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Proteomic investigation on *Anopheles gambiae* in Burkina Faso related to insecticide pressures from different climatic regions

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ABSTRACT

In Sub-Saharan Africa, *Anopheles gambiae* Giles (Diptera: Culicidae) largely contributes to malaria transmission, in direct relation to environmental conditions influencing the vector ecology. Therefore, we carried out a proteomic analysis on *An. gambiae sensu lato* (s.l.) mosquitoes to compare their metabolic state, depending on different pesticide pressures by selecting areas with or without cotton crops, in two climatic regions. Adult mosquitoes were collected, and the proteomes were analysed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). The data are available via ProteomeXchange with identifier PXD016300. From a total of 1,182 identified proteins, 648 were retained for further statistical analysis and were attributed to biological functions, the most important of which is energy metabolism (120 proteins) followed by translation-biogenesis (74), cytoskeleton (71), stress

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response (62), biosynthetic process (60), signalling (44), cellular respiration (38), cell redox homeostasis (25), DNA processing (17), pheromone binding (10), protein folding (9), RNA processing (9), other proteins (26) and unknown functions (83). The distribution of biological functions of all conditions was similar between climate zones or agricultural practices associated with different pesticide pressures. In the Sudano-Sahelian region, 421 (91.3%) proteins were found in samples from areas both with and without cotton crops. By contrast, in the Sahelian region, only 271 (55.0%) were common to both crop areas, and 233 proteins were up-regulated in samples from the cotton area. The focus was placed on differentially expressed proteins with putative roles in insecticide resistance, according to literature. This study provides the first whole-body proteomic characterisation of *An. gambiae* s.l. in Burkina Faso, as a framework to strengthen vector control strategies and understand the environment-vector interactions in different ecological sites.

Keywords: Proteomic, *Anopheles gambiae* s.l., insecticide pressures, Burkina Faso.

Statement of significance

In Burkina Faso, conventional cotton pest control involves at least three insecticide applications through the crop season. The energy costs of insecticide resistance have already been demonstrated to reduce the performance of insects in coping with changing environmental conditions, such as heat/cold treatments. We have compared the proteomes of *An. gambiae* s.l. according to varying insecticide pressures associated with cotton crops, in two climatic regions (Sudano-Sahelian and Sahelian) of Burkina Faso. The proteomic data shows no significant difference in biological functions according to insecticide pressure. Therefore, these results could show the similar metabolism of malaria vectors throughout these agricultural areas. However, in the Sahelian region, many proteins involved in insecticide resistance were up-regulated in cotton crop area. These results will allow the development of novel vector control strategies and a better understanding of the environment–vector interactions.

INTRODUCTION

In Sub-Saharan Africa, malaria is the main burden on public health, caused by several species in the *Plasmodium* genus (*P. falciparum, P. vivax, P. malariae, P. ovale* and *P. knowlesi*), with *P. falciparum* being responsible for 99.7% of malaria cases. [1] In Africa, *Anopheles gambiae* Giles (Diptera: Culicidae) is a major vector of the malaria agent besides *An. arabiensis, An. funestus, An. melas, An. moucheti* and *An. nili.* [1,2]

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In Burkina Faso, the epidemiological facies of malaria show increasing prevalence according to the variation in rainfall, from low to high, respectively in the Sahelian, Sudano-Sahelian and Sudanese climate regions. [3,4] Temperature, dry-season and rainfall impact the vector density and metabolism of *An. gambiae* (s.l.). [5,6] Supplementary studies are needed to compare the physiological state of the mosquito according to these ecological conditions. [7] Likewise, agricultural activities exert selection pressure on vectors due to insecticides. [8] In Burkina, conventional cotton pest control corresponds to at least three applications of organophosphates, organochlorines, carbamates and especially pyrethroids through the crop season. [9] This pressure generates resistant mosquito strains, characterised by increased metabolic detoxification of active molecules and/or by the insensitivity of target sites. [10] Insecticide resistance is widely listed as one of the major threats to sustainable malaria control. [11] Metabolic resistance to pyrethroids, organophosphates, organochlorines and carbamates is generally associated with elevated levels of enzymes, like cytochrome P450, monooxygenase, carboxylesterase and glutathione-S-transferase. [10,12] The energy costs of insecticide resistance have already been demonstrated to reduce the performance of insects in coping with changing environmental conditions, such as hot/cold treatments. [13,11]

In order to better understand several interacting mechanisms in mosquitoes, LC-MS/MS analysis focussed on the salivary glands, midgut and sensory organs in the most recent proteomic investigations on the *Anopheles* species. [14] Proteomics can also be another tool for the identification of adult mosquitoes at the genus level (*Aedes* spp., *Anopheles* spp., *Culex* spp.,). [15,16,17] Furthermore, it simultaneously allowed us to detect *Plasmodium* spp occurrence, [18] and identify blood meal sources in *An. gambiae* s.l.[19,20] Overall, quantitative proteomic investigations on *Anopheles* have improved the characterisation of the molecular basis of mosquito behaviour and immune responses. This could allow the identification of new targets for parasite or vector control and diagnostic biomarkers. [14,21]

Our study aimed to compare the proteomes of *An. gambiae* s.l. according to varying insecticide pressures associated with cotton crops and integrate different population origins from two climatic regions in Burkina Faso (the Sudano-Sahelian region, typically experiencing a cool/wet climate, and the Sahelian region, a hot/dry climate).

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MATERIALS and METHODS

Mosquito collection

All mosquito samples were collected using a mechanical aspirator based on the Prokopack design [22], between August and October 2017, from six replicate sites (three associated with a locality in a large cotton crop area and three with a locality in an area without cotton) in each of the two main climatic regions (Sudano-Sahelian and Sahelian) in Burkina Faso (**Figure 1**). Each *An. gambiae* s.l. was morphologically identified according to the identification keys of Gillies and Meillon (1968) [23] and Gillies and Coetzee (1987). [24] For each site, 15 non-blood engorged females were anaesthetised with chloroform. Mosquitoes were surface sterilised twice by washing with an alternation of ethanol 70% and sterile saline phosphate buffer. Samples were stored in -80°C prior to protein extraction.

Sample preparation

The 12 samples were ground under liquid nitrogen, and the proteins were extracted with an AllPrep® DNA/RNA/Protein Mini kit (Qiagen) according to the manufacturer's procedures. The protein pellets were resuspended in a 100 µl UT buffer (8 M urea and 100 mM Tris-HCl, pH 8.0). The protein content of each sample was quantified by the 'RC-DC Protein Assay' kit (Bio-Rad). The samples were then diluted in NH_4HCO_3 100 mM to get a final protein concentration of 20 µg/µL. Reduction and alkylation were performed by treating 1 µL of the samples with dithiothreitol (DTT), followed by iodoacetamide and DTT again, as described by Bauwens et al. (2013). [25] The proteins were then precipitated and washed of impurities, using the 2D-Clean up kit (GE Healthcare) according to the manufacturer's procedures. Samples were resuspended in NH_4HCO_3 100 mM and then digested using trypsin (Pierce MS grade, Thermo Scientific). After stopping digestion by adding trifluoroacetic acid (TFA) at 0.5% (v/v), samples were dried under vacuum using the SpeedVac (Thermo Scientific). Protein digests were then resuspended in water acidified with TFA 0.1%, and 1 µg peptides were injected into the LC system.

Mass spectrometry

LC-MS/MS analyses were performed on an Acquity M-Class UPLC (Waters), connected to a Q Exactive (Thermo Scientific), in nanoelectrospray positive ion mode. The trap column was a Symmetry C18 5 µm (180 µm x 20 mm), and the analytical column was an HSS T3 C18 1.8 µm (75 µm x 250 mm) (Waters). Samples were loaded at 20 µL/min on the trap column in 100% solvent A (formic acid 0.1% in water) for 3 minutes and subsequently

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separated on the analytical column; flow rate 600 nL/min, solvent A and solvent B (formic acid 0.1% in acetonitrile), linear-gradient 0 min, 98% A; 5 min, 93% A; 135 min, 70% A; 150 min, 60% A. Total run time was 180 min.

The mass spectrometer method was a TopN-MSMS method, where N was set to 12, meaning that the spectrometer acquires one full MS spectrum, selects the 12 most intense peaks in this spectrum (singly charged precursors excluded) and generates a full MS2 spectrum of each of these 12 compounds. The parameters for the MS spectrum acquisition were a mass range from 400 to 1,750 m/z, a resolution of 70,000, an automatic gain control (AGC) target of 1e6 and a maximum injection time of 50 ms. The parameters for the MS2 spectrum acquisition were an isolation window of 2.0 m/z, collision energy of 25 eV, a resolution of 17,500, an AGC target of 1e5 and a maximum injection time of 50 ms.

Data processing

Database searches were performed on the National Center for Biotechnology Information (NCBI) database restricted to *Anopheles* genus (232.271 sequences, downloaded on 19 October 2018), using the search engine Andromeda, via the software Maxquant vs 1.5.2.8, which allows for the normalisation of data and label free-quantification (LFQ) of proteins. Carbamidomethyl of cysteines (resulting from alkylation before digestion) and oxidation of methionine were set as variable modifications, with an MS/MS-FTMS (Fourier Transform Mass Spectrometry) tolerance of 10 ppm. Peptide mass tolerance was set at ±2 ppm and fragment mass tolerance at ±0.02 Da with a maximum of two missed cleavages. Significant identification was obtained when at least two peptides and one unique peptide were identified *per* protein hit, and the protein identification score was higher than 15. Protein identifications were filtered using a target–decoy approach at a false discovery rate (FDR) of 1%. The mass spectrometry proteomics data have been deposited into the ProteomeXchange Consortium via the PRIDE [26] partner repository with the dataset identifier **PXD016300**.

All the identified proteins were searched, from their accession number, in NCBInr/Protein Basic Local Alignment Search Tool (BLAST). The alignment of proteins was considered reliable when the BLAST score was higher than 200, and the sequence coverage was higher than 99.9%. Each protein was annotated regarding its biological function, using UniProtKB and Gene Ontology databases. Literature searches were performed to attribute some protein hits to a putative role in insecticide resistance.

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Data analyses

To compare *An. gambiae* s.l. proteomes in the 12 samples, the 'proteingroups.txt' file, generated by Maxquant, and the Perseus software were used (version 1.5.1.6). Contaminants and proteins identified with modified peptides were eliminated. The LFQ intensity ratios were transformed by $log_2(x)$. For each climatic region separately, samples were grouped according to the kind of agriculture practices, associated with cotton crop areas or not. Proteins were considered present in a group when at least two out of the three replicates showed a mass spectrometry signal, and proteins were considered absent in a group when none of the three replicates showed a signal. Based on these criteria, Venn diagrams were created to present the repartition of identified proteins between the two crop areas, for each climatic zone. Also, the proportion that proteins in different functional categories comprised of the total identified proteome was calculated for each group and presented in pie charts. And then, only proteins identified in all of the three replicates of each group were considered for differentially expressed proteomic analysis. For these proteins, a two-sample statistical test was performed between high and low insecticide pressure areas, with a significant threshold of $p < 0.05$ (Student's t-test), for each climate zone separately. Differentially expressed proteins between agricultural areas were presented in heatmap format and were clustered hierarchically. Clustering parameters were the following, for both rows and columns trees: the euclidian distance and average linkage; the numbers of clusters was 300; the maximum number of iterations was 10; the number of restarts was one. Eight-row clusters were automatically created, based on a distance threshold of 2.07, along with another manual, visual-based clustering. Finally, a permutation-based FDR correction was applied to target the differential proteins for which the likelihood of type I errors was minimised (protein hits were considered satisfying when $q < 0.10$). The whole workflow is summarised in **Figure 2**.

RESULTS

In total, 1,182 proteins were identified from *An. gambiae* s.l. mosquitoes, among which 648 identifications were considered reliable enough for further analysis according to insecticide pressure ('with or without cotton crops'), in the two climatic regions (for the Sahelian and Sudano-Sahelian regions, see **Supplementary Tables 1 and 2**, respectively). In the Sudano-Sahelian region, 421 (91.3%) proteins were found in samples from areas both with and without cotton crops (**Figure 3A**). By contrast, in the Sahelian region, only 271 (55.0%) were common to both crop areas, and 206 proteins were only found in samples from the cotton areas (**Figure 3B**).

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All proteins have been grouped according to their biological function, including energy metabolism, translation-biogenesis, cytoskeleton, biogenesis, stress response, biosynthetic process, signalling, cellular respiration, cell redox homeostasis, DNA processing, pheromone binding, protein folding and RNA processing. In both the Sudano-Sahelian (**Figure 4A**) and Sahelian (**Figure 4B)** regions, the distribution of whole proteome biological functions was almost similar between the 'with' or 'without cotton' areas. However, in the Sahelian region, the proportion that proteins from energy metabolism and cytoskeleton functional categories comprised of the total proteome was slightly higher in the 'without cotton crops' than in the 'with cotton' areas (**Figure 4B**). In the Sahelian region, the function distribution of the 233 up-regulated proteins in the cotton areas (**Figure 5**) did not show any remarkable disparity compared to function distributions presented in **Figure 4B**.

After literature searches, proteins involved in insecticide resistance have been found in both 'with cotton crop' and 'without cotton crop' areas, for both climatic regions. These proteins were mainly thioredoxin, glutathione (GSH) peroxidase, glutathione S-transferase, cytochrome, ester carboxylesterase, enoyl-CoA hydratase/isomerase, acyltransferase and acetyl-CoA carboxylase. In the Sahelian region, 16 proteins involved in insecticide resistance were up-regulated in the cotton crop area, while none of the up-regulated proteins in the non-cotton area could be attributed to a role in insecticide resistance (**Table 1)**. In the Sudano-Sahelian region, three and two proteins were up-regulated respectively, in the 'with cotton crops' and 'without cotton crops' areas **(Table 2**).

Heatmaps show the expression profiles and hierarchical clustering of 19 and 29 differentially expressed proteins in the Sudano-Sahelian and Sahelian regions, respectively (**Figure 6)**. Among differential proteins, 10 and 27 were up-regulated in the 'with cotton crop' areas, for the Sudano-Sahelian and Sahelian regions, respectively. The analysis of the clusters could allow the identification of dominant patterns of protein expression. In the Sudano-Sahelian region, three clusters could be attributed to one particular biological function. Two of them included only cytoskeleton proteins ('cluster c'), but with opposite expression trends. The third cluster ('cluster a') was composed of two proteins involved in energy metabolism that were up-regulated in the 'without cotton crop' areas (**Figure 6A**). In the Sahelian region, two clusters were exclusively formed by proteins involved in energy metabolism, up-regulated in the 'with cotton crop' areas. Finally, one cluster ('cluster b') included three proteins involved in response to stress, also up-regulated in the 'with cotton crop' areas (**Figure 6B**).

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DISCUSSION

This study presented the first investigation on the whole *An. gambiae* s.l. proteome in Burkina Faso, to identify protein expression patterns in a major malaria vector from areas of different insecticide pressures. We provided significant information on the impact of insecticide pressure from cotton crop areas on mosquito metabolism. Eventually, our results will allow a better understanding of interactions between the vector and its environment and the development of novel vector control strategies.

In our shotgun proteomic analysis, 1,182 proteins were identified from the mosquito's whole body. Previous studies have placed a lot of emphasis on the proteome of *Anopheles gambiae's* organs, hemolymph and saliva that are in direct contact with *Plasmodium*. [27] Then, 1,091 proteins were identified in the hemolymph of *An. stephensi,* [28] and 209 in *An. gambiae* s.l. [29] Likewise, 1,208 proteins were identified in the salivary glands of *Aedes aegypti* [30] and 159 in the saliva of *An. stephensi.* [31]

In this study, the most represented biological function is energy metabolism. Indeed, the proteome of *An. gambiae* s.l.'s salivary glands presented a large proportion of proteins involved in the metabolism of protein, carbohydrate and nucleic acid, transport or energy pathways. [32][33] Previous studies showed similar results concerning metabolism as the principal biological function, followed by cellular processes, biogenesis, biological regulation, and response to stress. [28,34] Furthermore, in *Aedes aegypti* (vector for dengue, chikungunya, Zika and yellow fever), similar results were found regarding the biological function of proteins. [30] However, each of these biological functions, mentioned above, has a role in the development of the mosquito or its adaptation to environmental conditions, such as insecticide pressure. Indeed, the acclimation of mosquitoes between the rainy and dry seasons can contribute to alternating the phenotypes that are accompanied by several changes in biological processes, including an increase in the hydrophobicity of the cuticle (cytoskeleton) in *An. coluzzii* and an increase in energy metabolism in *An. gambiae* s.l. [6] As the larvae of *An. gambiae* s.l. develop in temporary water produced by human activities and the rate of adult reproduction depends on the rainfall, there may also be post-transcriptional modifications for ecological adaptation. [35] Concerning *Anopheles* mosquitoes, signal transduction, energy metabolism, cytoskeleton, [36] signalling, translational regulation and stress response are functions potentially involved in blood feeding, biting behaviour and sporozoite–vector interactions. [31,37] Also, after a blood meal, there is an increase in cytoskeleton function and signal transduction associated with biogenesis, which could reflect the increased biosynthetic pathways associated with oogenesis. [38] Furthermore, higher

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expression of the genes involved in redox-metabolism and mitochondrial respiration were observed in *An. gambiae* s.l. refractory to *Plasmodium* infection than in susceptible ones. [39] However, the proportions of biological functions did not differ according to agriculture area, and no particular function could be highlighted when focussing on the 233 up-regulated proteins in the cotton areas of the Sahelian region.

Between the Sudano-Sahelian and Sahelian regions, the proportions of the proteins biological functions were almost identical. This relative similarity means that the climate characteristics were not major factors that affected the proteome of *An. gambiae* s.l. and their related metabolism state. However, the trends of our results greatly changed according to cotton crops in climatic zones from where the mosquitoes had been sampled. In the Sudano-Sahelian region, the proteome of *An. gambiae* s.l. was almost not impacted by the insecticide pressure that characterised the cotton areas. Indeed, 91.3% of identified proteins were common to both crop areas. By contrast, in the Sahelian region, only 55% of proteins were common to both areas, with 206 (41.8%) proteins being exclusive to the cotton crop area. It seems that mosquitoes subjected to insecticide pressure develop more resistance in a very hot region than in a colder one. Indeed, pyrethroid resistance to DDT (Dichloro diphenyl trichloroéthane) is augmented by heat shock in insecticide-resistant *An. arabiensis.* [40] Also, increasing the temperature increased the resistance to deltamethrin and bendiocarb of the susceptible *An. arabiensis.* [41] A possible explanation was that the detoxification enzyme systems had been affected by the temperature elevation. Therefore, the following discussion on differential proteins will focus on the Sahelian region.

In the Sahelian region, 16 proteins involved in insecticide resistance, such as thioredoxin, glutathione (GSH) peroxidase, GSH S-transferase, cytochromes, ester carboxylesterase, enoyl-CoA hydratase/isomerase, acyltransferase and acetyl-CoA carboxylase, [42,43] were up-regulated in the cotton crops areas. Previous studies have shown that GSH S-transferase, carboxylesterases and peroxidases were involved in the resistance to DDT and malathion, [42,44] while GSH S-transferases and cytochromes were associated with resistance to DDT, permethrin, deltamethrin, λ-cyhalothrin and malathion. [45,46,47] Thioredoxin peroxidase was associated with resistance to deltamethrin, [48] and acetyl-CoA acyltransferase, enoyl-CoA isomerase and glutathione S-transferase with resistance to organophosphate. [43] GSH S-transferase and cytochromes were the most impacted by insecticide pressures in both climate regions. In *An. gambiae*, these two enzymes were the most involved in detoxification. [42] Among the proteins of interest, GSH S-transferase was the only up-regulated protein in the cotton crop areas from the Sahelian region with a q-value

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below 0.10. Besides its involvement in insecticide resistance, the mosquito's GSH peroxidase is essential for *Plasmodium* transmission. [49] Similarly, the redox homeostasis system (thioredoxin, GSH peroxidase...) plays a vital role in the dynamics of symbiotic microbiota, which impacts *Plasmodium* transmission. [50] This would result in important implications for the design of strategies aiming at interfering with the GSH redox-system of the mosquito. [49]

Also, among the 233 up-regulated proteins in the cotton areas, GSH peroxidase, thioredoxin, acyl-CoA dehydrogenase, nitrile-specifier detoxification, deltamethrin and cathepsin B group could be related to insecticide metabolism (alterations in the levels or activities of detoxification proteins) or the reduction of the insecticide's ability to reach the target site. [51] Metabolic resistance, also called detoxification, is the set of biochemical mechanisms leading to degradation of the insecticide into metabolites. [52,53] These types of resistances have also been detected in rice-growing areas in south-western Burkina Faso, probably caused by heavy insecticide use [54] and long-lasting insecticidal nets (LLINs). [1] These results support the hypothesis of a metabolic resistance developed by mosquitoes confronted with higher insecticide pressure.

Because the FDR is a more stringent statistical test, only seven proteins in the Sahelian region were differentially expressed with a q-value lower than 0.10. All other differential proteins, with a p-value lower than 0.05 (Student's t-test) must be discussed with caution because of a high risk of false positives. In the Sahelian and Sudano-Sahelian regions, respectively, 29 and 19 differential proteins were considered for clustering. Among them, 27 and 11 were up-regulated in the cotton crop areas. They belonged to energy metabolism, cytoskeleton, stress response, signalling, biosynthetic process and cellular respiration. These results suggested that mosquitoes have higher metabolic activity due to agricultural practices with higher insecticidal pressure. However, only three biological functions, energy metabolism, cytoskeleton and stress response, had protein groups whose expression profiles generated clusters. In mosquitoes, energy metabolism and cytoskeleton were involved in sporozoite–vector interactions. [31,36,37] In addition, elevated energy metabolism and cytoskeleton functions are commonly associated with the mosquito's blood digestion. [55,38] In mosquito immune responses, stress response proteins were high, at the same level as the rate in immunity-related genes. [56] In the cotton areas of the Sahelian region, two clusters included up-regulated energy metabolism proteins (acyl-CoA dehydrogenases, crotonase/Enoyl-Coenzyme, isocitrate/isopropylmalate dehydrogenase and fatty acid oxidation complex), and one cluster included up-regulated proteins (formate-tetrahydrofolate ligase, acetoin dehydrogenase and mitochondrial carrier protein) involved in response to

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stress. More studies are needed to verify if such a pattern would be repeated in similar experimental conditions.

CONCLUSIONS

This study provides the first proteomic characterisation of *An. gambiae* s.l.'s whole body in Burkina Faso. In the Sahelian region, there were numerous up-regulated proteins in samples from the cotton crop areas, i.e. with high insecticide pressure. The identified proteins have been classified into many biological roles. However, no significant difference in biological functions was observed between crop areas. According to the literature, we highlighted several up-regulated proteins potentially implicated in insecticide resistance. Our results could be used for further in-depth research, in order to take environment and soil occupation into account in malaria vector control strategies.

Supporting information

Data generated or analyzed during this study are included in this published article and its supplementary information files. Data are available via ProteomeXchange with identifier **PXD016300**.

Authors' contributions

AAZ, AB and FF conceived and designed the study. AAZ, ZS and AB supervised the data and samples collection. AAZ performed practical work and wrote the manuscript. AAZ and LS analyzed and interpreted the results. AB, LS and FF revised the manuscript. All authors were the major contributors in writing the manuscript. All authors read and approved the final manuscript. We declare no conflicts of interest.

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Competing interests

The authors declare that they have no competing interests and no financial/commercial conflicts of interest.

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Ethics statement and consent to participate

This study was approved by the National Health Ethic Committee in Burkina Faso (reference number 2017-9-143 of September, 12th 2017). For mosquito collection in residential areas, written informed and consent was obtained from homeowners in each location.

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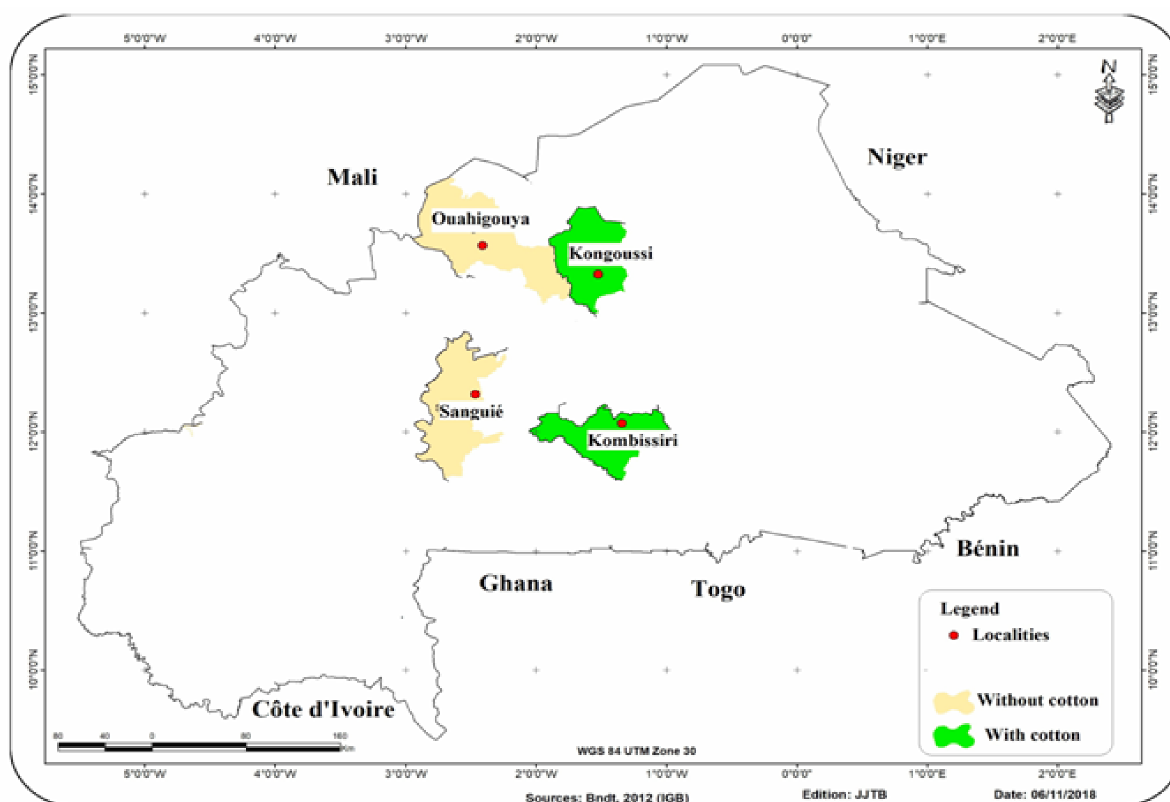

Figure 1. Map of *An. gambiae* s.l. collection locations: "cotton cropping area " (Kongoussi and Kombissiri) and "non cotton-cropping area" (Ouahigouya and Sanguié). Each locality had three replicates sites (15 mosquitoes per site).

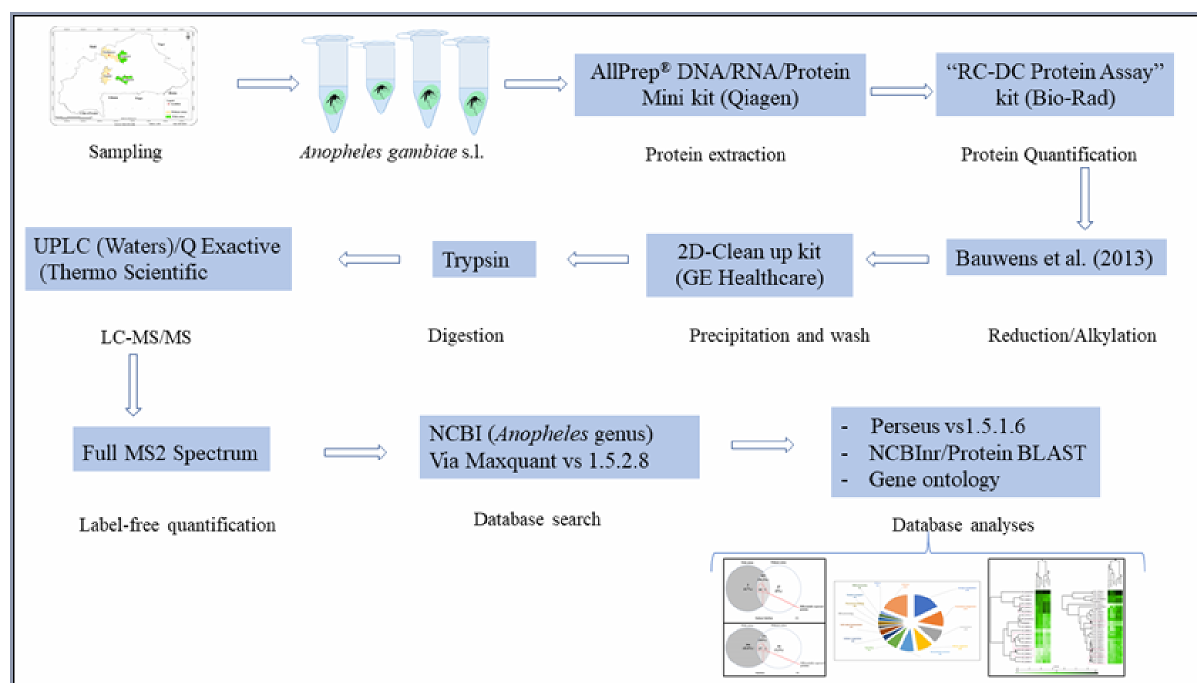

Figure 2. Shotgun proteomics workflow: from mosquitoes sampling to data analysis.

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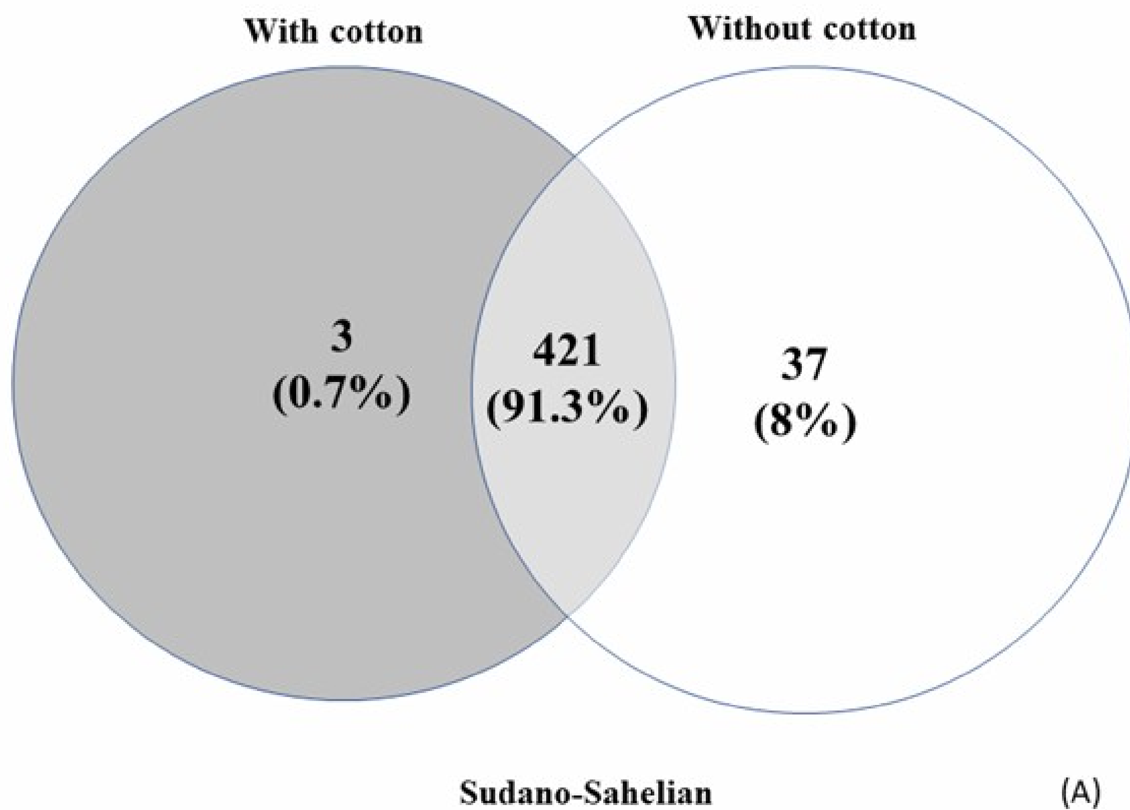

Figure 3. Distribution of identified proteins according to insecticide pressure in Sudano-Sahelian (A) and Sahelian (B) regions.

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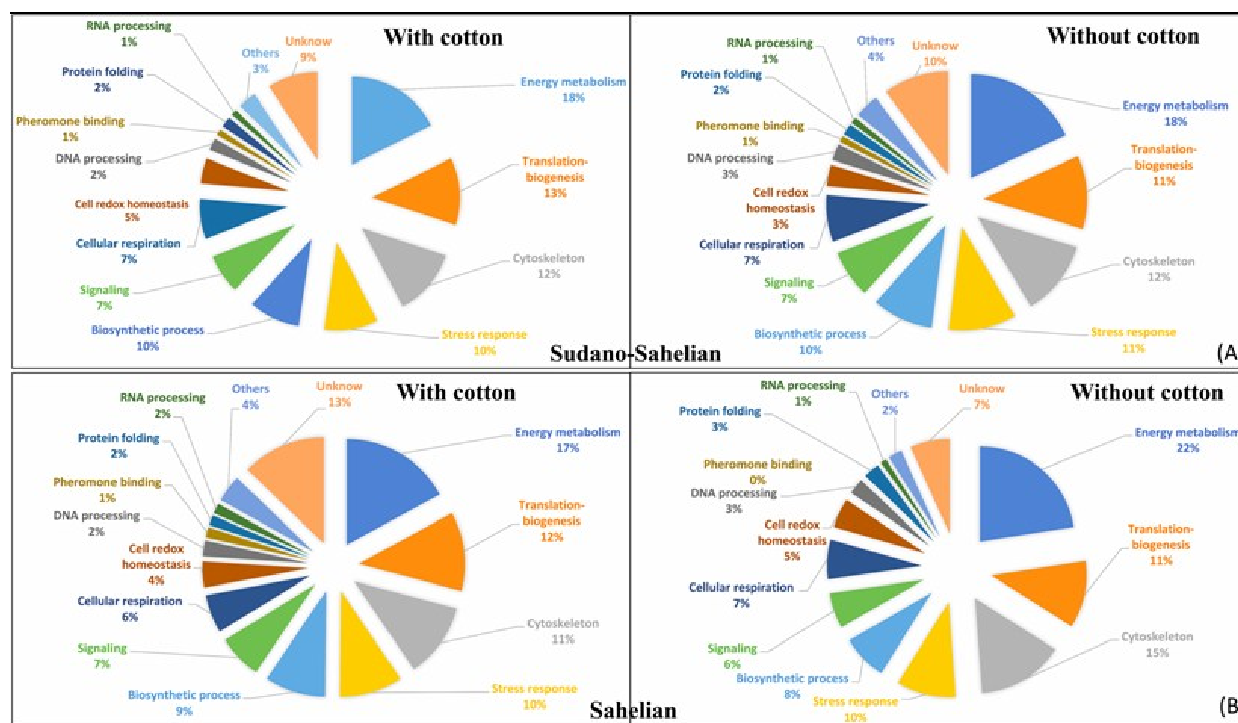

Figure 4. Distribution of biological functions according to crop area and climatic region: Sudano-Sahelian (A) and Sahelian (B).

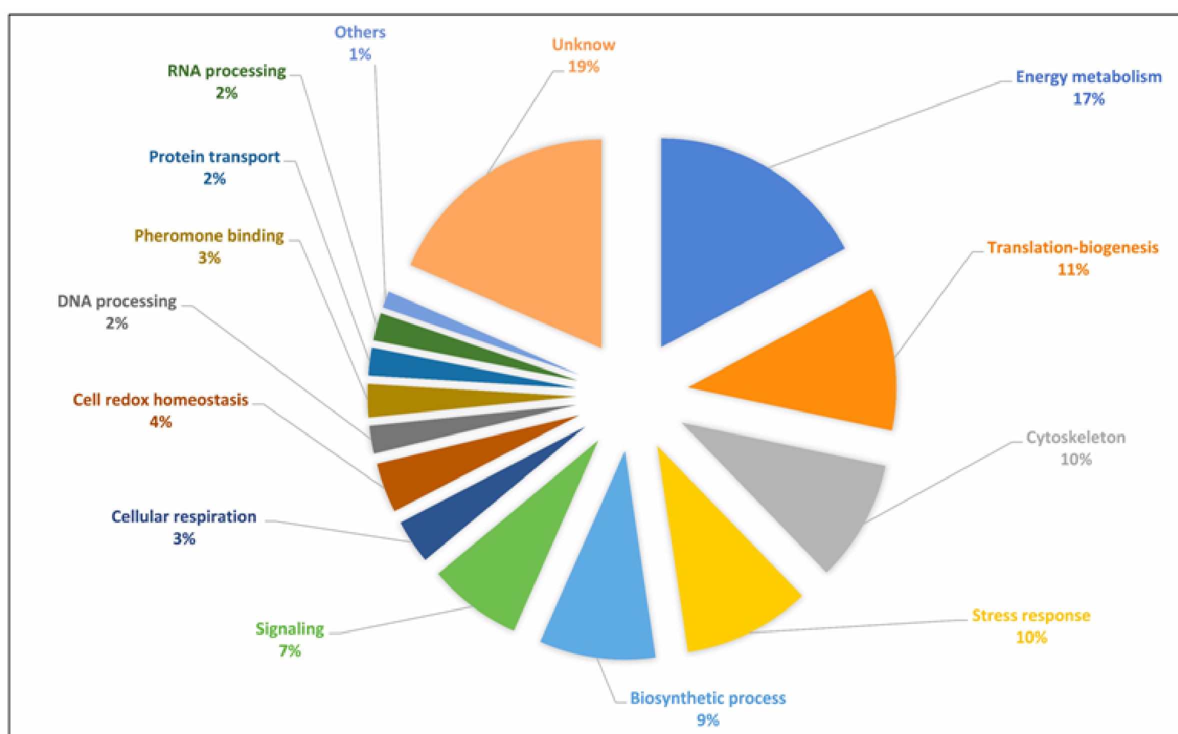

Figure 5. Biological functions distribution of differentially expressed proteins (233), in the cotton cropping area of the Sahelian region.

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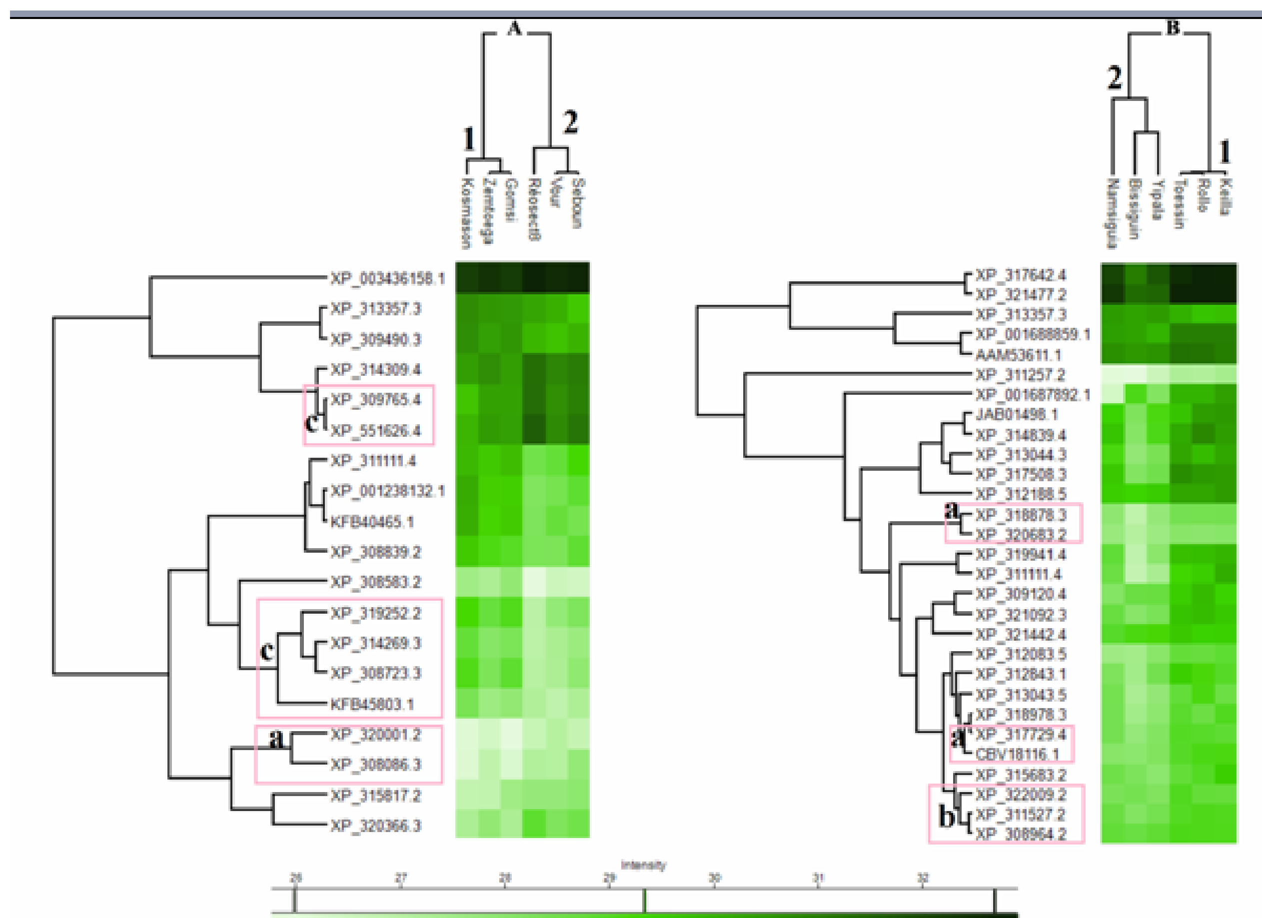

Figure 6. Hierarchical clustering of differentially expressed proteins (up-regulated) between agriculture areas, from Sudano-Sahelian (A) and from Sahelian (B) regions. **1** and **2** corresponds to areas with and without cotton crops, respectively. Proteins clusters: a = energy metabolism; b = stress response; c = cytoskeleton. Color scale reports log2-transformed values of LFQ intensities.

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Table 1: Identified proteins of *Anopheles gambiae* s.l. with putative role in insecticide resistance in Sahelian region. Color scale reports log2-transformed values of LFQ intensities. See references in text.

					Log2 (mean LFQ intensity)			
Fasta headers	**Species**	**Accession**	**Annotation**	**Biological function**	**With cotton**	**Without cotton**	***P* value**	***Q* value**
Proteins present only in with cotton area (Kongoussi)								
XP_321144.3 AGAP001919-PA	*Anopheles gambiae*	pfam00085	Thioredoxin	Cell redox homeostasis	25,6263	NS		
XP_313166.3 AGAP004247-PA	*Anopheles gambiae*	cd00340	Glutathione (GSH) peroxidase	Cell redox homeostasis	25,8472	NS		
XP_001238402.1 AGAP002113-PA	*Anopheles gambiae*	pfam00173	Cytochrome b5-like Heme/Steroid binding domain	Cytoskeleton	25,2088	NS		
AGK30034.1 glutathione S transferase class epsilon, partial	*Anopheles gambiae*	COG0625	Glutathione S-transferase	Stress response	26,4071	NS		
XP_001237970.1 AGAP007768-PA	*Anopheles gambiae*	cd00927	Cytochrome c oxidase subunit Vic	Cellular respiration	27,9145	NS		
AAU93513.1 thioredoxin-dependent peroxidase, partial	*Anopheles gambiae*	cd03015	Peroxiredoxin (PRX)	Cell redox homeostasis	25,9286	NS		
XP_315959.4 AGAP005929-PA	*Anopheles gambiae*	cd00925	Cytochrome c oxidase subunit Via	Celllular respiration	26,2722	NS		
XP_316821.4 AGAP000851-PA	*Anopheles gambiae*	cd00925	Cytochrome c oxidase subunit Via	Celllular respiration	27,1647	NS		
XP_307940.5 AGAP002245-PA	*Anopheles gambiae*	pfam02320	Ubiquinol-cytochrome C reductase hinge protein	Cellular respiration	28,4855	NS		
XP_310165.4 AGAP009526-PA	*Anopheles gambiae*	cd00928	Cytochrome c oxidase subunit VIIa	Cellular respiration	28,6239	NS		
XP_003436424.1 AGAP003238-PC	*Anopheles gambiae*	COG0596	Pimeloyl-[acyl-carrier protein] methyl ester carboxylesterase	Biosynthetic process	25,9410	NS		
JAB00403.1 putative cytochrome c oxidase subunit viia, partial	*Anopheles aquasalis*	cd00928	Cytochrome c oxidase subunit VIIa	Cellular respiration	26,1355	NS		
XP_312972.5 AGAP004097-PA	*Anopheles gambiae*	pfam16113	Enoyl-CoA hydratase/isomerase	Energy metabolism	25,2234	NS		
Proteins present both in with cotton (Kongoussi) and without cotton (Ouahigouya) areas								
XP_308583.2 AGAP007201-PA	*Anopheles gambiae*	pfam00085	Thioredoxin	Cell redox homeosta	27,0543	27,8875		

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XP_316990.3 AGAP008449-PA	*Anopheles gambiae*	pfam15955	Cuticle collagen 1	Cytoskeleton	28,7355	27,9232		
XP_309490.3 AGAP011159-PA	*Anopheles gambiae*	cd00923	Cytochrome c oxidase subunit Va	Cellular respiration	30,0179	29,7567		
XP_320347.4 AGAP012188-PA	*Anopheles gambiae*	pfam02271	Ubiquinol-cytochrome C reductase complex 14kD subunit	Cellular respiration	28,7929	27,4424		
XP_309490.3 AGAP011159-PA	*Anopheles gambiae*	cd00923	Cytochrome c oxidase subunit Va	Cellular respiration	30,0179	29,7567		
XP_314835.2 AGAP008724-PA	*Anopheles gambiae*	cd00924	Cytochrome c oxidase subunit Vb	Cellular respiration	30,2218	29,7859		
XP_003435734.1 AGAP013092-PA	*Anopheles gambiae*	cd00926	Cytochrome c oxidase subunit Vib	Cellular respiration	30,7142	29,2704		
XP_311546.4 AGAP010404-PA	*Anopheles gambiae*	cd03039	Glutathione S-transferase	Stress response	29,7691	29,8011		
XP_308018.4 AGAP002170-PA	*Anopheles gambiae*	pfam01257	Thioredoxin	Cell redox homeostasis	29,0353	27,1170		
XP_313049.1 AGAP004164-PA	*Anopheles gambiae*	cd03045	Glutathione S-transferase	Stress response	28,1881	27,0570		
XP_001689102.1 AGAP004710-PA	*Anopheles gambiae*	pfam05365	Ubiquinol-cytochrome C reductase, UQCRX/QCR9 like	Cellular respiration	27,6638	27,1694		
XP_001688099.1 AGAP006936-PB	*Anopheles gambiae*	pfam02167	Cytochrome C1 family	Energy metabolism	29,3847	29,2658		
XP_310154.1 AGAP009537-PA	*Anopheles gambiae*	COG3474	Cytochrome c2	Energy metabolism	30,3797	30,7127		
Differentially expressed proteins between with cotton and without cotton areas								
XP_314839.4 AGAP008727-PA	*Anopheles gambiae*	cd00922	Cytochrome c oxidase subunit IV	Cellular respiration	30,4579	28,9340	0,04	0,18
AAM53611.1 glutathione S-transferase S1-2, partial	***Anopheles gambiae***	**cd03039**	**Glutathione S-transferase**	**Stress response**	**31,1328**	**30,5775**	**0,00**	**0,07**
XP_319941.4 AGAP009176-PA	*Anopheles gambiae*	COG3321	Acyltransferase domain in polyketide synthase (PKS) enzymes	Biosynthetic process	29,8158	27,8612	0,02	0,11

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Table 2: Identified proteins of *Anopheles gambiae* s.l. with putative role in insecticide resistance in Sudano-Sahelian region. Color scale reports log2-transformed values of LFQ intensities. See references in text.

					Log2 (mean LFQ intensity)			
Fasta headers	**Species**	**Accession**	**Annotation**	**Biological function**	**With cotton**	**Without cotton**	***P* value**	***Q* value**
Proteins present only in with cotton area (Kombissiri)								
XP_321144.3 AGAP001919-PA	*Anopheles gambiae*	pfam00085	Thioredoxin	Cell redox homeostasis	25,9260	NS		
XP_313166.3 AGAP004247-PA	*Anopheles gambiae*	cd00340	Glutathione (GSH) peroxidase	Cell redox homeostasis	24,7225	NS		
XP_001238402.1 AGAP002113-PA	*Anopheles gambiae*	pfam00173	Cytochrome b5-like Heme/Steroid binding domain	Cytoskeleton	25,4136	NS		
Proteins present only in without cotton area (Sanguié)								
XP_003436424.1 AGAP003238-PC	*Anopheles gambiae*	COG0596	Pimeloyl-[acyl-carrier protein] methyl ester carboxylesterase	Biosynthetic process	NS	25,8229		
XP_312161.3 AGAP002761-PA	*Anopheles gambiae*	COG4799	Acetyl-CoA carboxylase, carboxyltransferase component	Energy metabolism	NS	25,1782		
Proteins present both in with cotton (Kombissiri) and without cotton (Sanguié) areas								
XP_311607.2 AGAP010337-PA	*Anopheles gambiae*	pfam02939	ubiquinol-cytochrome C reductase complex (cytochrome bc1 complex)	Cellular respiration	26,8818	26,8809		
AGK30034.1 glutathione S transferase class epsilon, partial	*Anopheles gambiae*	COG0625	Glutathione S-transferase	Stress response	25,9831	25,6387		
XP_001237970.1 AGAP007768-PA	*Anopheles gambiae*	cd00927	Cytochrome c oxidase subunit Vic	Cellular respiration	27,9143	27,9145		
XP_315959.4 AGAP005929-PA	*Anopheles gambiae*	cd00925	Cytochrome c oxidase subunit Via	Celllular respiration	25,5330	26,1930		
XP_316821.4 AGAP000851-PA	*Anopheles gambiae*	cd00925	Cytochrome c oxidase subunit Via	Celllular respiration	26,5036	26,6962		
XP_316990.3 AGAP008449-PA	*Anopheles gambiae*	pfam15955	Cuticle collagen	Cytoskeleton	28,1767	28,2717		
XP_309490.3	*Anophel*	cd0	Cytochrome c oxidase subunit	Cellular	30,25	29,627		

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AGAP011159-PA	*es gambiae*	0923	Va	respiration	64	3		
XP_314839.4 AGAP008727-PA	*Anopheles gambiae*	cd00922	Cytochrome c oxidase subunit IV	Cellular respiration	30,1596	30,4818		
XP_314835.2 AGAP008724-PA	*Anopheles gambiae*	cd00924	Cytochrome c oxidase subunit Vb	Cellular respiration	30,2136	29,6752		
XP_003435734.1 AGAP013092-PA	*Anopheles gambiae*	cd00926	Cytochrome c oxidase subunit Vib	Cellular respiration	30,8544	29,7032		
XP_311546.4 AGAP010404-PA	*Anopheles gambiae*	cd03039	Glutathione S-transferase	Stress response	29,9160	29,2029		
XP_308018.4 AGAP002170-PA	*Anopheles gambiae*	pfam01257	Thioredoxin	Cell redox homeostasis	28,6367	28,4139		
XP_313049.1 AGAP004164-PA	*Anopheles gambiae*	cd03045	Glutathione S-transferase	Stress response	27,4677	28,8571		
XP_001689102.1 AGAP004710-PA	*Anopheles gambiae*	pfam05365	Ubiquinol-cytochrome C reductase,	Cellular respiration	27,9964	28,0065		
AAM53611.1 glutathione S-transferase S1-2, partial	*Anopheles gambiae*	cd03039	Glutathione S-transferase	Stress response	31,4191	31,2363		
XP_001688099.1 AGAP006936-PB	*Anopheles gambiae*	pfam02167	Cytochrome C1 family	Energy metabolism	29,2048	29,2909		
XP_310154.1 AGAP009537-PA	*Anopheles gambiae*	COG3474	Cytochrome c2	Energy metabolism	30,8349	30,1921		
XP_319941.4 AGAP009176-PA	*Anopheles gambiae*	COG3321	Acyl transferase domain in polyketide synthase (PKS) enzymes	Biosynthetic process	29,5877	30,0144		
XP_307940.5 AGAP002245-PA	*Anopheles gambiae*	pfam02320	Ubiquinol-cytochrome C reductase hinge protein	Cellular respiration	28,3551	27,0396		
XP_310165.4 AGAP009526-PA	*Anopheles gambiae*	cd00928	Cytochrome c oxidase subunit VIIa	Cellular respiration	28,4509	28,3985		
Differentially expressed proteins between with cotton and without cotton areas								
XP_309490.3 AGAP011159-PA	*Anopheles gambiae*	cd00923	Cytochrome c oxidase subunit Va	Cellular respiration	30,2564	29,6273	0,01	0,89

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XP_308583.2 AGAP007201-PA	*Anopheles gambiae*	pfam00085	Thioredoxin	Cell redox homeostasis	27,3126	26,0925	0,01	1,00

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