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# Plant Protease Inhibitors: A Defense Mechanism Against Phytophagous Insects

**Dr. Manoj Kumar**

Assistant Professor, Department of Zoology, F.S. University Shikohabad Firozabad, Uttar Pradesh, INDIA.

Corresponding Author: manoj9412896264@gmail.com

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**ABSTRACT**

**In the realm of agriculture, the constant threat of pests and pathogens poses a significant challenge to crop yields. Traditional chemical pesticides, while effective, come with drawbacks such as lack of specificity and the development of resistance. This has spurred a growing interest in exploring alternative methods, with a focus on biodegradable biological control agents and natural products. One promising avenue is the use of Plant Protease Inhibitors (PPIs), which act as a defense mechanism against phytophagous insects. PPIs hinder the activity of insect gut digestive enzymes, leading to reduced protein digestion and impeding the growth and survival of insects. The article delves into the various types of PPIs, their mechanisms of action, and their effectiveness in plant defense. Specifically, it explores the Cystatin Superfamily, with a focus on Family–4 Cystatins known as Phytocystatins. These inhibitors, found in a variety of plants, exhibit potential as biopesticides due to their impact on insect proteolysis. The study also discusses the role of phytocystatins in controlling phytophagous arthropods by targeting their essential digestive proteases. In conclusion, the article emphasizes the significant value of phytocystatins in plant defense and suggests their potential integration as a novel tool in Pest Control Management, highlighting the need for improved policies to enhance their adoption in sustainable agriculture.**

***Keywords-*** Pest management, Biopesticides, Phytophagous insects, Chemical pesticides.

## I. INTRODUCTION

Plants face numerous challenges from pests and pathogens, leading to significant crop yield reductions. Chemical pesticides, employed for decades to mitigate damage, have drawbacks such as lack of specificity, development of resistance, and potential harm to human health due to residual toxicity. The agricultural industry relies on various pesticides, but the non-specific nature and the risk of resistance pose serious concerns. Consequently, there is a growing interest in exploring alternatives to these chemicals. Biodegradable biological control agents and natural products emerge as promising alternatives, being free of pollutant residues and less prone to microbial resistance. The increasing global demand for food necessitates smart technological applications in agriculture. Insect pests are a crucial factor limiting crop yields through consumption and destruction. Recent research focuses on compounds derived from plants with potential insecticidal properties. Plant Protease Inhibitors (PPIs) stand out as a noteworthy class of potential biopesticides, offering ecological friendliness and effective control against a broad spectrum of plant pests and pathogens.

## II. PROTEASE INHIBITORS

The defensive mechanisms plants employ against herbivores are diverse, dynamic, and operate through both direct and indirect means. Defensive compounds are produced either constitutively or in response to plant damage, impacting the feeding, growth, and survival of herbivores. Protease inhibitors (PIs) represent a prevalent class of defensive proteins in plants. PIs bind to insect gut digestive enzymes, hindering their activity and leading to reduced protein digestion. This results in amino acid shortages, slowing the development or causing starvation in insects (Azzouz et al., 2005). The

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defensive role of many PIs against insect pests, whether applied directly or expressed in transgenic plants to enhance insect resistance, has been extensively studied, particularly against lepidopteran (Dunse et al., 2005) and hemipteran insects (Azzouz et al., 2005). Protease inhibitors (PIs) are regulatory molecules present in various animal tissues, fluids, plants, and microorganisms. They control the activity of target proteases, sometimes inhibiting their excessive and uncontrolled activity (Ryan, 1990; Bode and Huber, 1992). Endogenous PIs primarily function to prevent unwanted proteolysis in normal physiological processes and pathological circumstances. This regulation of proteolytic activity involves coenzyme activation and the release of biologically active polypeptides (Laskowski and Kato 1980; Laskowski et al., 1986).

## III. PLANT PROTEIN PROTEASE INHIBITORS (PPIs)

Plant protease inhibitors (PIs) are innate proteins in plants that hinder the proteases of invading insect herbivores. Their effectiveness against insects depends not only on their potency against vulnerable insect systems but also on the insect's response to such challenges. PIs in plants have been extensively researched due to their physiological role in regulating endogenous proteases, storage functions, defense mechanisms against pathogenic infections, and their potential role as antifeedant compounds. This function protects plants from herbivorous insects by inhibiting digestive proteases (Valueva and Mosolov, 2004).

In seeds, tubers, and other storage tissues, PPIs make up around 10% of the total protein content, serving as sources of carbon, nitrogen, and sulfur required during germination (Mandal et al., 2002). Their occurrence in the aerial parts of plants has also been well-documented due to various stimuli (De Leo et al., 2002). The expression of these inhibitors varies based on maturation stage, tissue location, harvest and storage times, and plant variety. A single tissue or organ may have the coexistence of different classes of inhibitors and isoforms (Sels et al., 2008). Plants from the Solanaceae, Leguminosae (Fabaceae), and Gramineae (Poaceae) families often exhibit high levels of PPIs (Brzin and Kidric, 1996; Sin and Chye, 2004; Xu et al., 2001).

## IV. MECHANISM OF TOXICITY ACTION

The mechanism of action of these proteinase inhibitors has been a topic of extensive investigation (Barrett and Dalling, 1986; MacPhalen and James, 1987; Greenblatt et al., 1989). Understanding the mechanisms of protease action and their regulation, both in vitro and in vivo, across animals, plants, microorganisms, and more recently in viruses, has led to numerous practical applications of inhibitor proteins in medicine and agriculture. Baker et al. (1984) demonstrated that the secretion of proteases in insect guts is influenced by the midgut protein content rather than the volume of food consumed. Two mechanisms have been attributed to the secretion of proteases: a direct effect of food components (proteins) on the midgut epithelial cells, or a hormonal effect triggered by food consumption (Applebaum, 1985). Models proposed by Birk and Applebaum (1960) and Brovosky (1986) for the synthesis and release of proteolytic enzymes in insect midguts suggest that ingested food proteins stimulate the synthesis and release of enzymes from the posterior midgut epithelial cells. These enzymes are released from membrane-associated forms and sequestered in vesicles, which are, in turn, associated with the cytoskeleton.

## V. CYSTEINE PIS (CYS), THE CYSTATIN SUPERFAMILY

The cystatin superfamily encompasses various families of proteins that share structural and functional similarities with inhibitors of cysteine proteinase. These protein families, referred to as cysteine protease inhibitors or cystatins, inhibit the activity of cysteine proteases and are found widely across plants, animals, and microorganisms (Oliveira et al., 2003). These inhibitors are categorized into four families: Family-1 cystatins (stefin family), Family-2 cystatins (cystatin family), Family-3 cystatins (kininogen family), and Family–4 cystatins (phytocystatins). The classification is based on sequence relationships, molecular mass, and the numbers and arrangements of disulfide bonds (Turk and Bode, 1991; Barrett, 1987).

## VI. FAMILY–4 CYSTATINS (PHYTOCYSTATINS)

This family encompasses nearly all cysteine protease inhibitors found in plants, with identifications in various plants such as rice (Abe et al., 1987), maize (Abe et al., 1992), soybean (Hines et al., 1991), apple (Malus) fruit (Ryan et al., 1998), and other monocotyledonous and dicotyledonous plants (Pernas et al., 1998). Phytocystatins exhibit sequence similarities to stefins and cystatins but lack free cysteine residues (Fernandes et al., 1993). A distinctive characteristic of this superfamily is the highly conserved region containing the G58 residue with the glu-val-val-ala-gly (QVVAG) motif and a pro-trp (PW) motif. Studies on the papain inhibitory activity of oryzacystatin and its truncated forms have identified the QVVAG motif as a primary interaction region between the inhibitor and its corresponding enzyme. The PW motif is believed to function as a cofactor (Arai et al., 1991). Structurally, phytocystatins can be divided into two groups: single-domain proteins, which constitute the majority of these inhibitors, and multiple-domain

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proteins, including cysteine protease inhibitors isolated from potato tubers and tomato leaves (Bolter, 1993).

## VII. PHYTOPHAGOUS ARTHROPODS AND PHYTOCYSTATIN TARGETS

Insects and mites acquire essential nutrients through hydrolytic activities involved in the digestion process. Therefore, effective proteolysis of plant proteins is essential to generate free amino acids crucial for their survival. Given that many plant tissues have suboptimal protein content, nitrogen often becomes a limiting factor in the nutrition of numerous phytophagous arthropods. As digestive proteases play a key role in catalyzing protein breakdown, these enzymes become potential targets for controlling agricultural pests. Genes encoding proteases are highly expressed in gut tissues, subject to regulatory control during various developmental stages. Different phytophagous arthropods utilize distinct proteases for the digestion process based on their gut pH. This specificity in protease function provides an opportunity to develop specific strategies for pest control using Protease Inhibitors (PIs) (Figure 1). Expression levels and activity profiles of CysProt (Cysteine Proteases) in phytophagous arthropods vary throughout development and depending on the type of plant consumed. Quantitative changes in activity have been observed when comparing larvae and adults of the beetle Leptinotarsa decemlineata (Mello et al., 2002) and when larvae are fed on different plant hosts (Haq and Khan, 2003).

Larval midgut extracts from those that consumed eggplant leaves exhibited a limited number of protease forms, whereas extracts from larvae fed on potatoes and tomatoes revealed a multitude of forms, some of which were specific to particular diets. Similarly, Tetranychus urticae mites raised on maize displayed notably higher cathepsin B-like activity compared to those reared on beans (Alarcón et al., 2001). Transcriptome analysis indicated distinctive developmental expression patterns for both C1A and C13 Cysteine Proteases (CysProt) (De Leo et al., 2002), revealing that mites adjust their expression when feeding on different host plants (Sin and Chye, 2004).

## VIII. CONCLUSIONS

Phytocystatins play a crucial role in various physiological processes of plants, primarily by regulating endogenous or heterologous proteases. Their defensive capabilities have led researchers to view Phytocystatins as proteins of significant value, holding substantial potential for integration as a novel tool in Pest Control Management. While existing strategies have predominantly focused on their transgenic expression in plants, certain biochemical characteristics, such as their compact size and stability, have shifted attention toward their production as recombinant molecules. This approach helps minimize adverse environmental effects by reducing reliance on synthetic pesticides. To enhance the adoption of this scientific technology in countering the adverse impacts of pesticidal hazards, it is imperative to improve policies and provide recommendations on its increased utilization.

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