

Proteinaceous Inhibitors in Various Crops and Related Pests: A Review

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Abstract: Protease inhibitors participate in regulation of cell death during plant development and senescence. On the other hand, crop plant protease inhibitors like α -amylase, trypsin, chymotrypsin, carboxypeptidase, serine protease inhibitors, potato type I & II PIs, napin, soybean cysteine PI scN, aspartic PI pepstatin A, soybean Kunitz trypsin inhibitor KI have an important role in defence against crop plant pests and phytopathogenic microorganisms. The present review discusses the protease inhibitors of crop plants and pests. Still, perspective has several advantages over the standard method and is eco-friendly.

Keywords: Protease Inhibitors, α -amylase, trypsin, chymotrypsin, carboxypeptidase, Pest Management, plant defense, pests.

I. INTRODUCTION

Proteases or proteinases are proteolytic enzymes naturally found in all organisms. They are involved in a multitude of biological systems. Actions of proteases can be inhibited by proteolytic degradation and their inhibitors [Batista, I. F et.al 1996]. Pigeonpea [*Cajanuscajan*(L.) Millisp.] is a multipurpose grain legume grown by the resource poor farmers in the semi-arid tropics and subtropics. India produces more than 80% of the total production of pigeonpea [Mueller and Weder 1989]. Very little is known about the antioxidative defense system in pigeonpea (*Cajanuscajan*(L.) Millsp) leaves.

The difference in the levels of protein content and antioxidant enzymes activity at two stages of maturity, named young and mature in pigeonpea (*Cajanuscajan*(L.) mill sp) leaves. The results showed that detached pigeonpea mature leaves possessed higher activities of catalase (CAT) and peroxidase (POD) and lower activities of polyphenol oxidase (PPO) and ascorbate peroxidase (APX) as compared with young leaves. However, glutathione reductase (GR) showed in mature leaves no change in its activity was observed in pigeonpea [Goud & Kachole 2012].

Protease inhibitors such as trypsin and chymotrypsin inhibitors have been demonstrated to reduce the incidence of certain cancers and demonstrate potent anti-inflammatory properties. Angiotensin I-converting enzyme (ACE) inhibitor has been associated with a reduction in hypertension [Roy, Boye, Simpson, 2010].

Plant α -amylase inhibitors show great potential as tools to engineer resistance of crop plants against pests. M.V. Padul et.al. their study indicates that PIs are components of both constitutive and inducible defense and provide a ground for designing stronger inducible defense (PIs or other insect toxin based) in pigeonpea [Padul, Tak, Kachole, 2012]. Coexpression of potato type I and II protease inhibitors gives cotton plants protection against insect damage. [Dunse, K. M., et al. 2010] Additionally, serine proteinase inhibitors have anti-nutritional effects against several lepidopteran insect species. [Shulke and Murdock 1983] groundbreaking discovery of the wound-inducible production of protease inhibitors (PIs) that inhibit digestive herbivore gut proteases inspired the field of plant-insect interactions and became an iconic example of induced plant defenses [Green and Ryan 1972]. Carboxypeptidase inhibitors and serine protease inhibitors from potato and other plants have also been reported to have inhibitory effects against tumor cell growth [Blanco-Aparicio et.al 1998; Huang et.al 1997]. Napin was found to have antibacterial activity against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus cereus*, and *Bacillus megaterium* [Ngai, et.al 2004].



II. RESULTS AND DISCUSSION

R. Vijakumar studied quality nutrition through pigeonpea this aspect show that pigeonpea is capable to prevent and cure a number of human ailments such as bronchitis, coughs, pneumonia, respiratory infections, dysentery, menstrual disorders, sores, wounds, abdominal tumors, tooth ache, and diabetes [Saxena, Kumar, Sultana 2010]. Some believe that they have a role in controlling the endogenous proteases [Richardson M .1977]. Others have suggested that these inhibitors are involved in plant defense against insect pest attacks [Green and Ryan 1972]. Since these proteins are synthesized at about the same time as the seed storage proteins and are degraded during seed germination, these inhibitors may have a storage role. In fact Pusztai has suggested that they act as a sulphur depot because they are rich in sulphur containing amino acids as compared to the storage proteins in legumes which are usually deficient in sulpher containing amino acids.[Pusztai A ,1972]

Plant serine protease inhibitors are defense proteins crafted by nature for inhibiting serine proteases, article focuses on an entire array of plant serine protease inhibitors that have been explored in the past decade, their mode of action and biological implications as well as applications related pest control [Jamal, Pandey, Singh *et al*,2013]. In Specific protease inhibitors are being over expressed in certain transgenic plants to protect them against invaders. Most useful knowledge about plant protease inhibitors and their role in plant defense is briefly reviewed.[Huma **Habib and Khalid Majid Fazili** 2007] Volpicella give an overview of other families of plant PIs, active either against serine proteases or other class of proteases, describing their distribution, activity and main structural characteristics. Plant protease inhibitors (PIs) are generally small proteins present in high concentrations in storage tissues (tubers and seeds), and to a lower level in leaves [Volpicella, Mariateresa, 2011]. Protease inhibitors to protect transgenic plants against attack by herbivorous insects[Gatehouse & John, 2011]. The effects of purified SBTI and potato inhibitor II (an inhibitor of both trypsin and chymotrypsin) on the growth and digestive physiology of larvae of *Heliothis zea* and *Spodoptera exigua* and demonstrated that growth of larvae was inhibited at levels of 10% of the proteins in their diet[BROADWAY and DUFFEY, 1986]. A significant impact of OCI transgenic potato on larval mortality was obtained, with up to 53% mortality recorded in larvae reared on transgenic leaves.[Lecardonnell, et. al 1999]

Table 1.1: Effect of chemical inhibitors on Pests

Chemical Inhibitor	Specificity of the Inhibitor	Pests
cystatin	cysteine proteinases	fungus [Popovic,et.al, 2013]
Thermolysin	metallo proteinase,	<i>Galleria mellonella</i> [Wedde, Marianne, Weise et.al 2007]
Trypsin inhibitor	soybean Kunitz trypsin inhibitor	cotton boll weevil,[Octávio, et.al 2004]
Trypsin inhibitor	soybean Kunitz trypsin inhibitor	Brown planthopper[Lee, et.al. 1999]
Kunitz trypsin inhibitor	Trypsin inhibitor	H. Armigera [Srinivasan, Ajay, et al. 2005]
soybean cysteine PI scN, aspartic PI pepstatin A, soybean Kunitz trypsin inhibitor KI	cowpea bruchid gut proteases	<i>Callosobruchus maculatus</i> [Amirhusin,et.al,2007]
serine proteinase inhibitor	Buckwheat inhibitor	white wings butterfly [Khadeeva, et al. 2009]
ppI.PI-1	Subtilisin inhibitor	<i>Helicoverpa armigera</i> [Shaikh, et al. 2018]
Oryzacystatin I	Rice cystatin I	Colorado potato beetle(CPB)[Lecardonnell, et al. 1999]
ppI.PI-1	Arabidopsis Kunitz Trypsin	Spider Mites[Amaiz,2018]



Table 1.2: Plant protease inhibitors, their origin and related pests

PIs	Origin	Pest
A. thaliana Kunitz trypsin inhibitors (AtKTI4, AtKTI5)	Arabidopsis thaliana	spider mite[Arnaiz,2018]
AtSerp1	Arabidopsis thaliana	insect disease[Stuiver & Custers, 2001; Rustgi.et.al, 2017]
Kunitz type protease inhibitor (AtWSCP)	Arabidopsis thaliana	herbivore attack[Roberts, et.al, 2011; Rustgi.et.al, 2017]
Potato type I inhibitors	Solanumtuberosum	nematodes [Turra. et.al., 2009]
Bowman-Birk-type inhibitor	Oryzasativa	fungal disease[. Qu, L.J.; Chen, J.et.al 2003]
Potato carboxypeptidaseinhibitor (PCI)	Solanumtuberosum	fungal and insect disease[Quilis et.al.2007; Quilis. López-García.et.al. 2014]
Soybean Kunitz inhibitor (SKTI)	Glycine max	parasitic and insect disease,[Lee et.al. 1999; Azzouz,2005; Major: Constabel,2008]
Soybean Bowman-Birk inhibitor (SbBBI)	Glycine max	aphid parasitoids[Major & Constabel, (2008)]
Potato type I (StPin1A) inhibitor/Potato type II (NaPI) inhibitor	Solanumtuberosum Nicotianaalata	Helicoverpasp.[Dunse et.al , 2010]

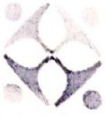
III. CONCLUSION

The use of recombinant protease inhibitors may also be an attractive way to protect plants from bacterial, fungal and viral pathogen. Several plants that express PIs have been produced and tested in order to increase the resistance against pathogenic organisms. Additionally other protease inhibitors from different families have been used to minimize the proteolysis of recombinant proteins expressed in plants. Studying plant defense responses and developing newer Eco-friendly strategies for protecting plants against crop pests and pathogens is one of the most dynamic area of research in plant science. The results obtained in this study suggest that protease inhibitors are involved in the defense response of the host plant against phytopathogens, viruses, bacteria, parasites, afids, microbes etc. Additionally they may have the potential use for as a non - cytotoxic clinical agents. This technique may not replace the use of chemical pesticides in near future but effectively complement it. Several successful examples of PIs which play role against pests have been mentioned in this review

REFERENCES

- [1]. Batista, I. F., Oliva, M. L., Araujo, M. S., Sampaio, M. U., Richardson, M., Fritz, H., & Sampaio, C. A. (1996) : "Primary structure of a Kunitz-type trypsin inhibitor from *Enterolobium contortisiliquum* seeds." *Phytochemistry*, 41, 1017-1022.
- [2]. R. Mueller and J. K. P. Weder, (1989): "Isolation and Characterization of Two Trypsin Chymotrypsin Inhibitors from Lentil Seeds (*Lens culinaris* Medik)," *Journal of Food Biochemistry*, Vol. 13, No. 1, pp. 39-63.
- [3]. 3.Prashanth B. Goud & Manvendra S. Kachole (2012) : Antioxidant enzyme changes in neem, pigeonpea and mulberry leaves in two stages of maturity, *Plant Signaling ,Behavior*, 7:10, 1258-1262,
- [4]. F. Roy, J.I. Boye. B.K. Simpson, (2010) : Bioactive proteins and peptides in pulse crops: Pea, chickpea and lentil, *Food Research International*, Volume 43, Issue 2, Pages 432-442, ISSN 0963-9969,

- [5]. Manohar V. Padul, Rajesh D. Tak, Manvendra S. Kachole, (2012) : Protease inhibitor (PI) mediated defense in leaves and flowers of pigeonpea (protease inhibitor mediated defense in pigeonpea, *Plant Physiology and Biochemistry*, Volume 52, Pages 77-82, ISSN 0981-9428,
- [6]. Dunse, K. M., et al. (2010): "Coexpression of potato type I and II proteinase inhibitors gives cotton plants protection against insect damage in the field." *Proceedings of the National Academy of Sciences* 107.34 , 15011-15015.
- [7]. SHULKE. R.H. and MURDOCK, L.L. (1983): Lipoxxygenase trypsin inhibitor and lectin from soybeans: effects on larval growth of *Manduca sexta* (Lepidoptera: Sphingidae). *Environmental Entomology*. vol. 12, p. 787-791.
- [8]. Green TR, Ryan CA (1972): Wound-Induced Proteinase Inhibitor in Plant Leaves: A Possible Defense Mechanism against Insects. *Science*. Feb 18; 175(4023):776-7.
- [9]. Blanco-Aparicio, C; Molina, MA; Fernández-Salas, E; Frazier, ML; Mas, JM; Querol, E; Avilés, FX; de Llorens, R. (1998): Potato carboxypeptidase inhibitor, a T-knot protein, is an epidermal growth factor antagonist that inhibits tumor cell growth. *J. Biol. Chem.* 273, 12370-12377.
- [10]. Huang, C; Ma, W-Y; Ryan, CA; Dong, Z. (1997): Proteinase inhibitors I and II from potatoes specifically block UV-induced activator protein-1 activation through a pathway that is independent of extracellular signal-regulated kinases, c-jun Nterminal kinases, and P38 kinase. *Proc. Natl. Acad. Sci. USA* , 94, 11957-11962.
- [11]. Ngai, PH; Ng, TB. (2004): A napin-like polypeptide from dwarf Chinese white cabbage seeds with translation-inhibitory, trypsin-inhibitory, and antibacterial activities. *Peptides* , 25, 171-176.
- [12]. KulBhushan Saxena, Ravikoti Vijaya Kumar Rafat Sultana (2010): Quality Nutrition Through Pigeonpea International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India;
- [13]. Richardson M (1977): The proteinase inhibitors of plants and microorganisms. *Phytochemistry* 16 159-169.
- [14]. Green T R, Ryan C A (1972): Wound-induced proteinase inhibitor in plant leaves: a possible defense mechanism against insects. *Science* 175 776-777.
- [15]. Pusztai A (1972): Metabolism of trypsin-inhibitory proteins in the germinating seeds of kidney bean (*Phaseolus vulgaris*). *Planta* 107 121-129.
- [16]. Jamal, F., Pandey, P.K., Singh, D. et al, (2013): Serine protease inhibitors in plants: nature's arsenal crafted for insect predators. *Phytochem Rev* 12, 1-34 (2013)
- [17]. Huma Habib and Khalid Majid Fazili (2007): Plant protease inhibitors : a defense strategy in plants Department of Biotechnology, The University of Kashmir, P/O Naseembagh, Hazratbal, Srinagar -190006, Jammu and Kashmir, India.
- [18]. Volpicella, Mariateresa; Leoni, Claudia; Costanza, Alessandra; De Leo, Francesca; Gallerani, Raffaele; R. Ceci, Luigi (2011): (Cystatins, Serpins and other Families of Protease Inhibitors in Plants.
- [19]. A Gatehouse, John. (2011): "Prospects for using proteinase inhibitors to protect transgenic plants against attack by herbivorous insects." *Current Protein and Peptide Science* 12.5, 409-416.
- [20]. BROADWAY, R.M. and DUFFEY, S.S. (1986): The effect of dietary protein on the growth and digestive physiology of larval *Heliothis zea* and *Spodoptera exigua*. *Journal of Insect Physiology*, a. vol. 32, p. 673-680.
- [21]. Anne Lecardonnel, Laura Chauvin, Lise Jouanin, Antony Beaujean, Geneviève Prévost, Brigitte Sangwan-Norreel, (1999): Effects of rice cystatin I expression in transgenic potato on Colorado potato beetle larvae. *Plant Science*, Volume 140, Issue 1, Pages 71-79, ISSN 0168-9452,
- [22]. Milica Popovic, Uros Andjelkovic, Lidija Burazer, Buko Lindner, Arnd Petersen, Marija Gavrovic-Jankulovic. (2013): Biochemical and immunological characterization of a recombinantly-produced antifungal cysteine proteinase inhibitor from green kiwifruit (*Actinidia deliciosa*), *Phytochemistry*, Volume 94, Pages 53-59, ISSN 0031-9422,
- [23]. Wedde, Marianne, Weise, Christoph, Nuck, Rolf, Altincicek, Boran and Vilcinskis, Andreas. (2007): "The insect metalloproteinase inhibitor gene of the lepidopteran *Galleria mellonella* encodes two distinct inhibitors" , vol. 388, no. 1, pp. 119-127.



- [24]. Octávio L. Franco, Simoni C. Dias, Claudio P. Magalhães, Ana C.S. Monteiro, Carlos Bloch, Francislete R. Melo, Osmundo B. Oliveira-Neto, Rose G. Monnerat, Maria Fátima Grossi-de-Sá. (2004): Effects of soybean Kunitz trypsin inhibitor on the cotton boll weevil (*Anthonomus grandis*), *Phytochemistry*, Volume 65, Issue 1, Pages 81-89, ISSN 0031-9422
- [25]. Lee, I.; Lee, S.H.; Koo, C.; Jin, C.H.; Lim, C.O.; Mun, H.; Han, S.Y.; Cho, J. (1999): Soybean Kunitz trypsin inhibitor (SKTI) confers resistance to the brown planthopper *Nilaparvata lugens* Stal in transgenic rice. *Mol. Breed.* 5, 1-9.
- [26]. Srinivasan, Ajay, et al. (2005): "A Kunitz trypsin inhibitor from chickpea (*Cicer arietinum* L.) that exerts anti-metabolic effect on podborer (*Helicoverpa armigera*) larvae." *Plant Molecular Biology* 57.3, 359-374.
- [27]. Bahagiawati Amirhusin, Richard E. Shade, Hisashi Koiwa, Paul M. Hasegawa, Ray A. Bressan, Larry L. Murdock, Keyan Zhu-Salzman, (2007): Protease inhibitors from several classes work synergistically against *Callosobruchus maculatus*, *Journal of Insect Physiology*, Volume 53, Issue 7, Pages 734-740, ISSN 0022-1910.
- [28]. Khadeeva, N. V., et al. (2009): "Use of buckwheat seed protease inhibitor gene for improvement of tobacco and potato plant resistance to biotic stress." *Biochemistry (Moscow)* 74.3, 260-267.
- [29]. Shaikh, Faiyaz K., et al. (2018): "Subtilisin inhibitor like protein 'pp LPI-1' from leaves of pigeonpea (*Cajanus cajan*, cv. BSMR 736) exhibits inhibition against *Helicoverpa armigera* gut proteinases." *Biotech* 8.1, 1-10.
- [30]. Lecardonnell, Anne, et al. (1999): "Effects of rice cystatin I expression in transgenic potato on Colorado potato beetle larvae." *Plant Science* 140.1, 71-79.
- [31]. Arnaiz, A.; Talavera-Mateo, L.; Gonzalez-Melendi, P.; Martinez, M.; Diaz, I.; Santamaria, M.E. (2018): Arabidopsis Kunitz Trypsin Inhibitors in Defense Against Spider Mites. *Front. Plant Sci.* 9, 986.
- [32]. Rustgi, S.; Boex-Fontvieille, E.; Reinbothe, C.; von Wettstein, D.; Reinbothe, S. (2017): Serpin1 and WSCP differentially regulate the activity of the cysteine protease RD21 during plant development in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA*, 114, 2212-2217.
- [33]. Stuijver, M.H.; Custers, J.H. (2001): Engineering disease resistance in plants. *Nature*, 411, 865-868.
- [34]. Roberts, T.H.; Ahn, J.W.; Lampl, N.; Fluhr, R. (2011): Plants and the study of serpin biology. *Methods Enzymol*, 499, 347-366.
- [35]. Rustgi, S.; Boex-Fontvieille, E.; Reinbothe, C.; von Wettstein, D.; Reinbothe, S. (2017): Serpin1 and WSCP differentially regulate the activity of the cysteine protease RD21 during plant development in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 2017, 114, 2212-2217.
- [36]. Turra, D.; Bellin, D.; Lorito, M.; Gebhardt, C. (2009): Genotype-dependent expression of specific members of potato protease inhibitor gene families in different tissues and in response to wounding and nematode infection. *J. Plant Physiol.* 166, 762-774.
- [37]. Qu, L.J.; Chen, J.; Liu, M.; Pan, N.; Okamoto, H.; Lin, Z.; Li, C.; Li, D.; Wang, J.; Zhu, G.; et al. (2003): Molecular cloning and functional analysis of a novel type of Bowman-Birk inhibitor gene family in rice. *Plant Physiol.* 133, 560-570.
- [38]. Quilis, J.; Meynard, D.; Vila, L.; Aviles, F.X.; Guiderdoni, E.; Segundo, B.S. (2007): A potato carboxy peptidase inhibitor gene provides pathogen resistance in transgenic rice. *Plant Biotechnol. J.* 5, 537-553.
- [39]. Quilis, J.; López-García, B.; Meynard, D.; Guiderdoni, E.; San Segundo, B. (2014): Inducible expression of a fusion gene encoding two proteinase inhibitors leads to insect and pathogen resistance in transgenic rice. *Plant Biotechnol. J.* 12, 367-377.
- [40]. Azzouz, H.; Cherqui, A.; Campan, E.D.; Rahbé, Y.; Dupont, G.; Jouanin, L.; Kaiser, L.; Giordanengo, P. (2005): Effects of plant protease inhibitors, oryzacystatin I and soybean Bowman-Birk inhibitor, on the aphid *Macrosiphum euphorbiae* (Homoptera, Aphididae) and its parasitoid *Aphelinus abdominalis* (Hymenoptera, Aphelinidae). *J. Insect Physiol.* 51, 75-86.



- [41]. Major, I.T.; Constabel, C.P. (2008): Functional analysis of the Kunitz trypsin inhibitor family in poplar reveals biochemical diversity and multiplicity in defense against herbivores. *Plant Physiol.* 146, 888–903.
- [42]. Dunse, K.M.; Stevens, J.A.; Lay, F.T.; Gaspar, Y.M.; Heath, R.L.; Anderson, M.A. (2010): Co-expression of potato type I and II proteinase inhibitors gives cotton plants protection against insect damage in the field. *Proc. Natl. Acad. Sci. USA*, 107, 15011–15015.