family I4) are the largest and most broadly distributed superfamily of protease inhibitors (Rawlings et al., 2004). Serpin-like genes have been identified in animals, plants, bacteria, and some viruses (Gettins, 2002). Most serpins are irreversible inhibitors of serine proteases of the chymotrypsin family, although some have evolved to inhibit other types of serine proteases, and a few are also able to inhibit cysteine protease (Schick et al., 1998, McGowan et al., 2006, Vercammen et al., 2006, Ong et al., 2007, Roberts and Hejgaard, 2008). Furthermore, some serpins have the ability to form complexes with very divergent proteases (Huntington, 2006). Serpins are involved in a number of fundamental biological processes, and a role in the protection of storage tissue against insects and pathogens has been proposed for plant serpins (Dahl et al., 1996; Rasmussen et al., 1996).

1 Proteinase inhibitors in plants

Plants have developed defense systems to combat various pathogens throughout their life cycle, from the seed stage until senescence, and it is particularly important to keep embryo freefrom infection. There are several embryonic defense mechanisms detected, including the production of plant lectins and pathogen-relatedproteins, in response to pathogen or insect attack (Ye et al., 2001; Guiderdoni et al., 2002). Serine proteinase inhibitors are expressedin developing seeds and are thought to play an important rolein inhibiting trypsin andchymotrypsin of external origin. Two major serine class of proteinase inhibitor shave been studied extensivelyin plants: Kunitz inhibitors and Bowman-Birk inhibitors (Ryan, 1990). Proteinase inhibitors of high molecular weight with low cystein content are termed as Kunitz type (Odani and Ikeneka, 1973). Bowman-Birk inhibitors (BBIs) are cystein rich proteins of about 8 kD to 16 kD with disulfide bonds. Serine proteinase inhibitors are universal and most studied class of proteinase inhibitors in plant kingdom (Haq et al., 2004, Mello et al., 2002). Although they are present in lower concentration in vegetative tissues, are primarily localized to storage tissues such as seeds and tubers rich in storage proteins.

Plant cystatins or phytocystatins are the second most studied class of inhibitors from plants, viz, cowpea, potato, cabbage, ragweed, carrot, papaya, apple fruit, avocado, chestnut, and Job's tears. Seed cystatins have been reported from wide range of crops including sunflower, rice, wheat, maize, soybean, and sugarcane (Kuroda et al., 2001; Yozaura et al., 2002; Connors et al., 2002). Squash inhibitor, member of highly potent canonical serine proteinase inhibitors with typical knottin fold, was isolated and characterized (Chiche et al., 2004).

2 Families of proteinase inhibitors and their distribution

A comprehensive system of classification has been proposed for facilitating the exchange, storage and retrieval of information about this group of proteins (Rawlings et al., 2004). On the basis of three-dimensional structures, 31 families are assigned to 26 clans. The term "Clan" is to designate a single evolutionary line of inhibitors defined by single type of protein fold. Leo et al (2002) developed PLANT –PIs database to facilitate retrieval of information on plant protease inhibitors and related genes (Table 1). Christeller and Laing (2005) identified eight families of serine proteinase inhibitors (Table 2), which matched previously identified eight families in MEROPS.

Soybean trypsin inhibitor was the first PI to be isolated and characterized. Since then many PIs have been characterized that are widely distributed throughout the plant kingdom (Konarev et al., 2004). Most of the plant PIs characterized are from Gramineae, Poaceae, Leguminosae, Fabaceae, and Solanaceae families (Brzin and Kidric, 1995). PIs are usually found in storage organs, such as seeds and

Table 1 Plant proteinase inhibitor family in *PLANT-Pis*

Plant protease inhibitor family	*PLANT-PIs* code
Bowmann-Birk serine proteinase inhibitors	BBI
Cereal trypsin / α-amylase inhibitors	BRI
Cysteine proteinase inhibitors	CYS
Metallo carboxypeptidase inhibitors	MCI
Mustard trypsin inhibitors	MSI
Potato type I inhibitors	PI1
Potato type II proteinase inhibitors	PI2
Serpin	SP1
Soybean trypsin inhibitors (kunitz)	KNI
Squash inhibitors	SQI

However, their occurrence in aerial part of plants,as a consequence of several stimuli, has also been documented (De Leo et al., 2002). PIs accumulate to about 1 to 10% of the total soluble proteins of storage tissues. Number of PIs has been reported from non-storage tissues, such as leaves, flowers and roots (Brzin and Kidric, 1995; Xu et al., 2001; Sin and Chye, 2004). Trypsin inhibitor in mung bean is found localized to cytosol of cotyledonary cells (Chrispeels and Baumgartner, 1978). Soybean trypsin inhibitor (SBTI) was found localized in cotyledonary cell walls and embryonic cells, and to lesser extent in protein bodies, cytoplasm and nuclei. Soybean Bowman- Brik inhibitor (SBBI) was found to occur in protein bodies, nuclei, and cytoplasm (Horisberger and Tacchini-Vonlanthen, 1983). In tomatoes, serine proteinase inhibitors I and II selectively accumulated in endosperm and secretory cells of root cap (Narwaez-Vasquez et al., 1993). Xu et al (2004) reported the expression of phloem specific PIN2 protein in S. americanum stems, roots, and leaves.

3 Properties and regulation of plant PIs

Plants PIs are typical polypeptides composed of L-amino acids linked through peptide bonds, widespread in both monocots and dicot species (Ryan, 1990). Although the molecular size of PIs varies from 4 to 85 kD, majority of them are in the range of 8 kD to 20 kD (Hung et al., 2003). Plant PIs usually have high cysteine residues that form disulfide bridges contributing significantly to the stability of the inhibitors. Bowman-Birk type of Trypsin inhibitor from Brassica campestris seed (BCTI) of molecular size 8 kD, was found to be thermo stable, which is apparently related to the presence of the disulfide bridge (Hung et al., 2003). Plant PIs are low in methionione, histidine and tryptophan but are often rich in aspartic acid, glutamic acid, serine, arginine and lysine. Glycosylated plant PIs similar to mammalian glycoprotein proteinase inhibitors have not been reported so far.

Systemic signals associated with translocation of wound response include systemin, abscisic acid (ABA), hydraulic (variation potentials) andelectrical signals (Malone and Alarcon, 1995). In tomato, it has been shown that protease inhibitor initiation factor (PIIF) is wound inducible, triggers the cascade of events leading to the synthesis of serine proteinase inhibitor in the whole plant (Bryant et al., 1976; Miège et al., 1976). This suggests the existence of signal that moves from injured tissue to all parts of the plant leading to systematic induction of PI gene expression. Systemin regulates the activation of over 20 defensive genes in tomato, in response to herbivore and pathogen attack, by activating lipid based signal transduction pathway- linolenic acid released from plant membranes is converted into signaling molecule, jasmonic acid (JA). Systemin, cell surface receptor of molecular size~160 kD, regulates intercellular cascade by (1) depolarization of the plasma membrane and (2) opening of ion channels, thus increasing the intracellular Ca^{2+}, which activates MAPK and phospholipase A. These rapid changes play vital role in intra cellular release of membrane linolenic acid and its conversion to JA, potent transcription activator of defense gene (Koiwa et al., 1997; Ryan, 2000). Plantinfected by pathogen trigger two possible signal pathways as its defense strategy- (1) recognition of penetration and colonization of pathogen through wound and (2) direct molecular recognition of the pathogen (Figure 1; Figure 2) (Cordero et al., 1994). Salicylic Acid (SA) and its methyl ester (MeSA) induce systemic acquired resistance in plants against pathogenic microorganisms (Hunt et al., 1996). Several jasmonic acid-dependent and independent wound signal transduction pathways have been identified and characterized. Components of these signaling pathways are similar to other signaling cascades that include reversible protein phosphorylation cascade, calcium/calmodulin- regulated events and production of reactive oxygen species (León et al., 2001). Stintzi et al (2001) demonstrated that in absence of JA, 1, 2-oxo- phytodienoic acid (OPDA), precursor of JA, elicits defense signal response. Induced rapid de novo synthesis of rice BBI was found in seedling/leaf

Table 2 Plant serine proteinase inhibitors family - MEROPS databases

Serine proteinase inhibitor family	Class	Target protease
Kunitz Family	13	Trypsin, Chymotrypsin, Plasma Kallikrein
SERPIN Family	14	Trypsin, Chymotrypsin, Elastase
Proteinase Inhibitor 1 (PP1) family	113	Chymotrypsin, Elastase, Subtilisin, Trypsin
Ragi Seed trypsin / alpha –amylase inhibitor Family	16	Trypsin, Plasma kallikrein, Factor X11a
Squash Family	17	Trypsin
Bowman Birk Family	112	Trypsin, Chymotrypsin, CathepsinG, Matriptase
Mustard seed trypsin inhibitor family	118	Trypsin, Chymotrypsin
Proteinase inhibitor 2 (PP2) family	120	Trypsin, Chymotrypsin, Elastase, CathepsinG, Pronase, Subtilisin

in response to cut, exogenous application of jasmonic acid (JA), and protein phosphatase 2A (PP2A) inhibitors and completely inhibited by cycloheximide (Rakwal et al., 2001). Studies on herbivore-induced synthesis of glucosinolates, trypsin inhibitors, and resistance to herbivory in Brassica suggest that induced levels of trypsin inhibitors vary with genotypes (Cippolini et al., 2003). Changes in expression of jasmonic acid (JA)-and salicylic acid (SA)-dependent defense genes was observed in potato in response to potato and green peach aphids infestation (Martinez et al., 2003). Saedler and Baldwin (2004) demonstrated the potential of VIGS to manipulate and silence the expression of two jasmonate-induced genes that mediate the expression of proteinase inhibitor in Nicotiana attenuata roots and shoots. Hypothetical model for defense gene activation involving JA has been proposed which involves PI genes and jasmonate as critical signals in dicot's defense responses (Doares et al., 1995; Sivasankar et al., 2000). Farmer et al (1992) hypothesized that lipases may be synthesized or activated in response to wounding and that linolenic acid released from membranes may initiate the intracellular transduction pathway leading to proteinase inhibitor synthesis. Free linolenic acid could be converted to jasmonic acid, which may be a key signaling component that may be very close to the trans-element(s) that regulate proteinase inhibitor gene transcription (Figure 1).

Early events of signaling cascade involve changes in protein phosphorylation pattern, which eventually regulate various cellular processes in eukaryotes (Hunter, 1995), including plant defense responses (Conrath et al., 1997). Phosphorylation of proteins is a transient process, can be regulated by using protein phosphatase inhibitors- cantharidin and endothall (Li et al., 1993; MacKintosh et al., 1994; Millward et al., 1999). JA is found highly effective in inducing defense-related and cellular defense proteins, including PRs (Agrawal et al., 2000; Rakwal et al., 1999). It is initiated with the interactions of local or systemic signal molecules (Abscisic acid, systemin, oligogalacturonic acid, chitosan, electrical, and hydraulic signals) and putative plasma membrane receptors (β-glucan-elicitor-binding protein and systemin-binding protein). *P*-chloromercuribenzene sulfonic acid (PCMBS) has been shown to inhibit phloem systemin translocation, and attenuate systemic induction of protease inhibitor (PI) gene expression. Wound signal is transduced by an unidentified lipase that facilitates the release of linolenic acid from membrane lipids, a process stimulated by ABA. Volicitin, which originates from oral secretion of insects, also function like linolenic acid (Alborn, 1997). *Def1* regulates the conversion of 13(S)- hydroperoxylinolenic acid (HPOTrE) to 12-oxo- phytodienoic acid (12-oxo-PDA). *Def1* tomato mutant sensitive to insect attack is found defective in octadecanoid pathway. Diethyldi-thiocarbamic acid (DIECA) reduces the conversion of HPOTrE to 13-hydroxylinolenic acid (HOTrE) and decreases the synthesis of jasmonic acid (Howe, 1996). Inhibitors of ethylene action (norbornardiene (NBD) and silver) and salicylic acid prevent jasmonic acid-stimulated induction of PI expression (Borella, 1996; O'Donnell, 1996). Okadaic acid, inhibitor of protein phosphatase1 and 2A, inhibits jasmonate-induced PI expression; suggest the involvement of these phosphatases downstream regulatory pathway of jasmonate. The protein kinase inhibitor, staurosporine, inhibits PI gene expression induced by ABA, specifically, but not jasmonate (Dammann, 1997) (Figure 1).

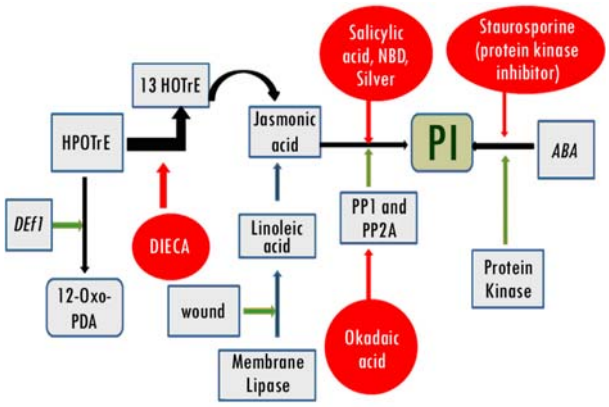

Figure 1 Regulation of proteinase inhibitor gene in plant
Note: Green arrow: activation; red arrow: inhibition; black/blue arrow: direction of reaction

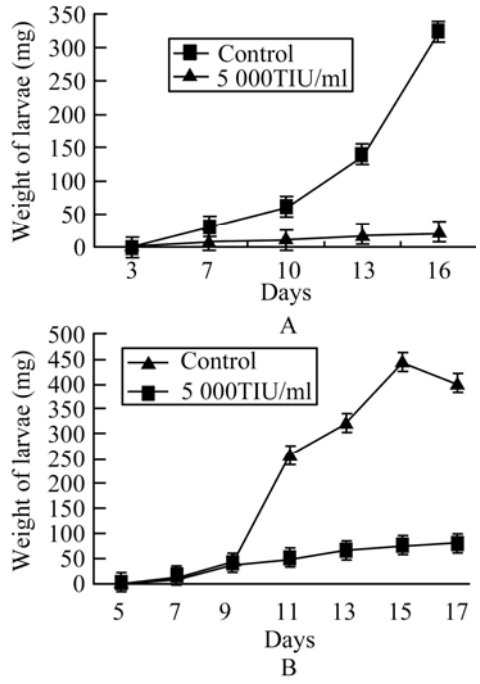

Figure 2 Larval growth curve of second (A) and third (B) instar Helicoverpa armigera larvae fed on artificial diet containing ChTI (5000 TIU/mL) and control on same diet without ChTI
Note: Each value represents the mean weight of 20 larvae from five independent replicates ± SE

4 Role of proteinase inhibitors in plants

Plant PIs are known to play important role in plant's defense against insect-pest and pathogens as well as regulation of endogenous proteinases (Mosolov et al., 2001; Birk, 2003; Shewry, 2003). They are of interest in plant biology as source of (1) resistance against pests and pathogens, (2) drugs with antiviral properties and (3) markers for studying plant diversity and evolution (Konarev et al., 2002; Lawrence and Koundal, 2002; Korsinczky et al., 2004).

4.1 In insect pest control

Serine proteinases have been identified in gut extracts of many lepidopteran insects (Houseman et al., 1989) and many of these enzymes are inhibited by proteinase inhibitors. The pH of lepidopteran guts are alkaline, ranging from 9.0-11.0 (Applebaum, 1985) wherein serine- and metallo-proteinases are most active. Hence, serine proteinase inhibitors show anti-nutritional effects against several lepidopteran insects (Shulke and Murdock, 1983; Applebaum, 1985). Nandeesha and Prasad (2001) have reported partially purified subabul trypsin inhibitor (STI) from *Leucaena leucacephala* with molecular weight of 15 kD that shows high-level thermo tolerance and pH stability. Bioassay reveals that STI is a strong inhibitor of *H. armigera*, extending larval growth period by 12 days and induces mortality by 40% at 20,000 TIU/mL concentrations. *Dimorphandra mollis* seed trypsin inhibitor (DMTI-Ⅱ) showed 67% mortality among bruchid, (*Callosobruchus maculates*) fed at 1% level in artificial diet (Macedo et al., 2002). Giri et al (2003) reported at least 14 trypsin inhibitors from *Psophocarpus tetragonolobus* seeds. WBTI-1 (28 kD) was identified as a potent inhibitor of HGP activity (94%). WBTI-2 (24 kD) and WBTI-4 (20 kD) inhibited HGP activity up to 85%. WBTI-3-5-7 showed limited inhibition of HGP as compared with trypsin. Barley (*Hordeum vulgare* L.) malt contains all the four classes of endoproteinases as well as low molecular weight peptides that inhibit many of these endoproteinases. These are chloroform/ methanol soluble peptides, possibly play a significant role in controlling the activity of barley proteinases during germination, and protecting seed and young plant from pathogens/pests (Jones and Fontanini, 2003). Cotton boll weevil, *Anthonomus grandis*, feeds on fruits and buds causing severe crop loss. Trypsin/ chymotrypsin inhibitor (BTCI) purified from Vigna unguiculata seeds showed low inhibitory activity against trypsin-like proteinases of adult weevils. The bioassay results strongly suggest that BTCI has potential to engineer crop plants for resistance to the cotton boll weevil (Franco et al., 2003). Peltophorum dubium seed trypsin inhibitor (20 kD), thermo stable kunitz type inhibitor showed 56% mortality in Anagasta kuehniella at 1.6% level (Rodrigues et al., 2003). Chougule et al (2003) analyzed seeds of 53

pigeon pea cultivars and wild genotypes, resistant and susceptible to insect-pests and pathogens, for the presence of proteinase inhibitors against Helicoverpa armigera gut proteinases (HGPs). PIs from pigeonpea cultivars showed complete inhibition of trypsin and chymotrypsin, and moderately towards HGPs. PIs of wild relatives showed stronger inhibition with HGPs. Telang et al (2003) purified bitter gourd (*Momordica charantia* L.) seed proteinase inhibitors (BGPIs) that strongly inhibit HGPs. Electrophoretic analysis revealed the presence of two major proteins (BGPI-1 and-2) and two minor proteins (BGPI-3 and-4) having inhibitory activity against trypsin and HGPs. BGPIs inhibited proteolytic activity of larvae fed on different host plants, artificial diet with or without PIs supplementation and proteinases excreted in fecal matter from respective samples. BGPIs were found to retard growth and development of *Helicoverpa armigera* and *Spodoptera litura*. Reports indicate that BGPIs mediated inhibition of insect gut proteinases directly affect fertility and fecundity of *H. armigera* and *S. litura*. Patricia et al (2003) used bioassays to investigate the effect of Bowman Birk and kunitz -type soybean trypsin inhibitor on growth pattern of Diatraea saccharalis moth using two diets- Diet 1 was less nutritious, with low protein content; and reduced minerals and essential aminoacids (cysteine, lysine and methionine) content while Diet 2 was richer and more complete. Food intake and utilization; larval development and mortality were monitored. When PI was supplemented, larval development was significantly altered in larvae fed with diet1, with reduced trypsin-like activity of midgut enzymes. Diet 2 fed larvae also showed reduced level of trypsin-like activity but it was less marked than diet 1. Similar feeding experiment was done with subabul high and low molecular weight trypsin inhibitor (HSTI, LSTI) using artificial diet, chickpea seeds and leaves. Larvae fed with artificial diet showed reduction in larval weight up to 21% (HSTI) and 43% (LSTI). However, larvae fed on seeds showed significant reduction in weight, 52.4% (HSTI) and 60.3% (LSTI), suggesting the diet also play vital role on the effectiveness of the inhibitors on larval growth and development (Bhavani et al., 2007). Franco et al (2004) suggested that SKTI can be an effective in developing transgenic plants against the cotton boll weevil, *Anthonomus grandis*. Cotton boll weevil gut digestive system contains serine proteinases. *In vitro* assay showed that SKTI inhibit these enzymes. Neonate larvae reared on an artificial diet containing SKTI showed reduction in larval weight of up to 64% and caused mortality and severe deformities of larvae, pupae and adult insects. *Oryza sativa* chymotrypsin inhibitor (OCPI1) transgenic positive plants showed higher grain yield and seed setting rate than the wild type and control under the severe drought stress conditions, whereas the potential yield of transgenic plants under normal growth conditions was not affected. Chymotrypsin- inhibitor activity assay from positive transgenic plants showed stronger inhibition. The decrease of total proteins in transgenic plants is less than the wild type under drought stress (Huang et al., 2007). The defensive role of PIs is based on their inhibitory activities towards proteolytic enzymes of insect gut and phytopathogens resulting either in a critical shortage of essential amino acids (Hilder et al., 1993; Jongsma and Bolter, 1997) or interfering with metabolic processes, such as the proteolytic activation of enzymes, molting of insects, or replication of viruses (Gutierrez-Campos et al., 1999). Direct evidence for the involvement of PIs in the plant defense system has come from studies on transgenic plants. Transgenic plants expressing PIs have been produced in the last two decades and tested for enhanced defense capacities, particularly against insect-pests (De Leo et al., 2002). Expression of cowpea trypsin inhibitor (CpTI) in transgenic tobacco was shown for the first time to confer resistance to feeding by tobacco budworm *Heliothis virescens* (Hilder et al., 1987). Plant proteinase inhibitors are known to confer natural protection against nematode attack (Atkinson et al., 2003; Cai et al., 2003; McPherson and Harrison, 2001). Nematode control with PIs expressed in transgenic tomato (Urwin et al., 1995), *Arabidopsis thaliana* (Urwin et al., 2000), and rice (Vain et al., 1998) has been well documented (Hepher and Atkinson, 1992).

PIs have the potential to enhance the current Bt toxin technology because they target a broader range of pests, including nematodes and fungi (Hilder and Boulter, 1999). There is a major concern that the effectiveness of *Bt* will be negated if field-evolved *Bt* resistance (Tabashnik et al., 2009) becomes a more widespread problem. A proposed management strategy for delaying insects' development of

resistance to plant-protection transgenes, such as *Bt* toxins, is to deploy multiple insect-control genes (such as PIs) with different modes of action in a single plant (Manyangarirwa et al., 2006). There is evidence that the combination of PIs with a sublethal-dose *Bt* toxin has a strong effect on the growth and development of insects (Zhu et al., 2007). Dunse et al (2010) coexpressed *Nicotiana alata* proteinase inhibitor (NaPI) and Ⅱ proteinase inhibitors and *Solanum tuberosum* potato type Ⅰ inhibitor (StPin1A). The combined inhibitory effect of NaPI and StPin1A on *H. armigera* larval growth in the laboratory was reflected in the increased yield of cotton bolls in field trials of transgenic plants expressing both inhibitors.

In our own study; Bhattacharjee et al (2009) showed that a thermotolerant monomeric trypsin inhibitor from *Cocculus hirsutus* (ChTI) caused 74% and 59.53% inhibition of bovine trypsin and *Helicoverpa* gut proteases respectively. The second and third instar larvae of *H. armigera* fed with ChTI (5000 TIU/mL) resulted in 84.59 and 58.71% reduction in mean larval weight respectively. An increase in the larval growth period was observed in ChTI fed larvae at all instars and inhibitor fed larvae could not complete their life cycle (Figure 2; Figure 3). In a recent study by Alvarez-Alfageme et al (2011) it was observed that AtSerpin1 (*Arbidopsis thaliana* Serine proteinase inhibitor) inhibited proteases from all pest and non-target species assayed. AtSerpin1 supplied in the artificial diet or by transgenic plants reduced the growth of *S. littoralis* larvae by 65% and 38%, respectively, relative to controls. Macedo et al (2010) suggested that trypsin inhibitor (ApTI) purified from *Adenanthera pavonina* have a potential antimetabolic effect when ingested by *Anagasta kuehniella*, a polyphagous pest that feeds on a wide variety of stored products. Larval and pupal developmental time of larvae fed on ApTI diet at 1% was significantly longer; the larval period was extended by 5 days and pupal period was 10 days longer, therefore delaying by up to 20 days and resulting in a prolonged period of development from larva to adult. The percentage of surviving adults (%S) decreased to 62%. The fourth instar larvae reared on a diet containing 1% ApTI showed a decrease in tryptic activity of gut and that no novel proteolytic form resistant to ApTI was induced. da Silva et al (2012) showed that 0.1% trypsin inhibitor (ApTI) have great toxic potential on the development of *Diatraea saccharalis*, a major sugarcane pest, causing damage to the stalks of sugarcane plants larvae. 0.1% ApTI produced approximately 67% and 50% decreases in weight and survival larval, respectively. The level of trypsin was significantly decreased (ca. 55%) in the midgut of larvae reared on a diet containing 0.05% ApTI and the trypsin activity in ApTI-fed larvae demonstrated sensitivity to ApTI.

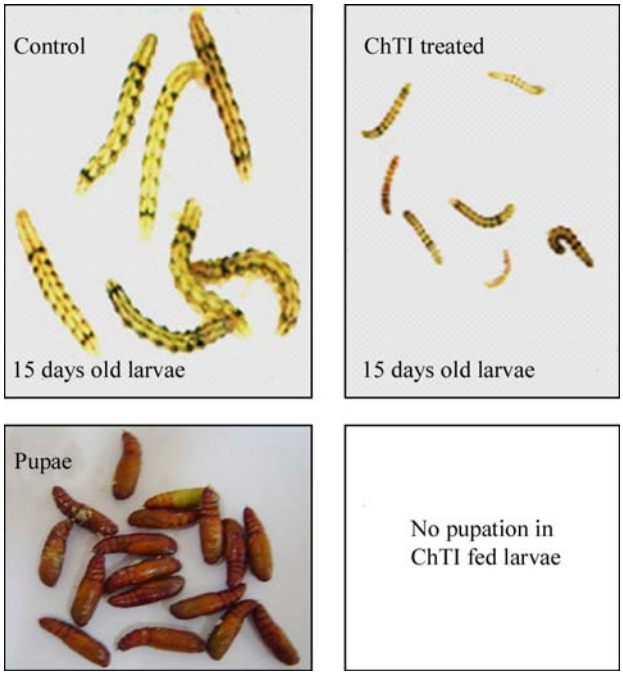

Figure 3 Effect of ChTI on growth of *Helicoverpa and Spodoptera* larvae
Note: The second star larvae were fed with artificial diet containing ChTI (5000 TIU) and control on same diet without ChTI

4.2 Proteinase inhibitor-antifungal and antibacterial property

Proteinase inhibitors in plants are able to suppress enzymatic activity of phytopathogenic microorganisms. Trypsin and chymotrypsin inhibitors of plant origin were shown to suppress activity of proteinases secreted by *Fusariumsolani* (Mosolov et al., 1976). Heat stable antimicrobial peptide, Potide G, completely suppress the proteolytic activity of trypsin, chymotrypsin and papain, in addition to growth inhibition of variety of bacterial and fungal strains (Kim et al., 2006). In tomato and potato tubers infected with the oomycete fungus, *P. infestans*, increase in trypsin and chymotrypsin inhibitor content was observed, which correlated with resistance to pathogen (Valueva et al., 1998, 2003). Mendieta et al (2004) reported that

trypsin inhibitor inhibited the spore germination of fungal pathogen *Sclerotinia sclerotiorum*. However, proteinase inhibitors induced in response to infection differed from inhibitors present in healthy plant (Geoffroy et al., 1990). Such induction in response to infection by pathogenic microorganisms is not limited to serine proteinase inhibitors, synthesis of cystatin like inhibitor was observed in chestnut leaves inresponse *B. cinerea* infection (Pernas et al., 2000). Kim et al (2005) purified potamin-1 (PT-1), a 5.6 kDa trypsin–chymotrypsin protease inhibitorfrom the tubers of the potato (*Solanum tuberosum*). PT-1 strongly inhibited pathogenic microbial strains, including *Candida albicans, Rhizoctonia solani*, and *Clavibacter michiganense* subsp. *Michiganinse*. This protease inhibitor, PT-1, was composed of polypeptide chains joined by disulfide bridge(s). Reduced PT-1 almost completely lost its activity against fungi and proteases indicating that disulfide bridge is essential for its protease inhibitory and antifungal activity. Wang et al (2006) purified 10 kDa proteinase inhibitor from mung bean (*Phaseolus mungo*) seeds. It exerted a potent inhibitory action toward a variety of fungal species including *Physalospora piricola*, *Mycosphaerella arachidicola*, *Botrytis cinerea*, *Pythium aphanidermatum*, *Sclerotium rolfsii* and *Fusarium oxysporum*, as well as an antibacterial action against *Staphylococcus aureus*. Our own study showed that ChTI exhibited strain specificity and inhibited growth and development of plant fungal pathogens. Bioassay studies on yeast strains indicated that ΔYNK and MNN1 are more sensitive to ChTI. The results suggest that phosphodiester linkage in cell wall components is likely to be the key determinants for binding of ChTI (Figure 4). It had no effect on bacteria (Bhattacharjee et al., 2009). Shilpa and Murugan (2010) purified a 14.3 kD proteinase inhibitor from *Coccinia grandis*. PI strongly inhibited pathogenic microbial strains, including *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Eschershia coli*, *Bacillus subtilis* and pathogenic fungus *Candida albicans*, *Mucor indicus*, *Penicillium notatum*, *Aspergillus flavus* and *Cryptococcus neoformans*. Examination by bright field microscopy showed inhibition of mycelial growth and sporulation. Morphologically, PI treated fungus showed a significant shrinkage of hyphal tips. Reduced PI completely lost its activity indicating that disulfide bridge is essential for its protease inhibitory and antifungal activity.

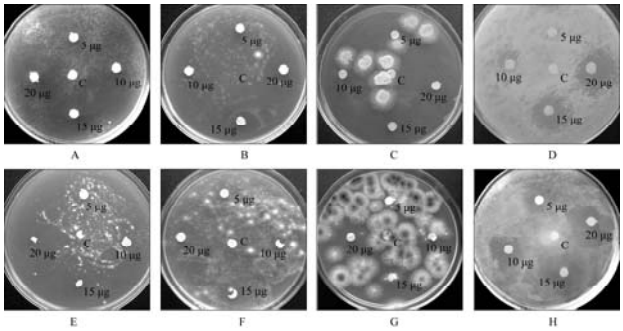

Figure 4 Antifungal assay using fungal isolates
Note: Zone inhibition assay of ChTI against Phytopathogens; A: *Alternaria alternate*; B: *Aspergillus flavus*; C: *Colletotrichumcapsici*; D: *Fusarium oxysporum*; E: *Fusarium solani (*crossandra isolated); F: *Fusariumsolani* (chick pea isolated); G: *Rhizoctonia oryzea*; H: *Sclerotia* sp.

4.3 Proteinase inhibitors in regulation of programmed cell death (PCD)

The compounds that were effective in controlling PCD in soybean- leupeptin, PMSF and AEBSF have inhibitory activity against proteases- leupeptin is an established inhibitor of cysteine proteases, whereas PMSF and AEBSF are inhibitors of serine proteases (Alonso et al., 1996). In plants, proteinase inhibitors are subject of regulation by intercellular signaling molecules, such as jasmonic acid (Farmer et al., 1992), salicylic acid (Doares et al., 1995) and systemin (Constabel et al., 1995). Salicylic acid was found to suppress the expression of cystatin (Doares et al., 1995). Salicylic acid also was shown to promote cell death induced by direct oxidative stress or pathogen attack leading to the possibility-(1) increased generation of H_2O_2 and (2) repressed cystatin expression (Shirasu et al., 1997). Controls of cellular fate through regulated expression of specific proteases in combination with the associated protease inhibitor genes provide additional plasticity to responses from outside stimuli. Plant cysteine proteases seem to play an important role in preventing PCD triggered by oxidative stress, wounding caused by insect chewing or during chilling-induced oxidative stress (Prasad et al., 1994; Solomon et al., 1999).

4.4 Proteinase inhibitors in health and disease control-medical and industrial aspects

Proteolytic enzymes are involved in numerous physiological processes in humans including digestion of food, tissue remodeling, host defense, blood coagulation and activation of proenzymes and prohormones. Proteinase inhibitors also play an

important physiological role in the regulation of enzymes. There are many examples of pathological conditions in which uncontrolled proteolytic activity of host enzymes leads to irreversible tissue destruction (e.g., in inflammatory processes, including rheumatoid arthritis and periodontitis), or to tumor growth and metastasis. In addition to host proteinases, exogenous proteinases from infectious agents such as bacteria, viruses or protozoa play a role in the onset and perpetuation of infection, suggesting the potentially therapeutic application for proteinase inhibitors in combating diseases.Therapeutic possibilities of plant PIs in the treatment of a wide range of disorders, such as pancreatitis, shock, allergy and inflammation associated with enhanced proteolytic activities had resulted in several kallikrein trypsin inhibitor-based drugs (Richardson, 1977; Park and Ohba, 2004). Epidemiological studies indicate decreased occurrence of breast, colon, and prostates cancers in vegetarian populations suggested the role of plant PIs in preventing these cancers (Birk, 1993), which has eventually led to extensive studies of plant PIs as cancer chemo preventive agents (Troll and Kennedy, 1993). Plant PIs are found active in regulating human physiological processes, e.g. cell signaling/migration, digestion, fertilization, growth, differentiation, immunological defense, wound healing and apoptosis, have great potential in therapeutic applications (Abdel-Meguid et al., 2000; Leung et al., 2000). Purified homodimeric trypsin inhibitor of 54kD from *Clausena lansium* seed inhibited trypsin with no inhibitory activity on chymotrypsin and proteinase K. However, *Clausena lansium* trypsin inhibitor inhibited the uptake of MTT by human leukemia HL60 and hepatoma Hep G2 cells. The activity of HIV-1 reverse transcriptase was reduced in the presence of the inhibitor (Ng et al., 2003).Two molecular species of oryzacystatin (OC), OC-I and OC-II were investigated to haveantiviral action against herpes simplex virus type 1 (HSV-1) *in vitro* and *in vivo*. In the mouse modelwith HSV-1-induced keratitis and encephalopathy, topical administration of OC-I to cornea produced a significant decrease in virusproduction and improvementin survival rates (Aoki et al., 1995). Kunitz trypsin inhibitors act on platelet aggregation, blood coagulation, fibrinolysis and inflammation. Due to its ability to block enzymes, plant Kunitz inhibitors are useful as tools in the study of biochemical processes of these phenomenon (Sampaio et al., 1996, Souza-Pinto et al., 1995). Serine proteinase inhibitor isolated from *Leucaena leucocephala* seeds has shown to inhibit plasmin, human plasma kallikrein, trypsin, chymotrypsin and Factor XIIa but not factor Xa (Oliva et al., 2000). Soybean derived KTI inhibits LPS-induced up-regulation of cytokine expression possibly through suppression of ERK1/2 and p38 kinase-mediated NFkappaB activation. These findings may identify anti-inflammatory properties of KTI at the level of gingival fibroblasts and suggest the use of KTI in modulating inflammation, including periodontal disease (Kobayashi et al., 2005).The Bowman-Birk trypsin-chymotrypsin inhibitor from soybean has been described as a potential chemopreventive agent of cancer. Effects of inhibitor were compared with two variants of pea seed protease inhibitors, rTI1B and rTI2B homologous to BBI but differing in inhibitory activity, on the growth of human colorectal adenocarcinoma HT29 cells. Significant and dose-dependent decrease in the growth of HT29 cells was observed using these proteinase inhibitors (Clemente et al., 2005).Squash family inhibitors are the smallest protein serine protease inhibitors, composed of 30 amino acid residues. Squash family inhibitors (from the seeds of bitter gourd, squash, gourd and luffa) were examined on serine proteases associated with blood coagulation. Five of them prolonged the activated thromboplastin time of human plasma. All inhibitors inhibited the amidolytic activities of factor XIIa, plasma kallikrein, factor Xa, but not to the same extent with factor XIa, IXa, VIIa and thrombin. The extension of activated partial thromboplastin time by inhibitors appeared to correspond to their inhibitory potencies for factor XIIa (Hayashi et al., 1994). Siritapetawee and Thammasirirak (2011) investigated biological effect of *Artocarpus heterophyllus* (jackfruit) latex on human blood coagulation. They demonstrated the serine protease inhibitory property of a heteromultimeric glycoprotein (HSGPL1) purified from jackfruit latex. This protein affects the intrinsic factors of human blood coagulation by prolonging the activated partial thrombin time (APTT) and inhibiting blood coagulation factors XIa and α-XIIa. In addition, this protein was provisionally identified as a

heat-shock/chaperone protein. These properties may be a medicinal benefit, e.g., in wound healing, blood coagulation and fibrinolysis.

Conclusion

The potential of PIs is quite evident and using PIs for developing transgenics can give durable resistance than using *Bt* genes alone. As such, the inhibitors can probably act not only as self-sufficient protective proteins, but can also protect other recombinant proteins from the deleterious effects of proteolytic enzymes. Apart from this, medicinal properties of proteinase inhibitors are being explored. Much importance and awareness are required to implement the same for health benefits in it's the most available forms throughout.

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Reference

Abdel-Meguid M., Von Der Helm K., Korant B.D., and Cheronis J.C., 2000, Proteases as targets for therapy, Springer, Berlin, Germany

Agrawal G.K., Jwa N.S., and Rakwal R., 2000, A novel rice (*Oryza sativa* L.) acidic *PR1* gene highly responsive to cut, phytohormones, and protein phosphatase inhibitors, Biochem. Biophys. Res. Commun., 274(1): 157-165 http://dx.doi.org/10.1006/bbrc.2000.3114 PMid:10903912

Alborn T., Turlings T.C.J., Jones T.H., Stenhagen G., Loughrin J.H., and Tumlinson J.H., 1997, An elicitor of plant volatiles from beet armyworm oral secretion, Science, 276(5314): 945-949 http://dx.doi.org/10.1126/science.276.5314.945

Alonso M., Hidalgo J., Hendricks L., and Velasco A., 1996, Degradation of aggrecan precursors within a specialized subcompartment of the chicken chondrocyte endoplasmic reticulum, Biochem. J., 316(Pt 2): 487-495 PMid:8687392 PMCid:1217376

Alvarez-Alfageme F., Maharramov J., Carrillo L., Vandenabeele S., Vercammen D., van Breusegem F., Smagghe G., 2011, Potential use of a serpin from Arabidopsis for pest control, PLoS One, 6(5): e20278 http://dx.doi.org/10.1371/journal.pone.0020278 PMid:21655276 PMC id: 3104999

Aoki H., Akaike T., Abe K., Kuroda M., Arai S., Okamura R., Negi A., and Maeda H., 1995, Antiviral effect of oryzacystatin, a proteinase inhibitor in rice, against herpes simplex virus type 1 *in vitro* and *in vivo*, Antimicrobial Agents and Chemotherapy, 39(4): 846-849 http://dx.doi.org/10.1128/AAC.39.4.846 PMid:7785982 PMCid:162640

Applebaum S.W., 1985, Biochemistry of Digestion, In: Kerkot G.A., and Gilbert L.I. (eds.), Comprehensive insect physiology; Biochemostry and Pharmacology, Pergamon Press, Oxford, UK, pp.279-311

Atkinson H.J., Urwin P.E., and Mcpherson M.J., 2003, Engineering plants for nematode resistance, Annual Review of Phytopathol, 41: 615-639 http://dx.doi.org/10.1146/annurev.phyto.41.052002.095737 PMid:12730388

Bhattacharjee C., Bhavani P., Mohan M.N., and Prasad D.T., 2006, Purification and characterization of trypsin proteinase inhibitor from *Cocculus hirsutus*, Plant and Animal Genomes XIV Conference, San Diego, 14~16, Januarary, CA, USA, pp. 927

Bhattacharjee C., Manjunath N.H., and Prasad D.T., 2009, Purification of a trypsin inhibitor from *Cocculus hirsutus* and identification of its biological activity, Journal of Crop Science and Biotechnology, 12(4): 253-256 http://dx.doi.org/10.1007/s12892-009-0094-8

Bhattacharjee C., and Prasad D.T., 2005, Purification and Characterization of trypsin proteinase inhibitor from *Cocculus hirsutus* and evaluation for antifungal activity, In: DAE BRNS Life Sciences symposium (LSS2005) on Molecular biology of Stress response and its application, 19~21, December, Bhava Atomic Research Centre, Bombay, India

Bhavani P., Bhattacherjee C., and Prasad D.T., 2007, Bioevaluation of Subabul (*Leucaena leucocephala*) proteinase inhibitors on *Helicoverpa armigera*, Arthropod-Plant Interactions, 1(4): 255-261 http://dx.doi.org / 10.1007/s11829-007-9020-5

Birk Y., 1993, Protease inhibitors of plant origin and role of protease inhibitors in human nutrition, In: Troll W., Kennedy A.R. (eds.), Protease inhibitors as cancer chemopreventive agents, Plenum Press, New York, USA, pp.97-106 http://dx.doi.org/10.1007/978-1-4615-2882-1_5

Birk Y., 2003, Plant protease inhibitors: significance in nutrition, plant protection, cancer prevention and genetic engineering, Springer, Berlin, Germany, pp.170

Borella M.A., Xu Y., Prabha T.N., Zhao Y., Narasimhan M.L., Wilson K.A., Nielsen S.S., Bressan R.A., and Hasegawa P.M., 1996, Different expression of soybean cystein proteinase inhibitor genes during development and response to wounding and methyl jasmonate, Plant physiol., 112(3): 1201-1210 http://dx.doi.org/10.1104/pp.112.3.1201 PMid:8938418 PMCid:158047

Bryant J., Green T.R., Gurusaddaiah T., and Ryan C.A., 1976, Proteinase inhibitor II from potatoes: isolation and characterization of its promoter components, Biochem., 15(16): 3418-3424 http://dx.doi.org/10.1021/bi00661a004 PMid:821519

Brzin J., and Kidric M., 1995, Proteinases and their inhibitors in plants: role in normal growth and in response to various stress conditions, Biotechnol. Genet. Eng. Rev., 13: 420- 467

Cai D.G., Thurau T., Tian Y.Y., Lange T., Yeh K.W., and Jung C., 2003, Sporamin-mediated resistance to beet cyst nematodes (*Heterodera schachtii* Schm.) is dependent on trypsin inhibitory activity in sugar beet (*Beta vulgaris* L.) hairy roots, Plant Mol. Biol., 51(6): 839-849 http://dx.doi.org/10.1023/A:1023089017906 PMid:12777044

Chiche L., Heitz A., Gelly J.C., Gracy J., Chau P.T., Ha P.T., Hernandez J.F. and LE-Nguyen D., 2004, Squash inhibitors: from structural motifs to macrocyclic knottins, Curr. Protein Pept. Sci., 5(5): 341-349 http://dx.doi.org/10.2174/1389203043379477 PMid:15551519

Chrispeels M.J., and Baumgartner B., 1978, Trypsin inhibitor in mung bean cotyledons: purification, characteristics, subcellular localization, and metabolism, Plant Physiol., 61(4): 617-623 http://dx.doi.org/10.1104/pp.61.4.617 PMid:16660348 PMCid:1091929

Christeller J. and Laing W., 2005, Plant serine proteinase inhibitors, Protein and Peptide Letters, 12(5): 439-447 http://dx.doi.org/10.2174/092986605439329 PMid:16029156

Chougule N.P., Hivrale V.K., Chhabda P.J., Giri A.P., and Kachole M.S., 2003, Differential inhibition of *Helicoverpa armigera* gut proteinases by proteinase inhibitors of pigeonpea (Cajanus cajan) and its wild relatives, Phytochem., 64(3): 681-687 http://dx.doi.org/10.1016/S0031-9422(03)00375-3

Cipollini D.F., Busch J.W., Stowe K.A., Simms E.L. and Bergelson J., 2003, Genetic variation and relationships of constitutive and herbivore-induced glucosinolates, trypsin inhibitors, and herbivore resistance in *Brassica rapa*, J. Chem. Ecol., 29(2): 285-302 http://dx.doi. org/10.1023/A:1022673726325 PMid:12737259

Clemente A., Gee J.M., Johnson I.T., Mackenzie D.A., and Domoney C., 2005, Pea (*Pisum sativum* L.) protease inhibitors from the Bowman-Birk class influence the growth of human colorectal

adenocarcinoma HT29 cells *in vitro*, J. Agric. Food Chem., 53(23): 8979-8986 http://dx.doi.org/10.1021/jf051528w PMid:16277391

Connors B.J., Laun N.P., Maynard C.A. and Powell W.A., 2002, Molecular characterization of a gene encoding a cystatin expressed in the stems of American chestnut, *Castanea dentate*, Planta, 215(3): 510-514 http://dx.doi.org/10.1007/s00425-002-0782-9 PMid:12111235

Constabel C.P., Bergey D.R., and Ryan C.A., 1995, Systemin activates synthesis of wound-inducible tomato leaf polyphenol oxidase via the octadecanoid defense signaling pathway, PNAS, 92(2): 407-411 http://dx.doi.org/10.1073/pnas.92.2.407

Conrath U., Silva H., and Klessig D.F., 1997, Protein dephosphorylation mediates salicylic acid-induced expression of PR-1 genes in tobacco, Plant J., 11(4): 747-757 http://dx.doi.org/10.1046/j.1365-313X. 1997. 11040747.x

Cordero M.J., Raventos D., and San Segundo B., 1994, Expression of a maize proteinase inhibitor gene is induced in response to wounding and fungal infection: systemic wound-response of a monocot gene, The Plant Journal, 6(2): 141-150 http://dx.doi.org/10.1046/j.1365-313X. 1994.6020141.x PMid:7920708

Dahl S.W., Rasmussen S.K., and Hejgaard J., 1996, Heterologous expression of three plant serpins with distinct inhibitory specificities, The Journal of Biological Chemistry, 271: 25083-25088 http://dx.doi. org/10.1074/jbc.271.41.25083 PMid:8810262

Dammann C., 1997, Abscissic acid and jasmonic acid activate wound inducible genes in potato through separate, organ specific signal transduction pathways, The Plant Journal, 11(4): 773-782 http:// dx.doi.org/10.1046/j.1365-313X.1997.11040773.x PMid:9161035

Da Silva W., Freire M. das G.M., Parra J.R.P., Marangoni S., Macedo M.L.R., 2012, Evaluation of the Adenanthera pavonina seed proteinaseinhibitor (ApTI) as a bioinsecticidal tool with potential for the control of *Diatraea saccharalis*, Process Biochemistry, 47(2): 257-263 http://dx.doi.org/10.1016/j.procbio.2011.11.002

De Leo F., Volpicella M.W., Licciulli F., Liuni S., Gallerani R., and Ceci L.R., 2002, PLANT-PIs: a database for plant protease inhibitors and their genes, Nucl. Acids Res., 30(1): 347-348 http://dx.doi.org/ 10.1093/nar/30.1.347 PMid:11752333 PMCid: 99076

Doares S.H., Narvaez-Vasquez J., Conconi A., and Ryan C.A., 1995, Salicylic acid inhibits synthesis of proteinase inhibitors intomato leaves induced by systemin and jasmonic acid, Plant Physiol., 108(4): 1741-1746 PMid:12228577 PMCid:157556

Dunaevskii Y.E., Gladysheva I.P., Pavlukova E.B., Beliakova G.A., Gladyschev D.P., Papisova A.I., Larionova N.I. and Belozersky M.A., 1997, The anionic protease inhibitor BBWI - 1 from buckwheat seeds. Kinetic properties and possible biological role, Physiologia Plantarum, 101(3), 483-488 http://dx.doi.org/10.1034/j.1399-3054.1997.1010305.x http://dx.doi.org/10.1111/j.1399-3054.1997.tb01027.x

Dunse K.M., Stevens J.A., Lay F.T., Gaspar Y.M., Heath R.L., and Anderson M.A., 2010, Coexpression of potato type I and II proteinase inhibitors gives cotton plants protection against insect damage in the field, PNAS, 107(34): 15011-15015 http://dx.doi.org/10.1073/pnas.1009241107 PMid:20696895 PMCid:2930582

Farmer E.E., Johnson R.R., and Ryan C.A., 1992, Regulation of expression of proteinase inhibitor genes by methyl jasmonate and jasmonic acid, Plant Physiol., 98(3): 995-1002 http://dx.doi.org/10.1104/pp.98.3.995 PMid: 16668777 PMCid:1080300

Franco O.L., Dias S.C., Magalhaes C.P., Monteiro A.C.S., Bloch Jr C., Melo F.R., Oliveira-Neto O.B., Monnerat R.G., and Grossi-de-Sa M.F., 2004, Effects of soybean *Kunitz trypsin* inhibitor on the cotton boll weevil *Anthonomus grandis*, Phytochem., 65(1): 81-89 http://dx.doi.org/ 10.1016/j.phytochem.2003.09.010 PMid:14697273

Geoffroy M., Legrand M., and Fritig B., 1990, Isolation and characterization of a proteinaceous inhibitor of microbial proteinases induced during the hypersensitive reaction of tobacco mosaic virus, Molecular Plant-Microbe Interaction, 3(5): 327-333 http://dx.doi.org/10.1094/ MPMI-3-327 PMid:2134857

Gettins P.G.W., 2002, Serpin structure, mechanism, and function, Chemical Review, 102(12): 4751-4804 http://dx.doi.org/10.1021/cr010170+ PMid:12475206

Giri A.P., Harsulkar A.M., Ku M.S., Gupta V.S., Deshpande V.V., Ranjekar P.K., and Franceschi V.R., 2003, Identification of potent inhibitors of *Helicoverpa armigera* gut proteinases from winged bean seeds, Phytochem, 63(5): 523-532 http://dx.doi.org/10.1016/S0031-9422 (03) 00181-X

Guiderdoni E., Cordero M.J., Vignols F., Garcia-Garrido J.M., Lescot M., Tharreau D., Meynard D., Ferriere N., Notteghem J.L. and Delseny M., 2002, Inducibility by pathogen attack and developmental regulation of the rice *Ltp1* gene, Plant Mol Biol., 49(6): 679-695 http://dx.doi.org/10. 1023/A:1015595100145

Gutierrez-Campos R., Torres-Acosta J.A., Saucedo-Arias L.J., and Gomez-Lim M.A., 1999, The use of cysteine proteinase inhibitors to engineer resistance against potyviruses in transgenic tobacco plants, Nature Biotech., 17: 1223-1226 http://dx.doi.org/10.1038/70781 PMid:10585723

Haq S.K., Atif S.M. and Khan R.H., 2004, Protein proteinase inhibitor genes in combat against insects, pests, and pathogens: natural and engineered phytoprotection, Arch. Biochem. Biophys., 431(1): 145-159 http://dx.doi.org/10.1016/j.abb.2004.07.022 PMid:15464737

Hayashi K., Takehisa T., Hamato N., Takano R., Hara S., Miyata T., and Kato H., 1994, Inhibition of serine proteases of the blood coagulation system by squash family protease inhibitors, J. Biochem., 116(5): 1013-1018 PMid:7896727

Hepher A., and Atkinson H.J., 1992, Nematode control with proteinase inhibitors, United States Patent, US006080920A

Hilder V.A., and Boulter D., 1999, Genetic engineering of crop plants for insect resistance-a critical review, Crop Prot., 18(3): 177-191 http://dx.doi.org/10.1016/S0261-2194(99)00028-9

Hilder V.A., Gatehouse A.M.R., and Boulter D., 1993, Transgenic plants conferring insect tolerance: protease inhibitor approach, In: Kung S.D., and Wu R. (eds.), Transgenic plants: engineering and utilization, Academic Press, New York, USA, pp.317-338

Hilder V.A., Gatehouse A.M.R., Sheerman S.E., Barker F., and Boulter D., 1987, A novel mechanism of insect resistance engineered into tobacco, Nature, 330: 160-163 http://dx.doi.org/10.1038/330160a0

Horisberger M., and Tacchini-Vonlanthen M., 1983, Ultrastructural localization of Bowman-Birk inhibitor on thin sections of Glycine max soybean cv. Maple Arrow by the gold method, Histochemistry, 77(3): 313-321 http://dx.doi.org/10.1007/BF00490894 PMid:6305885

Houseman J.G., Downe A.E.R., and Philogene B.J.R., 1989, Partial characterization of proteinase activity in the larval midgut of the European corn borer, Ostrinia nubilalis Hübner (Lepidoptera: Pyralidae), Can J. Zool., 67(4): 864-868 http://dx.doi.org/10.1139 /z89-127

Howe G.A., Lightner J., Browse J., and Ryan C.A., 1996, An octadecanoid pathway mutant (JL5) of tomato is comprised in signaling for defense against insect attack, The Plant cell, 8(11): 2067-2077 http://dx.doi.org/10.2307/3870413 PMid:8953771 PMCid:161335 http://dx.doi.org/10.1105/tpc.8.11.2067 PMid:8953771

Huang Y.M., Xiao B.Z., and Xionq L.Z., 2007, Characterization of a stress responsive proteinase inhibitor gene with positive effect in improving drought resistance in rice, Planta, 226(1): 73-85 http://dx.doi.org/ 10.1007/s00425-006-0469-8 PMid:17221232

Hung C.H., Huang C.C., Tsai W.S., Wang H.L., and Chen Y.L., 2003,

Purification and characterization of a trypsin inhibitor from *Brassica campestris* seeds, Journal of Yuanpei University of Science and Technology, 10: 13-22

Hunt M.D., Neuenschwander U.H., Delaney T.P., Weymann K.B., Friedrich L.B., Lawton K.A., Steiner H.Y., and Ryals J.A., 1996, Recent advances in systemic acquired resistance research-a review, Gene, 179(1): 89-95 http://dx.doi.org/10.1016/S0378-1119(96)00429-5

Hunter T., 1995, Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling, Cell, 80(2): 225-236 http://dx.doi.org/10.1016/0092-8674(95)90405-0

Huntington J.A., 2006, Shape-shifting serpins-advantages of a mobile mechanism, Trends in Biochemical Sciences, 31(8): 427-435 http://dx.doi.org/10.1016/j.tibs.2006.06.005 PMid:16820297

Jones B.L., and Fontanini D., 2003, Trypsin/alpha-amylase inhibitors inactivate the endogenous barley/malt serine endoproteinase SEP-1, Journal of Agricultural and Food Chemistry, 51(19): 5803-5814 http://dx.doi.org/10.1021/jf030075x PMid:12952437

Jongsma M. A. and Bolter C., 1997, The adaptation of insects to plant protease inhibitors, J. Insect Physiol., 43(10): 885-895 http://dx.doi.org/10.1016/S0022-1910(97)00040-1

Joshi B., Sainani M., Bastawade K., Gupta V.S. and Ranjekar P.K., 1998, Cysteine protease inhibitor from pearl millet: a new class of antifungal protein, Biochem. Biophys. Res. Commun., 246(2), 382-387 http://dx.doi.org/10.1006/bbrc.1998.8625 PMid:9610368

Kim J.Y., Park S.C., Kim M.H., Lim H.T., Park Y., Hahm K.S., 2005, Antimicrobial activity studies on a trypsin-chymotrypsin protease inhibitor obtained from potato, Biochem. Biophys. Res. Commun., 330(3): 921-927 http://dx.doi.org/10.1016/j.bbrc.2005.03.057 PMid:15809084

Kim M.H., Park S.C., Kim J.Y., Lee S.Y., Lim H.T., Cheong H., Hahm K.S. and Park Y., 2006, Purification and characterization of a heat-stable serine protease inhibitor from the tubers of new potato variety "Golden Valley", Biochem. Biophys. Res. Commun., 346(3): 681-686 http://dx.doi.org/10.1016/j.bbrc.2006.05.186 PMid:16777063

Kobayashi H., Yoshida R., Kanada Y., Fukuda Y., Yagyu T., Inagaki K., Kondo T., Kurita N., Suzuki M., Kanayama N., and Terao T., 2005, Suppression of lipopolysaccharide-induced cytokine production of gingival fibroblasts by a soybean, kunitz trypsin inhibitor, J. Periodontal Res., 40(6): 461-468 http://dx.doi.org/10.1111/j.1600-0765.2005.00824.x PMid:16302924

Koiwa H., Bressan R.A., and Hasegawa P.M., 1997, Regulation of protease inhibitors and plant defense, Trends in Plant Science, 2(10): 379-384 http://dx.doi.org/10.1016/S1360-1385(97)90052-2

Konarev A.V., Griffin J., Konechnaya G.Y., and Shewry P.R., 2004, The distribution of serine proteinase inhibitors in seeds of the *Asteridae*, *Phytochem*, 65: 3003-3020 http://dx.doi.org/10.1016/j.phytochem.2004.08.022 PMid:15504435

Konarev A.V., Tomooka N. and Vaughan D., 2002, Proteinase inhibitor polymorphism in genus *Vigna* subgenus *Ceratotropis* and its biosystematic implications, Euphytica, 123(2): 165-177 http://dx.doi.org/10.1023/A:1014920309710

Korsinczky M.L.J., Schirra H.J., and Craik D.J., 2004, Sunflower trypsin inhibitor-1, Current Protein and Peptide Science, 5(5): 351-364 http://dx.doi.org/10.2174/1389203043379594 PMid:15544530

Kuroda M., Kiyosaki T., Matsumoto I., Misaka T., Arai S., and Abe K., 2001, Molecular cloning, characterization, and expression of wheat cystatins, Bioscience, Biotechnology, and Biochemistry, 65(1): 22-28 http://dx.doi.org/10.1271/bbb.65.22 PMid:11272836

Lawrence P.K., and Koundal K.R., 2002, Plant protease inhibitors in control of phytophagous insects, Electronic Journal of Biotechnology, 5(1): 93-109 http://dx.doi.org/10.2225/vol5-issue1-fulltext-3

Leo F., De Volpicella M., Licciulli F., Liuni S., Gallerani R., and Ceci R., 2002, PLANT- PIs: a database for plant proteinase inhibitors and their genes, Nucleic Acid Research, 30(1): 347-348 http://dx.doi.org/10.1093/nar/30.1.347 PMid:11752333 PMCid:99076

León J., Rojo E., and Sanchez-Serrano J.J., 2001, Wound signalling in plants, Journal of Experimental Botany, 52(354): 1-9 http://dx.doi.org/10.1093/jexbot/52.354.1 PMid:11181708

Leung D., Abbenante G., and Fairlie D.P., 2000, Protease inhibitors: current status and future prospects, J. Med. Chem., 43(3): 305-341 http://dx.doi.org/10.1021/jm990412m PMid:10669559

Li Y.M., MacKintosh C., and Casadia J.E., 1993, Protein phosphatase 2A and its [3H] cantharidin/[3H] endothall thioanhydride binding site: inhibitor specifcity of cantharidin and ATP analogues, Biochem. Pharmacol., 46(8): 1435-1443 http://dx.doi.org/10.1016/0006-2952(93)90109-A

Macedo M.L.R., Durigan R.A., da Silva D.S., Marangoni S., Freire M. das G.M., and Parra J.R.P., 2010, *Adenanthera pavonina* trypsin inhibitor retard growth of *Anagasta kuehniella* (Lepidoptera: Pyralidae), Archives of Insect Biochemistry and Physiology, 73 (4): 213-231 PMid:20235154

Macedo M.L.R., Mello G.C., Freire M.d.G.M., Novello J.C., Marangoni S., and de Matos D.G.G., 2002, Effect of a trypsin inhibitor from *Dimorphandra mollis* seeds on the development of *Callosobruchus maculates*, Plant Physiol. Biochem., 40(10): 891-898 http://dx.doi.org/10.1016/S0981-9428(02)01441-9

MacKintosh C., Lyon G.D., and MacKintosh R.W., 1994, Protein phosphatase inhibitors activate anti-fungal defence responses of soybean cotyledons and cell cultures, The Plant J., 5(1): 137-147 http://dx.doi.org/10.1046/j.1365-313X.1994.5010137.x

Malone M., and Alarcon J.J., 1995, Only xylem-borne factors can account for systemic wound signalling in the tomato plant, Planta, 196: 740-746 http://dx.doi.org/10.1007/BF00197340 http://dx.doi.org/10.1007/BF01106769

Manyangarirwa W., Turnbull M., McCutcheon G.S., and Smith J.P., 2006, Gene pyramiding as a *Bt* resistance management strategy: how sustainable is this strategy? African Journal of Biotechnology, 5(10): 781-785

Martinez de Ilarduya O., Xie Q.G., and Kaloshian I., 2003, Aphid-induced defense responses in Mi-1-mediated compatible and incompatible tomato interactions, Mol. Plant-Microbe Interact., 16(8): 699-708 http://dx.doi.org/10.1094/MPMI.2003.16.8.699 PMid:12906114

McGowan S., Buckle A.M., Irving J.A., Ong P.C., Bashtannyk-Puhalovich T.A., Kan W.T., Henderson K.N., Bulynko Y.A., Popova E.Y., Smith A.I., Bottomley S.P., Rossjohn J., Grigoryev S.A., Pike R.N., Whisstock J.C., 2006, X-ray crystal structure of MENT: evidence for functional loop-sheet polymers in chromatin condensation, The EMBO Journal, 25: 3144-3155 http://dx.doi.org/10.1038/sj.emboj.7601201 PMid:16810322 PMCid:1500978

Mcpherson M.J., and Harrison D.J., 2001, Protease inhibitors and directed evolution: enhancing plant resistance to nematodes, Biochem. Soc. Symp., 68: 125-142 PMid: 11573343

Mello G.C., Oliva M.L.V., Sumikawa J.T., Machado O.L.T., Marangoni S., Novello J.C. and Macedo M.L.R., 2002, Purification and characterization of a new trypsin inhibitor from *Dimorphandra mollis* seeds, J. Protein Chem., 20(8): 625- 632 http://dx.doi.org/10.1023/A:1013764118579 PMid:11890203

Mendieta J.R., Giudici A.M., and de la Canal L., 2004, Occurrence of antimicrobial serin-proteinases in sunflower seeds, J. Phytopathol., 152(1): 43-47 http://dx.doi.org/10.1046/j.1439-0434.2003.00799.x

Miège M., Mascherpa J., Royer-Spierer A., Grang A., and Miège J., 1976, Analyse des crops proteiquesisoles de *Lablab Pupureus* (L.) sweet:

localizationintracellulaire des globulines proteases et inhibiteurs de la trypsire, Planta, 131(1): 81-86 http://dx.doi.org/10.1007/BF00387349

Millward T.A., Zolnierowicz S., and Hemmings B.A., 1999, Regulation of protein kinase cascades by protein phosphatase 2A, Trends in Biochemical Science, 24(5): 186-191 http://dx.doi.org/10.1016/S0968-0004(99)01375-4

Mosolov V.V., Grigoreva L.I., and Valueva T.A., 2001, Plant proteinase inhibitors as multifunctional proteins (review), Appl. Biochem. Microbiol., 37(6): 643-650 http://dx.doi.org/10.1023/A:1012323705646 http://dx.doi.org/10.1023/A:1012352914306

Mosolov V.V., Loginova M.D., Fedurkina N.V., and Benken I.I., 1976, The biological significance of proteinase inhibitors in plants, Plant Sci. Lett. 7(2): 77-80 http://dx.doi.org/10.1016/0304-4211(76)90074-2

Nandeesha P., and Prasad D.T., 2001, Characterization of serine proteinase inhibitor from subabul (*Leucaena leucocephala* L.) seeds, J. Plant Biochem. Biotechnol., 10(1): 75-78

Narwaez-Vasquez J., Franceschi V.R., and Ryan C.A., 1993, Proteinase-inhibitor synthesis in tomato plants - evidence for extracellular deposition in roots through the secretory pathway, Planta, 189(2): 257-266

Ng T.B., Lam S.K., and Fong W.P., 2003, A homodimeric sporamin-type trypsin inhibitor with antiproliferative, HIV reverse transcriptase-inhibitory and antifungal activities from wampee (*Clausena lansium*) seeds, Biol. Chem., 384(2): 289-293 http://dx.doi.org/10.1515/BC.2003.032 PMid:12675522

Odani S., and Ikenaka T., 1973, Studies on soybean trypsin inhibitors VIII. disulfide bridges in soybean bowman-birk proteinase inhibitor, J. Biochem., 74(4):697-715 PMid:4797072

O'Donnell P.J., Calvert C., Atzorn R., Wasternack C., Leyser H.M.O., and Bowles D.J., 1996, Ethylene as a signal mediating the wound response of tomato plants, Science, 274(5294): 1914-1917 http://dx.doi.org/10.1126/science.274.5294.1914 PMid:8943205

Oliva M.L.V., Souza-Pinto J.C., Batista I.F.C., Araujo M.S., Silveira V.F., Auerswald E.A., Mentele R., Eckerskorn C., Sampaio M.U., and Sampaio C.A.M., 2000, *Leucaena leucocephala* serine proteinase inhibitor: primary structure and action on blood coagulation, kinin release and rat paw edema, Biochimica et Biophysica Acta, 1477(1-2): 64-74 http://dx.doi.org/10.1016/S0167-4838(99)00285-X

Ong P.C., McGowan S., Pearce M.C., Irving J.A., Kan W.T., Grigoryev S.A., Turk B., Silverman G.A., Brix K., Bottomley S.P., Whisstock J.C., and Pike R.N., 2007, DNA accelerates the inhibition of human cathepsin V by serpins, J. Biol. Chem., 282(51): 36980-36986 http://dx.doi.org/10.1074/jbc.M706991200 PMid:17923478

Park S.S., and Ohba H., 2004, Suppressive activity of proreinase inhibitors from buckweed seeds against human T-acute lymphoblastic leukemia cell lines, Appl. Biochem. Biotechnol., 117(2): 65-74 http://dx.doi.org/10.1385/ABAB:117:2:065

Patrícia P., Maria C.F., Jose Roberto P.P. and Marcio C.S., 2003, Coupling diet quality and Bowman-Birk and Kunitz-type soybean proteinase inhibitor effectiveness to *Diatraea saccharalis* development and mortality, Entomologia Experimentalis et Applicata, 109(3): 217-224 http://dx.doi.org/10.1046/j.0013-8703.2003.00107.x

Pernas M., Sanchez-Monge R., and Salcedo G., 2000, Biotic and abiotic stress can induce cystatin expression in chestnut, FEBS Letter, 467(2-3): 206-210 http://dx.doi.org/10.1016/S0014-5793(00)01157-1

Prasad T.K., Anderson M.D., Martin B.A., and Stewart C.R., 1994, Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide, The Plant Cell, 6(1): 65-74 http://dx.doi.org/10.2307/3869675 PMid:12244221 PMCid:160416 http://dx.doi.org/10.1105/tpc.6.1.65 PMid:12244221

Rakwal R., Agrawal G.K., and Yonekura M., 1999, Separation of proteins from stressed rice (*Oryza sativa* L.) leaf tissues by two-dimensional polyacrylamide gel electrophoresis: induction of pathogenesis related and cellular protectant proteins by jasmonic acid, ultraviolet irradiation and copper chloride, Electrophoresis, 20(17): 3472-3478 http://dx.doi.org/10.1002/(SICI)1522-2683(19991101)20:17<3472::AID-ELPS3472>3.0.CO;2-0 http://dx.doi.org/10.1002/(SICI)1522-2683(19991101)20:17<3472::AID-ELPS3472>3.3.CO;2-S

Rakwal R., Agarwal K.G., and Jha N.S., 2001, Characterization of a rice (*Oryza sativa* L.) Bowman-Birk proteinase inhibitor: tightly light regulated induction in response to cut, jasmonic acid, ethylene and protein phosphatase 2A inhibitors, Gene, 263(1-2): 189-198 http://dx.doi.org/10.1016/S0378-1119(00)00573-4

Rasmussen S.K., Dahl S.W., Norgard A., and Hejgaard J., 1996, A recombinant wheat serpin with inhibitory activity, Plant Molecular Biology, 30(3): 673-677 http://dx.doi.org/10.1007/BF00049343 PMid:8605317

Rawlings N.D., Tolle D.P., and Barrett A.J., 2004, MEROPS: the peptidase database, Nucleic Acids Research, 32 (Suppl. 1), D160-D164 http://dx.doi.org/10.1093/nar/gkh071 PMid:14681384 PMCid:308805

Richardson M., 1977, The protease inhibitors of plants and micro-organisms, Phytochem., 16(2): 159-169 http://dx.doi.org/10.1016/S0031-9422(00)86777-1

Roberts T.H., and Hejgaard J., 2008, Serpins in plants and green algae, Functional and Integrative Genomics, 8: 1-27 http://dx.doi.org/10.1007/s10142-007-0059-2

Rodrigues M.L., Machado Freire M.G., Cabrini E.C., Toyama M.H., Novello J.C., and Marangoni S., 2003, A trypsin inhibitor from *Peltophorum dubium* seeds active against pest proteases and its effect on the survival of *Anagasta kuehniella* (Lepidoptera: Pyralidae), Biochimica et Biophysica Acta-General Subject, 1621(2): 170-182

Ryan C.A., 1989, Insect-induced chemical signals regulating natural plant protection responses, In: Denno R.F. and Mcclure M.S. (eds.), Variable plants and herbivores in natural and managed system, Academic Press, New York, USA, pp.43-60

Ryan C.A., 1990, Protease inhibitors in plants: genes for improving defenses against insects and pathogens, Annual Review of Phytopathology, 28: 425-449 http://dx.doi.org/10.1146/annurev.py.28.090190.002233 http://dx.doi.org/10.1146/annurev.phyto.28.1.425

Ryan C.A., 2000, The systemin-signaling pathway: differential activation of plant defensive genes, Biochimica et Biophysica Acta, 1477(1-2): 112-121 http://dx.doi.org/10.1016/S0167-4838(99)00269-1

Saedler R., and Baldwin I.T., 2004, Virus-induced gene silencing of jasmonate-induced direct defences, nicotine and trypsin proteinase-inhibitors in *Nicotiana attenuate*, J. Exp Bot., 55: 151-157 http://dx.doi.org/10.1093/jxb/erh004 PMid:14623900

Sampaio C.A.M., Oliva M.L.V., Sampaio M.U., Batista I.F.C., Bueno N.R., Tanaka A.S., Auerswald E.A., and Fritz H., 1996, Plant serine proteinase inhibitors. Structure and biochemical applications on plasma kallikrein and related enzymes, Immunopharmacology, 32(1-3): 62-66 http://dx.doi.org/10.1016/0162-3109(96)00073-2

Satheesh L.S., and Murugan K., 2011, Antimicrobial activity of protease inhibitor from leaves of *Coccinia grandis* (L.) Voigt, Indian J. Exp. Biol., 49(5): 366-374 PMid:21615062

Schick C., Brömme D., Bartuski A., Uemura Y., and Schechter N., 1998, The reactive site loop of the serpin SCCA1 is essential for cysteine proteinase inhibition, PNAS, 95(23): 13465-13470 http://dx.doi.org/10.1073/pnas.95.23.13465

Shewry P.R., 2003, Tuber storage proteins, Ann. Bot., 91(7): 755-769 http://dx.doi.org/10.1093/aob/mcg084 PMid:12730067

Shilpa L.S., and Murugan K., 2011, Antimicrobial activity of protease inhibitor from leaves of *Coccinia grandis* (L.) Voigt, Indian journal of

experimental Biology, 49(5): 366-374 PMid:21615062

Shirasu K., Nakajima H., Rajasekhar V.K., Dixon R.A., and Lamb C., 1997, Salicylic acid potentiates an agonist-dependent again control that amplifies pathogen signals in the activation ofdefense mechanisms, The Plant Cell, 9(2): 261-270 http://dx.doi.org/10.2307/3870546 PMid:9061956 PMCid:156916 http://dx.doi.org/10.1105/tpc.9.2.261 PMid:9061956

Shulke R.H., and Murdock L.L., 1983, *Lipoxygenase trypsin* inhibitor and lectin from soybeans: effects on larval growth of *Manduca sexta* (Lepidoptera: Sphingidae), Environmental Entomology, 12(3): 787-791

Sin S.F., and Chye M.L., 2004, Expression of proteinase inhibitor Ⅱ proteins during floral development in *Solanum americanum*, Planta, 219(6): 1010-1022 http://dx.doi.org/10.1007/s00425-004-1306-6 PM id:15197 596

Siritapetawee J., and Thammasirirak S., 2011, Purification and characterization of a heteromultimeric glycoprotein from *Artocarpus heterophyllus* latex with an inhibitory effect on human blood coagulation, Acta Biochim. Pol., 58(4): 521-528 PMid: 22132372

Sivasankar S., Sheldrick B., and Rothstein S.J., 2000, Expression of allene oxide synthase determines defense gene activation in tomato, Plant Physiol., 122(4): 1335-1342 http://dx.doi.org/10.1104/pp.122.4.1335 PMid:10759530 PMCid:58969

Smith C.M., and Boyko L.V., 2006, The molecular bases of plant resistance and defense responses to aphid feeding: current status, Entomologia Experimentalis et Applicata, 122(1): 1-16 http://dx.doi.org/10.1111/j.1570-7458.2006.00503.x

Solomon M., Belenghi B., Delledonne M., Menachem E., and Levine A., 1999, The involvement of cysteine proteases and protease inhibitor genes in the regulation of programmed cell, The Plant Cell, 11(3): 431-443 http://dx.doi.org/10.1105/tpc.11.3.431 http://dx.doi.org/10.2307/3870871 PMid:10072402 PMCid:144188

Souza-Pinto J.C., Remacle-Volon G., Sampaio C.A.M., and Damas J., 1995, Collagenase-induced oedema in the rat paw and the kinin system, European Journal of Pharmacology, 274(1-3): 101-107 http://dx.doi.org/10.1016/0014-2999(94)00723-K

Stintzi A.W.H., Weber H., Reymond P., Browse J., and Farmer E.E., 2001, Plant defense in the absence of jasmonic acid: the role of cyclopentenones, PNAS, 98(22): 12837-12842 http://dx.doi.org/10.1073/pnas.211311098 PMid:11592974 PMCid:60140

Tabashnik B.E., Van Rensburg J.B., and Carrière Y., 2009, Field-evolved insect resistance to *Bt* crops: Definition, theory, and data, J Econ Entomol., 102(6): 2011-2025 http://dx.doi.org/10.1603/029.102.0601 PMid: 20069826

Telang M., Srinivasan A., Patankar A., Harsulkar A., Joshi V., Damle A., Deshpande V., Sainani M., Ranjekar P., Gupta G., Birah A., Rani S., Kachole M., Giri A., and Gupta V., 2003, Bitter gourd proteinase inhibitors: potential growth inhibitors of *Helicoverpa armigera* and *Spodoptera litura*, Phytochem., 63(6): 643-652 http://dx.doi.org/10.1016/S0031-9422(03)00239-5

Troll W., and Kennedy A.R., 1993, Protease inhibitors as cancer chemopreventive Agents, Plenum Press, University of Michigan, USA, pp.313 http://dx.doi.org/10.1007/978-1-4615-2882-1

Urwin P.E., Atkinson H.J., Waller D.A., and Mcpherson M.J., 1995, Engineered oryzacystatin-I expressed in hairy roots confers resistance to *Globodera pallida*, Plant J., 8(1): 121-131 http://dx.doi.org/10.1046/j.1365-313X.1995.08010121.x PMid: 7655503

Urwin P.E., Levesley A., Mcpherson M.J., and Atkinson H.J., 2000, Transgenic resistance to the nematode *Rotylenchulus reniformis* conferred by Arabidopsis thaliana plants expressing proteinase inhibitors, Mol. Breed., 6(3): 257-264 http://dx.doi.org/10.1023/A:1009669325944

Vain P., Worland B., Clarke M.C., Richard G., Beavis M., Liu H., Kohli A., Leech M., Snape J., Christou P., and Atkinson H., 1998, Expression of an engineered cysteine proteinase inhibitor (Oryzacystatin-I delta D86) for nematode resistance in transgenic rice plants, Theor. Appl. Genet., 96(2), 266-271 http://dx.doi.org/10.1007/s001220050735

Valueva T.A., Revina T.A., Kladnitskaya G.V., and Mosolov V.V., 1998, Kuntz-type proteinase inhibitors from intact and Phytophthora infected potato tubers, FEBS Letter, 426(1): 131-134 http://dx.doi.org/10.1016/S0014-5793(98)00321-4

Valueva T.A., Revina T.A., Gvozdeva E.L., Gerasimova N.G., and Ozeretskovskaya O.L., 2003, Role of proteinase inhibitors in potato protection, Russian Journal of Bioorganic Chemistry, 29(5): 454-458 http://dx.doi.org/10.1023/A:1026053626260

Vercammen D., Belenghi B., van de Cotte B., Beunens T., Gavigan J.A., Rycke R.D., Brackenier A., Lnze D., Harris J.L., Breusegem F.V., 2006, Serpin 1 of *Arabidopsis thaliana* is a suicide inhibitor for metacaspase 9, Journal of Molecular Biology, 364(4): 625-636 http://dx.doi.org/10.1016/j.jmb.2006.09.010 PMid:17028019

Wang S., Lin J., Ye M., Ng T.B., Rao P., and Ye X., 2006, Isolation and characterization of a novel mung bean protease inhibitor with antipathogenic and anti-proliferative activities, Peptides, 27 (12): 3129-3126 http://dx.doi.org/10.1016/j.peptides.2006.07.013 PMid:16971020

Williamson V.M. and Hussey R.S., 1996, Nematode pathogenesis and resistance in plants, Plant Cell, 8(10): 1735-1745 http://dx.doi.org/10.2307/3870226 PMid:8914324 PMCid:161311 http://dx.doi.org/10.1105/tpc.8.10.1735 PMid:8914324

Xu Z.F., Qi W.Q., Ouyang X.Z., Yeung E., and Chye M.L., 2001, A proteinase inhibitor II of *Solanum americanum* is expressed in phloem, Plant Mol. Biol., 47(6): 727-738 http://dx.doi.org/10.1023/A:1013623628857 PMid:11785934

Xu Z.F., Teng W.L., and Chye M.L., 2004, Inhibition of endogenous trypsin- and chymotrypsin-like activities in transgenic lettuce expressing heterogeneous proteinase inhibitor SaPIN2a, Planta, 218(4): 623-629 http://dx.doi.org/10.1007/s00425-003-1138-9 PMid:14574575

Ye X.Y., Ng T.B., Tsang P.W., and Wang J., 2001, Isolation of a homodimeric lectin with antifungal and antiviral activities from red kidney bean (*Phaseolus vulgaris*) seeds, Journal of Protein Chemistry., 20(5), 367-375 http://dx.doi.org/10.1023/A:1012272518778 PMid:11732686 http://dx.doi.org/10.1023/A:1012276619686 PMid:11 732688

Yozaura S., Yaguchi M., Haraguchi K., and Ohtsubo K., 2002, Molecular cloning and functional expression of cDNA encoding a cysteine proteinase inhibitor, cystatin, from Job's tears (*Coix lacryma-jobi* L. var. Mayuen Stapf), Biosci. Biotechnol. Biochem, 66 (10): 2287-2291 PMid:12450152

Zhu Y.C., Abel C.A., and Chen M.S., 2007, Interaction of Cry1Ac toxin (*Bacillus thuringiensis*) and proteinase inhibitors on the growth, development and midgut proteinase activities of the bollworm, *Helicoverpa zea*, pestic, Biochem. Physiol., 87(1): 39-46 http://dx.doi.org/10.1016/j.pestbp.2006.05.004