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First report on CRISPR/Cas9 based *in vitro* restriction of *yellow* gene in the oriental fruit fly, *Bactrocera dorsalis*

SANGEETA DASH[1]*, ASOKAN R[2] and JEMLA NAIK D[3]

ICAR-Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru, Karnataka 560 089, India

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ABSTRACT

Bactrocera dorsalis (Hendel) is a serious pest that can cause significant financial losses. CRISPR/Cas9 is a reliable and effective approach for site-directed genome editing. The CRISPR/Cas9 system modifies DNA in a stable and heritable manner. Due to their complementary nature to the target gene, these artificially created single guide RNAs (sgRNAs) instruct the Cas9 endonuclease to produce double strand breaks in the gene sequence. This causes a functional disruption in target gene expression in the organism. The present study was carried out during 2020–2022 at ICAR-Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru, aimed at the validation of genetic targets for genetic biological control of this notorious pest via CRISPR/Cas9 mediated genome editing at 560089. Gene *yellow* crucial for melanin pigmentation and body colouration in the insect was cloned followed by the synthesis of two (sg692 and sg915) off-target minimised guide RNA. The designed gRNAs were used for restriction assay to check the cleavage efficiency of sgRNAs. A successful *in vitro* cleavage of the gene was witnessed. Thus, our research proves the effectiveness of CRISPR/Cas9 mutagenesis as a suitable genetic control strategy for the management of pests of quarantine and economic significance.

Keywords: *Bactrocera dorsalis*, CRISPR/Cas9, Genetic control, Single guide RNAs, *Yellow* gene

Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is a worldwide horticultural pest causing substantial economic losses. It was originally recorded from Taiwan, China, in 1912 (Wan *et al.* 2012). The genus *Bactrocera*, comprises 440 species (Liu *et al.* 2019). Quick adaptation, better climate tolerance, faster dispersal abilities, and high polyphagia are the prime reasons for the rapid invasion of the pest over Asia-Pacific region, Africa, Oceania, America, etc. (Zheng *et al.* 2019). *B. dorsalis* infests over 250 host plant species (Meng *et al.* 2019), including mango, banana, guava, orange, papaya, etc. (Zhu *et al.* 2022). The economic losses range from 25–30% of the crop produced. Moreover, the pest is categorized as a high-risk quarantine species. It completes 3–5 generations/ year in tropical climates (Chen and Ye 2007). CRISPR/ CRISPR-associated protein 9 (Cas9) is unique genome editing tool. Of late, genetic biocontrol attained by pgSIT by the CRISPR/Cas9 system is popularised in insects *Aedes aegypti*, *Anopheles gambiae*, *Drosophila suzukii*, *Zeugodacus cucurbitae*, and *B. dorsalis*. CRISPR/Cas9 system was primarily discovered as a prokaryotic adaptive immunity system. The mechanism involves mature crRNA and the trans-activating crRNA (tracrRNA), forming the sgRNA, which directs the Cas9 endonuclease to the target by identifying a PAM (Protospacer-Adjacent Motif) (Deltcheva *et al.* 2011). Due to the double-strand break caused by this, the error-prone NHEJ (Nonhomologous End-Joining) pathway and the error-free HDR (homology-directed repair) pathway are activated, initiating the cellular DNA repair system (Moon *et al.* 2022). The *yellow* gene has been essential for DOPA-melanin production (Andersen 2012). As in *Bombyx mori* (Linnaeus) and *Tribolium castaneum* (Herbst), functional disruption of the *yellow* gene encoding Drosophila DCE results in a lack of incorporation of melanin as well as a yellowish overall appearance of the insect body. The phylogenetic diversity of the *yellow* genes was examined in this study and the *in vitro* restriction of the *yellow* gene at multiple sites was successfully demonstrated for the first time to our best knowledge, using a multiplex of two sgRNAs and CRISPR/Cas9 editing tool in *B. dorsalis*.

[1]ICAR-Indian Agricultural Research Institute, Pusa campus, New Delhi; [2]ICAR-Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru, Karnataka; [3]College of Agriculture, University of Agricultural Sciences, Gandhi Krishi Vigyan Kendra, Bengaluru, Karnataka. *Corresponding author email: sangeetadash031@gmail.com

MATERIALS AND METHODS

Insect colony maintenance: The present study was carried out during 2020–2022 at ICAR-Indian Institute of

Horticultural Research (13.13°N, 77.49°E), Hessaraghatta, Bengaluru, Karnataka. Since mango is a costly and seasonal host, ripe banana fruits (cv Ney Poovan, Yelakki) were used for larval feeding and as oviposition substrate. By evaluating morphological characteristics and DNA-barcoding of the mitochondrial COI gene, the identity of the insect was verified. *B. dorsalis* was cultured in cuboidal chambers measuring 30 cm × 30 cm × 30 cm, bound by a wire mesh on 1 side, glass on 3 sides, a cloth which has an opening to handle flies on the 1 side, and a wooden floor. The environment of the culture room was maintained at 14 h light-to-dark photoperiod and controlled temperature and humidity levels (25±1°C and 75±1%, respectively). The adult flies were fed with 1:1 mixture of yeast and sugar hydrolysate powder, along with a ball of cotton which has been soaked in a 1:5 solution of a vitamin mixture that was made locally using distilled water and syrup (A to Z NS multivitamin syrup; Alkem Laboratories) (Rajan *et al.* 2023).

Molecular cloning and analysis of B. dorsalis Bd-yellow *gene*: Five seven-day-old pupae from the *B. dorsalis* laboratory population have been used to extract total RNA, using RNAiso Plus (Takara Bio Inc.) as per the instructions of the manufacturer. Using NanoDrop (Thermo Fisher Scientific), the RNA's quality was assessed, and 1.5% agarose gel electrophoresis was done to confirm the RNA's integrity. Following the manufacturer's instructions, 1µg of extracted total RNA was converted into single-stranded cDNA by utilizing the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). Using the PCR, the overall coding region was amplified using *yellow* gene-specific primers (Forward primer: GTTGTACGCAAGGAAACGG, Reverse primer: CTCTTGTCATCTGCCAGTTC). The cycling conditions included 2 min of initial denaturation at the temperature of 95°C, 30 sec of annealing at the temperature of 54°C, 2 min and 30 sec of extension at the temperature of 68°C, and 10 mins of final extension at the temperature of 68°C. The PCR amplicon which has been cloned to the *pTZ57R/T* cloning vector (Thermo Fisher Scientific) was gel-purified by utilizing the Nucleo Spin Extract II kit (Machery Nagel). By utilizing M13 universal forward as well as reverse primers, isolated plasmids "from transformed *E. coli* DH5-α colonies were subjected to Sanger sequencing (ABI prism® 3730 XL DNA Analyzer; Medauxin) to verify gene identity. Using 1000 bootstrap repetitions, ClustalW multiple alignments of the attained *yellow* gene sequences with database reference gene sequence was carried out in BioEdit (version 7.2.6.1). Using MEGA11.Ink, a phylogenetic tree was constructed. With RStudio (version 2022.12.0+353), the pairwise sequence similarity matrix along with the sequence difference count matrix has been generated.

Preparation of sgRNA: The sgRNAs have been designed for the *yellow* gene by utilizing the online design tool of CRISPR, CHOPCHOP V3 (Montague *et al.* 2014). The gRNA region was identified from the functional domain of the concerned protein. Two off-target minimized guide RNA sequences, in exon 2 (5'-CCCCGATCCATTGCGTGGTGACT-3' and 5'-TTTGTGGTGCTGGATGAACGTGG-3') were selected for *yellow* gene by performing NCBI-BLAST to check off-target effect. A reverse complement of the gRNA was also designed.

sgRNA hybridization and PCR amplification of sgRNA cassette: The gRNA and its reverse complement were hybridized (Thermo Scientific sgRNA hybridization kit). For *in vitro* single guide RNA synthesis, *yellow* gene-specific primers were used for the first PCR reaction, and hybridization of the oligonucleotide of primers of sgRNAs was done. Vector, *pBSK* was digested by using restriction endonuclease enzyme, Hind III and then the sgRNAs were ligated to the restriction site. Cloning and sequencing were done to confirm the ligated sgRNAs.

In vitro *transcription of sgRNA*: The amplified product was eluted from gel by utilizing the NucleoSpin Extract II kit (Machery Nagel) as per the protocol of the manufacturer. Two micrograms have been utilized as a template for an *in vitro* transcription reaction, which was then treated with DNase1 (Thermo Fisher Scientific) and then incubated at the temperature of 37°C for one hour after being treated with 5X transcription buffer, T7 RNA polymerase (30U), 10mM NTP mix, and RiboLock RNase inhibitor (50U) in a 150µL total reaction volume. The resultant *in vitro* transcribed sgRNA had been hybridised, purified, as well as quantified utilizing NanoDrop (Thermo Fisher Scientific) and NEB (New England Bio Lab)'s Monarch RNA Clean Up Kit.

In vitro *restriction assay*: To evaluate restriction effectiveness of the designed sgRNAs, an *in vitro* restriction test was performed. NEB r3.1 buffer (10X), *yellow* gene-specific CDS, 30 nM of EnGen Spy Cas9 NLS enzyme (New England Bio Lab), 30 nM of *in vitro* transcribed sgRNAs (sg692 and sg915), and 5 mM of KCl were used to set up a reaction mixture in a 20 µL reaction volume. After assembling the reaction mixture without the *yellow* CDS on ice, it was incubated for span of 30 min at the temperature of 25°C in a water bath. The combined mixture was then given a 1 h incubation period at the temperature of 25°C in a water bath after adding *yellow* CDS. The identical mixture composition minus the sgRNAs was used to set up the negative control. The entire reaction mixture was loaded onto a 1.5% agarose gel, after a 1 h incubation period. Different-sized digested products were produced by the reaction mixture containing the *yellow* gene-specific CDS, depending on the sgRNA cut site position in the *yellow* CDS. Using specifically designed sgRNAs, the digested size of the band had been compared to the anticipated size of the band of digestion from the Cas9 cut site to the ends of the *yellow* CDS. The negative control showed no signs of digestion.

RESULTS AND DISCUSSION

The yellow gene is typically involved in melanin synthesis in insects. When compared to the abdomen, the *yellow* gene's expression is greater in the compound eyes (head region) of 7–12-day-old pupae (Bai *et al.* 2019).

Similar to *D. melanogaster*, where *yellow-y* has been greatly expressed in pupal stages as well as peaks in the 2nd half of pupal growth, *B. tryoni yellow* exhibited the highest level of expression at the time of the development of pupa. The yellow phenotype is caused by mutations in the yellow gene's coding and regulatory sequence, which causes the fly to lose melanin overall and have a continuous yellowish body colour for the duration of its life (Wittkopp *et al.* 2002). Removing yellow-y from the screw-worm fly in the New World Aussie sheep blowflies with *Cochliomyia hominivorax* Strong yellowish body colour is caused by *Lucila cuprina* at eclosion (Paulo *et al.* 2022). In the housefly *Musca domestica*, loss of *yellow-y* expression also impacts melanin production throughout the body (Heinze *et al.* 2017); however, in the coleopteran *Tribolium castaneum*, it solely impacts the pigmentation of the hindwing (Arakane *et al.* 2010). Significant drops in eclosion rates and the proportion of fliers immediately following emergence were seen in the Bd_*yellow* altered flies. The yellow strain exhibited more locomotor activity, and these alterations did not affect copula latency, duration, or mating probability. In comparison to wild-type flies, yellow flies lived about 10-days less long in both sexes (Nguyen *et al.* 2021). Since melanin formation depends on the *yellow* gene, the decreased expression of *Bd-yellow1* in mutants cause reduction in dark pigmentation in head spots (Bai *et al.* 2019). For the first time, this work offers proof that the *white* and *yellow* genes may be involved in cuticle colouring (Bai *et al.* 2019). Thus, *yellow* gene editing can have a significant impact on the survival and longevity of *B. dorsalis* providing opportunities for sustainable pest management. Additionally, the gene can be employed in SIT (Sterile Insect Technique) programmes as a marker. For SIT, substituting yellow body markers for dyes can streamline the production process, remove a step that has been shown to lower fly quality, eliminate potentially dangerous dyes from the manufacturing process, allow for precise separation from wild flies, and increase cost-effectiveness (Nguyen *et al.* 2021).

The CRISPR/Cas9 genome editing tool is a useful equipment for studying gene function in non-model organisms and inducing gene-specific mutagenesis (Wang *et al.* 2017, Choo *et al.* 2018, Paulo *et al.* 2022). Using a single sgRNA, this technique has been successfully utilized for mutagenesis in tephritid fruit flies such as *Anastrepha suspensa* (Li and Handler 2019), *Ceratitis capitata* (Meccariello *et al.* 2017, Sim *et al.* 2019), *B. tryoni* (Choo *et al.* 2018), as well as *B. dorsalis* (Zheng *et al.* 2019). More recently, *Z. cucurbitae* (Paulo *et al.* 2022) has also been successfully exploited. Using double sgRNA and embryonic RNP microinjections, we were able to successfully disrupt numerous locations in the *yellow* gene of *B. dorsalis* in our work.

Characterization and diversity analysis of B. dorsalis yellow *gene*: Sanger sequencing followed by NCBI-BLAST confirmed the identity of the *yellow* gene in *B. dorsalis*. The sequenced results showed 94.87% identity to the predicted *B. dorsalis* protein *yellow* mRNA in NCBI (XM_011216478.3 → XP_011214780.2) (BioProject: PRJNA851394). The genomic area with the greatest match was extracted from nucleotide database of the NCBI, and the locations of the exons and introns were determined (NC_064306.1). The putative *yellow* gene 1686 bp coding sequence was made up of two exons that together coded for 553 amino acids across a 16,740 bp genomic sequence. Using the CHOPCHOP online tool, two gRNAs (sg692, sg915) were designed in exon 2 of the *B. dorsalis yellow* gene (Fig. 1A). The *yellow* gene nucleotide sequence of *B. dorsalis* (XM_011216478.3) was translated into the amino acid sequence. Available *yellow* gene sequences of closely related tephritids like *Bactrocera neohumeralis* (XM_050474451.1), *Bactrocera latifrons* (XM_018946934.1), *Bactrocera oleae* (XM_036365790.1), *Zeugodacus cucurbitae* (XM_011184078.3), *Bactrocera tryoni* (XM_040106768.1), *Anastrepha obliqua* (XM_054871608.1), *Ceratitis capitata* (XM_004521040.3), and *Anastrepha ludens* (XM_054092633.1) were taken from the NCBI database, converted to amino acid sequences, and then used the Clustal W algorithm in BioEdit with 1000 bootstrap replicates to align them with the amino acid sequences of *B. dorsalis*. The *yellow* protein of *B. dorsalis* formed a distinct clade and has different amino acid sequences from other tephritid flies, according to the phylogenetic tree (Fig. 1B). With 88.4% nucleotide sequence similarity to the *yellow* gene of *B. oleae*, 87.7% to *B. neohumeralis*, 87.3% to *B. latifrons*, 84.7% to *Z. cucurbitae*, 77.6% to *B. tryoni*, 72.2% to *Anastrepha ludens*, 70.9% to *Ceratitis capitata*, and 59.9% to *Anastrepha obliqua,* the *yellow* gene of *B. dorsalis* exhibits a high degree of homology. The pairwise nucleotide sequence difference matrix depicted that *B. dorsalis yellow* gene nucleotide sequence has 906 nucleotide sequence variations with *yellow* gene of *Anastrepha obliqua*, 679 with *C. capitata*, 646 with *Anastrepha ludens*, 506 with *B. tryoni*, 350 with *Z. cucurbitae*, 287 with *B. latifrons*, 277 with *B. neohumeralis* and 261 with *B. oleae* [Fig. 1D (b)]. In a similar manner, the difference count matrix and similarity matrix of amino acid sequences were plotted (Fig. 1E).

Designing of single guide RNA (sgRNA): Designed gRNAs had no potential off-target sites, which were confirmed by NCBI-BLAST. Both the PAM (protospacer adjacent motif) and seed region (the last 12 nucleotides of the target sequence within sgRNA) sequences matched the target sequence exactly. Studies by Cong *et al.* (2013) revealed that the seed region of sgRNA as well as PAM sequences play a vital role in initiating efficient restriction in the target region. Mismatch in these seed regions and absence of PAM site effects in non-recognition of a target site.

In vitro *restriction assay for* yellow *gene*: The ability of used sgRNAs to restrict the gene was validated by an *in vitro* restriction assay using the designed sgRNAs, either in isolation or in combination (Fig. 1C). Sample 1 (*Yellow* CDS + sg692 + sg915 + Cas9), sample 2 (*Yellow* CDS + sg915 + Cas9), sample 3 (*Yellow* CDS + sg692 + Cas9) were used. The results revealed that, in sample 1 (*Yellow* CDS + sg915 + sg692 + Cas9), the *Yellow* CDS (1.6kb)

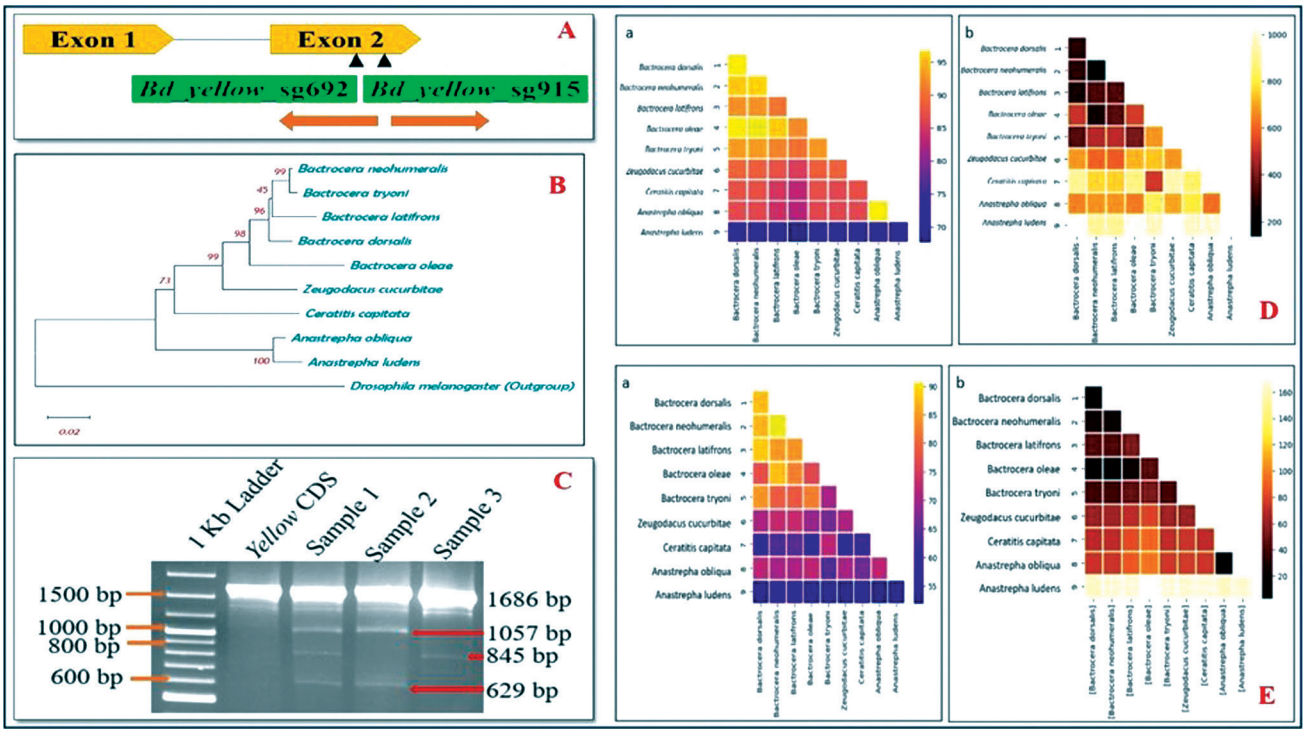

Fig. 1 (A), Schematic representation of position of single guide RNA; (B), Bayesian phylogenetic tree of the yellow protein of different species of Tephritidae; (C), *In vitro* restriction assay of yellow sgRNAs. The samples are, Sample 1 (*Yellow* CDS +sg692+sg915+Cas9), sample 2 (*Yellow* CDS +sg915+Cas9), sample 3 (*Yellow* CDS +sg692+Cas9); (D) (a), Pairwise nucleotide sequence similarity matrix, (b), Pairwise nucleotide sequence difference count matrix of *yellow* gene of different species of Tephritidae; (E) (a), Pairwise amino acid sequence similarity matrix, (b), Pairwise amino acid sequence difference count matrix of *yellow* gene of different species of Tephritidae.

was restricted by Cas9 to generate four digested products of size 1057 bp, 845 bp, 841 bp, and 629 bp. Likewise, on digestion with Cas9, sample 2 (*Yellow* CDS + Cas9 + sg915) produced two digested products of 1057 bp and 629 bp, and sample 3 (*Yellow* CDS + Cas9 + sg692) produced two digested products of 841 bp and 845 bp. The above results on CRISPR/Cas9-based *in vitro* digestion showed that the single guide RNAs that we designed are compatible with the Cas9 enzyme and ready for editing of the *yellow* gene of mango fruit fly, *B. dorsalis.* Thus, these sgRNAs can be further used in the delivery of ribonucleoprotein (RNP) complex into embryos of *B. dorsalis*.

In this work, we effectively disrupted the *yellow* gene of *B. dorsalis* by targeting many sites within the gene at once with double multiplexed sgRNA. Double sgRNA cleaves the target gene at many places simultaneously, which is more effective (Dong *et al.* 2019). Various sgRNA molecules have different cleavage effectiveness, and using double sgRNA could aid in cleaving a gene substantially without sacrificing numerous other traits, whereas individual sgRNAs may fail to achieve this (Zhang and Reed 2017).

When two sgRNA are used simultaneously for embryo microinjection, the target gene is more successfully cleaved at several places than when sgRNA is used alone (Zhang and Reed 2017, Dong *et al.* 2019). Double sgRNA microinjection into embryos was effective in causing a mutation in the *white* gene in *B. oleae* (Meccariello *et al.* 2020), *B. tryoni* (Choo *et al.* 2018), and *B. dorsalis* (Bai *et al.* 2019). According to Meccariello *et al.* (2017), RNPs (Cas9 + sgRNA) were microinjected into embryos using CRISPR-Cas9 to disrupt the *white* gene which is present in *C. capitata*. The *white* gene had significant fragment deletions when 2 sgRNAs had been applied simultaneously to target two different sites. Within the eyes of the G0 individuals that survived, a high prevalence of somatic mosaics had been noted (Meccariello *et al.* 2017). The efficacy of sgRNA cleavage varies amongst molecules; using double sgRNA could aid in cleaving a significant portion of gene while using single sgRNA might result in a tiny deletion that may be filled in by the DNA repair pathway. Thus, to significantly alter the gene, we employed double sgRNA (Pradhan *et al.* 2023). Additionally, using double sgRNAs enhances the likelihood of lengthy deletions with the loss of several additional phenotypes and improves mutagenesis efficiency, something that single sgRNAs occasionally fail to do, according to researchers (Chen *et al.* 2014, Zhang and Reed 2017). Thus, our study has provided concrete evidence of disruption of the *yellow* gene present in *B. dorsalis* using multiple sgRNAs which can be further delivered *in vivo* to study the mutagenic effect on the insect.

In the present investigation, CRISPR/Cas9 proved to be a revolutionary tool, for the effective generation of mutations with reduced off-target effects. *B. dorsalis* is an essential horticultural pest of numerous fruits as well as

vegetables worldwide. The *yellow* gene in *B. dorsalis* is primarily responsible for black melanin pigmentation. It is highly expressed adults and seven-day-old pupae compared to the other immature stages of tephritid fruit flies. Loss of the gene's functionality will thus lead to the development of colourless cuticles in insects. The present study has thus generated a comprehensive protocol of how CRISPR/Cas9 can disrupt the *yellow* gene present in *B. dorsalis*, confirmed by *in vitro* restriction studies. The application of twin sgRNAs to modify the *yellow* gene of *B. dorsalis* significantly affected the loss of pigmentation on the head and body. By using CRISPR/Cas9-based *in vitro* mutagenesis to *B. dorsalis*, it is possible to eliminate functional genes that control the physiology as well as the behaviour of this species of fly and develop novel approaches to managing an economically significant pest.

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REFERENCES

Andersen S O. 2012. Cuticular sclerotization and tanning. *(In) Insect Molecular Biology and Biochemistry,* pp. 167–92.

Arakane Y, Dittmer N T, Tomoyasu Y, Kramer K J, Muthukrishnan S, Beeman R W and Kanost M R. 2010. Identification, mRNA expression and functional analysis of several yellow family genes in *Tribolium castaneum*. *Insect Biochemistry and Molecular Biology* **40**(3): 259–66.

Bai X, Zeng T, Ni X Y, Su H A, Huang J, Ye G Y, Lu Y Y and Qi Y X. 2019. CRISPR/Cas9-mediated knockout of the eye pigmentation gene white leads to alterations in colour of head spots in the oriental fruit fly, *Bactrocera dorsalis*. *Insect Molecular Biology* **28**: 837–49.

Chen P and Ye H. 2007. Population dynamics of *Bactrocera dorsalis* (Diptera: Tephritidae) and analysis of factors influencing populations in Baoshanba, Yunnan, China. *Entomological Science* **10**: 141–47.

Choo A, Crisp P, Saint R, O'Keefe L V and Baxter S W. 2018. CRISPR/Cas9-mediated mutagenesis of the white gene in the tephritid pest *Bactrocera tryoni*. *Journal of Applied Entomology* **142**(1–2): 52–58.

Cong L, Ran F A, Cox D, Lin S, Barretto R, Habib N, Hsu P D, Wu X, Jiang W, Marraffini L A and Zhang F. 2013. Multiplex genome engineering using CRISPR/Cas systems. *Science* **339**(6121): 819–23.

Deltcheva E, Chylinski K, Sharma C M, Gonzales K, Chao Y, Pirzada Z A, Eckert M R, Vogel J and Charpentier E. 2011. CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. *Nature* **471**: 602–07.

Dong Z, Qin Q, Hu Z, Chen P, Huang L, Zhang X, Tian T, Lu C and Pan M. 2019. Construction of a one-vector multiplex CRISPR/Cas9 editing system to inhibit nucleopolyhedrovirus replication in silkworms. *Virologica Sinica* **34**: 444–53.

Heinze S D, Kohlbrenner T, Ippolito D, Meccariello A, Burger A, Mosimann C, Saccone G and Bopp D. 2017. CRISPR-Cas9 targeted disruption of the yellow ortholog in the housefly identifies the brown body locus. *Scientific Reports* **7**(1): 4582.

Li J and Handler A M. 2019. CRISPR/Cas9-mediated gene editing in an exogenous transgene and an endogenous sex determination gene in the Caribbean fruit fly, *Anastrepha suspensa*. *Gene* **691**: 160–66.

Liu H, Zhang D, Xu Y, Wang L, Cheng D, Qi Y, Zeng L and Lu Y. 2019. Invasion, expansion, and control of *Bactrocera dorsalis* (Hendel) in China. *Journal of Integrative Agriculture* **18**: 771–87.

Meccariello A, Tsoumani K T, Gravina A, Primo P, Buonanno M, Mathiopoulos K D and Saccone G. 2020. Targeted somatic mutagenesis through CRISPR/Cas9 ribonucleoprotein complexes in the olive fruit fly, *Bactrocera oleae*. *Archives of Insect Biochemistry and Physiology* **104**(2): e21667.

Meccariello A, Monti S M, Romanelli A, Colonna R, Primo P, Inghilterra M G, Del Corsano G, Ramaglia A, Iazzetti G, Chiarore A and Patti F. 2017. Highly efficient DNA-free gene disruption in the agricultural pest *Ceratitis capitata* by CRISPR-Cas9 ribonucleoprotein complexes. *Scientific Reports* **7**(1): 10061.

Meng L W, Yuan G R, Lu X P, Jing T X, Zheng L S, Yong H X and Wang J J. 2019. Two delta class glutathione S-transferases involved in the detoxification of malathion in *Bactrocera dorsalis* (Hendel). *Pest Management Science* **75**: 1527–38.

Montague T G, Cruz J M, Gagnon J A, Church G M and Valen E. 2014. CHOPCHOP: A CRISPR/Cas9 and TALEN web tool for genome editing. *Nucleic Acids Research* **42**: 401–07.

Moon T T, Maliha I J, Khan A A M, Chakraborty M, Uddin M S, Amin M R and Islam T. 2022. CRISPR-Cas genome editing for insect pest stress management in crop plants. *Stresses* **2**: 493–514.

Nguyen T N, Mendez V, Ward C, Crisp P, Papanicolaou A, Choo A, Taylor P W and Baxter S W. 2021. Disruption of duplicated yellow genes in *Bactrocera tryoni* modifies pigmentation colouration and impacts behaviour. *Journal of Pest Science* **94**: 917–32.

Paulo D F, Cha A Y, Kauwe A N, Curbelo K, Corpuz R L, Simmonds T J, Sim S B and Geib S M. 2022. A unified protocol for CRISPR/ Cas9-mediated gene knockout in tephritid fruit flies led to the recreation of white eye and white puparium phenotypes in the melon fly. *Journal of Economic Entomology* **115**: 2110–15.

Pradhan S K, Karuppannasamy A, Sujatha P M, Nagaraja B C, Narayanappa A C, Chalapathi P, Dhawane Y, Bynakal S, Riegler M, Maligeppagol M and Ramasamy A. 2023. Embryonic microinjection of ribonucleoprotein complex (Cas9 + sgRNA) of white gene in melon fly, *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae) produced white eye phenotype. *Archives of Insect Biochemistry and Physiology* **114**(4): e22059.

Rajan V V, Kumar H, Parvathy M, Bhargava C, Ashok K, Pradan S K, Anu C, Aravintharaj R and Asokan R. 2023. Modifying oviposition behaviour of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) to obtain uniform G0. *Pest Management in Horticultural Ecosystems* **29**(1).

Sim S B, Kauwe A N, Ruano R E, Rendon P and Geib S M. 2019. The ABCs of CRISPR in Tephritidae: Developing methods for inducing heritable mutations in the genera *Anastrepha, Bactrocera* and *Ceratitis*. *Insect Molecular Biology* **28**(2): 277–89.

Wan X, Liu Y and Zhang B. 2012. Invasion history of the oriental fruit fly, *Bactrocera dorsalis*, in the Pacific-Asia region: Two main invasion routes. *PLOS One* **7**: 36176.

Wang J, Wang H, Liu S, Liu L, Tay W T, Walsh T K, Yang Y

and Wu Y. 2017. CRISPR/Cas9 mediated genome editing of *Helicoverpa armigera* with mutations of an ABC transporter gene HaABCA2 confers resistance to *Bacillus thuringiensis* Cry2A toxins. *Insect Biochemistry and Molecular Biology* **87**: 147–53.

Wittkopp P J, True J R and Carroll S B. 2002. Reciprocal functions of the Drosophila yellow and ebony proteins in the development and evolution of pigment patterns. *Development* **129**: 1849–58.

Zhang L and Reed R D. 2017. A practical guide to CRISPR/Cas9 genome editing in Lepidoptera. *Diversity and Evolution of Butterfly Wing Patterns: An Integrative Approach* **24**: 155–72.

Zheng W, Li Q, Sun H, Ali MW and Zhang H. 2019. Clustered regularly interspaced short palindromic repeats (CRISPR)/ CRISPR-associated 9-mediated mutagenesis of the multiple edematous wings gene induces muscle weakness and flightlessness in *Bactrocera dorsalis* (Diptera: Tephritidae). *Insect Molecular Biology* **28**: 222–34.

Zhu Y, Qi F, Tan X, Zhang T, Teng Z, Fan Y, Wan F and Zhou H. 2022. Use of age-stage, two-sex life table to compare the fitness of *Bactrocera dorsalis* (Diptera: Tephritidae) on northern and southern host fruits in China. *Insects* **13**: 258.