CRISPR-cas9: A Nascent Approach to Tackle Insect Pests

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Abstract
Globalization, ease of doing trade across the world brings exotic or non-native pests into the new boundary. Immediate action is needed under such circumstances to check the invasion otherwise it could cause chaos in the new environment cause lack of natural resistance. With the advancement in the field of genome editing using multiple scientific tools, opens a new venture in the area of pest management. Modification of insect genome to create a gene drive or by bringing back its susceptibility to chemicals can minimize the pest problem. In genome editing, Clustered regularly interspaced short palindromic repeats (CRISPR) and the CRISPR-associated gene Cas9 represents a vital tool because of its simple and easy to handling nature, even to the non-model insects. Thus, the integration of new technologies in pest management has been imperative for achieving pest control strategies in quicker ways.

Introduction
Precise gene changes in an organism (intentionally) by the way of insertion, deletion or replacement of gene by the use of molecular scissors and manipulating the cells repair mechanism is called genome editing. Majorly genome editing tools have been in use for the identification of target gene function in a variety of organisms. Before the advent of CRISPR/Cas9, zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) were used for gene editing. ZFNs and TALENs are more complex and labour intensive which requires the use of the variety of nucleases; the off-target effects like cellular toxicity were appropriately replaced by the CRISPR/Cas9 system. The feasibility of ‘gene drive’ mechanism to the subsequent generations in CRISPR/Cas9 is found to be interesting which is not possible in RNA interference (RNAi) unless the dsRNA is supplied continuously makes one of the invaluable gene-editing tools in functional genomic studies.

Mode of Action
The CRISPR-cas9 perhaps is an immune system for bacteria. They use this mechanism to fight against invaders like the bacteriophages. The bacterial cells produce certain enzymes to protect them from the phages. While eliminating the phages, certain other enzymes of the cell collect up the remains of the virus genetic code and stores it in the CRISPR spacer system. Each spacer segment of CRISPR is uniquely collected from variable phages. Along with the CRISPR certain other genes also embedded, they are called
as CRISPR associated genes or cas genes. Cas genes produce cas proteins. These cas proteins are helicases, they unwind the double helix of invaded DNA and the nucleases, they cut the DNA. Whenever the bacteria face the threat from the bacteriophages, the cas genes transcribe and translate to cas9 enzyme it combines with the transcribed spacer DNA called crRNA along with trans-acting crRNA (tracrRNA). When this crRNA matches with the viral DNA, the cas9 enzyme cut the virus nucleic acid to stop the menace.

CRISPR/Cas9- A Novel Tool

By adopting the above technique, researchers come up with a new genome-editing tool CRISPR/cas9. For genome editing the crRNA is make fused with tracrRNA to form tracrRNA- crRNA chimaera also known as single guide RNA (sgRNA). This sgRNA guides the cas9 (from Streptococcus pyogenes) to cut the target gene adjacent to the protospacer adjacent motif to create double-stranded breaks (DSB). After DSB the cell tries to fix it by either non-homologous end joining (NHEJ), it brings two free ends together and re-join them which is error-prone or homology-directed repair (HDR), it uses the sister chromatid as a template to replace the breaks (Figure 1). Thus, being a simple components system, it is easily programmable for mutating specific genomic targets for either insects or plants, to plan a new control strategy.

Application in Insect Pest Control

• CRISPR/cas9 makes functional genomic studies easier even in non-model insects because of less time and effort requirements.
• Gene drive mechanism of CRISPR/cas9 targets the gene of interest which causes either functional abnormalities or sterility can pass through generation after generation faster than mendelian inheritance. Mosquitoes, the major vectors for many fatal ailments to humans, can be easily eradicated from a locality within a shorter span of generations by utilizing this technique.
• Silencing off the genes confer resistance against insecticide, by using CRISPR/cas9 will enable to use of the same insecticides which already got resistance in the field level. This technique would stop the over dumping of pesticide load in the environment.
• Functional abnormalities can be induced on the economically important insect pests with regards to agriculture. In Spodopteralitura, CRISPR/cas9 mediated gene targeting on abdominal gene-A makes the abnormal segmentation which made them more vulnerable.

• Researchers have investigated that virus-induced CRISPR/cas9 system with cas9 nuclease being activated upon infection with viruses. This mechanism will help in the development of transgenic silkworm resistant to nuclear polyhedrosis virus.
• CRISPR/Cas9 has provided valuable information regarding wing colour mimicry of butterflies and social behaviour in ants.

Conclusion

CRISPR/cas9 technology has been proven as an effective and precise tool in functional genomics studies. Its promising effects on insect pests will open a new window for pest management. However, the knowledge about the gene drive on the ecology is not completely understood. There is a need for the systemic scientific approach to move from uncertain risks on ecology to quantifiable hazards.

References

