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Identification of Potent Inhibitors of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) Gut Proteinase from Plant Gum PIs

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ABSTRACT

We report on the efficacy of proteinase inhibitors (PIs) from six host plants *Zea mays, m35 sorghum*, *Sorghum bicolour*, *Saccharum spp*., *Setaria italia* and *Pennisetum glaucum (L)* stem tissue and five non-host *Acacia nilotica, Acacia leucophloea, Azardirachta indica, Mangifera indica* and *Terminalia spp.* plant gum extracts PIs, in retarding the growth of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) larvae, a devastating pest of important crop plants. Enzyme assays and electrophoretic analysis of interaction of *C.partellus* gut proteinases (CPGPs) with PIs revealed that non-host PIs inhibited CPGPs activity efficiently whereas host PIs were ineffective. In the electrophoretic assay, trypsin inhibitor activity bands were detected in all of the host and non-host plants, but CPGPs inhibitor activity bands were present only in non-host plants. *C. partellus* larvae reared on a diet containing non-host PIs showed growth retardation, reduction in total and trypsin like proteinase activity. In present study potent PIs identified against *C. partellus* linsects.

Keywords: *Chilo partellus (Swinhoe) (Lepidoptera: Pyralidae) insects; CPGPs; Host and non-host PIs; BApNA; GXCPS*

1. INTRODUCTION

Lepidopteron stem borers are the most injurious insect pests. Insect pest represents one of the most important biotic stresses to agricultural crops such as maize, sorghum and sugarcane. They are responsible for severe reduction in crop yields. Nearly 32% of the maize and sorghum crop is lost due to insect pests in India (Borad and Mittal, 1983; Kfir,1994; Jotwani and Young, 1972). *C. partellus* is a major pest in Asia, eastern and southern Africa (Jotwani *et al*., 1980). These crops are reduced greatly because of many reasons, among them, the losses caused by is important of the stem borer.

The use of genetic engineering technology for the transformation of crop plants for insect resistance has created access to genes that were otherwise beyond the scope of conventional breeding. The *Bacillus thuringiensis* (Bt) endotoxin gene has been successfully expressed in several crops to impart resistance against herbivorous insects (Jouanin *et al*., 1998; Schuler *et al*., 1998). However, insects have developed resistance to Bt endotoxin by producing a proteinase(s) that inactivates the toxin (Oppert *et al*., 1997; Michaud, 1997), or by lacking the proteinase allele required for activation of Bt protoxin (Oppert *et al*.,1996).

The development of pest-resistant transgenic plants expressing gene of PIs, amylase inhibitors, and lectins of plant origin is another approach that needs further exploration (Jouanin *et al*., 1998; Schuler *et al*., 1998; Ryan, 1990; Boulter, 1993; De Leo *et al*., 1998). The use of PIs in developing insect resistance in transgenic plants is of dual benefit, as they inhibit insect mid-gut proteinases, thereby protecting other defense proteins from proteolytic degradation (Michaud, 1997). PIs block digestive proteinases in insect guts and starve them of essential amino acids (Ryan, 1990; Broadway and Duffey, 1986). They also affect a number of vital processes, including proteolytic activation of enzymes and molting (Hilder *et al*., 1993). PIs are present in the leaves and storage tissues, and are shown to be induced upon wounding, thereby significantly reducing the insect attack (Green and Ryan, 1972; Howe *et al*., 1996).

In an effort to identify potential inhibitors of *C. partellus*, we screened several non-host plants and found that *Acacia nilotica, Acacia leucophloea, Azardirechta indica, Mangifera indica, Moringa oleofera and Terminalia spp.* Are good sources of plant gum PIs. The present work was aimed, identification of potent inhibitors of *C.partellus* gut proteinases from plant gum PIs. The host group of plants selected for this study included *Sorghum bicolor*, m35 sorghum, *Sorghum bicolour* (L), *Pennisetum glaucum* and *Setaria italia*. The activity and in vitro stability studies of host and non-host plant PIs on the basis of their inhibition potential toward *C.partellus* were carried out. Feeding assays were performed to ascertain the potency of the inhibitors in inhibiting growth of *C.partellus* insects. The results provide the basis for the selection of a non-host PIs and developing *C.partellus* resistant transgenic plants.

2. MATERIALSAND METHODS

2.1 Extraction of Fresh *Chilo partellus* Gut Proteases (CPGPs)

Larvae of *C. partellus* were collected from sorghum fields. *C. partellus* gut of 3rd instar larvae were carefully dissected out and stored at -20°C until further use. Proteinases from the gut tissues of CPGPs were extracted by homogenizing the tissue with 0.1M Glycine-NaOH buffer pH 9.6 in 1:6 w/v ratio and kept at 6°C for 2h for extraction. The suspension was then centrifuged at 10,000g for 10 min at 6°C. The resulting supernatant was analyzed for proteinase activity in assays on gels and purification of proteinase. *C. partellus* crude gut proteins was estimated using (Lowry *et al* 1951).

2.2 Plant Gum Protease Inhibitor Extraction

1.0 gm of plant gum powder was homogenized in 15 ml of the MQ water (1:15 w/v) and allowed to stand for 24h at room temperature. These suspensions were then filtered through cintered glass funnel having a processing such as the spore size (40-90 and 5-15 microns). The filtrate was sterilised by passing through 0.2µm filters. 5 ml of clean filtrate and 25 ml chilled acetone (1:5 v/v), these suspensions were later centrifuged. The precipitate was collected and dried at room temperature. Finally dried precipitate was suspended in the 10% PVP at a ratio (1:3 w/v) and stored for 24h at 4ºC. The suspension was then centrifuged at 14,000g for 10min at 6ºC. The upper clear supernatant containing water soluble protein was used for detection of trypsin and chymotrypsin inhibitors. Gum proteins in crude extract were estimated using (Lowry *et al* 1951).

2.3 Proteinases and PIs Assay

CPGPs activity were estimated using chromogenic substrate BApNA with concentration of 1.0 mM in 1.0 ml assay buffer (After initial solubilization in DMSO) otherwise stated 0.1M Glycine- NaOH, pH 9.6 buffer was used for enzyme assay. Assays were carried out 37°C for 30 min. The reactions were terminated by adding 200µl of 30% acetic acid (Patankar *et al*., 2001; Telang *et al*., 2003) and absorbance was measured at 410nm. 0.5ml of supernatant was added to 0.5ml of 1.0M NaOH. The absorbance of this solution was measured at 410 nm and increase in 1.0 OD per min was defined as one proteinase unit. Assays were run in duplicate with appropriate blanks. Casein assay was carried out using 0.5% casein as substrate, CPGPs was added to 2ml of 0.5% casein, incubated for 30 min at 37°C and the reaction was terminated by adding 2ml of 5% trichloroacetic acid. The precipitated proteins were centrifuged at 10,000g, for 10min and supernatant was measured at 410nm. The protease inhibitory activity was measured using a caseinolytic assay (Belew and Porath,1970), as well as by using the synthetic substrate Benzoyl- arginyl- p-nitro-anilide (Erlanger *et al*.,1964). PIs activity were determined by mixing 20µg of trypsin. Equivalent amount of the enzyme was used in the study with sufficient amount of PIs. Defined amount of enzyme and inhibitor were mixed and incubated at 37ºC for 10min and the residual activity was measured at 410nm on a spectrophotometer. One unit of proteinase activity was defined as the amount of enzyme that caused an increase in one optical density unit at 410nm in the 5% TCA soluble products of casein hydrolysis per minute. One PIs unit was defined as the amount of inhibitor that inhibited one unit of proteinase inhibitor activity.

2.4 Visualization of Isoforms of CPGPs and PIs by GXCP Method

Vertical slab gel electrophoresis was carried out according to (Davis, 1964). After electrophoresis the gel was processed for proteases activity by the gel X-ray film contact print method (GXCP). Visualization of *C. partellus* gut, after PAGE was carried out using the X-ray film contact print technique (Pichare and Kachole, 1994). After electrophoresis gel was incubated in 0.1M Glycine-NaOH buffer, pH 9.6 for the 7-8 min, then gel was placed on undeveloped X-ray film and removed after 45 min depending on the extent of gelatine hydrolysis and the X-ray film was washed with either tap or warm water. *C. partellus* gut protease bands appeared as unhydrolyzed gelatin against the background of hydrolyzed gelatin. The X-ray film was then developed. After developing, the protease bands on radiographic film became translucent against the dark opaque background.

Vertical slab gel electrophoresis was carried out according to (Davis, 1964). After electrophoresis the gel was processed for protease inhibitory activity by the gel X-ray film contact print (GXCP) method. 10% native PAGE was washed in 0.1M Tris- HCl buffer pH 7.8 for 4-5 min followed by incubation in 1.0mg/ml trypsin for 7-8 min at room temperature. The gel was then briefly rinsed in 0.1M tris-HCl buffer, pH 7.8 to remove the excess trypsin. The gel and X-ray film were placed in the tray and incubated at 37°C. The gel was placed on the undeveloped X-ray film and was removed after 10 min and extent of hydrolysis of gelatine was monitored visually depending on extent of gelatine hydrolysis. The X-ray film was washed with warm water. The same gel was overlaid 3 to 4 times with different undeveloped X-ray films. Protease inhibitory bands appeared as unhydrolyzed gelatine against the background of hydrolyzed gelatine. Same procedure was followed for chymotrypsin inhibitors.

2.5 Inhibition of CPGPs by Host and Non Host Plant PIs

Gel X-ray film contact print (GXCP) method was employed to check interaction of plant protease inhibitors

against CPGPs. Appropriate volume of gut extracts and plant extract PIs. CPGP was incubated with host and non-host plant PIs for 30min at room temperature. After 30min above suspensions were loaded on native PAGE, after electrophoresis, gel was placed in 0.1MGlycine - NaOH buffer pH 9.6 for 10min for equilibration. The gel was placed on an undeveloped X-ray film for 35-45 min and gelatine hydrolysis was monitored visually. The X-ray film was washed with warm water, dried and scanned.

2.6 Feeding Assay

In vivo efficacy of host and non host plant PIs belonging to various families were studied by feeding assays using *C. partellus* insects. The artificial diet was prepared as reported (Onyango and Oching-Oders, 1994). Feeding assay was conducted with added different concentrations of PIs in the artificial diet. 0.63gm of each of diet containing PIs were mixed with yeast 0.57gm, ascorbic acid 0.06gm, sorbic acid 0.03gm, methyl-p-hydroxy benzoate 0.05gm, vitamin E capsules 0.05gm, sucrose 0.88gm, agar powder 0.32gm and formaldehyde (5%) 0.05ml were added, mixed thoroughly, and poured into trays. Cubes of feed were cut and used for feeding experiments. In control group artificial diet without PIs was used. The larvae were reared on this diet and any gain or loss in weight was recorded on every day until pupation. Also different concentrations of PIs work added to artificial diet feeding to *C. partellus* insects.

3. RESULTS AND DISCUSSION

3.1 Inhibition of CPGPs by Host and Non-Host Plant PIs

Several host and non-host plant PIs were analyzed for inhibition of CPGPs activity and only one non-host plant PIs showed complete inhibition. Inhibition of CPGPs was studied at pH 9.6 because of proteinases showing activity at specific pH 9.6. Table 1, **Table 1.** Inhibition potential of host and non-host plant PIs against gut proteinase activity of *C. partellus*. Activity assays were performed at pH 9.6; five different concentrations of inhibitor extract were used to assess the potential of each inhibitor for inhibiting CPGPs activity. The maximum possible inhibition of CPGPs due to respective PIs is shown as follows. Each value is an average of three replicates ± SE.

Table 1. Inhibition potential of host and non-host plant PIs against gut proteinase activity of *C. partellus*. Activity assays were performed at pH 9.6; five different concentrations of inhibitor extract were used to assess the potential of each inhibitor for inhibiting CPGPs activity. The maximum possible inhibition of CPGPs due to respective PIs is shown as follows. Each value is an average of three replicates ± SE.

Plants PIs	Maximum inhibition of CPGPs activity in pH 9.6 (%)
Control	0.0 ± 0.0
Non host plants	
Azadirachta indica Plant gum extracts	41.23 ± 1.3
Acacia leucophoea Plant gum extracts	92.00 ± 1.0
Mangifera indica Plant gum extracst	38.25 ± 0.8
Acacia nilotica Plant gum extracts	33.33 ± 1.0
Moringa oleofera Plant gm extracts	22.32 ± 1.1
Terminalia spp plant Gum extracts	18.75 ± 0.6
Host plants	
Sorghum bicolor (L) stem tissue extracts	19.00 ± 1.2
M35 sorghum stem tissue extracts	22.33 ± 1.0
Sorghum bicolor stem tissue extracts	53.10 ± 1.0
Pennisetum glaucum(L) stem tissue extracts	18.00 ± 1.1
Setaria italia stem tissue extracts	17.67 ± 0.9
Saccharum spp. stem tissue extracts	29.00± 1.3

gives an account of the efficiency of inhibition of CPGPs activity by various plant PIs. A close examination of the data shown in Table 2,

Table 2. In vitro stability of host and non-host plant PIs against CPGPs Inhibitors were preincubated with CPGPs for 30min at 37°C and then assayed for their inhibitory activity toward CPGPs as described in "Materials and Methods". Each value is an average of three replicates ± SE.

Plant PIs	Inhibition of CPGPs activity in 30 min. (%)
Control	0.0 ± 0.0
Non host plants	
Azadirechta Indica Plant gum extracts	33.33 ± 1.3
Acacia leucophloea Plant gum extracts	67.92 ± 0.8
Mangifera indica Plant gum extracst	31.25 ± 1.0
Acacia nilotica Plant gum extracts	33.33 ± 1.12
Moringa oleofera Plant gm extracts	22.22 ± 0.53
Terminalia spp plant Gum extracts	18.75 ± 0.9
Host plants	
Sorghum bicolour (L) stem tissue extracts	9.00 ± 0.13
M35 sorghum stem tissue extracts	03.33 ± 1.13
Sorghum bicolor (Jawar) stem tissue extracts	45.10 ± 0.03
Pennisetum glaucum (L) stem tissue extracts	0.33 ± 0.4
Setaria italia stem tissue extracts	16.67 ± 0.23
Saccharum spp. stem tissue extracts	25.00± 1.1

revealed that PIs from the host group of plants comprising *Sorghum bicolor*, m35 sorghum, *Pennisetum glaucum (L)* and *Sorghum biocolor (L)* showed 45%, 12% 25%, 10% and 14% inhibition of CPGPs activity at pH 9.6. *pennisetum glaucum (L)* PIs did not inhibit CPGPs activity. On the other hand, PIs from the non-host plants of *Acacia leucophoea* showed total inhibition of CPGPs activity, which inhibited CPGPs activity up to 89%. Another approach of electrophoretic visualization of inhibition of CPGPs isoforms by host and non-host plant PIs indicated that major CPGPs were insensitive to sorghum bicolor, *Setaria italia* PIs (Fig 1, lanes 8 and 7).

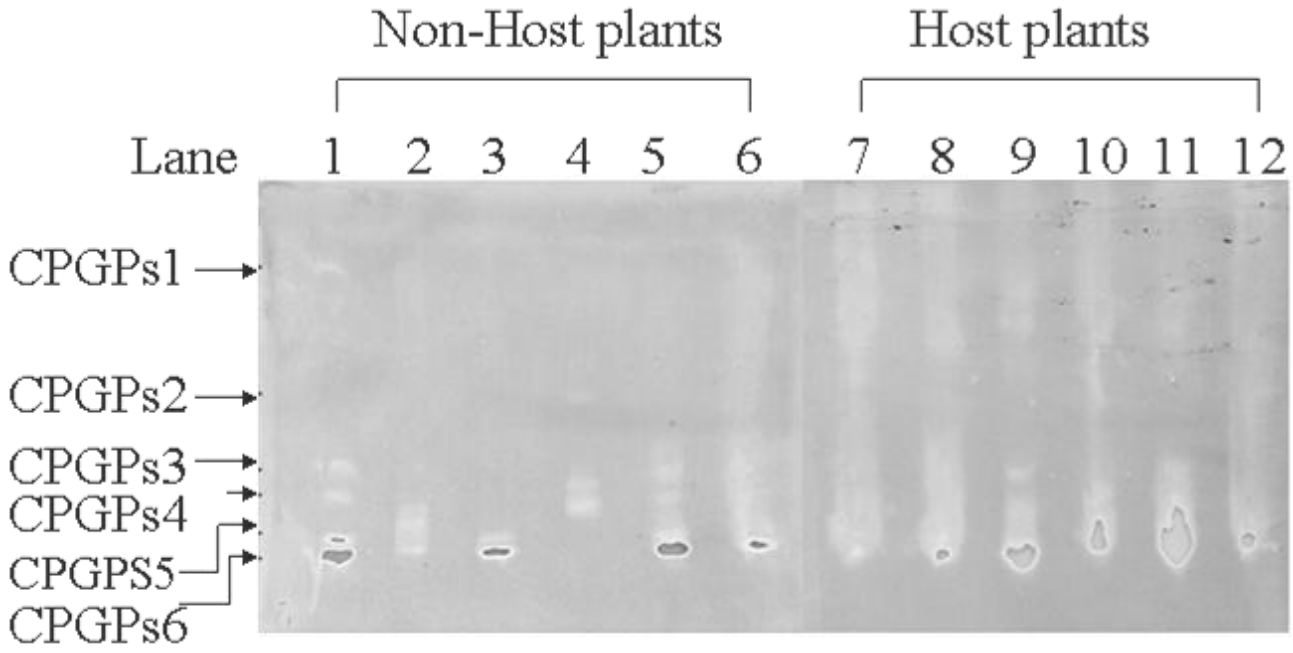

Figure 1. CPGPs profiles of host and non-host plants.

CPGPs bands were visualized as described in "Materials and Methods". Equal PIs units were loaded on both gels. Lanes 1, crude CPGPs; lanes 2, *Azrdirachts indica;* lanes 3, *Acacia leucophoea*; lanes 4, *Mangifera indica*; lanes 5, *Acacia nilotica;* lanes 6, *Terminalia app.*; lanes 7, *Pennisetum glaucum (L)*; lanes 8, *Sorghum bicolor*; lanes

9, *Sorghum bicolour*; lane 10, m35 sorghum ;lane 11, *Zea mays* and lanes 12, *Saccharum spp*. PIs. Among the non-host PIs, those from *Acacia leucophoea* effectively inhibited all CPGPs isoforms (Fig 1 electrophoretic, lane 3), whereas *Azardirachta indica* PIs and *Acacia nilotica* PIs inhibited 33% CPGPs activity (Fig 1, lanes 2 and 5). Based on the data shown in Table 1 and Fig 1, it can be concluded that non-host PIs are able to inhibit total proteinases activity and almost all of the isoforms of CPGPs effectively compared with the host plant PIs, which are poor inhibitors against CPGPs.

3.2 Effect of Host and Non-Host Plant PIs on Growth and Development of *C.partellus* Larvae

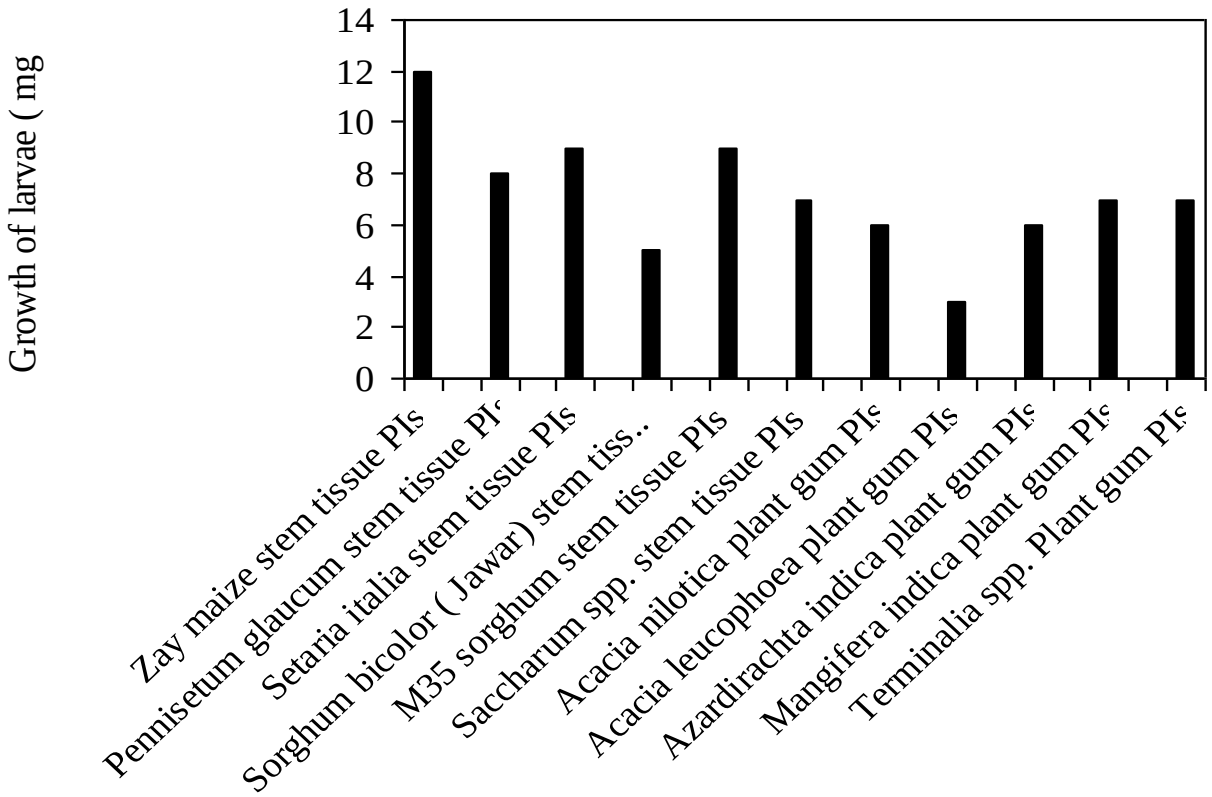

Figure 2. Development of *C. partellus* reared on artificial diet containing host plant PIs and non-host plant PIs. Weight of larvae grown on diet containing host plant PIs and non host plant PIs. Weights of larvae were critically measured on every day.

To estimate the in vivo effects of host and non-host plant PIs on the development of *C. partellus* larvae, feeding trials were conducted with the appropriate controls. Typical development of larvae reared on a diet containing *Zea mays* host PIs and on a diet containing *Acacia leucophoea* non-host PIs. There was a 2 to 4 fold reduction in weight gain in larvae fed with non-host PIs. Food intake was drastically reduced in the larvae showing growth retardation. Furthermore, pupation was also delayed for more than 8days in larvae showing growth. It was observed that the instar stage of *C. partellus* larvae was critical for assessing potential of dietary inhibitors. From the above results, it can be suggested that the inhibitor concentration of non-host PIs used in the diet was sufficient to inhibit growth of larvae.

3.3 In Vivo Inhibition of Gut Proteinases in *C. partellus* Larvae Reared on PIs

To understand the in vivo effect of non-host PIs, *C. partellus* larvae fed on a control diet on PIs of *Zea mays* were dissected after 8 days and the midgut proteinase activity was estimated (Table 3). **Table 3.** Gut proteinase activity of *C. partellus* larvae reared on host and non-host plant PIs. Larvae fed on control diet and PI-containing diet were dissected after 8days and proteinase activity was estimated using casein and BA*p*NA substrates as described in "Materials and Methods." Values in parentheses are the percentages of the proteinase activity of control. Each value is an average of three replicates ± SE.

Table 3. Gut proteinase activity of *C. partellus* larvae reared on host and non-host plant PIs. Larvae fed on control diet and PI-containing diet were dissected after 8days and proteinase activity was estimated using casein and BA*p*NA substrates as described in

"Materials and Methods." Values in parentheses are the percentages of the proteinase activity of control. Each value is an average of three replicates ± SE.

CPGPs	Estimable proteinase activity per 5 guts	
	Caseinase	BApNAase
		%
Control	2.72 ±0.63(100)	8.4 ±0.72(100)
Sorghum bicolor(Jawar)	1.80 ±0.42(66.17)	5.36±0.50 (63.80)
Acacia leucophoea	1.05 ±0.28(38.60)	2.59 ±0.31(30.83)

Proteinase activity of control larvae was considered as 100% and the proteinase activity of PI-fed larvae was calculated accordingly. The larvae fed on jawar (*Sorghum bicolor)* PIs showed 66% caseinolytic nature, whereas in non-host PI-fed larvae the caseinolytic activity was 39%. BA*p*NAase activity, which measures trypsin-like proteinases, was found to be lowest (31%) in *Acacia leucophloea* PI-fed larvae, and 64% in *Sorghum bicolor* PI-fed larvae. Larvae reared on non-host PIs showed a significant decrease in estimable proteinase activity, suggesting that native inhibitors or their fragments were active in the larval gut. The inhibition potential of maximum amount (concentration greater than that required to inhibit total proteinase activity of control CPGPs). The larvae fed with *Sorghum bicolor* showed 45% inhibitor activity, while those fed *Acacia leucophoea* showed only 92% inhibitor activity. *Acacia leucophloea* PIs was able to inhibit 92% of the CPGPs activity. To determine if the complement of gut proteinases of *C.partellus* changed following PI ingestion, gut extracts were separated through PAGE. The gut proteinases of larvae fed on control diet and who were fed on non host plant PIs diet containing. Gut proteinases of larvae reared on *Sorghum bicolor* PIs could no show the effect of CPGP-1, CPGP-2 and CPGP-3. However, CPGP-4 and CPGP-5 showed a trend similar to that of larvae fed on control diet. CPGP-1, CPGP-2, CpGP-3, CPGP-4 and CPGP-5 could not be detected in the gut of larvae fed on *Acacia leucophloea* PIs. The above results indicate that there are non host PIs inhibiting the proteinases and larval growth of *C.partellus* larvae.

3.4 Trypsin inhibitors of Host and Non-Host Plants

Fig 3, shows the electrophoretic profiles of TIs of host and non-host plants. TI activities were present in both the host and non-host plants. *Sorghum bicolor* and *Acacia leucophloea* TI bands (Fig. 3, lanes 1 and 2).

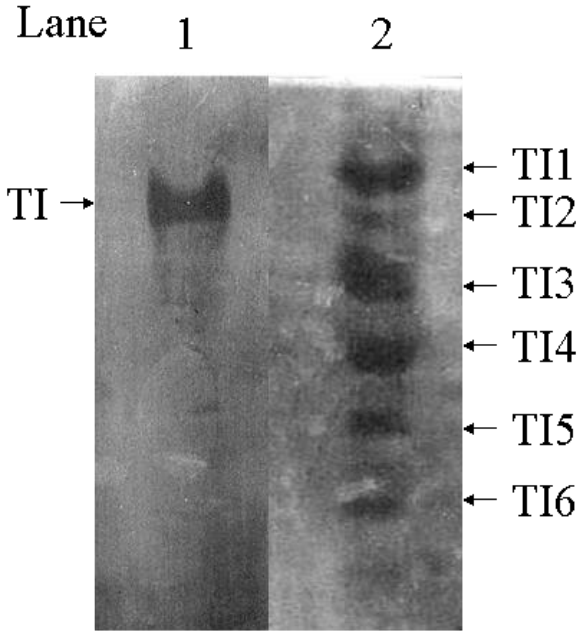

Figure 3. Electrophoretic profiles of *Sorghum bicolor* (lane 1) and *Acacia leucophoea* trypsin inhibitors (lane 2).

Showed that *Sorghum bicolor,* one TI was present (Fig. 3, lane 1), while in *Acacia leucophloea*, six TI bands could be observed (Fig. 3, lane 2). In the non-host group, *Acacia leucophoea* showed six TI bands, which possessed CPGPs activity. The TI bands of *Azadirachta indica, Acacia nilotica, Terminalia spp.,* and *Moringa oleofera* did not have inhibitory activity against CPGPs. The results indicated that no potential inhibitor(s) of CPGPs could be used in *Azadirachta indica, Acacia nilotica, Terminalia spp., Moringa oleofera.* this is the first study in which specific insect PIs have been detected using an in gel assay.

4. DISCUSSION

In our study, earlier we detected the six trypsin inhibitors in *Acacia leucophloea*. This suggests that the *Acacia leucophloea* contains a broad range of PIs which will

make good candidates for developing *C.partellus*-resistant transgenic plants. However, it is necessary to check the activity of these individual purified PIs against gut proteinases of larvae fed on different host plants. It is an apparent paradox that insects feed on plants in spite of the fact that PIs are ubiquitous, especially in case of legumes. Insect pests adapt to host plant PIs by synthesizing proteinases that are either insensitive to inhibitors (Broadway, 1995, 1996, 1997; Jongsma *et al*., 1995). or have the capacity to degrade them (Michaud, 1997; Girard *et al*.,1998a; Giri *et al*.,1998). In a stabilized host-pest complex, insects have evolved and adapted to overcome the effect of PIs of their host plants (Broadway, 1995, 1996, 1997; Bolter and Jongsma, 1995; Jongsma *et al*., 1996). It is therefore necessary to study non-host plant PIs as potential sources to overcome the host inhibitor-insensitive proteinases of insect pests. The present work evaluates non-host PIs to establish their potential against CPGPs through a series of in vitro and in vivo experiments. For the first time to our knowledge, specific inhibitors of insect gut proteinases have been identified.

4.1 Insect Proteinase and Plant PIs

Proteolytic activity of insect guts comprises many isoforms having diverse properties and specificities (Johnston *et al*., 1991; Bown *et al*., 1997; Zhu *et al*.,1997). The presence of proteinases of different specificities in the midgut has great significance for survival and adaptation of phytophagous insects on several host plants. The adaptation of pests to host plant PIs probably results from the selection pressure acting on an entire insect population when they encounter PIs of their host plants (Laskowski *et al*.,1988). Proposed that the structural compatibility between the plant PIs and the insect proteinases determines the level of inhibitory activity against specific proteinases. Structural variation occurring in gut proteinases followed by selection against host plant PIs may modify insect proteinases which, although are of the same class, have insensitive to host plant PIs. In order to survive, plants also must evolve their inhibitor proteins to effectively inhibit insect proteinases. A struggle at the molecular level appears to be a course of evolution in which the proteinases and their inhibitors are variable hotspots of evolutionary changes (Laskowski *et al*.,1988). Both pests and plants have therefore been evolving new forms of enzymes and inhibitors to counteract each other's defence mechanisms (Bown *et al*., 1997; Ishimoto and Chrispeels, 1996). In fact, a few studies have demonstrated that plant inhibitor genes are prone to mutations (Ishimoto and Chrispeels, 1996).

4.2 *Acacia leucophloea* Non-Host Plant PIs are potent inhibitors of CPGPs and retard growth of *C.partellus* larvae

The present study has demonstrated the efficacy of *Acacia leucophloea* plant gum PIs in inhibiting the proteinases and larval growth of *C.partellus*. The *Acacia leucophloea* PIs inhibited total proteinase activity in enzyme assays, and all of the CPGPs isoforms in electrophoretic detection (Table 3). The electrophoretic profiles of PIs in host and non-host plants revealed a number of isoforms differing in intensity and mobility. Most of the CPGPs activity is trypsin like. Plant defense proteins can be potentially recognized as substrates by insect gut proteinases. The following reports have demonstrated that insect gut proteinases neutralize the effect of PIs by degrading them: multicystatin of potato tubers by *Diabrotica* larval proteinases (Orr *et al*., 1994), and oryzacystatin by black vine weevil proteinases (Michaud *et al*., 1995; Michaud *et al*., 1996). Insects derive dual benefits by the digestion of PIs namely through the restoration of gut proteinase activity and availability of valuable, sulfur-rich amino acids. The stability of PIs in proteolytic environment of gut is thus an important criterion for selecting candidate PIs. Total inhibition of CPGPs by non-host plant PIs in the solution assay indicates their stability against the gut proteinases (Table 3). To evaluate the in vivo effects of *Acacia leucophloea* PIs on *C.partellus*, feeding assays were conducted with added inhibitor protein in the artificial diet. Larval growth and development were dramatically reduced when insects were fed on *C.partellus* PI diet. Reduced feeding of larvae was observed in case of PI-incorporated diet as compared to control diet. Feeding studies show that PIs of *Acacia leucophloea*, inhibit the growth of nearly all of *C.partellus* larvae. However, as evident from significant growth retardation, the larvae were suffering from loss of proteinase activity because of the dietary non-host PIs (Table 3, Fig 2.). Recently (Broadway, 1997), speculation has been made that insects might possess specific mechanisms for regulation of individual proteinases controlled by a monitor peptide.

5. CONCLUSION

The present study has demonstrated the efficacy of *Acacia leucophloea* plant gum PIs in inhibiting the proteinases and larval growth of *C.partellus*. The *Acacia leucophloea* PIs inhibited total proteinase activity *C. partellus* larvae. Identification of potent inhibitors against *C. partellus* with plant gum PIs is first study. *Acacia leucophloea* plant gum PIs potent against *C. partellus* larvae was identified in the present study and their proposed successive use has potential in developing *C. partellus* resistant transgenic plants.

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