Transcriptome Analysis of Detoxification-Related Genes in *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Haoliang Chen,1,4,* Minghui Xie,1 Lulu Lin,1 Yongzhi Zhong,1 Feng Zhang,2,3 and Weihua Su1

1Anhui-CABI Joint Laboratory for Agricultural Pest Control, Institute of Plant Protection and Agro-Products Safety, Anhui Academy of Agricultural Sciences, Hefei 230031, China, 2MARA-CABI Joint Laboratory for Bio-safety, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China, 3CABI East & South-East Asia, Beijing 100081, China, and 4Corresponding author, e-mail: chenhaoliang@aaas.org.cn

Subject Editor: Amr Mohamed

Received 30 August 2021; Editorial decision 14 December 2021

Abstract

*Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is an important pest on maize, and it can cause large yield losses. As *S. frugiperda* has invaded many developing countries in Africa and Asia in recent years, it could impact food security. Pesticides remain the main method to control *S. frugiperda* in the field, and this pest has developed resistance to some pesticides. In this study, we used second-generation sequencing technology to detect the gene expression change of *S. frugiperda* after treatment by LC20 of three pesticides, lufenuron, spinetoram, and tetrachloroamide, which have different modes of actions. The sequence data were first assembled into a 60,236 unigenes database, and then the differential expression unigenes (DEUs) after pesticide treatment were identified. The DEU numbers, Gene Ontology catalog, and Kyoto Encyclopedia of Genes and Genomes pathway catalog were analyzed. Finally, 11 types of unigenes related to detoxification and DEUs after pesticide treatment were listed, and Cytochrome P450, Glutathione S-transferase, and ATP-binding cassette transporter were analyzed. This study provides a foundation for molecular research on *S. frugiperda* pesticide detoxification.

Key words: transcriptome, fall armyworm, differential expression unigene, pesticide detoxification

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is a destructive pest of maize and is a native species of tropical and subtropical origin in the Western Hemisphere (Sparks 1979). Because *S. frugiperda* has a high reproductive rate of approximately 1,000 eggs per female and a relatively short generation time of about 30 d, the voracious feeding of the larva, significantly reduces grain yield (Johnson 1987). *S. frugiperda* is highly polyphagous. It can feed on 353 species from 76 plant families, including corn, rice, sorghum, sugarcane, vegetables, cotton, and other crops (Montezano et al. 2018). *S. frugiperda* can quickly destroy a maize crop due to its heavy feeding, and, without timely control it may destroy other crops. The yield of corn in Brazil was reduced by 34% after *S. frugiperda* attack, and the annual loss caused by *S. frugiperda* reached 400 million US dollars (Figueiredo et al. 2005, Lima et al. 2010). In the nine southeastern states of the United States, *S. frugiperda* caused economic losses ranging from 39 to 297 and averaged 60 million dollars annually from 1975 to 1983 (Sparks 1986). Since 2016, *S. frugiperda*, as an invasive pest, has been found in Sub-Saharan Africa, and South and Southeast Asia. It may cause a serious impact on food security because of the less development and the high population density in the newly invaded countries.

Various methods have been developed to control *S. frugiperda* in its native Americas and other countries after it spread to Africa and Asia, such as planting Bt Maize (Bunting et al. 2004, Storer et al. 2012), agro-ecological approaches (Harrison et al. 2019), biological control (Molina-Ochoa et al. 2003, Kenis et al. 2019, Koffi et al. 2020), pheromone-based trapping (Batista-Pereira et al. 2006, Unbehend et al. 2014), and chemical pesticides (Pitre 1986, Yu 1991). Using synthetic insecticides to control *S. frugiperda* is the most widely used method to minimize the damage of maize fields, and also it is the most important method in integrated pest management to control this pest (Andrews 1988, Sisay et al. 2019). Due to the use of various chemical insecticides in the North and Central America, *S. frugiperda* has developed resistance to at least 40 active ingredients including organophosphorus, carbamates, pyrethroids, and Bt protein (https://www.pesticideresistance.org/display.php?page=species&arId=200). *S. frugiperda* can develop resistance very rapidly. For example, in Puerto Rico, flubendiamide, chlorantraniliprole, spinosad, and spinetoram were considered as effective pesticides to control *S. frugiperda* in 2012, but by 2018, *S. frugiperda* had developed high levels of resistance to a variety of new insecticides in Puerto Rico, such as flubendazole (500 times) and
chlorantraniliprole (160 times) (Belay et al. 2012, Gutiérrez- Moreno et al. 2019).

With the development of second-generation sequencing technology, it’s gives us a chance to comprehensively understand gene expression after pesticides are used to treat the insect, and to detect the genes that may be involved in detoxification. Tetrachloroamide, lufenuron, and spinetoram with the target of ryanodine receptor, chitin synthase, and nicotinic acetylcholine receptors, respectively, were considered effective against S. frugiperda, and also were recommended by the Ministry of Agriculture and Rural Affairs of the People’s Republic of China to control S. frugiperda in 2020–2021. In this study, sublethal doses of these three pesticides were used to treat S. frugiperda larvae, and gene expression was detected by RNA-Seq. The different expression unigenes (DEUs) after pesticide treatment and the unigenes related to detoxification were analyzed. The results of this study will provide a basic knowledge of what kind of genes may be involved in the pesticide detoxification of S. frugiperda.

Materials and Methods

Insect Rearing

S. frugiperda were first obtained from Chizhou, Anhui province, China (117.04 E, 30.48 N) in 2019, then reared about 10 generations on corn leaves in a walk-in chamber at 25 ± 1°C, 75% RH, and a photoperiod of 16:8 (L:D) h. Later instar larvae were collected from the cornfield and reared individually in a small plastic cup with a cover (4.5 cm diameter by 4 cm high). After pupation, the pupae were put into a petri dish without cover and then placed into a 4-mesh metal cage (length: 40 cm, width: 25 cm, higher: 20 cm). A piece of wax paper was covered on the top of the metal cage, and a moist towel was pressed on the paper. The adults of S. frugiperda would lay eggs on the wax paper after mating, then the paper with eggs was removed and put into a plastic cage (length: 24 cm, width: 16 cm, higher: 8 cm) with some corn leaves. Finally, 80 mesh nylon net with rubber band was used to cover the plastic cage. About one week later, the larvae had grown to the third instar, and larvae were then reared individually in a small plastic cup with a cover (diameter: 4.5 cm, height: 4 cm) until pupation.

Pesticide Treatment and RNA Isolation

Three technical products of pesticides were obtained from Shenyang Research Institute of Chemical industry (Tetrachloroamide 91.6%), Shandong Renjie Biotechnology Co., Ltd (Lufenuron 96.7%), and Shandong Lifa Biotechnology Co., Ltd (Spinetoram 95.6%).

The LC_{50} of the three pesticides were determined by the method of leaf-dip bioassays modified from the study of Ismail and Wright (Ismail et al. 1991). Briefly, acetone was used to dissolve the active ingredient insecticides to 10,000 mg/L, and then dilute with water to test concentration gradually. The corn leaves were cut into 3 cm x 3 cm squares and immersed into the solution for 5 s. After removal, the leaf strips were put in the tray for air drying. The dried corn leaves were put into a 6-well cell culture plate, and one 3rd instar larva was placed on each corn leaf. The 3rd instar larvae were starved for 4 h in advance, and each treatment had 90 larvae in 3 replicates. Corn leaves immersed into the water for 5 s were used as a control. After treatment, the test insects were placed in the insect rearing room, the temperature was set at 25°C, and a photoperiod of 16:8 (L:D) h. After 72 h, the mortality was recorded. The death of the larva was determined by lack of movement when the brush touched the larva. The mortality and corrected mortality were calculated, and the LC_{50} of each period was calculated by Probit Model in SPSS 16.0 (SPSS Inc., Chicago, IL, USA) (Supp File 1 [online only]). The LC_{50} of tetrachloroamide, spinetoram, and lufenuron were 0.177 mg/L, 0.094 mg/L, and 7.547 mg/L, respectively. S. frugiperda 3rd instar larvae were treated with a LC_{50} concentration of pesticides for 72 h, and one live larva was collected for RNA extraction, each treatment has 3 replicates.

RNA extraction was performed by a Total RNA Isolation System Kit (SV Total RNA Isolation System Kit, Promega Corporation, Madison, WI) according to manufacturer’s protocol. Deoxyribonuclease (Dnase I: Fermentas Inc., Burlington, ON, Canada) was used to remove residual genomic DNA in RNA samples. The values of RIN (RNA integrated number) for RNA samples detected by Agilent 2100 were from 8.9 to 9.5 (Supp File 2 [online only]).

CDNA Library Preparation and RNA-Seq

Each RNA sample prepared in the previous step was treated by a standard protocol to synthesize CDNA first and then sequenced. Briefly, total RNA was treated by magnetic beads with Oligo dT to enrich the mRNA with PolyA tail. Enriched mRNA was segmented by using the interrupt buffer, and then the random N6 primers were used for reverse-transcription to synthesize the double-strand cDNA. Synthesized double-stranded cDNA were flattened and phosphorylated at the 5’ end. The 3’ end formed a sticky end protruding ‘A’, and then was ligated with bubble adapter. The ligation products were amplified by PCR with specific primers, and then the PCR products were heat-denatured into single-strand DNA. Bridge primers were used to cyclize the single-strand DNA to a circular DNA library. Finally, the BGISEQ-500 sequencing platform from Beijing Genomics Institute (BGI; Shenzhen, China) was used to sequence the prepared library. The raw reads were deposited in the NCBI Sequence Read Archive database under the accession number from SRR14476538 to SRR14476549 with BioProject number PRJNA728479.

Unigene Assembly, Analysis, and Annotation

Clean reads were obtained after filtering the reads with low quality, contaminated adapters, and more than 5% unknown nucleotides. After that, the reference unigenes database was assembled by all samples’ clean reads. De novo assembly method and function annotation were used method described in Wei et al. (2019). Briefly, all sample clean data were put together to get the de novo assembly unigene database using Trinity (http://trinityrnaseq.sourceforge.net/) (Grabherr et al. 2011). The unigene completeness was evaluated by BUSCO v5.1.2 (Seppey et al. 2019). Coding sequences (CDs) of unigenes were predicted using TransDecoder (v5.0.1) (https://github.com/TransDecoder/TransDecoder). MISA (v2.1) was used to detect the SSR in unigenes (Beier et al. 2017). The assembled unigene database was annotated with the NCBI nucleotide (NT), Nonredundant protein sequences (NR), Swiss-Prot, and Cluster of Orthologous Groups function (COG) databases. BLAST2GO was used to assign the unigenes into three different Gene Ontology (GO) classifications: molecular functions, biological processes, and cellular components (https://www.blast2go.com/) (Conesa et al. 2005). Finally, unigenes were mapped into the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto 2000). The unigenes related to detoxification were analyzed.

Differential Expression Unigenes (DEUs) Detecting

The expression of each unigene was calculated by fragments per kilobase of transcript per million mapped reads (FPKM) value (Mortazavi et al. 2008). DEUs between samples were detected...
according to the FPKM value; a FPKM value ratio between two treatments > 2 or < 0.5 was considered to be significant. Use FPKM value ratio between different samples to detected DEUs were proved effective in the previous studies (Chen et al. 2017, Chen et al. 2019, Wei et al. 2019). The DEUs’ GO functional group and KEGG mapping were analyzed (Kanehisa and Goto 2000; Conesa et al. 2005). The number of DEUs classified into each GO catalog and KEGG pathway catalog after different pesticides treatments were calculated in comparison with the control, and the most 20 enrichment of GO terms and KEGG pathways were also analyzed.

**Results**

**Result of RNA Sequencing and Assembled Unigenes**

In this study, each sample had more than 42.70 million clean reads and 6.40 Gb clean bases, the percentage of clean reads Q20 (error probability less than 0.01) was higher than 97.84%, and the ratio of clean reads was more than 97.44%; all of these statistics showed the high quality of sequencing (Supp File 3 [online only]). All samples’ clean data were used to assemble the unigene database by Trinity software, and 60,236 unigenes with a mean length of 1747 bp and N50 (50% of unigenes are longer than this length) of 1153 bp were produced (Table 1). The GC percentage of all of the assembled unigenes was 39.84% (Table 1). The number of unigenes between 200 and 499 bp was the largest group, and the number of unigenes decreased with an increase in the unigene length (Fig. 1). Among the assembled unigenes, 31,252 with CDSs were detected; the group of unigenes with CDSs of length between 500 and 999 bp was the largest, and then the number group of unigenes with CDSs decreased with the increase in the unigene length, except for those of length 200–499 bp (Fig. 1). The percentage of unigenes with CDSs in all assembled unigenes within the same length range increased first and then decreased as the unigene length increased (Fig. 1). The highest percentage of the unigenes with CDSs were those between 1,000 and 1,499 bp in length, and the percentage was 93.63% (Fig. 1). BUSCO results showed 94.72% of the genes in BUSCO database were completely matched, and the genes in which only the fragments or missing matched were 4.62% and 0.66%, respectively. SSR detection in the unigenes is listed in Supp File 4 (online only); the number of unigenes decreased with the increase of duplicate nucleotides.

**Annotation to Public Database**

The number and percentage of unigene annotations to the different public databases are shown in Table 2. More than 50% of the unigenes could be annotated in NR, NT, and KEGG database, and overall 78.96% of the unigenes could be annotated in at least one database. In total, 62.67% of assembled unigenes had annotated results after comparison against the NT database, which is the largest group when annotating to public databases. In total, 30,120 unigenes could map into 44 KEGG pathway categories, and only 33.28% assembled unigenes could assign into GO terms, which was the smallest group when compared against the public databases (Table 2).

**DEUs After Pesticide Treatments**

Pesticide treatment affected the unigenes expression in *S. frugiperda*. Figure 2 shows the number of up- and down-regulated unigenes that changed more than two fold between pesticide-treated samples and the control. About one-third of unigenes changed in expression after pesticide treatment. For detail, 10,833 unigenes were up-regulated after lufenuron treatment, while 10,144 were down-regulated; after tetrachloroamide treatment, 10,594 unigenes were up-regulated and 10,099 were down-regulated; 7,536 unigenes were up-regulated and 9,949 were down-regulated after spinetoram treatment (Fig. 2). Comparing the DEUs with the pesticide treatments and the control, the expressions of 6,720 unigenes were synchronously changed after the three pesticide treatments, which was the largest group in Fig. 3. The common DEUs of the two comparisons were from 10,057 to 12,170, and the unique DEUs after pesticide treatment were from 4,082 to 5,470 (Fig. 3).

**DEUs’ GO Identification and KEGG Pathway Mapping**

For the lufenuron treatment compared to the control, 7,794 DEUs could be cataloged in 26,469 GO terms; for tetrachloroamide treatment, 7,579 DEUs were cataloged in 26,385 GO terms; and for spinetoram treatment, 6,764 DEUs in 22,951 GO terms. The pattern of DEUs numbers classified into the GO catalog in the three comparisons looked similar (Fig. 4A, C, and E). For all three comparisons, ‘catalytic activity’ and ‘binding’ from the main GO category ‘molecular function’ were the largest two GO catalog groups, followed by ‘membrane part’ and ‘membrane’ from the main GO category ‘cellular component’, and ‘cellular process’ and ‘metabolic process’ from the main GO category ‘biological process’ (Fig. 4A, C, and E). For all three comparisons, GO term of ‘integral component of membrane’ followed by ‘extracellular region’ and ‘transmembrane transport’ enriched the greatest number of unigenes. For lufenuron and

---

**Table 1. Parameters of the assembled unigenes**

| Sample        | Total number | Total length | Mean length | N50   | GC (%) |
|---------------|--------------|--------------|-------------|-------|--------|
| Assembled unigene | 60,236       | 1.05E+08     | 1747        | 1153  | 39.84  |

---

**Fig. 1.** Effect of unigene length on assembled unigene number, number of unigenes with coding sequences (CDSs), and the percentage of unigenes with CDSs. The x-axis means the unigene length, the left y-axis means the number of unigene, including assembled unigene number and the number of unigene with CDSs, and right y-axis means the percentage of unigenes with CDSs in all assembled unigenes within the same length range.
### Table 3. Result of assemble unigenes annotation to public database

| Values          | Total   | NR     | NT      | Swissprot | KEGG    | COG     | GO      | Overall |
|-----------------|---------|--------|---------|-----------|---------|---------|---------|---------|
| Number          | 60,236  | 36,843 | 37,751  | 28,116    | 30,120  | 78.96   | 33.28   | 78.96   |
| Percentage (%)  | 100     | 61.16  | 62.67   | 46.68     | 50.00   | 45.02   | 33.28   | 78.96   |

Table 3

- **NT**: Numbers in each circle mean the DEU number of the pesticides treated versus the control.
- **LU**: Lufenuron; **SP**: Spinetoram; **TE**: Tetrachloroamide.

**Fig. 2.** Up- and down-regulated unigenes after different pesticide treatments. **CK**: Control; **LU**: Lufenuron; **SP**: Spinetoram; **TE**: Tetrachloroamide.

**Fig. 3.** DEUs comparison between pesticide treatments and the control. The number in each circle means the DEU number of the pesticides treated versus control. **CK**: Control; **LU**: Lufenuron; **SP**: Spinetoram; **TE**: Tetrachloroamide.

### Discussion

In this study, 60,236 assembled unigenes were generated. This was less than the 71,425 contigs assembled in the Silva-Brandão’s (2017) study, but 47,565 (78.96%) of unigenes got annotation results in this study, which is higher than Silva-Brandão’s (2017) study (45,036 contigs, 63.05%). The mean length of unigenes in our study was 1747 bp, which was much longer than Silva-Brandão’s (937 bp). While the GC% of assembled unigenes in both studies was similar (39.84% in this study and 40.40% in Silva-Brandão (2017) study).

The numbers of cytochrome P450 in *S. frugiperda* were variable. In our study, 190 unigenes were identified as cytochrome P450, but in Xiao’s (2020) study, they identified 169 cytochrome P450, while in Gouin’s (2017) study, they obtained 117–176 cytochrome P450 for *S. frugiperda* in different strains. In Xiao’s (2020) study, they analyzed the gene number of the CYP clades, and the largest group clade was the CYP4 clade, followed by the CYP3 clade, Mito clade, and CYP2 clade. This is different from our study (Supp File 9 [online only]) and Gouin’s (2017) study, which CYP3 clade is the largest clade, followed by clade 4, Mito clade, and CYP2 clade, although in this study and Gouin’s (2017) study the number of cytochrome P450 genes in each clade is different. *S. frugiperda* is a polyphagous lepidopteran pest, which means it should develop a powerful detoxification system to metabolize toxic chemicals from host plants (Gatehouse, 2002; Desprès et al., 2007). As the major detoxification enzymes in insects, usually, the number of cytochrome P450 is around 100 in insect genomes (Feyereisen 1999), but the number of cytochrome P450 genes in *S. frugiperda* is much more than in other species because of its polyphagous. Cytochrome P450 genes are also considered related to insecticide resistance and detoxification in *S. frugiperda* (do Nascimento et al. 2015, Giraudo et al. 2015, Zhang et al. 2020). As an insect with high resistance to conventional pesticides (Zhang et al. 2020), it may have a large group of cytochrome P450 genes that deal with detoxification. In our study, Cytochrome P450-related unigenes are not only the largest group in considered detoxification-related genes but also the largest group of DEUs after all three pesticide treatment in considered genes (Table 3). In this

### Unigenes Related to Detoxification

The numbers of 11 types of unigene related to detoxification in *S. frugiperda* are listed in Table 3. The name of the unigenes and their expression after the three pesticide treatments compared with the control are listed in Supp Files 5–8 (online only). The assembled sequences of Cytochrome P450, GST, and ABC transporter were deposited in GenBank, and the accession number for each unigene was listed in Supp Files 5 (online only). The classification of Cytochrome P450, GST, and ABC transporter were listed in Supp Files 9–11 (online only). Cytochrome P450 had the largest unigene group in the considered gene type, and it also had the largest group of DEUs in all three pesticide treatments versus the control. The group of unigene, which was considered cuticle protein in de novo database, ranked third among the 11 detoxification-related gene types, but after pesticide treatment, it had the highest percentage of gene expression changes, and it was the second largest group of DEUs in all three comparisons (Table 3). In the group of cuticle protein, more than 85% of DEUs in all three comparisons were down-regulated (Table 3). Trypsin was the second largest group in the detoxification-related genes, and more than 50% of trypsin unigenes’ expression was changed (Table 3).
study, nine unigenes of Cytochrome P450 were always upregulated after three pesticides treatment, five of which were from CYP6B subfamily, and other four genes were from subfamily CYP6A, CYP4G, CYP321, and CYP337. Those nine cytochrome P450 gene were belonged to CYP3 clade except for one CYP4G subfamily gene, from CYP4 clade. CYP3 clade were considered to be related to xenobiotic metabolism and insecticide resistance, and also can be inducible by pesticides (Feyereisen 2006). Three out of six significantly increased CYP450 genes were from CYP6B subfamily of *S. frugiperda* treated with Noposion Yihao 5% EC (Hafeez et al. 2021).

GSTs are a large family of enzymes in insects with multi-functional activity, and they play an important role in the detoxification of both endogenous and xenobiotic compounds including insecticides (Enayati et al. 2005). We identified 30 unigenes related to GST in this study, which was less than the 59 GST genes identified by Xiao’s (2020) study, but similar to Gui’s (2020) study, which had

---

**Fig. 4.** The number of DEUs classified into the GO catalog (A, C, and E) and GO enrichment of top 20 GO terms (B, D, and F) using different pesticides in comparison with the control. (A) and (B): treatment with lufenuron versus control, (C) and (D): treatment with tetrachloroamide versus control, and (E) and (F): treatment with spinetoram versus control.

**Fig. 5.** The number of DEUs mapped into the KEGG pathway catalog (A, C, and E) and KEGG enrichment of the top 20 KEGG pathways (B, D, and F) using different pesticides compared to the control. (A) and (B): treatment with lufenuron versus control, (C) and (D): treatment with tetrachloroamide versus control, and (E) and (F): treatment with spinetoram versus control.
Table 3. The unigenes related to detoxification and DEGs after pesticide treatments

| Gene name                                      | Unigenes number in de novo database | LU vs CK | TE vs CK | SP vs CK |
|------------------------------------------------|------------------------------------|----------|----------|----------|
| Glutathione S-transferase                      | 30                                 | 8        | 6        | 9        |
| Carboxylesterase                               | 32                                 | 5        | 12       | 9        |
| Cytochrome P450                                 | 190                                | 48       | 60       | 63       |
| NADH dehydrogenase                             | 36                                 | 2        | 9        | 2        |
| Trypsin                                        | 97                                 | 21       | 29       | 31       |
| Superoxide dismutase                           | 10                                 | 3        | 1        | 5        |
| ABC transporter/ATP-binding cassette transporter| 63                                 | 19       | 12       | 5        |
| Cuticle protein                                | 82                                 | 4        | 64       | 8        |
| UDP-glucuronosyltransferase                    | 74                                 | 24       | 13       | 24       |
| Acetylcholine receptor                         | 15                                 | 4        | 1        | 4        |
| Chloride channel                               | 9                                  | 0        | 3        | 0        |

Up: CK: control; LU: Lufenuron; SP: Spinetoram; TE: Tetrachloroamide.

29 GST genes, while in Gouin’s (2017) study, they reported 45 GST genes. There were 8–38 GST genes in the list of 15 insect species in Fang’s (2012) study, and 23–45 GST genes for 6 insect species in Gouin’s (2017) study. Only 7 out of 15 species had all six (Delta, Epsilon, Omega, Sigma, Theta, and Zeta) classes. In this study, Theta and Zeta classes were missing in S. frugiperda (Supp File 10 [online only]), while in Gouin’s (2017) study, there have one and two gene in Theta and Zeta classes respectively. Epsilon is the largest GST group in S. frugiperda, and this group gene is usually considered to be related to the metabolism of organophosphate and organochlorine insecticides (Huang et al. 1998, Wei et al., 2001, Ortelli et al., 2003). This may indicate that S. frugiperda has a strong capacity to metabolize pesticides, and it is easy to develop pesticide resistance. The expression of four GST genes in lufenuron resistant strain was significantly higher than that in lufenuron susceptible strain in S. frugiperda indicate the GST may be involved in detoxification of lufenuron (do Nascimento et al. 2015). At least one-third of GST-related unigenes changed expression after treatment with the three pesticides indicating that the GSTs were involved in metabolism of all three pesticides (Table 3). In the DEUs, Unigene11892 and Unigene22019 changed expression after all three pesticide treatments, but both of them were up-regulated after being treated by lufenuron and tetrachloroamide and both of them were down-regulated after being treated by spinetoram. This may be because of the different modes of action of these three pesticides.

ABC transporters are a group of transmembrane proteins in eukaryotic cells, which can excrete transport substrates and allocrites from the cells (Gott et al. 2017). In insects, ABC transporters also can excrete chemicals including pesticides and be involved in pesticide resistance (Srinivas et al. 2004, Porretta et al. 2008, Strycharz et al. 2013). ABC transporter genes were considered involved in pesticide resistance in S. frugiperda and other insects (Merzendorfer 2014). In this study, we identified 63 unigenes as ABC transporter in S. frugiperda, and the number is between Xiao’s (2020) and Gui’s (2020) studies, the numbers in their studies were 79 and 58, respectively. While in Gouin’s (2017) study, they reported 46 and 47 ABC transporter genes for corn strain and rice strain respectively. In this study, ABC transporter unigenes were classified into six ABC gene subfamilies, but no unigenes were classified into the subfamily of E and H (Supp File 11 [online only]). In most insect species, there was only one gene in the ABC transporter subfamily E (Merzendorfer 2014), which may be missed during sequencing. Several insect species listed in Merzendorfer’s study (Merzendorfer 2014) missed subfamily H, which may indicate not all insect species have eight ABC transporter subfamilies. The ABC transporter subfamily G is the largest group in our study, and in the 11 species listed in Merzendorfer’s study (Merzendorfer 2014), ABC transporter subfamily G of 5 species is the largest group of the subfamily. There were only 9 ABC transporter unigenes in DEUs after tetrachloroamide treatment, which was less than 31 and 24 DEUs after lufenuron and spinetoram treatments, respectively (Table 3). The expression of three same unigenes was changed after all three pesticide treatments and CL2487. Contig1 was the most up-regulated unigenes in ABC transporters in all three pesticide treatments.

After exposure to the three pesticides, the expression of 17,485–20,977 unigenes changed (Fig. 2), which accounted for one-third of all assembled unigenes and indicated that treatment with the three pesticides had a great influence on insect metabolism. The synchronous changed unigenes after the three pesticide treatments were the largest group (Fig. 3), indicating that many genes were regulated by the stimulation by exogenous substances. The similar patterns of DEGs’ GO and KEGG catalog after pesticide treatment (Figs. 4 and 5) means that the unigenes regulated by pesticides have similar function and pathways involved in detoxification. There were 4,082–5,470 (23.35%–26.08%) unigenes regulated by unique pesticides in this study, and this may be because the three pesticides had different modes of action.

Conclusions
We provided information about the genes of S. frugiperda involved in the response to the modes of action of three pesticides: lufenuron, spinetoram, and tetrachloroamide. The DEUs’ GO catalog and KEGG pathway can provide more information for understanding the pesticide tolerance and detoxification mechanisms of S. frugiperda. The detailed analysis of Cytochrome P450, Glutathione S-transferase, and ATP-binding cassette transporter in S. frugiperda and the information about DEUs of 11 types of detoxification-related genes could lay a foundation for further study on pesticide tolerance and detoxification.

Supplementary Data
Supplementary data are available at Journal of Insect Science online.
Supplementary File 1: LC_{50}, LC_{90}, and 72h bioassay results of LC_{50} for three pesticides; Supplementary File 2: The quality values of RNA samples; Supplementary File 3: The quality statistics of clean reads; Supplementary File 4: SSR detection of the unigenes; Supplementary File 5: Unigenes related to detoxification; Supplementary File 6:
DEUs of lufenuron versus control; Supplementary File 7: DEUs of tetrachloroamide versus control; Supplementary File 8: DEUs of spinetoram versus control; Supplementary File 9: P450 Clade classification based on annotated results; Supplementary File 10: GST classification based on annotated results; Supplementary File 11: ABC transporters classification based on annotated results.

Acknowledgments
We thank Guangling Zhang for technical assistance. This research was funded by Major Science and Technology Projects in Anhui Province (201903a06020027), the National Key R&D Program of China (2019YFD0300105), the Team Project of Anhui Academy of Agricultural Sciences (2022YJ1026), and China’s donation to CABI Development Fund (VM10051).

Author Contributions
H.C., F.Z., and W.S. conceived research, M.X., I.L., and Y.Z. conducted the experiments and conducted statistical analyses, H.C. and M.X. wrote the manuscript. All authors read and approved the manuscript.

References Cited
Andrews, K. L. 1988. Latin American Research on Spodoptera frugiperda (Lepidoptera: Noctuidae). Fla. Entomol. 71: 630–633.
Batista-Pereira, L. G., K. Stein, A. F. de Paula, J. A. Moreira, I. Cruz, M. d. e. L. Figueiredo, J. Perri, Jr. and A. G. Corrêa. 2006. Isolation, identification, synthesis, and field evaluation of the sex pheromone of the Brazilian population of Spodoptera frugiperda. J. Chem. Ecol. 32: 1085–1099.
Beier, S., T. Thiel, T. Münch, U. Scholz, and M. Mascher. 2017. MISA-web: a web server for microsatellite prediction. Bioinformatics. 33: 2583–2585.
Belay, D., R. Huckaba, and J. Foster. 2012. Susceptibility of the fall armyworm, Spodoptera frugiperda (Lepidoptera: Noctuidae), at Santa Isabel, Puerto Rico, to different insecticides. Fla. Entomol. 95: 476–478.
Bunton, G. D., K. L. Flanders, and R. E. Lynch. 2004. Assessment of experimental Bt events against fall armyworm and corn earworm in field corn. J. Econ. Entomol. 97: 259–264.
Chen, H., L. Lin, M. Xie, G. Zhang, and W. Su. 2017. Using next-generation sequencing to detect differential expression genes in BradyDia odoriphaga after exposure to insecticides. Int. J. Mol. Sci. 18: 2445.
Chen, H., L. Lin, F. Ali, M. Xie, G. Zhang, and W. Su. 2019. Genome-wide analysis of developmental stage-specific transcriptome in Bradydia odoriphaga. Comp. Biochem. Physiol. Part D. Genomics Proteomics. 30: 45–54.
Conesa, A., S. Götz, J. M. García-Gómez, J. Terol, M. Talón, and M. Robles. 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics. 21: 3674–3676.
Després, L., J. P. David, and C. Gallet. 2007. The evolutionary ecology of insect resistance to plant chemicals. Trends Ecol. Evol. 22: 298–307.
do Nascimento, A. R., P. Fresa, F. L. Consoli, and C. Omoto. 2015. Comparative transcriptome analysis of lufenuron-resistant and susceptible strains of Spodoptera frugiperda (Lepidoptera: Noctuidae). BMC Genomics. 16: 985.
Enayati, A. A., H. Ranson, and J. Hemingway. 2005. Insect glutathione transferases and insecticide resistance. Insect Mol. Biol. 14: 3–8.
Fang, S. 2012. Insect glutathione S-transferases: a review of comparative genomic studies and response to xenobiotics. Bull. Insectol. 65: 265–271.
Feyereisen, R. 2006. Evolution of insect P450. Biochem. Soc. Trans. 34: 1252–1255.
Feyereisen, R. 1999. Insect P450 enzymes. Annu. Rev. Entomol. 44: 507–533.
Figueiredo, M. L. C., A. M. Penteado-Dias, and I. Cruz. 2005. Danos provocados por Spodoptera frugiperda na producao de materia seca nos rendimentos de graos, na cultura do milho. Sete Lagosas, MG: Embrapa, CNPM5, 130. https://ainfo.cnptia.embrapa.br/digital/bitstream/CNPM5/18881/1/Com_130.pdf.
Gatehouse, J. A. 2002. Plant resistance towards insect herbivores: a dynamic interaction. New Phytol. 156: 145–169.
Giraud, M., F. Hilliou, T. Fricaux, P. Audant, R. Feyereisen, and G. Le Goff. 2015. Cytochrome P450s from the fall armyworm (Spodoptera frugiperda): responses to plant allelochemicals and pesticides. Insect Mol. Biol. 24: 115–128.
Gott, R. C., G. R. Kunkel, E. S. Zobel, B. R. Lovett, and D. J. Hawthorne. 2017. Implicating ABC transporters in insecticide resistance: research strategies and a decision framework. J. Econ. Entomol. 110: 667–677.
Grabherr, M. G., R. Haas, M. Yassour, J. Z. Levin, D. A. Thompson, I. Amit, X. Adiconis, L. Fan, R. Raychowdhury, Q. Zeng, et al. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat. Biotechnol. 29: 644–652.
Gui, F., T. Lan, Y. Zhao, W. Guo, Y. Dong, D. Fang, H. Liu, H. Li, H. Wang, R. Hao, et al. 2020. Genomic and transcriptomic analysis unveils population evolution and development of pesticide resistance in fall armyworm Spodoptera frugiperda. Protein Cell. 10. doi:10.1007/s13238-020-00795-7.
Gutiérrez-Moreno, R., D. Mota-Sanchez, C. A. Blanco, M. E. Whalon, H. Terán-Santófimo, J. C. Rodriguez-Maciel, and C. Di Fonzo. 2019. Field-evolved resistance of the fall armyworm (Lepidoptera: Noctuidae) to synthetic insecticides in Puerto Rico and Mexico. J. Econ. Entomol. 112: 792–802.
Hafeez, M., X. Li, Z. Zhang, J. Huang, L. Wang, J. Zhang, S. Shah, M. M. Khan, F. Xu, G. M. Fernández-Grandon, et al. 2021. De novo transcriptomic analyses revealed some detoxification genes and related pathways responsive to Noposion Yihaogong® 5% EC (lambda-cyhalothrin 5%) exposure in Spodoptera frugiperda third-instar larvae. Insects. 12: 132.
Harrison, R. D., C. Thierfelder, F. Baudron, P. Chinwada, C. Midega, U. Schaffner, and J. van den Berg. 2019. Agro-ecological options for fall armyworm (Spodoptera frugiperda JE Smith) management: providing low-cost, smallholder friendly solutions to an invasive pest. J. Environ. Manage. 243: 318–330.
Huang, H. S., N. T. Hu, Y. E. Yao, C. Y. Wu, S. W. Chiang, and C. N. Sun. 1998. Molecular cloning and heterologous expression of a glutathione S-transferase involved in insecticide resistance from the diamondback moth, Plutella xylostella. Insect Biochem. Mol. Biol. 28: 651–658.
Ismail, F., and D. J. Wright. 1991. Cross-resistance between acylinea insect growth regulators in a strain of Plutella xylostella L. (Lepidoptera: Yponomeutidae) from Malaysia. Pest. Sci. 33: 359–370.
Johnson, S. J. 1987. Migration and the life history strategy of the fall armyworm, Spodoptera frugiperda in the Western Hemisphere. Int. J. Trop. Insect Sci. 8: 543–549.
Kanelisa, M., and S. Goto. 2000. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 28: 27–30.
Kenis, M., H. du Plessis, J. Van den Berg, M. N. Ba, G. Goergen, K. E. Kwaadjo, I. Baona, A. Buddie, G. Cafa, L. Offord, et al. 2019. Telosmos remus, a candidate parasitoid for the biological control of Spodoptera frugiperda in Africa, is already present on the continent. Insects. 10: 92.
Koffi, D., R. Kyrerematen, V. Y. Eziah, K. Agboka, M. Adom, G. Goergen, and R. L. Meagher, Jr. 2020. Natural enemies of the fall armyworm, Spodoptera frugiperda (Lepidoptera: Noctuidae) in Ghana. Fla. Entomol. 103: 85–90.
Lim, M. S., P. S. L. Silva, O. F. Oliveira, K. M. B. Silva, and F. C. L. Freitas. 2010. Corn yield response to weed and fall armyworm controls. Planta Daninha. 28: 103–111.
Merzendorfer, H. 2014. Chapter one - ABC transporters and their role in protecting insects from pesticides and their metabolites. Adv. Insect Physiol. 46: 1–72.
Molina-Ochoa, J., J. E. Carpenter, E. A. Heinrichs and J. E. Foster. 2003. Parasitoids and parasites of Spodoptera frugiperda (Lepidoptera: Noctuidae) in the Americas and Caribbean basin: an inventory. Fla. Entomol. 86: 254–289.
Montezano, D. G., A. Specht, D. R. Sosa-Gómez, V. F. Roque-Specht, J. C. Sousa-Silva, S. V. Paula-Moroes, J. A. Peterson and T. E. Hunt. 2018. Host plants of Spodoptera frugiperda (Lepidoptera: Noctuidae) in the Americas. Afr. Entomol. 26: 268–300.
Mortazavi, A., B. A. Williams, K. McCue, L. Schaeffer, and B. Wold. 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat. Methods. 5: 621–628.

Ortelli, F., L. C. Rossiter, J. Vontas, H. Ranson, and J. Hemingway. 2003. Heterologous expression of four glutathione transferase genes genetically linked to a major insecticide resistance locus from the malaria vector Anopheles gambiae. Biochem. J. 373: 957–963.

Pitre, H. N. 1986. Chemical control of the fall armyworm (Lepidoptera: Noctuidae): an update. Fla. Entomol. 69: 3.

Porreta, D., M. Gargani, R. Bellini, A. Medici, F. Punelli, and S. Urbanelli. 2008. Defense mechanisms against insecticides temephos and diflubenzuron in the mosquito Aedes caspius: The P-glycoprotein efflux pumps. Med. Vet. Entomol. 22: 48–54.

Seppey, M., M. Manni, and E. M. Zdobnov. 2019. BUSCO: assessing genome assembly and annotation completeness. Methods Mol. Biol. 1962: 227–245.

Silva-Brandão, K. L., R. J. Horikoshi, D. Bernardi, C. Omoto, A. Figueira, and M. M. Brandão. 2017. Transcript expression plasticity as a response to alternative larval host plants in the speciation process of corn and rice strains of Spodoptera frugiperda. BMC Genom. 18: 792.

Sisay, B., T. Tefera, M. Wakgari, G. Ayalew, and E. Mendesi. 2019. The efficacy of selected synthetic insecticides and botanicals against fall armyworm, Spodoptera frugiperda, in maize. Insects. 10: 45.

Sparks, A. N. 1979. A review of the biology of the fall armyworm. Fla. Entomol. 62: 82–87.

Sparks, A. N. 1986. Fall armyworm (Lepidoptera, Noctuidae): potential for area-wide management. Fla. Entomol. 69: 603–614.

Srinivas, R., S. S. Udikeri, S. K. Jayalakshmi, and K. Sreeramulu. 2004. Identification of factors responsible for insecticide resistance in Helicoverpa armigera. Comp. Biochem. Physiol. C. Toxicol. Pharmacol. 137: 261–269.

Storer, N. P., M. E. Kubiszak, J. Ed King, G. D. Thompson, and A. C. Santos. 2012. Status of resistance to Bt maize in Spodoptera frugiperda: lessons from Puerto Rico. J. Invertebr. Pathol. 110: 294–300.

Strycharz, J. P., A. Lao, H. Li, X. Qu, S. H. Lee, W. Sun, K. S. Yoon, J. J. Doherty, B. R. Pittendrigh, and J. M. Clark. 2013. Resistance in the highly DDT-resistant 91-R strain of Drosophila melanogaster involves decreased penetration, increased metabolism, and direct excretion. Pestic. Biochem. Physiol. 107: 207–217.

Unbehend, M., S. Hänniger, G. M. Vásquez, M. L. Juárez, D. Reisig, J. N. McNeil, R. L. Meagher, D. A. Jenkins, D. G. Heckel, and A. T. Groot. 2014. Geographic variation in sexual attraction of Spodoptera frugiperda corn- and rice-strain males to pheromone lures. Plos One. 9: e89255.

Wei, N., Y. Zhong, L. Lin, M. Xie, G. Zhang, W. Su, C. Li, and H. Chen. 2019. Transcriptome analysis and identification of insecticide tolerance-related genes after exposure to insecticide in Sitobion avenae. Genes, 10: 951.

Wei, S. H., A. G. Clark, and M. Syvanen. 2001. Identification and cloning of a key insecticide-metabolizing glutathione S-transferase (MdGST-6A) from a hyper insecticide-resistant strain of the housefly Musca domestica. Insect Biochem. Mol. Biol. 31: 1145–1153.

Xiao, H., X. Ye, H. Xu, Y. Mei, Y. Yang, X. Chen, Y. Yang, T. Liu, Y. Yu, W. Yang, et al. 2020. The genetic adaptations of fall armyworm Spodoptera frugiperda facilitated its rapid global dispersal and invasion. Mol. Ecol. Resour. 20: 1050–1068.

Yu, S. J. 1991. Insecticide resistance in the fall armyworm, Spodoptera frugiperda (J. E. Smith). Pestic. Biochem. Physiol. 39: 84–91.

Zhang, B., X. Su, C. Zhen, L. Lu, Y. Li, X. Ge, D. Chen, Z. Pei, M. Shi, and X. Chen. 2020. Silencing of cytochrome P450 in Spodoptera frugiperda (Lepidoptera: Noctuidae) by RNA interference enhances susceptibility to chlorantraniliprole. J Insect Sci. 20: 12.