# Functional loss of CHS2 confers high levels resistance to *Bacillus thuringiensis* Vip3Aa, but with significant fitness costs in five lepidopteran species

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# Abstract

*Bacillus thuringiensis* (Bt) crops, engineered to produce insecticidal proteins such as Vip3Aa and Cry toxins, have revolutionized pest management by providing a sustainable alternative to chemical pesticides. However, the development of resistance to these toxins has driven the investigation of underlying mechanisms to better understand how pests evade toxicity and to develop more effective resistance management strategies. Recently, a laboratory-selected *Spodoptera frugiperda* strain exhibited a high level of resistance to Vip3Aa (resistance ratio: 5562-fold), with the mutation of the chitin synthase gene, *SfCHS2*, identified as a key factor. In this study, we extend these findings to additional lepidopteran species, including *Spodoptera exigua* and *Agrotis ipsilon*. Our results show that *CHS2* knockout strains lack the peritrophic matrix (PM), while the resistant Sfru_R3 strain retains its PM. Knockout of this gene in five lepidopteran species resulted in high levels resistance to Vip3Aa. Notably, homozygous knockout strains suffered significant fitness costs and had difficulty surviving on corn leaves, in contrast to the Sfru_R3 strain, which showed no such survival issues. These findings further validate the role of the *CHS2* gene in Vip3Aa resistance and highlight its potential as a target for resistance management in lepidopteran pests. This study advances our understanding of the molecular mechanisms behind resistance to Vip3Aa and supports the development of strategies to delay resistance in Bt crop management.

# Keywords

Vip3Aa resistance; peritrophic matrix; midgut; CHS2; lepidopteran


# 1. Introduction

Genetically modified (GM) plants that produce toxins derived from the bacterium *Bacillus thuringiensis* (Bt) are known as Bt crops and represent one of the most successful applications of genetic engineering in agriculture. These transgenic plants (eg: cotton, maize and soybean) can produce toxic proteins to disrupt the digestive system of many key lepidopteran and coleopteran pests, thus have revolutionized pest management by offering an efficient, environmentally friendly alternative to chemical pesticides. The most widely used are crops expressing Cry toxins, which have been commercialized and widely grown around the world for decades(Hutchison et al., 2010; Lu et al., 2012). However, intensive planting of these Bt crops has created a growing number of resistance in target insect species (Dhurua and Gujar, 2011; Orozco-Restrepo et al., 2024; Tabashnik et al., 2023; Yang et al., 2022a; Zhang et al., 2016). The Vip3Aa toxin, which is produced in the vegetative stage of Bt, was introduced in crops alone, or with other Cry toxins together, to confer higher protection and delay insect resistance (Kurtz et al., 2007; Niu et al., 2021; Rabelo et al., 2020; Yang et al., 2022a).

Understanding the mechanism of Vip3Aa toxicity and resistance is essential

for effective pest management (Wu, 2014; Yang et al., 2022b). The protein sequence of Vip3Aa shows no homology with Cry proteins (Estruch et al., 1996), and current evidence suggests no cross-resistance between the two (Bachler et al., 2024; Chakroun et al., 2016; Kurtz et al., 2007; Liu et al., 2024; Sena et al., 2009), indicating a different resistance mechanism for Vip3Aa. Although the resistance to Vip3Aa in field pest populations is rare (Amaral et al., 2020; Yang et al., 2021a), several laboratory-isolated strains have shown medium-to-high resistance, providing valuable resources for detecting the underlying mechanism (Bachler et al., 2024; Bernardi et al., 2016; Carrière et al., 2023; Jin et al., 2023; Kerns et al., 2023; Liu et al., 2024; Pickett et al., 2017; Roy et al., 2025; Yang et al., 2020; Yang et al., 2021b).

Only a few genes have been identified as being associated with Vip3Aa resistance in vivo. In *Helicoverpa armigera*, *HaVipR1* was identified as a crucial determinant of Vip3Aa resistance from two field-derived resistant lines, with its role in mediating resistance confirmed through knock-out experiments (Bachler et al., 2024). The involvement of this gene in Vip3Aa resistance was further validated in *Spodoptera frugiperda* (Zhang et al., 2024). Recently, two genes associated with Vip3Aa resistance in *S. frugiperda* were identified from two distinct field-derived populations in our group. The downregulation of *SfMyb* gene in DH-R strain confers 206-fold resistance (Jin et al., 2023), while a naturally occurring mutation of a chitin synthase gene (*SfCHS2*) in Sfru_R3 strain confers 5560-fold resistance (Liu et al., 2024).

The *CHS2* gene is primarily expressed in insect gut epithelial cells and produces chitin for the formation of the peritrophic matrix (PM) (Merzendorfer and Zimoch, 2003). However, the relationship between PM formation and Vip3Aa resistance has not been well defined (Jiang et al., 2023). Following the knock-out of *CHS2* genes in *S. frugiperda*, *Spodoptera litura*, and *Mythimna separata* in our last paper (Liu et al., 2024), we here extend the knock-out to two additional lepidopteran species—*Spodoptera exigua*, and *Agrotis ipsilon*. The results showed high levels resistance to Vip3Aa and the absence of PM in all knock-out strains, while the PM was retained in the Sfru_R3 strain. These findings provide further evidence of *CHS2* gene's association with Vip3Aa resistance in lepidopteran insects in general.

# 2. Materials and methods

## 2.1. Insect strains and rearing

The *S. frugiperda*, *S. litura*, and *M. separata* strains were described in our previous work (Liu et al., 2024). The Se-WT strain of *S. exigua* was first collected in Yantai City, Shandong Province, China, in 2023. The Ai-WT strain of *A. ipsilon* was purchased from Keyun Biological Co., Ltd. (China) in 2024. The wild-type strains and their corresponding knock-out strains, SeCHS2-KO and AiCHS2-KO, were maintained at 26 ± 2 °C and 70 ± 10% relative humidity (RH) under a 14 h light:10 h dark photoperiod. The larvae were fed with a

wheat germ based diet (Jin et al., 2021). Adults were provided with 10% sucrose solution.

## 2.2. Design and preparation of sgRNAs

The *SeCHS2* and *AiCHS2* genes were identified by using the protein sequence of SfCHS2 (protein_id: WXB24377.1) to perform a BLAST search against the *S. exigua* and *A. ipsilon* data base. The sgRNA target sites of *SeCHS2* and *AiCHS2* were then identified using the sgRNAcas9 (V3.0) software (www.biootools.com/software). Two sgRNAs were used for each insect species (Se-sgRNA1 targeting exon21 of *SeCHS2*: 5'-TGTCCACCACTAAATTGGATTGG-3'; Se-sgRNA2 targeting exon22 of *SeCHS2*: 5'- ACGACTGAACACCGACGACCTGG-3'; Ai-sgRNA1 targeting exon21 of *AiCHS2*: 5'- TCTCTACCACGCATCTCAACTGG-3'; Ai-sgRNA2 targeting exon22 of *AiCHS2*: 5'- ACGTCTGAACACAGACGACTTGG-3'. PAM sequences are underlined). The GeneArt Precision gRNA Synthesis Kit (Thermo Fisher Scientific, cat. no. A29377) was used to synthesize and purify the sgRNAs according to the manufacturer's instructions.

## 2.3. Embryo collection and microinjection

Freshly laid eggs (within 1 hour after oviposition) were collected and fixed onto a glass slide with double-sided tape. A mixture of sgRNAs (200 ng/μl) and Cas9 protein (500 ng/μl) (TrueCut™ Cas9 Protein v2, Thermo Fisher Scientific,

cat. no. A36497) was injected into individual eggs using a FemtoJet 4i and InjectMan 4 microinjection system (Eppendorf, Hamburg, Germany) within 1 hour. Injected eggs were placed at 26 ± 2℃ with 70 ± 10% relative humidity under a 14 h light:10 h dark photoperiod until hatching.

## 2.4. Mutagenesis detection and homozygous screening

Genomic DNA was extracted from the hind legs of adults prior to mating. Primers flanking the target sites were used to detect mutagenesis (Table S1), and the PCR product was sequenced by Sangon Biotech (Shanghai, China). Microinjected $G_0$ individuals were single paired with wild-type individuals to generate $G_1$ progeny. Heterozygous $G_1$ adults with the same mutation were crossed to generate $G_2$ progeny. The homozygous $G_2$ individuals were then crossed to establish a knock-out strain.

## 2.5. Bt toxin and bioassays

The Vip3Aa protoxin was purchased from Beijing Generalpest Biotech Research Co., Ltd. (Beijing, China). Diet overlay bioassays were conducted as previously described (Liu et al., 2024). Briefly, diet was dispensed into 24-well plates, and different concentrations of Vip3Aa (0, 200, 400, and 800μg /$cm^2$) were evenly applied to the surface and allowed to dry. A single unfed neonate was transferred to each well. After 7 days, mortality was recorded (larvae were considered dead if they had died or remained in the first or second instar).

SPSS18.0 was used to calculate the $LC_{50}$. $LC_{50}$ values with non-overlapping 95% confidence intervals (CIs) were considered significantly different. Resistance ratios were calculated by dividing the $LC_{50}$ of the tested strain by the $LC_{50}$ of the wild-type or susceptible strain (SS).

For homozygous knock-out individuals that suffered severe fitness cost and could not reach the adult stage, progeny from heterozygous crosses were used for the overlay bioassay. Survivors were subjected to PCR testing to determine their mutagenesis types.

## 2.6. Effect of corn leaves on fitness cost

Neonates and final instar larvae of the three *S. frugiperda* strains were used to test their fitness cost when fed non-Bt corn leaves. For neonates, 20 individuals were pooled in a single petri dish and fed with fresh leaves. There were six replicates, totaling 120 neonates per strain. The number of survivors were recorded daily. For final instar larvae, they were placed individually in 1oz plastic cups and fed with non-Bt corn leaves until they pupated. Sixty replicates were used for each strain. The final instar duration and weights of the pupae were recorded. In contrast, larvae of the three strains were also fed with artificial diet starting from the first instar. Their larval developmental time, pupal weight, and survival rate were recorded. The number of samples used

for each strain was 126 for Sfru-SS, 126 for Sfru_R3, and 88 for the SfCHS2-KO-A strain, respectively

All data were analyzed with SPSS18.0. The Kaplan-Meier method was used to assess the impact of strain on survival probability when neonates were fed with corn leaves, and Log-Rank test was used for pairwise comparisons. Kruskal-Wallis test was used detect the impact of strain on larval duration and pupal weight when treated with artificial diet, and Mann-Whitney test was used for post-hoc comparisons.

## 2.7. Dissection and histological analysis

The final instar larvae were used for dissection in all strains. The midgut was fixed with Bouin's solution to observe the presence of PM. For histological analysis of the midgut, the samples were fixed in 4% paraformaldehyde and sent to Servicebio (Wuhan, China) for paraffin embedding. Briefly, the samples were dehydrated using a serial grades of ethanol, followed by clearing with xylene. The pretreated midgut was embedded in paraffin (Sigma-Aldrich, USA), and the midgut samples were cross-sectioned into 5 μm thick slices. The paraffin sections were then deparaffinized through successive baths of xylene and rehydrated through serial grades of ethanol, stained with hematoxylin–eosin (Servicebio, Wuhan, China), and finally mounted with neutral resin (Servicebio, Wuhan, China). The slides were examined under a

digital pathology system (Pannoramic MIDI, 3DHISTECH Int.) to assess the morphological characteristics of the midgut samples.

# 3. Results

## 3.1. Knockout of *CHS2* resulted in high levels resistance to Vip3Aa toxin in *S. exigua* and *A. ipsilon*.

The *CHS2* gene sequences of *S. exigua* (GenBank ID: DQ912929.1) and *A. ipsilon* (GenBank ID: OL826754.1) were used to design sgRNAs to induce mutations at the target sites. Two strains with frameshift mutations were obtained and designated as SeCHS2-KO and AiCHS2-KO (Fig. S1). Each strain carries a deletion of 5 or 146 base pairs, respectively. The altered sequence of SeCHS2 introduced a stop codon in exon 21, yielding a truncated protein consisting of 1350 amino acids. Similarly, the modified *AiCHS2* sequence introduced a stop codon in exon 23, producing a truncated protein of 1,396 amino acids.

The bioassay results showed that SeCHS2-KO strain survived well on a diet overlaid with 800 μg/cm$^2$ Vip3Aa toxin, exhibiting a resistance ratio of >33,333-fold compared to the wild-type susceptible strain (Table 1). However, the homozygous mutants of *A. ipsilon* experienced a very high fitness cost, with only a few individuals reaching the adult stage and no offspring obtained. Alternatively, we used the progeny of heterozygous crosses

in the bioassay. Survivors were observed in all treatments, with 13 out of 72 progressing beyond the second instar when exposed to ≥200 μg/cm² (maximum: 800 μg/cm$^2$) Vip3Aa toxin (Table 2). PCR analysis showed that all these survivors were homozygotes (Fig. S2). The proportion of survivors did not significantly differ from the expected 25% homozygous ratio, as determined by a one-sample z-test for proportions ($p$ = 0.174). These results suggest that the resistance ratio of homozygous mutants of *A. ipsilon* is >11,268-fold compared to the wild-type susceptible strain.

Table 1 Responses of CHS2-knockout strains to Vip3Aa

| Strain | n[a] | $LC_{50}$ (95% CI)[b] | Slope ± SE | RR [c] |
|---|---|---|---|---|
| Sf-SS [d] | 192 | 0.13 (0.11 - 0.16) | 4.0 ± 0.6 | 1.0 |
| SfCHS2-KO-A [d] | 80 | > 1600[d] | NA | > 12,000 |
| Sl-SS [d] | 96 | 0.016 (0.0030 - 0.086) | 4.1 ± 0.8 | 1.0 |
| SlCHS2-KO [d] | 96 | >1600[d] | NA | > 100,000 |
| Ms-SS [d] | 168 | 1.20 (0.97 - 1.48) | 6.6 ± 1.0 | 1.0 |
| MsCHS2-KO [d] | 96 | >1600 [d] | NA | > 1,300 |
| Se-SS | 192 | 0.024(0.016 - 0.034) | 4.13 ± 0.78 | 1.0 |
| SeCHS2-KO | 96 | >800 | NA | >33,333 |
| Ai-SS | 144 | 0.071(0.032 - 0.10) | 3.68 ± 0.98 | 1.0 |

[a] Number of neonates tested.

[b] Median lethal concentration ($LC_{50}$); concentration that caused 50% of neonates to die or fail to advance to the third instar in 7 days and its 95% CI in

μg Vip3Aa per $cm^2$ diet.

[c] Resistance ratio: $LC_{50}$ for a strain or progeny from a cross divided by the $LC_{50}$ for SS.

[d] Data from (Liu et al., 2024).

CI, confidence interval; SS, susceptible strain.

Table 2 Bioassay with offspring from heterozygous crosses of *Agrotis ipsilon*

| Vip3Aa concentration ($\mu g/cm^2$) | Initial number | Survived number |
|---|---|---|
| 0 | 24 | 23 |
| 200 | 24 | 6 |
| 400 | 24 | 3 |
| 800 | 24 | 4 |

## 3.2. The peritrophic matrix was absent in the *CHS2* knockout strains.

Since *CHS2* is responsible for chitin synthesis in the midgut of lepidopterans, the five knockout strains (SfCHS2-KO-A, SlCHS2-KO, MsCHS2-KO, SeCHS2-KO, and AiCHS2-KO), and their respective wild-type strains, along with the resistance strain of *S. frugiperda* (Sfru_R3), were dissected at their final instar of larval stage. All samples were fixed with Bouin's solution, which imparts a bright yellow coloration to enhance visibility.

The dorsal view of the digestive tract in the three *S. frugiperda* strains revealed the presence of PM in the Sfru-SS and Sfru_R3 strains, but not in the SfCHS2-KO-A strain (Fig 1 a-c). Observing the midgut in isolation further emphasized this difference. Since the midgut and PM have different contraction ratios, the PM protrudes from the midgut when excised, providing a clear indicator of its presence (Fig 1 d). The results confirmed that the PM was absent in all five knockout strains, but present in the corresponding wild-type and Sfru_R3 strains (Fig. 1).

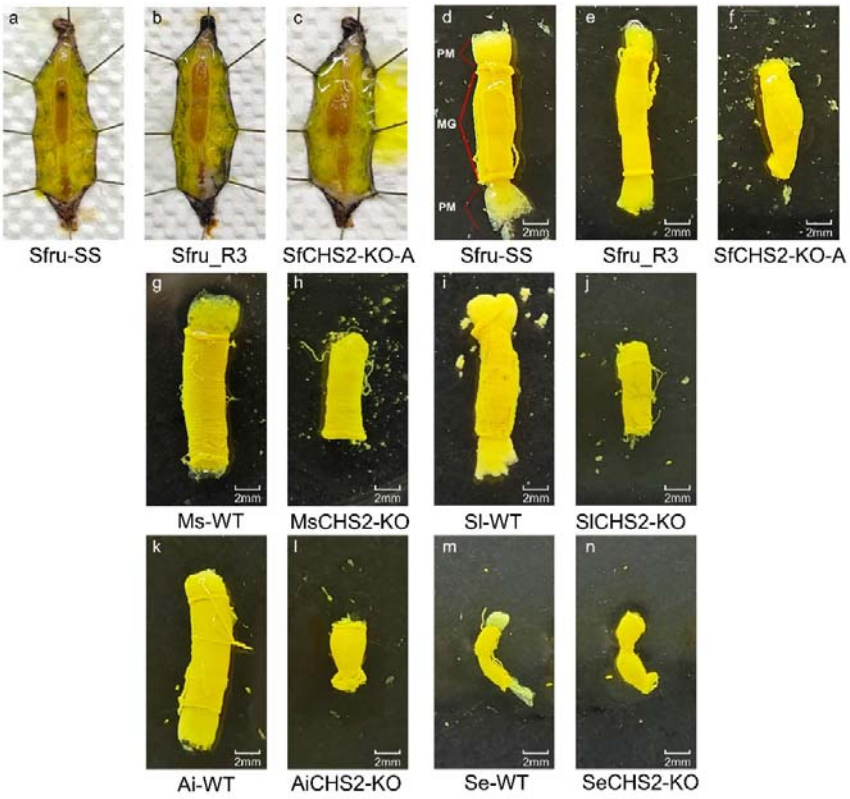


Fig. 1 The peritrophic matrix was absent in the CHS2 knockout strains. Dorsal view of the digestive tract in the three *S. frugiperda* strains (a-c) and excised midgut showing the presence of PM in different strains (d-n). Dissection of final instar larvae clearly showed that the PM was absent in all CHS2-KO strains

but present in Sfru_R3 and all wild-type strains. MG, midgut; PM, peritrophic matrix.

### 3.3. Histological structure analysis further confirmed the absence of PM in knockout strains of two species.

Strains of *S. frugiperda* and *A. ipsilon* were selected for histological structure analysis. Transverse sections of the midgut showed that the PM was absent in both knockout strains, SfCHS2-KO-A and AiCHS2-KO, but present in Sfru_R3 and wild-type susceptible strains (Fig. 2).

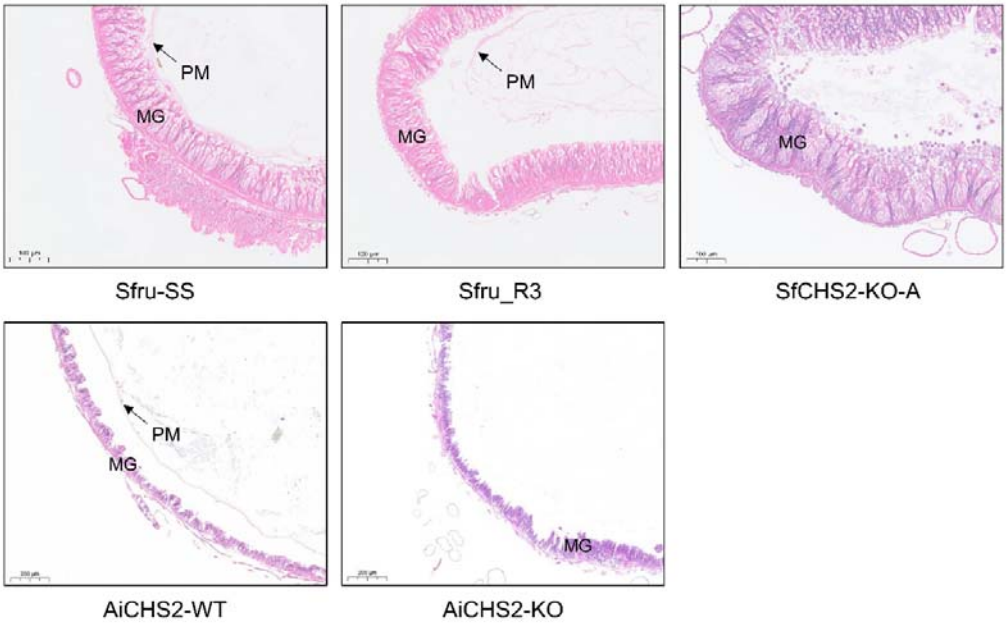


Fig. 2 Histological analysis of transverse sections through the midgut of S. frugipeda, and A. ipsilon. The results showed that the PMs were absent in SfCHS2-KO-A and AiCHS2-KO strains, but present in the other strains. MG, midgut; PM, peritrophic matrix.

### 3.4. Fitness costs and survival potential of chs2 mutant strains in different diet conditions

To assess survival chance of the *CHS2* mutant strains in the field, the first- and final-instar larvae of the *S. frugiperda* knockout strain SfCHS2-KO-A and the resistance strain Sfru_R3 were tested on fresh non-Bt corn leaves. All SfCHS2-KO-A neonates died within four days, while Sfru_R3 neonates exhibited a significantly higher mortality than the Sfru-SS strain during the first seven days (Fig 3 A). In final instar larvae, we found that the SfCHS2-KO-A strain was unable to pupate when fed on corn leaves, whereas both the Sfru-SS and Sfru_R3 strains successfully pupated, each with an 85% pupation rate. However, Sfru_R3 larvae took significantly longer to pupate than Sfru-SS ($p < 0.001$) (Fig. 3 B) and had a significantly lower pupal weight ($p < 0.001$) (Fig. 3 C).

In contrast, all three strains survived on an artificial diet, with survival rates from neonates to pupae of 91.27%, 96.03%, and 92.05% for Sfru-SS, Sfru_R3, and SfCHS2-KO-A, respectively. Sfru_R3 larvae had a significantly longer developmental time compared to the other strains (Fig. S3 A). Additionally, pupal weights differed significantly: SfCHS2-KO-A larvae were the heaviest, followed by Sfru_R3, with Sfru-SS being the lightest (Fig. S3 B).

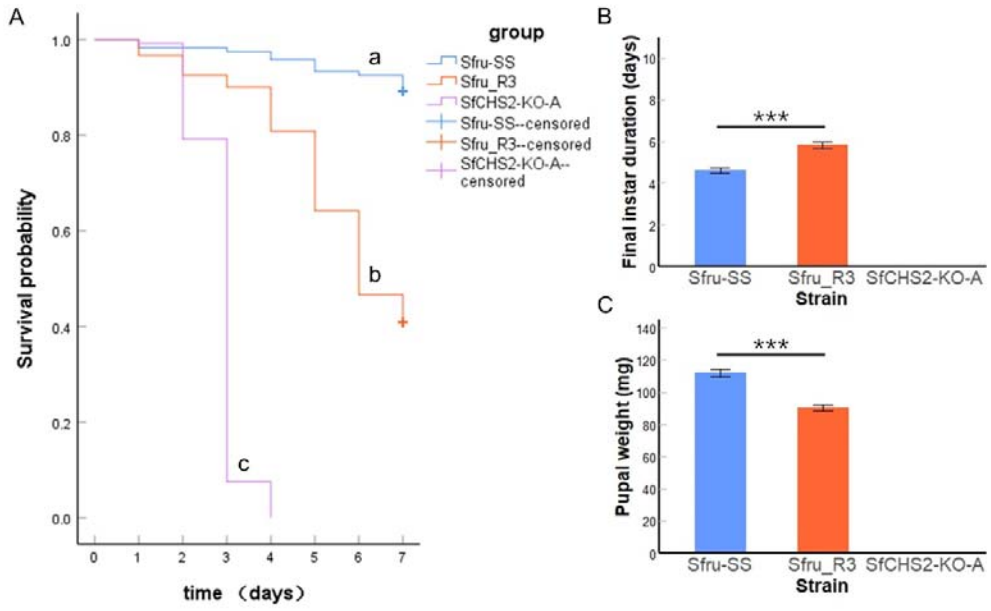


Fig. 3 Fitness costs and survival potential of *sfchs2* mutant strains on non-Bt corn leaves. (A) Survival curve of neonates fed on non-Bt corn leaves. (B) Final instar duration and (C) average pupal weights of *S. frugiperda* strains fed on non-Bt corn leaves from the last instar. Different letters indicate significant differences between groups ($p < 0.05$). ***, $p < 0.001$.

## 3.5. Histological structure analysis of the midgut in *S. frugiperda* strains after feeding on non-Bt corn leaves.

The transverse section through the midgut of the last instar larvae after feeding on non-Bt corn leaves revealed distinct structural differences among the strains. In the Sfru-SS and Sfru_R3 strains, the PM was intact and corn leaf fragments were well contained, forming a well-organized barrier. In contrast, the SfCHS2-KO-A strain lacked a PM, allowing direct contact between leaf fragments and epithelia cells (Fig 4).

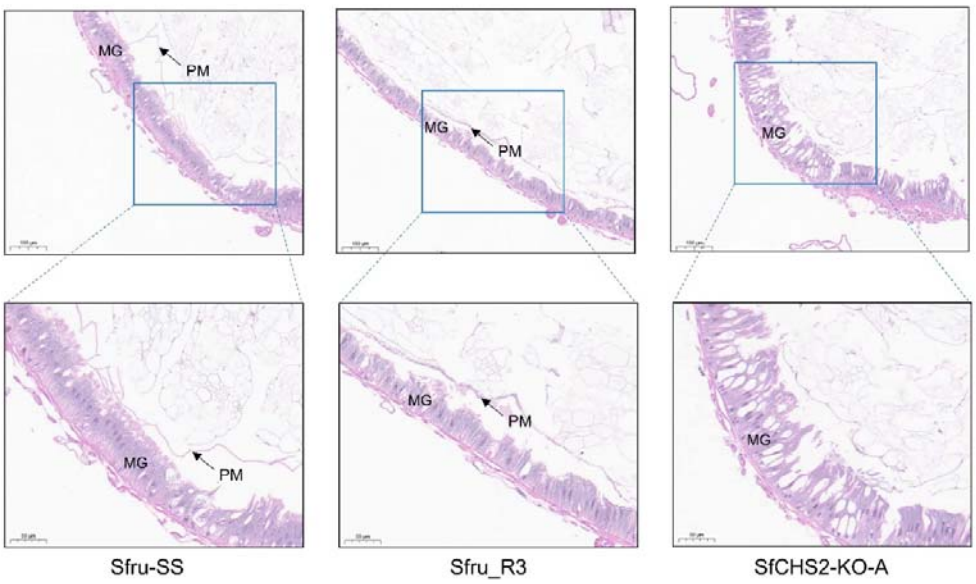


Fig. 4 Histological structure analysis of the midgut of three *S. frugiperda* strains after feeding on non-Bt corn leaves. Direct contact between leaf fragments and epithelia cells was observed in SfCHS2-KO-A strain. MG, midgut; PM, peritrophic matrix.

# 4. Discussion

In our previous work, we identified a retrotransposon (Yaoer)-mediated disruption of *CHS2* in *S. frugiperda*, which conferred high resistance to Vip3Aa (Liu et al., 2024). Knockout of this gene in *S. frugiperda*, as well as in two other lepidopteran species, *S. litura* and *M. separata*, resulted very high resistance to Vip3Aa. In this study, we generated knockout strains for two additional species, *S. exigua* and *A. ipsilon*, both of which also exhibited high resistance to Vip3Aa (Table 1 and Table 2). The role of CHS2 in determining Vip3Aa susceptibility across five species from three different noctuid tribes suggests a conserved function in Lepidoptera.

The *CHS2* knockout strains experienced significant fitness cost when maintained on artificial diets, except for the *S. frugiperda* SfCHS2-KO-A strain, which demonstrated robust survival on a wheat germ based diet (Fig S3). In contrast, the high mortality of homozygous individuals of the remaining four knockout strains may have resulted from heightened sensitivity to dietary composition, environmental conditions or pathogenic infections. These homozygous cultures were particularly prone to rapid collapse, making it necessary to maintain these strains predominantly in a heterozygous state to ensure their viability. Our efforts to establish a homozygous strain of *A. ipsilon* were unsuccessful, as only a few homozygous individuals reached adulthood, and their offspring failed to hatch. Additionally, efforts to develop a knockout strain of *H. armigera* were hindered by the strain's rapid collapse before any formal testing could be conducted.

The PM in insects functions as a permeable barrier between the food bolus and the midgut epithelium, facilitating digestive processes while protecting the brush border from mechanical damage and attacks by toxins and pathogens (Tellam, 1996). The PM is composed of a complex mixture of chitin, proteins, glycoproteins and proteoglycans (Wang and Granados, 2001). Our study demonstrates that knocking out the *CHS2* gene resulted in the absence of the PM in all five species during the larval stage, as shown in both dissection

results (Fig 1) and histological structure analysis (Fig 2). Transverse section images of the midgut showed that, in the absence of the PM, food debris directly contacted the epithelium, potentially causing mechanical damage (Fig 4). Many homozygous individuals died during the larval stage, suggesting impaired digestive capability.

The PM in the Sfru_R3 strain, on the other hand, remained intact (Fig 1), likely due to the reduced yet persistent presence of wild-type *SfCHS2* transcripts (Liu et al., 2024). It appears that the level of wild-type *SfCHS2* transcripts is negative correlated with the resistance ratio to Vip3Aa and, consequently, with the chitin content in the PM. Recent research has shown that the binding of Domain V of Vip3Aa toxin to the PM via glycan-binding activity contributes to Vip3Aa's insecticidal activity, while domains II-III of Vip3Aa were involved in binding to brush border membrane vesicles (BBMV) proteins (Infante et al., 2024; Jiang et al., 2023). However, the cytotoxicity of Vip3A against Sf9 and Sf21 cells indicates that while the presence of the PM is necessary for Vip3Aa toxicity, it is not solely sufficient for its full effect on larvae (Jiang et al., 2018a; Jiang et al., 2018b; Singh et al., 2010).

Assessing the survival potential of resistant and mutant strains on crops is crucial for effective pest resistance management. Our findings indicate that even the SfCHS2-KO-A strain, which thrives on an artificial diet, is unable to

complete development in either the first or sixth instar when fed corn leaves (Fig 3). One possible explanation is the damage caused by leaf debris to gut epithelial cells (Fig 4), which may increase vulnerability to pathogens and plant secondary metabolites (Chen et al., 2021; Zhu et al., 2023). In contrast, the Sfru_R3 strain successfully survived on corn leaves, underscoring the critical role of an intact PM for survival in the field. The fact that a laboratory-selected resistant strain can persist on crops raises concerns that high levels of Vip3Aa resistance could evolve in field populations, highlighting the need for vigilant resistance management. Notably, the same *SfCHS2* mutation found in the Sfru_R3 strain was detected in a sample collected from the field of China in 2020 (Liu et al., 2024).

Understanding how Vip3Aa interacts with its receptor and functions as an efficient insecticide is crucial for countering resistance in the field. It has been proposed that proteins such as scavenger receptor-C (Sf-SR-C), fibroblast growth factor receptor-like protein (Sf-FGFR), and Prohibitin II serve as Vip3Aa receptors in Sf9 cell line (An et al., 2022; Jiang et al., 2018a; Jiang et al., 2018b). However, knockout experiments using CRISPR/Cas9 in *S. frugiperda* failed to confirm Sf-SR-C and Sf-FGFR as functional receptors (Shan et al., 2022). To date, three genes have been reported as being associated with Vip3Aa resistance in *S. frugiperda*: *SfMyb*, *SfCHS2* and *SfVipR1* (Jin et al., 2023; Liu et al., 2024; Zhang et al., 2024). Additionally, a recent study indicates

that three distinct genetic loci are involved in Vip3Aa resistance in five resistant *H. zea* strains (Yang et al., 2024). Collectively, these findings indicate that resistance to Vip3Aa is likely determined by multiple factors, with mutations in *CHS2* having greater potential to confer high levels of resistance.

In summary, our findings demonstrate here that knocking out *CHS2* in five lepidopteran species confers high levels resistance to Vip3Aa toxin, with the phenotype closely linked to the absence of the PM. These results highlight the essential role of *CHS2* in maintaining PM integrity and its association with Vip3Aa susceptibility. Further investigation into the involvement of the PM in Vip3Aa resistance presents an intriguing research avenue. A deeper understanding of this mechanism could provide valuable insights into the mode of action of Vip3Aa and contribute to the development of innovative strategies for sustainable pest management.

# Data availability

All data generated and analyzed in this study are available within the article and the supplementary information.

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# Declaration of competing Interests

The authors declare no competing interests.

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