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HISTOLOGY AND BIOGENIC AMINES LOCALIZATION OF SALIVARY GLAND OF THE ADULT RED PALM WEEVIL, *RHYNCHOPHORUS FERRUGINEUS* (COLEOPTERA: DRYOPHTHORIDAE)

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ABSTRACT

This study describes the histology and biogenic amines localization of salivary glands of the adult Red Palm Weevil (RPW), *Rhynchophorus ferrugineus*. The observation were done by using light microscopy of Zeiss Image Analyzer with Axio Vision software and Zeiss Axioscope with iSolutionlite software. The histology involves staining procedures of haematoxylin and eosin staining method while the immunohistochemistry analysis involves serotonin and dopamine of the biogenic amines. The salivary gland of the RPW adult is a single tubular gland. The single gland lies dorsally on the alimentary tract of the insect that begins in the head and extends through the thorax into the abdomen. The reservoir region is located in the mid to fore region of the gland, while the secretary region is found in the mid to rear

part of the gland. The reservoir region of the RPW tubular gland seems to probably secrete only water and ions but do not secrete enzymes. The distribution of biogenic amines suggest that dopamine mainly stimulates the production of water-based saliva in the secretory cells of the reservoir region, while serotonin stimulates the production of protein-rich saliva in the secretory cells of the secretory region.

Keywords: histology, biogenic amines, salivary gland, *Rhynchophorus ferrugineus*

ABSTRAK

Kajian ini menerangkan tentang histologi kelenjar liur dan taburan amina biogenik pada kelenjar liur Kumbang Merah Palma (RPW), *Rhynchophorus ferrugineus.* Pemerhatian dijalankan dengan menggunakan mikroskop cahaya Penganalisis Imej Zeiss dengan perisian Axio Vision dan Zeiss Axioscope dengan perisian iSolutionlite. Histologi dikaji dengan menggunakan kaedah pewarnaan hematoksilin dan eosin manakala analisis immunohistokimia melibatkan serotonin dan dopamin sebagai amina biogenik kajian. Kelenjar liur RPW merupakan kelenjar tubul yang hadir secara tunggal. Kelenjar tersebut terletak pada bahagian dorsal saluran penghadaman serangga ini, yang bermula pada bahagian kepala mengunjur ke bahagian toraks hingga ke abdomen. Kawasan reservoir terletak pada bahagian hadapan hingga ke tengah kelenjar, manakala kawasan rembesan terletak pada bahagian tengah hingga ke hujung kelenjar tersebut. Kawasan reservoir kelenjar tubul RPW berkemungkinan hanya terlibat dalam proses perembesan air dan ion tetapi tidak terlibat dalam perembesan enzim. Taburan amina biogenik menunjukkan bahawa dopamin adalah perangsang utama dalam penghasilan air liur bebas protein pada kawasan reservoir, manakala serotonin merangsang penghasilan air liur berprotein pada kawasan rembesan.

Kata kunci: histologi, amina biogenik, kelenjar liur, *Rhynchophorus ferrugineus*

INTRODUCTION

Red Palm Weevil (*Rhynchophorus ferrugineus*, Olivier 1790) that is known as RPW is an economically important, notorious tissue-boring pest of palm trees worldwide. This pest feeds primarily on Arecaceae such as dates palms, coconut tree, sago palm as well as oil palm. Besides, it has also been recorded on Agavaceae (century plant), and Poaceae (sugar cane) (Malumphy and Moran 2009). RPW distributed most widely within tropical Asia that extends from Pakistan, through Southeast Asia to Melanesia (Murphy and Briscoe 1999). The range expansion of this insect was then further facilitated through the growing of commercialization of coconut and oil palm within tropical Asia which particularly involves the development of plantation monocultures (Nirula et al. 1953). Despite of the outrageous facts of RPW, there is a lack of information regarding the biology of this pest especially on the salivary gland that contribute mainly to its feeding activity.

Salivary glands are glands associated with the mouth or oral cavity and produce secretions known as saliva that are mixed with the food during feeding and are ingested along with the food. Insect salivary gland are ectodermal origin and there are four glands that are associated with the oral cavity of insects although all four generally not present in the same insect; the mandibular, maxillary, hypopharyngeal and labial glands (Baptist 1941). Generally, these glands involve in the lubrication of the mouthparts as well as formation of bolus (Walker 2009). The gland that usually associated with the production of saliva in insect is the labial gland (Chapman 1998, Resh and Carde 2009, Romoser and Stoffolano 1998). The labial glands are of two main types that are the simple structure of tubular gland and the more complex structure of acinar gland (Ali 1997).

The control of insect salivary gland is achieved either by direct innervation or via neurohormones (Ali 1997). In the nervous system and in various peripheral organs of vertebrates and invertebrates, biogenic amines act as neurotransmitter, neurohormones, or neuromodulators (Blenau and Baumann 2001, Baumann et al. 2003) In insect, both serotonin and dopamine appear to be the most prominent amines associated with the salivary gland control.

The structure and function of the RPW salivary glands as well as the nature its control is very important towards the investigation of its feeding habits and mechanism that hopefully can be applied and used in the prospects of Integrated Pest Management (IPM). Due to the high curiosity on the salivary gland characters as well as its control mechanisms, the primary aims of this study are: 1) to illustrate and describe the morphology and histology of the salivary gland of adult weevil of RPW and to 2) to localize the biogenic amine, serotonin and dopamine, the chemical compounds that may controls the secretions of saliva in the adult weevils of RPW.

MATERIALS AND METHODS

Morphological Observation

The digestive tract of RPW was removed through dissection in phosphate buffer (PBS). The dissection process was done using a surgical microscope Stemi 2000-C Carl Zeiss. Microphotograph of the digestive tract then taken by using Zeiss Image Analyzer with Axio Vision software. For storage purposes, the digestive tract was fixed in 10 % formalin solution.

Histological Procedure

Samples for sectioning and light microscopy observation were fixed in formalin solution. The tissues were washed with 70% ethanol to remove the excess fixative and put through a series of ethanol solutions (70-100%) for dehydration followed by xylene

substitute. Tissues were embedded in wax and labelled. Blocks of waxed tissues were then sectioned (3µm) using a Reichert microtome. Four to five sections were placed on a slide and dried overnight. Sectioned tissues were stained per haematoxylin and eosin staining method.

The first step of haematoxylin and eosin staining method is to put the slides with the tissue into xylene substitute solution for 15 minutes. Then the slides were put in a series of alcohol: 100 %, 95%, 80% and 70, 3 minutes for each solution. Then the slides were rinsed in distilled water before being put into haematoxylin solution for 5 minutes. Next, the slides were rinsed in running tap water and placed in eosin solution for 3 minutes and dipped in 95% alcohol four times before being put in 100% alcohol solution for 2 minutes. The final step in staining was to put the slides in xylene substitute solution. Lastly, the slides were mounted and microphotography was observed by light microscopy using Zeiss Axioscope with iSolutionlite software.

Immunohistochemical Staining

Slides of tissues sections were taken through a series of processes include dewaxing, chromogenic staining, dehydration and clearing, and mounting. In dewaxing process, the slides were put into xylene twice, alcohol 100%, 80%, 70% and rinse in running tap water, 3 minutes for each solution. The slides were then undergone chromogenic staining process in which they were incubated with hydrogen peroxide for 6 minutes and rinse with running tap water for 3 minutes before being put into antigen-retrieval buffer for 40 minutes. The slides were rinsed again with running tap water for 3 minutes, and rinsed with PBT+N three times before being incubated with the primary antibody (serotonin/dopamine) for 30 minutes. Next, slides were rinsed triple of times with PBT+N and incubated with the secondary antibody, the HRP Polymer for 30 minutes. Once again, the slides were rinsed triple times with PBT+N and incubated with DAB Chromogen for 7 minutes and then rinsed in running tap water

for one minute. The slides were then counterstained with haematoxylin for 5 minutes and rinsed in running tap water. In dehydration and clearing process, the slides were put into a series of alcohol (80%, 90%, and 100%) and xylene each for 3 minutes. Then, the slides were mounted and viewed.

RESULTS AND DISCUSSION

The salivary gland of RPW is an unpaired elongated tube that lies dorsally along the alimentary tract of the insect. The epithelial layer of the gland is unicellular and is covered by a basement membrane, similar to that reported by House and Ginsborg (1985). The gland is about 10mm long and 0.3mm wide and consists of two main regions, the reservoir and the secretory region (Fig. 1a). The weevil's secretory region comprises a long distal section located mostly in the abdomen. Epithelial cells in the secretory region contain granules (Fig. 1c).

According to Resh and Carde (2009), cells in the distal section serve their function to synthesize and secrete salivary enzymes and another salivary component (Fig. 1d). Epithelial cells in reservoir region of RPW line an enlarged lumen, that may contained the secreted products (Fig. 1b), suggesting that secretions from the secretory region are stored in the lumen of the reservoir before being secreted out during feeding. Even though, the cells in the reservoir region are generally similar in appearance to those in secretory region, they do not contain secretory granules. This suggesting that the proximal region of RPW tubular gland seems to probably secrete only water and ions but do not secrete enzymes.

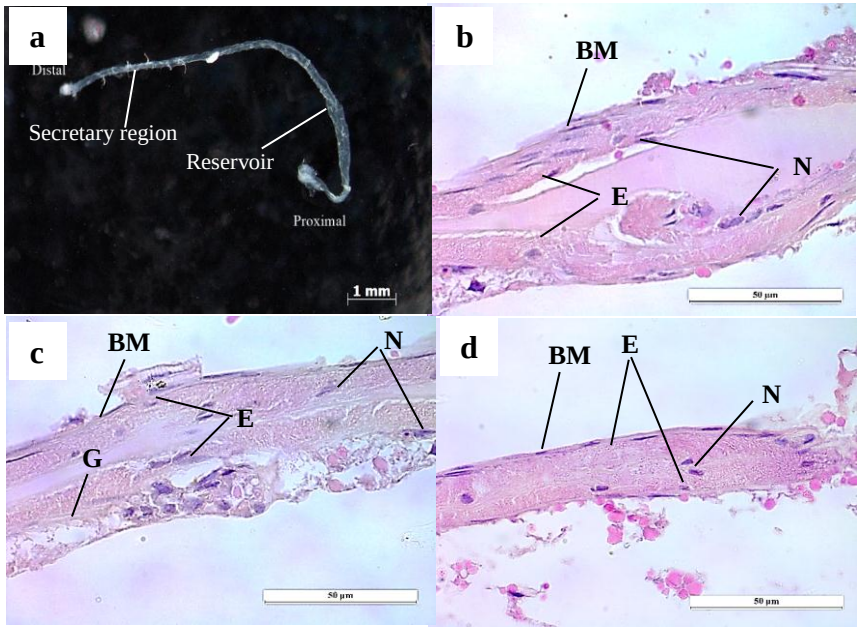

Figure 1 The adult salivary gland structure of RPW (a) the morphology of the tubular gland, (b) the longitudinal section of the reservoir region (100X), (c) the longitudinal section of the secretory region (100X), (d) the longitudinal section of the distal region of the gland (100X). BM: basement membrane, E: epithelium, G: granules, N: nucleus.

In this study, the immunohistochemical result for serotonin and dopamine revealed that the salivary gland of adult RPW are not directly innervated since there are no nerve-like structure immunoreactivity found in the salivary gland. This result is in line with the one reported by Oschman and Berridge (1970). In their study, they found that the tubular gland is not innervated to the same extent as acinar gland. The tubular gland

of RPW appear to be under neurohormonal control. This finding has been reported in *Calliphora* where serotonin acts neurohormonally to increase salivation via cyclic AMP and inositol triphosphate second messenger systems (Berridge 1970, Berridge and Heslop 1981, Berridge and Patel 1968).

According to Just and Walz (1996), serotonin stimulates the production of a protein-rich saliva, while dopamine stimulates the production of protein-free saliva. The result from this study revealed that both serotonin and dopamine were present in the secretory cells of the secretory region (Fig. 2c and Fig. 2d). However,only dopamine present in the secretory cells of the reservoir region while serotonin are nearly absent in this region. The histology result showed that the secretory region involves in the production of protein-rich saliva as there are secretory granules present, hence it was stimulated by serotonin. In contrast, dopamine present in the reservoir region as it stimulates the production of water-based saliva in this region.

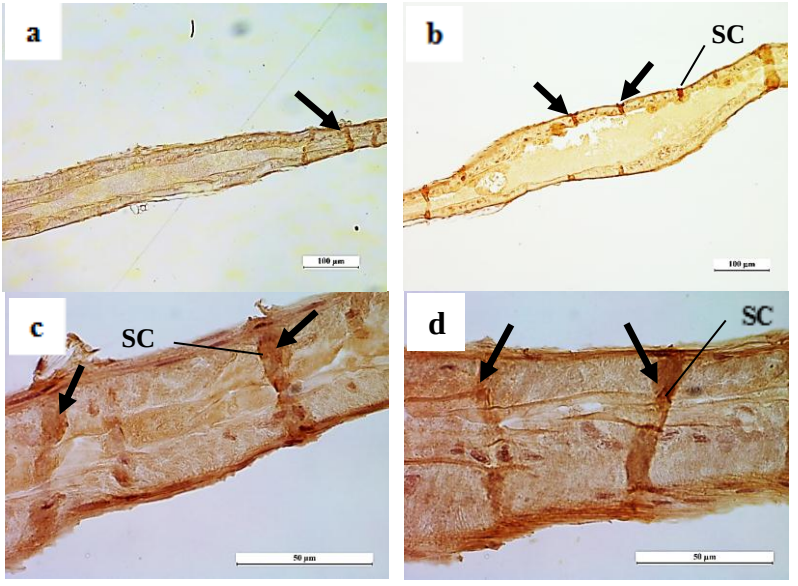

Figure 2 The immunohistochemical analysis of adult salivary gland of RPW (a) the secretory cells in the reservoir region shows negative immunoreactivity towards serotonin (20X), (b) the secretory cells in the reservoir region shows positive immunoreactivity towards dopamine (arrows) (20X), (c) the secretory cells in the secretory region shows positive immunoreactivity towards serotonin (arrows) (100X), (d) the secretory cells in the secretory region shows positive immunoreactivity towards dopamine (arrows) (100X). SC: secretory cell.

CONCLUSION

The tubular salivary gland of adult weevils of RPW consists of two main regions, the reservoir and secretory region. The reservoir region probably involves in the production of water-based saliva that is stimulated by dopamine. In contrast, the secretory region probably involves in the production of protein-rich saliva that is controlled mainly by serotonin. Further detailed

studies should be conducted to collect more information and evidence to prove the statements above.

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REFERENCES

Malumphy, C. & Moran, H. 2009. Red Palm Weevil, *Rhynchophorus ferrugineus*. *Plant Pest Fact Sheet, The Food and Environment Research Agency (FERA).*

Murphy, S. T. & Briscoe, B. R. 1999. The red palm weevil as an alien invasive: biology and the prospects for biological control as a component of IPM. *Biocontrol News and Information,* 20(1): 35-46.

Nirula, K. K., Anthony, J., Menon, K. P. V. 1953. The red palm weevil and its control. *Proceedings 40th Session Indian Science Congress*, 147-148.

Baptist, B. A. 1941. The morphology and physiology of the salivary glands of Hemiptera-Heteroptera. *The Quarterly Journal of Microscopical Science*, 2:91–139.

Walker, G. P. 2009. Salivary glands. *Encyclopedia of Insects Second Edition. Academic Press, Elsevier, Amsterdam,* pp 897-901.

Chapman, R. F. 1998.The Insects: Structure and Function. *Cambridge University Press*.

Resh, V. H. &. Carde, R. T. 2009. Encyclopedia of Insects. *Academic Press*.

Romoser, W. S. & Stoffolano, J. G. 1998. The Science of Entomology Fourth Edition. *McGraw-Hill, Boston.*

Ali, D. W. 1997. The aminergic and peptidergic innervation of insect salivary glands. *Journal of Experimental Biol*ogy, 200:1941–1949.

Blenau, W. & Baumann, A. 2001. Molecular and pharmacological properties of insect biogenic amine receptors: lessons from *Drosophila melanogaster* and *Apis mellifera*. *Archives of Insect Biochemistry and Physiology,* 48:13–38.

Baumann, A., Blenau, W. & Erber, J. 2003. Biogenic amines. *Encyclopedia of Insects. Academic Press*, pp 91-94.

House, C. R. & Ginsborg, B. L. "Salivary gland," in *Comprehensive Insect Physiology and Pharmacology Vol. 11*, edited by G. A. Kerkut and L. I. Gilbert (Oxford, Pergamon Press, 1985), pp 195–224..

Oschman, J. L. & Berridge, M. J. 1970. Structural and functional aspects of salivary fluid secretion in *Calliphora. Tissue and Cell*, 2:281–310.

Berridge, M. J. 1970. The role of 5-hydroxytryptamine and cyclic AMP in the control of fluid secretion by isolated salivary glands. *Journal of. Experimental. Biology,* 53:171–186.

Berridge, M. J. & Heslop, J. P. 1981. Separate 5-hydroxytryptamine receptors on the salivary gland of the blowfly are linked to the generation of either cyclic adenosine 3',5'-monophosphate or calcium signals. *British Journal of Pharmacology*, 73:729–738

Berridge, M. J. & Patel, N. G. 1968. Insect salivary glands: stimulation of fluid secretion by 5-hydroxytryptamine and adenosine-3',5'-monophosphate. *Science*, 162:462–463.

Just, F. & Walz, B. 1996. The effects of serotonin and dopamine on salivary secretion by isolated cockroach salivary glands. *Journal Experimental Biology*, 199: 407–413.