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Lipoxygenase - trypsin inhibitor activity axis induction in the host plants of muga silkworm, *Antheraea assamensis* Helfer by feeding

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Abstract

The muga silkworm, *Antheraea assamensis* Helfer bears great economic importance, producing golden yellow muga silk which is in global demand for both the textile and biomedical industries. The insect's larval stages are maintained in the field by farmers on its primary host plant *Persea bombycina* Kost in Assam located in the North-East part (25.57 ° N, 93.25 ° E) of India. If continuous feeding by *A. assamensis* is inducing any direct defense in *P. bombycina*, the aspect is not yet questioned. We used the activity of lipoxygenases (LOX) and trypsin inhibitors as markers for determining defense responses in *P. bombycina* and *Litsea monopetala* Roxb, the two primary host plants of the silkworm due to insect feeding. The induction of anti-herbivore trypsin inhibitor by insect feeding was examined by studying their effect on the midgut trypsin activity of *A. assamensis* larvae. This is the first report showing that the LOX- trypsin inhibitor axis is differentially activated in the two host plants and greater induction of the defense response is exhibited in *P. bombycina*, the most commercially used host plant in muga silkworm rearing.

Key words: Feeding induced defense, LOX–trypsin inhibitor, muga silkworms

Introduction

Antheraea assamensis Helfer has great economic importance, producing golden yellow muga silk which is in global demand for both the textile and biomedical industries. The insect is cultured in a semi-domesticated way. The larval stages are maintained on host plants in outdoor fields. *A. assamensis* is endemic to the north-east part (25.57 ° N, 93.25 ° E) of India and is raised largely on its primary host plant, *Persea bombycina* Kost. The insect uses its gustatory sensilla for host plant preference (Bora *et al*. 2015, 2016). Upon herbivory, the plants may activate their defense response pathways. Literature reveals that a specialist herbivore generally wins the evolutionary arms race (Jongsma & Bolter 1997; Mello & Silva-Filho 2002) and can substantially damage plants. *A. assamensis* is oligophagous as it prefers plants in the Lauraceae family and occupies a position between a generalist (polyphagous) and a specialist (monophagous). Neither the host plant nor *A. assamensis* is reported to be even involved in any arms race. But if the race has been on, the host plants might have been producing defensive factors antagonistic to *A. assamensis*. The very question of the possibility of defense induction in the popular host plant is raised in the present work from a popular belief among the muga farmers of the state of Assam (26.20 ° N, 92.94 ° E) located in North East India. The authors noted, during personal interactions with the local silk growers of Assam, that they invariably reported about unsuccessful rearing and greater loss of cocoon production if they used the same host plants consecutively for several generations. The muga silk worm growers carry the seeds produced in one area of Assam to another area for producing the next generation of the silkworms and never go for continuous production of several generations in the same area. It is well

established now that continuous stress on host plants by herbivory may cause defense induction in the host plants. Cases of indirect defense in *P. bomycina* in the form of volatile organic chemical has already been reported (Bora *et al*. 2012; Deka & Dipsikha 2014). The question, if continuous feeding induces defense response in the host plants of silk worms and *A. assamensis* is being inhibited by host plant defense responses is neither known nor to date has any attempt been made to understand this ecologically and economically significant trophic interaction which can be used in the conservation of the species.

Plant defense proteins and other molecules, such as protein inhibitors of insect digestive enzymes, amino acid deaminases (Chen *et al*. 2005; Lomate *et al*. 2013), enzymes like lipoxygenase, peroxidase, and catalase (Bi *et al*. 1994; Felton *et al*. 1994), deter insect feeding or interfere with insect growth and development as an effective mechanism of plant defense. Differential expression of genes, induction of signaling pathways such as the octadecanoid pathway, jasmonic acid pathway, shikimic acid pathways, and production of toxic secondary metabolites in plants in response to pathogens and insect attack are well documented (Heidel & Baldwin 2004; Karban & Baldwin 1997; Xia *et al*. 2014). Lipoxygenases (LOX) are nonheme iron-containing enzymes and catalyze the oxygenation of polyunsaturated fatty acids such as linoleic and linolenic acid to fatty acid hydroperoxides (Brash 1999). It is the key enzyme initiating the octadecanoid pathway, whose end product is the hormone jasmonic acid that triggers the production of anti-herbivore protease inhibitors, ubiquitously present molecules evolved in plants to defend against pathogens and herbivores (Farmer & Ryan 1992; Steppuhn & Baldwin 2007). The C-6 volatile pathway producing green leafy volatiles and the signaling molecules detected by the third trophic level predators and parasitoids of the herbivores (Bora *et al*. 2012) is also initiated by LOX. The LOX activity thus constitutes an induced defense strategy of plants against herbivory. Insect oral secretions (regurgitant and saliva) are reported to contain elicitors that trigger plant defense responses (Alborn *et al*. 1997; Musser *et al*. 2002; Turlings *et al*. 1993). Considering that LOX enzyme activity is the first step of the lox defense pathway of plants and trypsin inhibitor is the end product of this pathway, we have hypothesized that the induced LOX enzyme activity and trypsin inhibitors should do well as a measure of activation for plant defenses in response to herbivory.

In this study, we examined the leaves of *P. bombycina* and *Litsea monopetala* both with and without *A. assamensis* herbivory, for the induction of LOX and anti-herbivore trypsin inhibitor to understand if differential induction of defense responses occur between host plants due to forced continuous association with *A. assamensis* for the commercial production of muga silk.

Materials and methods

Biological material

Disease-free cocoons were procured from government sericulture farms and reared in a greenhouse in the Department of Life Sciences, Dibrugarh University, Assam, India, on leaves of *P. bombycina* and *L. monopetala*. The greenhouse was maintained at 24 ± 1°C, 80 ± 5% relative humidity, and with a 14 h light/10 h dark photoperiod. Disease-free 5th instar larvae were selected for feeding experiments conducted on 2–3-year-old young primary host plants, *P. bombycina* and *L. monopetala*.

Feeding bioassay

The young plants of the two species were exposed to 5th instar larvae (n = 5) of *A. assamensis* for 2, 6, 24, and 48 h, after which the fed leaves along with leaves in the close vicinity were collected and stored at −20°C. An unfed control was maintained simultaneously.

Mechanical damage (MD)

When the lepidopteran larvae feed, they bite and chew the leaves with their mandibulate mouthparts and thereby cause mechanical damage to the plant; therefore we investigated whether manually created mechanical damage could induce LOX activity. Mechanical damage was caused by creating five 1 cm^2 wounds per leaf by scraping the leaves of *P. bombycina* and *L. monopetala* plants with a blade. The leaves were then collected after 2, 6, 24, and 48 h. Scraped plants whose leaves were collected immediately were used as controls. Another plant whose leaves were not scraped was used as absolute control. All samples were assayed for LOX activity. This experiment was conducted to eliminate the effect of mechanical damage caused by insect feeding.

Regurgitant collection and processing

The regurgitant was collected from the 5th instar larvae by placing the larvae between the palms and shaking them vigorously. The larvae were then allowed to regurgitate the intestinal contents reflexively on a glass plate which was collected with a micropipette and stored immediately at −20°C until further use.

Before use, the regurgitant was processed by heating at 95°C for 30 min to inactivate hydrolytic enzymes and centrifuged at 11,000 g for 10 min to remove solids (Turlings *et al*. 1993).

Application of regurgitant (R)

Leaves of *P. bombycina* and *L. monopetala* plants were mechanically damaged as described above and 10 μL of processed regurgitant was applied to each wound. The leaves were then collected after 2, 6, 24, and 48 h for assay of LOX activity. Scraped and regurgitant applied young plants whose leaves were collected immediately were used as control plants. Three to five plants were taken for obtaining each pooled sample.

Preparation of enzyme extract and LOX activity assay

The leaves were homogenized in 0.2 M ice cold phosphate buffer (pH 8) and centrifuged for 10 minutes at 14,000 rpm and 0°C. The supernatant was stored at −20°C for assay of lipoxygenase enzyme activity. The enzyme activity was evaluated following the method of Koch *et al*. (1992) with modification using the synthetic substrate linoleic acid. The reaction mixture consisted of 10 μL of plant leaf extract, 10 μL of 10 mM linoleic acid, and 980 μL of 50 mM phosphate buffer (pH 6.5). The mixture was incubated for 30 seconds at 25°C and absorbance was measured at 234 nm using a Shimadzu UV–Vis spectrophotometer. The assay was performed in triplicate.

The lipoxygenase activity was calculated using the following equation-

$$\text{Lipoxygenase activity} = \frac{\Delta\text{Abs} \times \text{total volume} \times \text{dilution factor}}{\varepsilon \times \text{vol of enzyme} \times \text{time}}$$

Where ε is the molar extinction coefficient for the product linoleic acid hydroperoxide which is equal to 25,000 $LM^{-1}\ cm^{-1}$.

1 U/L of LOX activity is defined as the amount of enzyme which converts 1 μmole of substrate per minute into the product (linoleic acid hydroperoxide).

Trypsin activity assay and determination of percentage of trypsin acitivity inhibition

(i) **Trypsin activity**: The silkworm gut (0.5 g) was crushed in ice cold condition using 50 mM ice cold phosphate buffer (pH 8)(10 ml) and used for measuring trypsin activity. Three to five insects were taken for obtaining the pooled gut extract. Nα-Benzoyl-DL-arginine 4-nitroanilide hydrochloride (BAPNA) is a synthetic peptide and was used as substrate for measuring Trypsin activity. 2 mM BAPNA substrate was incubated with gut extract for 10 minutes at 25°C and pH 10. The absorbance of all three assays was measured at 400 nm using a UV–Vis Spectrophotometer (Shimadzu). Protein level was estimated in the 50 mM phosphate buffer (pH 8) extracted gut tissue following the method of Lowry *et al*. (1951)

(ii) **Activity of trypsin inhibitor:** Inhibition studies were conducted following the methods of Kakade *et al*. (1969) and Lin and Jeng (1977) with modification. Since we used only insect gut extract for assay of trypsin activity, we needed to confirm that the prepared extract contained trypsin enzyme. Therefore, we used a standard trypsin inhibitor (soybean) to detect the presence of trypsin enzyme in silkworm midgut extract. For the purpose, the gut extract was first incubated with a standard soybean trypsin inhibitor of different concentrations: 2.5, 5, 7.5, 10, and 12.5 μg/mL for 10 minutes at 25°C in 50 mM NaOH- glycine buffer, pH 10. BAPNA was added as substrate as described above for determining trypsin activity. Incubated the mixture for 10 minutes at 37°C and pH 10, followed by measurement of absorbance at 400 nm using a UV–Vis spectrophotometer. The assay was performed in triplicate. The results given as supplementary material showing linearity in increase in inhibition with higher amount of standard trypsin inhibitor allowed us to continue with the work using crude gut trypsin (Fig. S1). For determining the trypsin inhibition activity of control and fed leaves (0.5 g) of *P. bombycina* and *L. monopetala*, standard, control, and sample assays were performed as per the above methods. The absorbance of all three assays was measured at 400 nm using a UV–vis spectrophotometer. The percentage of inhibition was calculated using the formula of Lin and Jeng (1977) with modification.

$$\frac{(\text{Standard} + \text{Control}) - \text{Sample}}{\text{Standard}} \times 100$$

where, in the standard assay, 0.125 mL silk worm gut extract (trypsin extract) was added to 0.125 mL buffer and 0.875 mL Nα-Benzoyl-DL-arginine 4-nitroanilide hydrochloride (BAPNA), and incubated for 10 min at 25°C at pH 10. In the control assay, 0.125 mL *P. bombycina*/*L. monopetala* trypsin inhibitor (TI) (crude leaf extract) extracts, 0.125 mL buffer, and 0.875 mL BAPNA were added, and incubated for 10 min at 25°C at pH 10. In sample assay, the gut extract was first incubated with 20 fold diluted *P. bombycina*/*L. monopetala* leaf extract (fed) and BAPNA for 10 minutes at 25°C in 50 mM NaOH- glycine buffer, pH 10.

Statistical Analysis

All the tests were repeated three times. For the assay of LOX and trypsin inhibitor activity, a total of five saplings, and for gut protein and trypsin activity, a total of five insects were used

independently for each replication. The effects of feeding on LOX and trypsin inhibitor activity of the induced plants were examined *via* analysis of variance (one-way ANOVA). Post-hoc mean separation was executed by LSD test at $p < 0.05$ and $p < 0.01$. The statistical analysis was performed using SPSS version 18. (SPSS Inc., Chicago, IL, USA).

Results

LOX activity in *P. bombycina* and *L. monopetala* due to insect feeding, mechanical damage, and regurgitant application

The LOX activity of the *P. bombycina* leaves challenged by 5th instar *A. assamensis* larvae increased by multiples of 2.68, 2.69, 1.68, and 2.73 at 2, 6, 24 and 48 h of herbivory respectively, in comparison to the controls ($p = 0.001$). In the case of *L. monopetala*, LOX activity increased by 1.22 after 2 h feeding, but afterward, the activity decreased to less than that of the control ($p = 0.008$) (Tables 1 and 2). We found that LOX activity in mechanically damaged *P. bombycina* leaves was increased by a multiple of 4.69 compared to the undamaged control (0 h UD). This increase continued up to 24 h but decreased by 48 h ($p < 0.0001$). Although we examined whether there was a change in LOX activity with time after mechanical damage at zero hours, no specific pattern of LOX activity could be detected until 48 h. In the case of *L. monopetala*, LOX activity in the leaves of undamaged plants was higher compared to that in leaves inflicted with mechanical damage at every time point ($p < 0.0001$) (Table 2). Considering that the regurgitant released by larvae at the site of feeding might have a different effect on LOX activity, we examined LOX activity by applying regurgitant onto mechanically created lesions. We found increased LOX activity till 48 h on the leaves so treated, when compared to those with mechanical damage and regurgitant treatment in the case of *P. bombycina* ($p < 0.0001$) (Table 1). In the case of *L. monopetala*, there was a decrease in LOX activity in all MD and MD + R applied leaves as compared to that in the undamaged leaves, but as compared to the leaves with mechanical damage and regurgitant, the regurgitant caused an increase in activity by 1.01–1.23 folds ($p < 0.0001$) (Table 2).

Trypsin inhibitor activity in *P. bombycina* and *L. monopetala* due to insect feeding

When we compared the trypsin inhibitor activity (TI) of unfed and feeding challenged leaves with the gut trypsin activity of silkworm larvae fed with the defense induced leaves we found a negative correlation between plant trypsin inhibitory activity and gut trypsin activity. After 2 h of feeding of *P. bombycina* by *A. assamensis*, there was a significant increase in leaf LOX activity (times 2.68), in trypsin inhibition to 74.35%, and a decrease in gut trypsin activity by 30%. At 24 h, TI activity increased accompanied by a significant decrease (69.01%) in gut trypsin activity ($p < 0.0001$). At 48 h, the TI activity increased further higher to 89.74% followed by reduction in trypsin activity (Fig. 1). In the case of *L. monopetala*, there was only a 1.22-fold increase in LOX activity after 2 h of feeding which then decreased. Simultaneously, trypsin inhibitor activity decreased from 96.15% at 0 h up to 46.15% at 24 h and 73.07% at 48 h. The Pearson correlation carried out to examine the correlation between LOX activity and trypsin inhibition percentage revealed the correlation to be significant in the case of *P. bombycina* ($r = 0.403$, $p < 0.05$) but not in the case of *L. monopetala* ($r = 0.327$, $p > 0.05$). Pearson correlations performed to examine the correlation between percent trypsin inhibitor activity and gut trypsin activity showed significant negative correlation in the case of both *P. bombycina* ($r = -0.672$, $p < 0.01$) and *L. monopetala* ($r = -0.394$, $p < 0.05$), although the level of significance was different. Thus, in insects feeding on leaves with low trypsin inhibitory activity, we found their gut trypsin activity to have increased ($p = 0.009$) (Fig. 1), but the activation of the LOX–trypsin inhibitor axis by the feeding of *A. assamensis* was more pronounced in *P. bombycina*.

Discussion

Among the different factors responsible for the selection of a host plant as food, plant biochemical characters play a vital role in host plant selection as well as sustenance on the selected host plant. In our study, uninfested *P. bombycina* leaves with low LOX activity showed no trypsin inhibitory activity while uninfested *L. monopetala* leaves with a high LOX activity caused 100% inhibition of silkworm gut trypsin. This may explain why *P. bombycina* leaves have always been preferred over *L. monopetala* by *A. assamensis* larvae. The preferential behavior of *A. assamensis* larvae in favor of *P. bombycina* leaves was also reported by Neog *et al.* (2011) where they referred to the involvement of plant secondary metabolites like chlorogenic acid, without any mention of the involvement of trypsin inhibitor. Plants may opt for reconfiguration of primary and secondary metabolism to tolerate insect herbivory and the primary metabolites may act as a signaling molecule in defense responses (Schwachtje & Baldwin 2008). A preferred plant may not be the best host for the growth and development of an insect herbivore for infinity. In a similar study, feeding by *A. assamensis* larvae for 2 h, enhanced the leaf proteins of *P. bombycina* and *L. monopetala* (Mech 2019) and such observations corroborate with the significant induction of the defense-related LOX

Table 1 LOX activity (U/L ± SE) in *Persea bombycina* (PB) 2, 6, 24 and 48 h after feeding by *Antheraea assamensis*, mechanical damage (MD), and mechanical damage (MD) + regurgitant (R) application (MD + R)

	Mode of damage				
	Feeding		Mechanical damage (MD)	Mechanical damage (MD) and regurgitant (R) application (MD + R)	
Time after damage	(LOX activity) U/L ± SE	Folds of increase to control (UD)	(LOX activity) U/L ± SE	(LOX activity) U/L ± SE	Folds of increase to MD + R
0 h(UD)	258.66 ± 21.33[a]	NA	258.66 ± 21.33[b]	258.66 ± 21.33[a]	NA
0 h(MD)	NA	NA	1213.33 ± 113.92[a]	1213.33 ± 113.9[b]	NA
0 h(MD+R)	NA	NA	NA	158 ± 18.29[a]	NA
2 h	693.33 ± 55.87[b]	2.68	1432.00 ± 248.38[a]	1,146 ± 123.21[b]	7.25
6 h	696.00 ± 16.00[b]	2.69	896.00 ± 116.57[a]	1,552 ± 147.44 [c]	9.82
24 h	434.66 ± 101.33[c]	1.68	1317.33 ± 9.61[a]	1,256 ± 52.54[b]	7.94
48 h	706.66 ± 35.87[b]	2.73	232.00 ± 12.22[b]	264 ± 9.23[a]	1.67
$CD_{0.05}$	174.80		375.03	288.464,	
SE	78.45		172.20	136.713	

U/L ± SE with different letters indicates that the LOX activity is significantly different from each other in post-hoc LSD test ($p < 0.05$); **CD,** Critical difference; **SE,** Standard error of means; **'–'** indicates decrease as compared to control; **NA**, Not applicable; **1 U/L** indicates the amount of enzyme that converts 1 μmole of substrate per minute; **UD**, Undamaged; **MD**, Mechanical damage; **MD+R**, Mechanical damage (MD) and regurgitant (R) application.

Table 2 LOX activity (U/L ± SE) in *Litsea monopetala* (LM) - 2, 6, 24 and 48 h after feeding by *Antheraea assamensis*, mechanical damage (MD), and mechanical damage (MD) + regurgitant (R) application.(MD + R)

	Mode of damage				
	Feeding		Mechanical damage (MD)	Mechanical damage (MD) and regurgitant (R) application (MD + R)	
Time after damage	(LOX activity) U/L ± SE	Folds of increase to control (UD)	(LOX activity) U/L ± SE	(LOX activity) U/L ± SE	Folds of increase to MD + R
0 h (UD)	602.66 ± 13.08^{a}	NA	602.66 ± 13.08 ^{c}	602.66 ± 13.08^{a}	NA
0 h (MD)	NA	NA	216.00 ± 24.33^{a}	256 ± 24.33^{b}	NA
0 h (MD+R)	NA	NA	NA	288.00 ± 27.71 ^{bc}	NA
2 h	736.00 ± 12.22^{b}	1.22	357.33 ± 53.92^{b}	354.66 ± 30.05 ^{c}	1.23
6 h	536.00 ± 65.48^{a}	-	317.33 ± 11.62^{ab}	293.33 ± 7.05 ^{bc}	1.01
24 h	584.00 ± 56.19^{a}	-	314.66 ± 23.24^{ab}	328.00 ± 12.22 ^{bc}	1.13
48 h	450.66 ± 27.84^{c}	-	344.00 ± 24.00^{bc}	322.00 ± 22.71 ^{bc}	1.1
$CD_{0.05}$	130.16		84.167	68.35	
SE	58.45		38.646	32.09	

U/L ± SE with different letters indicates that the LOX activity is significantly different from each other in post-hoc LSD test ($p < 0.05$). **CD,** Critical difference; **SE,** Standard error of means; **'–'** indicates decrease as compared to control; **NA**, Not applicable; **1 U/L** indicates the amount of enzyme that converts 1 μmole of substrate per minute; **UD**, Undamaged; **MD**, Mechanical damage; **MD+R**, Mechanical damage (MD), and regurgitant (R) application.

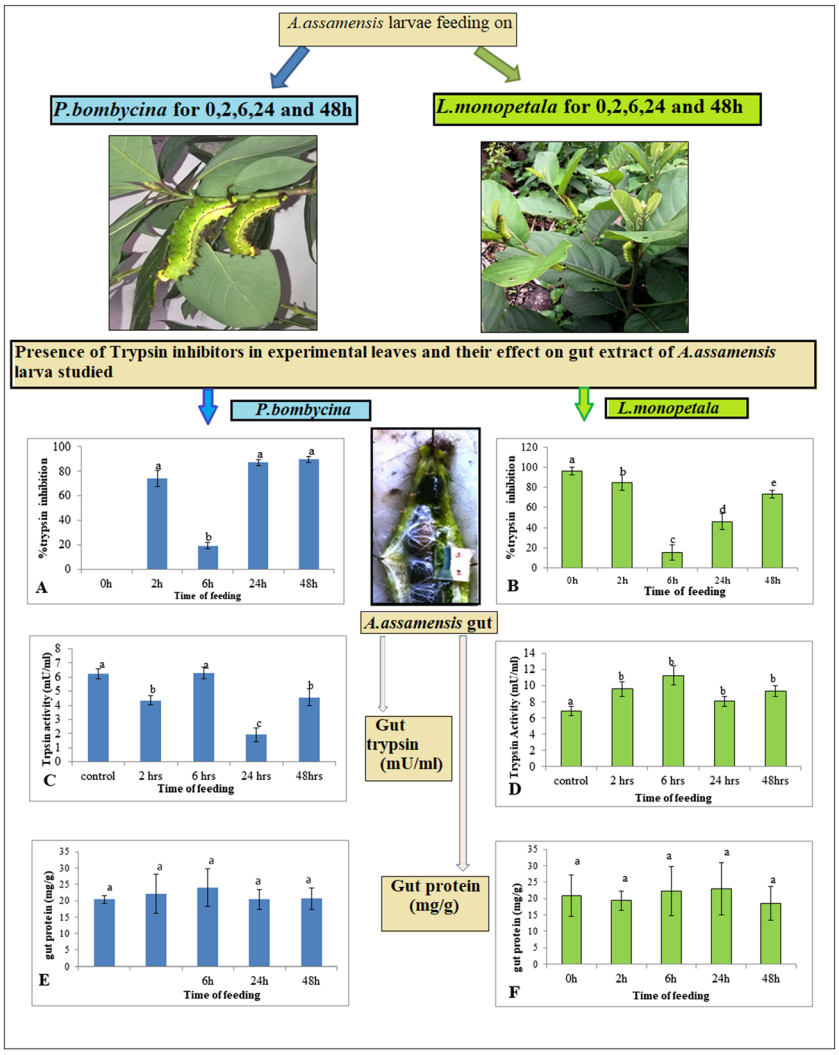

Figure 1 A & B show percent trypsin inhibitory activity (% trypsin inhibition) of the 2, 6, 24, and 48 h fed *P. bombycina* and *L. monopetala* leaves on *A. assamensis* midgut. C & D show trypsin activity (mU/mL) of *Antheraea assamensis* gut extract fed on 0, 2, 6, 24, and 48 h previously fed *P. bombycina* and *L. monopetala* leaves (defense induced leaves). E & F show the protein content of *A. assamensis* larval gut tissue fed on induced (0, 2, 6, 24, 48 h previously fed) *P. bombycina* and *L. monopetala* plants respectively. Error bars indicate ±SE. Different letters indicate that % trypsin inhibition activity, trypsin activity, and gut protein at different durations of feeding are significantly different from each other by the LSD test ($p < 0.05$).

enzyme in both the host plants. In another study performed using tomatoes LOX activity exhibited a peak within 2 h of approximately 1.84 times more than that of the untreated leaves, but a decline in activity was recorded thereafter (Hu *et al*. 2011).

Multiple herbivore-associated molecules present in insect oral secretions act synergistically or independently to modulate various defense responses in host plants (Basu *et al*. 2017). For instance, the glucose oxidase present in the oral secretions of *Heliothis zea* suppressed defenses in *Nicotiana tabacum* (Tobacco) (Musser *et al*. 2002) while the enzyme from the oral secretion of both *H. zea* and *Ostrinia nubilalis* induced defense responses in tomato (*Solanum lycopersicum*). The enzyme oxidized glucose to gluconic acid and hydrogen peroxide (H_2O_2) and the resultant H_2O_2 is modulated plant defense responses to biotic stress (Louis *et al*. 2013). In the case of *O. nubilalis*, saliva induced or suppressed plant defensive responses by upregulation of lipoxygenase (LOX) and *12*-oxo-phytodienoic acid (OPR) genes (Louis *et al*. 2013; Musser *et al*. 2002). In the current study, the regurgitant enhanced LOX activity only in *P. bombycina*. In *L. monopetala*, both mechanical damage and the insect regurgitant suppressed LOX activity as compared to undamaged. As regurgitant can mimic the action of insect feeding, the result showing suppression of LOX activity in *L. monopetala* post-application of regurgitant reinforces the fact that LOX pathway was not activated by the insect's feeding in *L. monopetala*.

The defensive chemicals of the host plant may exert an inhibitive effect on insect growth. The 2 h fed *P. bombycina* and *L. monopetala* leaf extract exhibited 74.35% and 84.87% inhibition of gut trypsin activity (*in-vitro*) respectively in *A. assamensis* larvae. As shown in Fig 1, trypsin activity in the gut of these silkworms as compared with control decreased

when fed with defense induced *P. bombycina* and increased when fed with defense induced *L. monopetala*. In the later hours of defense induction, *P. bombycina* gut trypsin activity decreased after 6 h onwards while in the case of *L. monopetala*, it increased or was similar to the control. This indicates that the trypsin enzyme in *A. assamensis* was inhibited and had not developed tolerance to the protease inhibitor produced in *P. bombycina*, probably interfering with proper utilization of protein due to the suppression of trypsin activity. In the case of *L. monopetala*, the trypsin inhibitor activity was higher in control leaves as compared to *P. bombycina* and on feeding by *A. assamensis* there was reduction in TI activity. The reduction in TI activity in *L. monopetala* may indicate depression or non-induction of lipoxygenase pathway which is reflected in the non-significant variation of LOX activity in *L. monopetala* on feeding except for 2 h (Table 2).

Lepidopterans exhibit different isoforms of trypsin enzyme and may prefer tolerant proteases as an evolutionary strategy rather than adopting an avoidance strategy using enhanced trypsin activity (Christellar *et al*. 1992; Srinivasan *et al*. 2006; Zhu-Salzman *et al*. 2008; Jongsma & Beekwilder 2011). So, the insect in the current study is not yet adapted to the defensive chemical, trypsin inhibitor of *P. bombycina*, or the other way round, the insect is adversely affected by the defense response of *P. bombycina*. Therefore, defense induction was more pronounced in terms of the activity of LOX and production of trypsin inhibitor in the case of *P. bombycina*. In the case of *L. monopetala*, the LOX-TI axis was not significantly activated as indicated by their non-significant variation in trypsin activity (Fig. 1) and suppression of LOX activity discussed above. Thus, the LOX–trypsin inhibitor axis was differentially activated in the two host plants, and greater induction of the defense response was exhibited in *P. bombycina*, the most commercially used host plant in muga silkworm rearing. That handling of different stress by plants cannot be generalized and often varies with the plant-insect system, has been reviewed recently by Duy *et al*. (2016). Plant tolerance may act as selection pressure against herbivory and this is a beneficial trait for crop protection, but when the herbivore is an economically important organism, it is important to understand the differential expression of defense induction for the benefit of the herbivore for greater productivity. This is the first report showing that the LOX–trypsin inhibitor axis is differentially activated in the two host plants and greater induction of the defense response is exhibited in *P. bombycina*, the most commercially used host plant in muga silkworm rearing. Further research using pathway inhibitor, gene expression studies *etc*. is required to throw light on the use of an alternate strategy of host plant feeding to combat host plant defense strategies.

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Conflict of interest

The authors declare no conflict of interest.

Authors contribution

DSB contributed to the conceptualization of the work, analysis, and manuscript editing, AM contributed towards experimental works, analysis, and manuscript writing. KKC contributed towards experimenting and maintaining the biological materials in greenhouse.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig S1. Effect of soybean trypsin inhibitor (SBTI) on the gut trypsin activity in *Antheraea assamensis* larvae. Error bars indicate ±SE. Commercial soybean trypsin inhibitor significantly inhibited the trypsin like activity of the gut extract, confirming the presence of trypsin.