Role of CRISPR/Cas9 in Insect Resistance

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Insect pests pose a significant threat to global agriculture, leading to substantial economic losses and reduced crop yields. Initially, for pest management, several breeding approaches were applied which have now been gradually replaced by genome editing (GE) strategies as they are more efficient and less laborious. The genome editing technique results in base substitutions and/or insertions/deletions (indels) in the target DNA. It includes several techniques, for instance, the use of zinc finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs), and the recently established clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated nuclease 9 (Cas9) system. In contrast to TALENs and ZFNs, the CRISPR/Cas system is more direct and easier to handle as it requires a single guide RNA (gRNA) for target determination with the Cas9 nuclease. This article explores the application of CRISPR technology in developing insect-resistant crop varieties. We examine the mechanisms by which CRISPR can enhance resistance to insect pests, the regulatory landscape, and the implications for future agricultural practices. Insect pests are responsible for the loss of approximately 18% of global crop production annually, highlighting the urgent need for sustainable pest management strategies. CRISPR technology offers a transformative solution by enabling precise edits in the genomes of crops, thereby enhancing their resistance to pests in a more efficient manner.

What is CRISPR-Cas9?

CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated system) was originally identified as an adaptive immune system in bacteria. Through scientific advancements, it has evolved into a powerful tool of genome editing to precisely alter DNA within organism.

The technology works by using a guide RNA to target a specific DNA sequence in the genome of an organism. The Cas9 enzyme then cuts the DNA at the targeted location, allowing scientists to add, remove, or alter genetic material. This precision and flexibility make CRISPR-Cas9 a promising solution for developing insect pest resistance in crops.

A. Components of the CRISPR/Cas9 System

1. **Guide RNA (gRNA):** This RNA molecule is designed to match a specific target DNA sequence within the insect genome. It consists of two parts: a scaffold region that binds to the Cas9 protein and a spacer sequence that complements the target DNA.
2. **Cas9 Protein:** Cas9 is an endonuclease enzyme that introduces double-strand breaks (DSBs) in the DNA at the location specified by the gRNA.

B. Mechanism of Action

1. **Target Recognition:** The gRNA binds to the target DNA sequence in the insect genome through base pairing. The specificity of this binding is crucial; mismatches can lead to off-target effects.
2. **DNA Cleavage:** Once the gRNA is bound to the target sequence, the Cas9 protein is recruited to the site. Cas9 then induces a double-strand break in the DNA, effectively cutting both strands.
3. **Cellular Repair Mechanisms:** The cell's natural repair mechanisms respond to the DSB:

I. **Non-Homologous End Joining (NHEJ):** This is the most common repair pathway. It quickly rejoins the broken DNA ends but often introduces small insertions or deletions (indels) at the break site. These changes can disrupt gene function, effectively knocking out genes of interest.

II. Homology-Directed Repair (HDR): If a donor DNA template is provided, the cell can use this template to repair the break accurately. This method allows for precise editing, such as introducing specific mutations or inserting new genetic material.

How CRISPR-Cas9 Works in Pest Resistance?

1. **Gene Knockout in Pests:** CRISPR-Cas9 can be used to knock out specific genes in insect pests to suppress their population or reduce their capacity to damage crops. Mechanisms include:

i. **Disrupting reproductive genes:** Targeting genes critical for reproduction can lead to sterile or infertile insects, reducing the pest population over time.
ii. **Disabling vital functions:** Genes involved in development, feeding, or digestion can be knocked out to hinder pest survival or performance.
iii. **Altering pest behaviour:** Genes that influence insect attraction to crops can be modified to divert pests away from agricultural plants.

2. Gene Drives for Pest Population Control

CRISPR-based gene drives are used to spread genetic alterations rapidly through pest populations, ensuring that beneficial traits (e.g., reduced fertility, inability to digest crops) are passed down at a higher-than-normal rate. This can:

i. **Suppress pest populations:** Target genes critical for survival, leading to a population crash over several generations.
ii. **Control pest-borne diseases:** In cases where pests also transmit diseases (e.g., insects that spread plant viruses), gene drives can prevent the insects from becoming effective vectors.

3. Enhancing Crop Resistance to Insects

CRISPR can be applied to modify the genomes of crops to make them more resistant to insect pests. Mechanisms include:

i. **Modifying plant defense pathways:** CRISPR can be used to enhance natural defense systems in plants, such as by increasing the production of insect-repelling toxins or chemicals that harm insects when ingested.
ii. **Improving physical barriers:** Altering the structure of plant tissues, such as thickening cell walls, to make it more difficult for insects to feed on them.
iii. **Editing resistance genes**: Introducing or modifying genes that produce proteins toxic to insects, similar to how Bt (Bacillus thuringiensis) crops work, but with greater specificity.

4. Resensitizing Pests to Insecticides

Insects often develop resistance to chemical pesticides through mutations in detoxification enzymes or target receptors. CRISPR can be used to:

i. Re-sensitize insect pests to pesticides by targeting the resistance-conferring genes. This can restore the effectiveness of existing chemical controls.
ii. Block detoxification pathways: Disabling genes that allow insects to neutralize pesticides can increase the efficacy of those pesticides.

5. Biocontrol *via* Symbiotic Organisms

Some insects rely on symbiotic microbes for survival or reproduction. CRISPR can be employed to:

i. Disrupt symbiotic relationships by editing the genes of symbiotic bacteria or fungi within the insect, weakening the pest's ability to thrive or reproduce.
ii. Modify symbiotic bacteria to make them produce compounds that are harmful to their insect host, thus serving as a biocontrol agent.

6. Developing Insect-Resistant Plants via RNA Interference (RNAi)

CRISPR can be used to enhance RNA interference (RNAi) mechanisms in crops, which can silence essential genes in insect pests. This technology involves:

i. Producing dsRNA in crops that, when ingested by insect pests, silences critical genes in the pests, leading to their death or reduced fitness.
ii. Targeting pest-specific genes with CRISPR to ensure that RNAi mechanisms only affect pests and not beneficial insects or other organisms.

7. Creating Transgenic or Hybrid Crops with Insect Resistance

CRISPR can be used to introduce specific traits into crops that confer insect resistance:

i. Engineering crops to express insecticidal proteins, like those from Bt, but with greater precision and less likelihood of resistance development.
ii. Enhancing pest-resistance traits from wild relatives by transferring or enhancing natural resistance traits found in wild plant species into cultivated crops.

Benefits of CRISPR-Cas9 in Insect Pest Control

1. **Targeted and Precise:** CRISPR-Cas9 offers unparalleled precision, allowing scientists to target specific genes, minimizing off-target effects

and reducing the risk of unintended consequences.

2. **Environmentally Friendly:** CRISPR-Cas9-engineered crops can reduce reliance on chemical insecticides, minimizing their environmental impact and promoting sustainable agricultural practices.
3. **Durable Resistance:** CRISPR-Cas9-mediated resistance is more durable than traditional methods, as it targets the insect pest's genome directly, making it more challenging for pests to develop resistance.
4. **Faster Development:** CRISPR-Cas9 allows for quicker development of insect-resistant varieties compared to traditional breeding methods, accelerating the deployment of solutions to pressing agricultural challenges.

Challenges and Considerations

1. **Off-target effects:** CRISPR modifications must be carefully monitored to prevent unintended consequences in both plants and pests.
2. **Resistance development:** Just as pests have evolved resistance to pesticides, there is concern that they could eventually evolve to resist CRISPR-induced changes.
3. **Environmental and ecological impacts:** Reducing or eliminating specific pest species could disrupt ecosystems, impacting other organisms that depend on them.

Regulatory Considerations

The use of CRISPR technology in agriculture raises important regulatory questions. Different countries have adopted varied stances on the classification of CRISPR-edited crops. For example, the United States has a more permissive regulatory environment compared to the European Union, which often treats CRISPR-edited organisms similarly to GMOs. This disparity can affect the speed at which insect-resistant varieties reach the market.

Conclusion

CRISPR technology holds great promise for the development of insect-resistant crop varieties, offering a sustainable solution to a pressing agricultural challenge. As research continues and the regulatory landscape evolves, the potential for CRISPR-edited crops to transform pest management strategies in agriculture is significant. Continued collaboration between researchers, regulatory bodies, and the agricultural community will be crucial to harnessing this technology for the benefit of global food security.

References

Mohan K.S., (2017). An area-wide approach to pink bollworm management on Bt cotton in India–a dire necessity with community participation. *Current Science*, 112:2016-2020.

Naik V.C.B., Pusadkar P.P., Waghmare S.T., Raghavendra K.P., Kranthi S., Kumbhare S., Nagrare V.S., Kumar R.,Prabhulinga T., Gokte-Narkhedkar N. and Waghmare V.N., (2020). Evidence for population expansion of cotton pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) in India. *Scientific Reports.* 10:1-11.

Li, J. J., Shi, Y., Wu, J. N., Li, H., Smagghe, G., & Liu, T. X. (2021). CRISPR/Cas9 in lepidopteran insects: Progress, application and prospects. *Journal of insect physiology*, 135, 104325.

Taning, C. N. T., Van Eynde, B., Yu, N., Ma, S., & Smagghe, G. (2017). CRISPR/Cas9 in insects: Applications, best practices and biosafety concerns. *Journal of insect physiology*. 98: 245-257.

Singh, S., Rahangdale, S., Pandita, S., Saxena, G., Upadhyay, S. K., Mishra, G., & Verma, P. C. (2022). CRISPR/Cas9 for insect pests management: a comprehensive review of advances and applications. *Agriculture.* 12(11): 1896.

Tang, X., Lowder, L. G., Zhang, T., Malzahn, A. A., Zheng, X., Voytas, D. F., & Qi, Y. (2017). A CRISPR–Cpf1 system for efficient genome editing and transcriptional repression in plants. *Nature plants.* **3**(3): 1-5.

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