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Gene disruption technology for management of stored grain insect pest

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Introduction

After crop harvesting agricultural products are to be stored for various future uses like seed, feed, grain, industrial raw material and for processing as valuable products so during storage period these agricultural products can be attacked by various stored grain insect pests which results in significant losses to the farmers. The infestation of insect pest while storage leads to deterioration in quantity and quality of stored product as well as causes reduction in percentage germination in seeds. In India, the traditional methods of management like sun drying, application of vegetable oil and mixing of botanical material is commonly used practices. All these methods were cheap, easy and eco-friendly but results are slow as compare to synthetic insecticide and fumigants. The fumigation method was most extensively used for preventing insect pests in storage because it gives quick as well as maximum prevention against all the stages of insect pests. The commonly used fumigants are phosphine, methyl bromide, cyanogens, sulfuryl fluoride. The main limitations with these fumigants where they leave residue, develop resistance and adverse effect on environment and human health. Keeping in view the limitation of traditional as well as synthetic insecticides, the new gene disrupting technology such as RNA interference (RNAi) and Clustered Regulatory Interspaced Short Palindromic Repeat (CRISPR) were used as effective tool for management of storage insect pest.

Major insect pest of storage grain

Common name	Pest	Family	Order
Rice weevil	*Sitophilus oryzae, S. zeamais, S. granarius*	Curculionidae	Coleoptera
Lesser grain borer	*Rhyzopertha dominica*	Bostrychidae	Coleoptera
Angoumois grain moth	*Sitotroga cerealella*	Gelechiidae	Lepidoptera
Pulse beetle	*Callosobruchus chinensis, C. maculatus*	Bruchidae	Coleoptera
Cigarette beetle	*Lasioderma sericorne*	Anobiidae	Coleoptera
Drug store beetle	*Stegobium paniceum*	Anobiidae	Coleoptera
Tamarind Beetle	*Pachymeres gonagra*	Bruchidae	Coleoptera
Sweet Potato weevil	*Cylas formicarius*	Anobiidae	Coleoptera
Potato tuber moth	*Phthorimoea operculella*	Gelechiidae	Lepidoptera
Arecanut beetle	*Araecerus fasciculatus*	Anthribidae	Coleoptera
Red flour beetle	*Tribolium castaneum*, *Tribolium confusum*	Tenebrionidae	Coleoptera
Indian meal moth	*Plodia interpunctella*	Phycitidae	Lepidoptera
Fig moth or almond moth	*Ephestia cautella*	Phycitidae	Lepidoptera
Rice moth	*Corcyra cephalonica*	Galleriidae	Lepidoptera
Khapra beetle	*Trogoderma granarium*	Dermestidae	Coleoptera

Most of the available publication on gene disruption in the agricultural field has focused on the red flour beetle, *Tribolium castaneum*, the current genetic model for coleopteran stored grain pests [13]. Therefore, in this article, we will focus on *T. castaneum*, and the advances made for potential agricultural use of two gene disruption technologies, RNAi and CRISPR.

RNA interference (RNAi)

In nature, RNAi initiates when long double stranded RNA (dsRNA) is introduced into an organism via infection. Once the dsRNA is introduced, the endoribonuclease Dicer cleaves the dsRNA into 21–23 nucleotide fragments, which known as short interfering RNA (siRNA). The unwound single-stranded guide strand of the siRNA is incorporated into an RNAi-induced silencing complex (RISC) that targets and degrades RNA with complementary sequence. It was first discovery in *Caenorhabditis elegans* [10], whereby the induced dsRNA moves from cell to cell throughout the entire body via a systemic response. The successful RNA knockdown is dependent on factors like length and concentration of the dsRNA fragment, nucleotide sequence specificity, life stage and genetic background of the test organism [14,11]. In *T. castaneum,* directly injecting dsRNA at any life stage can result in gene silencing [12].

CRISPR

CRISPR and the endonuclease CRISPR-associated protein 9 (Cas9) system (CRISPR-Cas9) originated from the innate immune system of bacteria. Unlike RNAi, which disrupts gene expression, CRISPR-Cas9 is a effective DNA editing technology that not only disrupts gene expression, but also alters or even inserts coding sequences. In bacteria, foreign DNA sequences integrated into DNA are targeted by the CRISPR-Cas9 system as part of a defense mechanism that enables bacteria toward off infections from viruses and bacteria [7]. CRISPR-Cas9 uses a guide RNA (gRNA) to detect and form base pairs with a DNA target sequence, and binds the Cas9 endonuclease which cuts the double stranded DNA (dsDNA) very precisely (Fig 1) [6,8,9]. The DNA break is repaired by non-homologous end joining (NHEJ) or homology-directed repair (HDR) by endogenous cellular machinery. When dsDNA breaks are repaired by NHEJ, a single or multiple nucleotide insertion or deletion (INDELS) often occurs, and can shift the reading frame of the gene sequence, effectively turning the gene off. When no INDELS occur, the DNA is restoring to its original state and no change occurs. HDR needed the incorporation of an additional template component containing the desired altered sequence, flanked by sequences homologous to either side of the cut site. In HDR, homologous recombination is utilized to incorporate new sequences to repair or introduce genes. CRISPR genome editing is simpler, more cost effective, faster, and easier to use than already existing genome editing technologies, like transcription activator-like effector nucleases (TALEN) and zinc finger nucleases, and facilitates precise and efficient targeting, editing, modification, and regulation of cells and organisms [1].

CRISPR technology holds gene-editing promise for insect model organisms such as *T. castaneum* [4]. The first and only report of CRISPR technology utilized in a stored product pest was in *T. castaneum* for gene targeting and transgene replacement [5], with more reports anticipated to follow. The development of a system that uses the *T. castaneum* U6 promoter (or other more effective promoters) for expression in plasmid delivery systems will further advance CRISPR studies in *T. castaneum.*

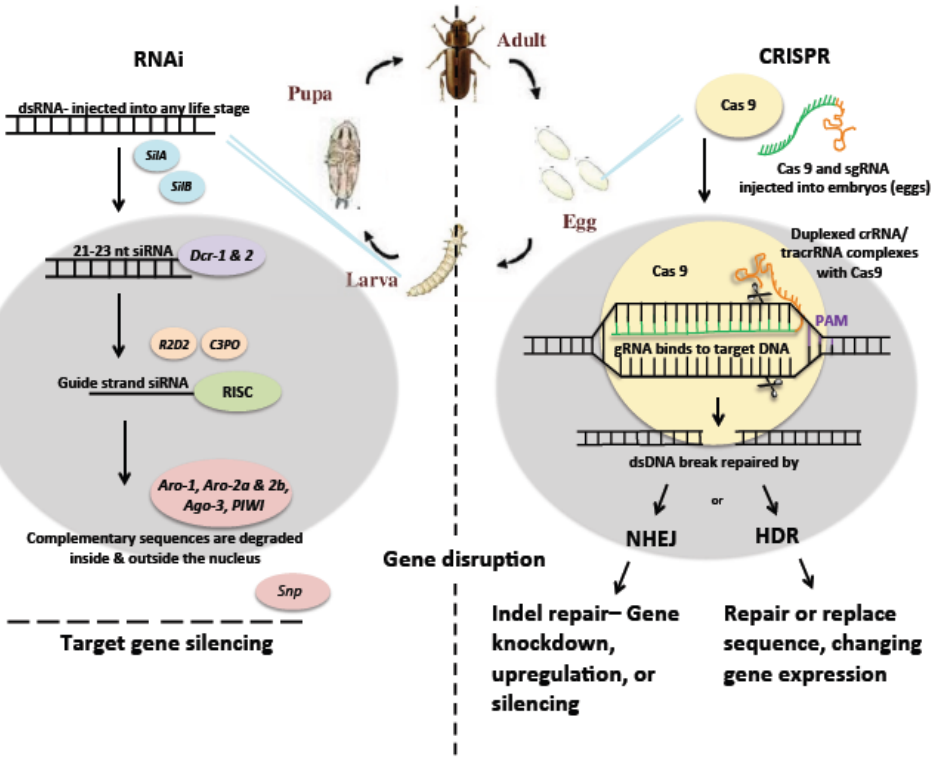

Fig 1: Diagrammatic representation of RNA interference (RNAi) and CRISPR-Cas technology in *T. castanem.*

Difference between RNAi and CRISPR

CRISPR edits the DNA of the cell, thereby changing gene expression permanently if it is a stable transformation. RNAi interferes with existing gene expression and has diminishing effects unless dsRNA is continuously administered, while in some cases a parental RNAi effect has been reported [2]. A modification of the RNAi technology called CRISPR interference (CRISPRi) has reversible effects, but targets DNA instead of RNA. CRISPRi uses a catalytically deactivated Cas9 (dCas9) that reversibly binds to target DNA to inhibit gene expression [3]. CRISPR does not interfere with the endogenous cellular machinery, which is limitation of siRNAs or short hairpin RNAs (shRNA) that may cause cell death [1]. While there are advantages of CRISPR for permanent gene alteration, RNAi has advantages in applied use, whereas CRISPR technology has thus far been restricted by delivery methods as well as biosafety containment considerations. RNAi and CRISPR gene disruption technologies supplement each other in gene function research.

Conclusion

RNAi and CRISPR both are new molecular based technology for insect pest management. These technologies are target specific so don't have any adverse effect on humans and environment. The new gene disruption techniques succeed in dealing with major limitation of traditional methods and synthetic insecticide used for management of stored grain pests. RNAi and CRISPR technologies have generated the potential for novel and yet unpractised stored product pest control methods, gene expression modification, gene editing, and gene modification. These gene disruption technologies also show compatibility with integrated pest management technology.

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