North Asian International Research Journal of Multidisciplinary

IRJIF IMPACT FACTOR:6.019

ISSN: 2454-2326 Vol.11, Issue, 2 February-2025

Index Copernicus Value: 58.12 Indian Citation Index

NAIRJC A Peer Reviewed Refereed Journal

doiglobal.org/doilink/10.2024/67b6076437fc5

ADVANCES IN GENETIC CONTROL STRATEGIES FOR *BACTROCERA* AND RELATED FRUIT FLY SPECIES IN INDIA

***MUMINA KHAN**

**College of Temperate Sericulture Mirgund, SKUAST- Kashmir, J&K*
Email: muminaagri@gmail.com

ABSTRACT

India has nearly 200 species of fruit flies, representing approximately 5% of the global tephritid fauna. The subfamily Dacinae, particularly the genus Bactrocera, includes economically significant pests such as B. dorsalis, B. correcta, B. zonata, and B. cucurbitae. These species inflict severe damage to fruit crops, with B. dorsalis causing substantial yield losses in mango and guava production. In Bangalore, mango losses range from 2.5% to 59%, depending on the season, cultivar, and region. Traditional management practices include orchard sanitation, pheromone traps, pre-harvest insecticide sprays, and post-harvest treatments. Integrated pest management (IPM) strategies have achieved control rates of up to 100%.Recent advancements in molecular biology have revolutionized pest management through genome-editing tools such as CRISPR-Cas9 and gene-drive technology. Gene drives introduce genetic elements that bias inheritance, facilitating the suppression or replacement of target insect populations. This innovative approach offers precision, simplicity, cost-effectiveness, and the ability to impact populations across generations without contributing to environmental pollution or chemical resistance. Successful gene-drive systems have been developed for Drosophila melanogaster, Aedes aegypti, and Anopheles gambiae. Incorporating genetic control tools for B. dorsalis presents an opportunity to complement existing strategies. Potential genetic targets have been identified, paving the way for the development of effective control technologies. This review consolidates current knowledge on B. dorsalis management, highlights advancements in genetic engineering, and evaluates the potential of gene-drive systems for sustainable pest control in India.

KEYWORDS:

INTRODUCTION

Tephritid fruit flies are a highly diverse and intriguing group of insects, often referred to as "Peacock flies" due to their characteristic wing-strutting and vibrating behavior (*Kapoor, 1993).* The larvae of most *tephritid* species develop within the seed-bearing organs of plants, with around 35% of these species targeting soft fruits, including a wide range of commercially important fruits and vegetables (*White & Elson-Harris, 1992*). Of the three subfamilies within the *Tephritidae* family, Dacinae holds significant economic importance. Within this subfamily, the genus *Bactrocera* includes key pest species such as *Bactrocera dorsalis* (Hendel) and *B. zonata* (Saunders). Economically relevant species like *B. cucurbitae* belong to the subgenus *Zeugodacus.* Gravid females of *Bactrocera* lay their eggs on ripened fruits, and the larvae, upon hatching, feed on the fruit's edible tissues, leading to decay and secondary infections. The larvae undergo three instar stages before dropping to the ground to pupate. Remarkably, the larvae can leap to locate a suitable pupation site. After emerging, adult flies often require protein from plant surface bacteria for egg maturation (*Drew, 1989; Drew & Lloyd, 1989; Lloyd, 1991*). Globally, there are over 4,000 species of fruit flies (*Norrbom et al., 1998),* with approximately 5% of them found in India (*Ramani, 1998).* The Andaman and Nicobar Islands host 21 species of fruit flies (*Ranganath & Veenakumari*, 1999; *Ramani, 1998*). Interestingly, most species found on mainland India, except *B. cucurbitae* (Coquillett) and *B. tau* (Walker), have not been recorded on these islands. However, some Dacinae fruit fly pests are currently restricted to the islands, necessitating vigilant monitoring to prevent their spread to mainland India.

FRUIT FLY IN INDIA

India, as a leading producer of tropical and subtropical fruits, faces significant losses due to fruit flies, particularly B. dorsalis. This pest is a major challenge, causing yield losses that range from 5% to 80% in mangoes and 10% to 80% in guavas. The substantial economic impact of *B. dorsalis* underlines the need for effective management strategies to improve fruit productivity. Over the years, various control measures have been implemented to mitigate fruit fly infestations, with ongoing research aimed at eradicating these economically harmful pests.

ORIENTAL FRUIT FLY, *BACTROCERA DORSALIS*

The oriental fruit fly, *Bactrocera dorsalis*, is widely distributed across India. In northern India, the species undergoes hibernation during the winter months, whereas it remains active year-round in southern regions, particularly when summer temperatures rise (*Butani, 1979; Nair, 1995; Verghese & Sudha Devi*, 1998). Previously, *B.dorsalis* was reported to infest over 150 host plants. However, after the resolution of the dorsalis species complex, its host range has been narrowed to 117 species, spanning 76 genera and 37 families (*Allwood et al., 1999*). Additionally, this fruit fly has been observed infesting the weed Solanum *indicum L.* in eastern India (Agarwal, 1985).

Table 1. Percentage crop loss caused by *Bdorsalis* in mango and guava.

Host	% Crop loss	Reference
Mango	27	*Kumar et al. 1994*
Mango	31–86	*Mann 1996*
Mango	1–3	*Shukla et al. 1984*
Mango	5–7	*Tandon & Verghese 1996*
Guava	60–80	*Jalaluddin et al. 1999*

Guava 19–42 *Arora et al. 1998*

BIOLOGY AND LIFE CYCLE

The life cycle of Bactrocera dorsalis is akin to that of other fruit-feeding species within the Bactrocera genus (EPPO, 2021). Unlike Bactrocera oleae, which typically mates only twice in its lifespan, female B. dorsalis are polyandrous, mating multiple times (Wee et al., 2000). Copulation occurs at dusk and lasts between 2 and 12 hours, occurring approximately every 4-5 days (Capinera, 1978; Mutamiswa et al., 2021). The species demonstrates high fecundity, producing between 1,400 and 3,000 eggs over a lifespan of around 80 days (Capinera, 1978; Mutamiswa et al., 2021). Eggs are deposited in the mesocarp of fruits following penetration of the peel using the ovipositor, a specialized structure composed of the last three abdominal segments (Aye, 2018; Loomans et al., 2019). The eggs hatch within 1-2 days, depending on environmental conditions such as temperature and humidity. B. dorsalis is a holometabolous insect, with the duration of its immature stages being highly temperature-dependent (Mutamiswa et al., 2021). The larval stage consists of three instars, lasting about seven days under optimal temperatures (28-35°C) but extending up to 36 days at lower temperatures (15°C) (EPPO, 2021; Mutamiswa et al., 2021). Larvae feed on fruit pulp before reaching the third instar, at which point they drop to the ground and burrow into the soil for pupation (Mutamiswa et al., 2021). The pupal stage lasts between 10-14 days under favorable temperatures, though it can extend up to 34 days at 15°C (Ekesi et al., 2006; EPPO, 2021; Mutamiswa et al., 2021). Upon emergence, adults attain sexual maturity within 6-12 days, continuing the reproductive cycle (Capinera, 1978; Loomans et al., 2019; Mutamiswa et al., 2021).

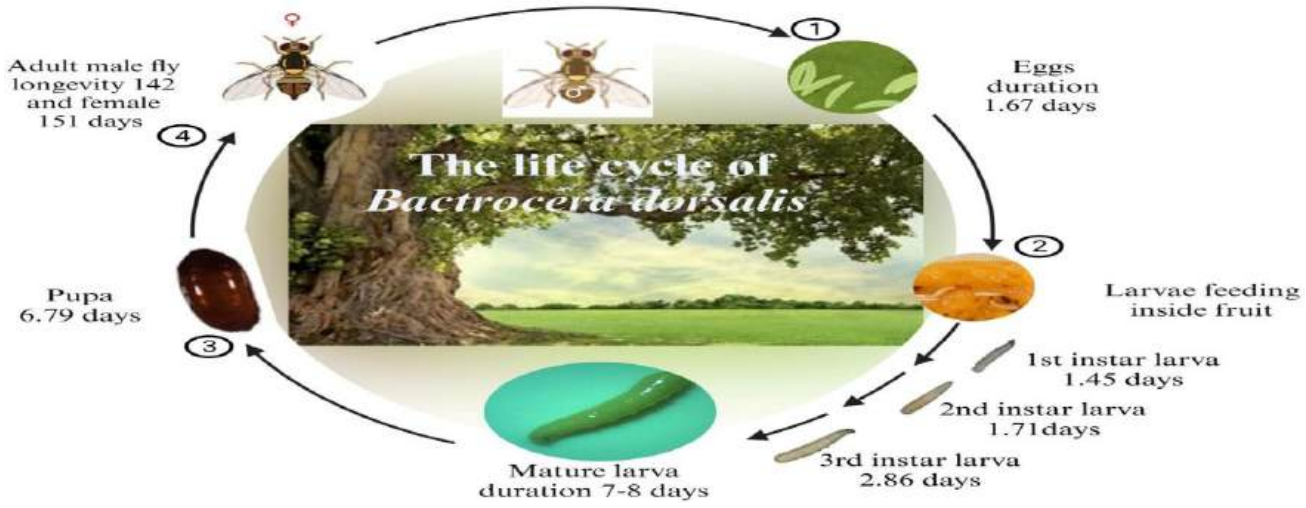

Figure 1: Life cycle of the Oriental fruit fly(Jaffar et al., 2023) . (1) After mating, gravid female B. dorsalis (2) lays its eggs in the mesocarp of the host fruit (e.g., mango) after stinging. (3-4) the eggs hatch 1-2 days later and the larvae develop in the fruit pulp for 7-12 days. (4-5) once reached the last larval stages, larvae fall to the ground and bury themselves in the soil to develop into pupae. (5-6) pupal life lasts about 10-14 days and results in the emergence of adult flies that reach sexual maturity after 6-12 days.

GENOME OF BACTROCERA DORSALIS

The genome of B. dorsalis consists of six chromosome pairs—five autosomal pairs and one pair of sex chromosomes (XX or XY) (Zacharopoulou, Augustinos, & Franz, 2011; Augustinos et al., 2014). Autosomes are numbered II to VI in descending size order, with chromosomes II, III, V, and VI classified as sub-metacentric, while chromosome IV is metacentric. The sex chromosomes are smaller, with the Y chromosome being the smallest (Zacharopoulou, Augustinos, & Franz, 2011; Augustinos et al., 2014). The genome size is estimated to be between 415 and 530 Mb (USDA-ARS, 2014; CAIQ, 2021; CASCEMPS, 2022). Presently, three genome sequences of B. dorsalis are available (ASM78921v2, ASM2028386v1, and ASM2337382v1), with the reference genome (ASM78921v2) comprising approximately 13,026 genes, 19,921 transcripts, and 19,018 mRNAs, as documented in the NCBI Eukaryotic Genome Annotation database (NCBI, 2017).

BREEDING AND OVIPOSITION STIMULATION IN BACTROCERA DORSALIS

Maintaining B. dorsalis colonies under controlled laboratory conditions enables research into various biological aspects such as development, physiology, behavior, gene expression, and genetic modification (Ekesi et al., 2007; Jandt et al., 2015). Genetic modifications typically require microinjections of transgenic DNA into freshly laid eggs, followed by screening for transgenic lines. Thus, a stable laboratory colony is essential for these experiments. Successful rearing of B. dorsalis requires optimal environmental conditions, including a temperature range of 26-28°C, relative humidity of 75-80%, and a photoperiod of 14 hours of light and 10 hours of darkness (Ali et al., 2017; Dong et al., 2017; Wei et al., 2018; Zhang et al., 2020; Chen et al., 2021; Li et al., 2022).
The ideal artificial diet for adult flies includes yeast extract (2.5%), sugar (7.5%), honey (2.5%), agar (0.5%), and water (87%) (Ali et al., 2017; Dong et al., 2017; Sohail et al., 2019; Zheng et al., 2019). Flies typically reach sexual maturity within 6-12 days after emergence, with mating occurring at dusk (Capinera, 1978). Oviposition in gravid females can be stimulated using an oviposition device, typically a perforated plastic bottle (500 mL, 6 cm diameter, 23 cm length), preferably yellow, as it is more attractive to females (Ekesi et al., 2007; Jayanthi et al., 2017).

A cellulose sponge (2 cm^3) soaked with a stimulating solution—either fruit juices (mango, banana) or synthetic compounds (1-octen-3-ol, ethyl tiglate, benzothiazole, or γ-ocatalactone)—is placed inside the device.Among these, γ-ocatalactone has shown the highest stimulation efficacy for oviposition (Jayanthi et al., 2017). Eggs and larvae are maintained in an artificial medium containing sodium benzoate, nipagen, sugar, brewer's yeast, citric acid, and water (Table: 1) (Chang et al., 2009; Liu et al., 2015; Anato et al., 2017). After hatching, larvae feed on this medium until reaching the third instar, at which point they are transferred to a tray containing sterilized, humidified beach sand (72°C for 72 hours) to facilitate pupation. Adults then emerge from pupae within designated cages (Liu et al., 2015; Anato et al., 2017).

Table 1: Composition of an artificial larval medium for Bactrocera dorsalis raring

Ingredients	Proportion (%)
Sodium benzoate	0.09
Nipagen	0.09
Sugar	5.96
Brewer's yeast	11.52

Citric acid	1.26
Water	81.08

CHEMICAL CONTROL OF BACTROCERA DORSALIS

fruit flies until the late 19th century, their excessive use has led to the development of resistance within pest populations, posing a significant threat to horticultural protection (Vontas et al., 2011; Hsu et al., 2012).The resistance mechanisms in B. dorsalis primarily involve enhanced detoxification, facilitated by upregulation of detoxifying enzymes such as Chemical insecticides remain the primary method for managing Bactrocera dorsalis and other Tephritidae pests worldwide (Vontas et al., 2011; Mutamiswa et al., 2021). Key techniques include the male annihilation method, which employs insecticide-laced male attractant pheromone traps, the baiting strategy using food-based traps combined with insecticides, and direct pesticide application (Vontas et al., 2011; Mutamiswa et al., 2021).Initially, organochlorines (OCL), organophosphates (OP), and carbamates (CARB) were widely used before the adoption of pyrethroids and spinosad. While these chemicals were effective against cytochrome P450 monooxygenases (P450s), glutathione S-transferases (GSTs), carboxylesterases (CarEs), and acetylcholinesterase (AChE). Additionally, behavioral resistance is evident as the pest avoids treated areas upon detecting toxic substances (Vontas et al., 2011; Liu et al., 2019).Reports of B. dorsalis resistance to insecticides have emerged from various countries. China and Taiwan have documented resistance to organophosphates, pyrethroids, carbamates, and spinosad (Liu et al., 2019). Similarly, Pakistan has reported resistance to organophosphates and spinosad (Azhar, Khan, and Akram, 2018). In the Americas, resistance to spinosad has been observed in Hawaii (Scott, 2008). In Africa, Pyrethroids and organophosphates remain the most commonly used insecticides, often leading to overuse and potential resistance development. However, no formal insecticide resistance monitoring programs for B. dorsalis have been reported (Mutamiswa et al., 2021). Nonetheless, genetic resistance to commonly used insecticides has been identified in other insect species, such as Anopheles and Aedes mosquitoes (Vontas, Katsavou, and Mavridis, 2020)

BIOLOGICAL CONTROL

Sterile Insect Technique (SIT)

The sterile insect technique (SIT) is a well-established biological control method for managing Bactrocera species, with a history of successful implementation in various countries. As an environmentally friendly and sustainable pest control strategy, SIT complements other management approaches and provides long-term population suppression of economically significant insect pests.

This technique has been effectively used for decades, including for Bactrocera dorsalis (oriental fruit fly), to mitigate its impact on agricultural production. SIT Involves mass-rearing male insects, sterilizing them through irradiation, and subsequently releasing them into the wild. When sterile males mate with wild females, the resulting eggs fail to hatch, leading to a gradual decline in the pest population (Diouf, E.G et al.,).The technique was initially introduced in the 1950s to combat the screwworm (Cochliomyia hominivorax) in the southern United States, and since then, it has been successfully applied against various insect pests worldwide. Several countries, including China, Australia, and Hawaii (Pérez-Staples et al., and Cladera, J.L et al.,), have employed SIT to manage fruit fly infestations. For example, Hawaii implemented SIT in the early 2000s to control oriental

fruit fly outbreaks in the agricultural sector, leading to a substantial reduction in pest populations (Mau, R.; Jang, E., et al.,) Similarly, in Australia, SIT has been integrated into pest management programs to control Ceratitis capitata (Mediterranean fruit fly) in the horticulture industry (Haq, I.U.; Abd-Alla et al.,).A key advancement in SIT involves the development of genetic sexing strains (GSS), which help manipulate population sex ratios to enhance the efficiency of pest management. This method allows for the selective identification and separation of male and female fruit flies using genetic markers, ensuring that only sterile males are released into the environment. By eliminating the need for chemical insecticides (Ramírez-Santos et al.,), GSS further enhances the eco-friendly nature of SIT. This technique has also been applied to manage fruit flies in other countries, including Australia and Thailand (Haq, I.U et al., and Aluja, M et al., and Hendrichs et al.,).These studies highlight the effectiveness of genetic sexing strains as a valuable component of integrated pest management strategies for controlling tephritid fruit flies.

GENETIC CONTROL STRATEGIES

Genetic control methods involve introducing targeted genetic modifications into insect populations to disrupt their reproductive capacity, survival, or ability to cause harm (Mudziwapasi et al., 2021; Quinn et al., 2020; Verkuijl et al., 2022). These modifications can render insects sterile, increase mortality, or alter their natural behavior (Leftwich et al., 2021). Understanding the biology of Bactrocera dorsalis and its host-fruit interaction is essential for identifying genetic control targets. One challenge in applying genetic control to B. dorsalis is its polyandrous mating system, where females mate with multiple males per reproductive cycle. This behavior complicates strategies like the Sterile Insect Technique (SIT), which relies on the release of sterile males. Genetic interventions can be designed to achieve either population replacement or population suppression, each utilizing different approaches.

POPULATION REPLACEMENT

The goal of population replacement is to substitute wild populations with genetically modified, less harmful counterparts (Legros et al., 2021). This strategy involves introducing genetic traits that disrupt key biological functions responsible for the species' negative impact (Marshall et al., 2016). For disease-transmitting insects like Anopheles gambiae and Aedes aegypti, one approach is to disrupt their ability to carry and spread pathogens (Marshall et al., 2016). In B. dorsalis, the damage stems from the larvae developing within and feeding on fruit pulp. A potential replacement strategy would be to genetically alter the insect’s interaction with its host fruit. One promising target for modification is the olfactory system, which plays a central role in guiding flies to their host fruit (Liu et al., 2020).

Altering olfactory cues could prevent flies from locating and infesting fruit, thereby reducing economic damage. However, there is a risk that the modified population might adapt to a new host or experience population decline, inadvertently shifting towards a population suppression strategy.

IMPAIRING FEMALE FERTILITY

Fertility is a critical factor in sustaining pest populations, and disrupting this function can lead to population decline. In Anopheles gambiae, targeted mutations in specific genes, such as AGAP011377, AGAP005958, and AGAP007280, have been shown to reduce female fertility (Hammond et al., 2016).In Bactrocera dorsalis, several

genes with similar functions have been identified. The Bdtud gene is essential for ovarian development, and its knockdown has been shown to reduce both mating success and fertility (Xie et al., 2019). Additionally, suppression of the BdNanos gene results in failed ovary development (Hou et al., 2021). Another study found that inhibiting the Bddsxf transcript of the doublesex gene disrupted yolk protein production, negatively affecting ovarian development and fertility (Chen et al., 2008).

SKEWING SEX RATIOS

Altering the natural sex ratio of a population can lead to population decline and eventual extinction. One method involves to induce a programmed reproductive sex ratio distortion towards males by eliminating female spermatozoa during male spermatogenesis process (Papathanos, Windbichler, &Akbari, 2014; Galizi et al., 2016; Simoni et al., 2020). This approach has been successfully implemented in Anopheles gambiae using site-specific nucleases like I-PpoI or CRISPR-Cas9, which target repeated sequences in the ribosomal genes of the X chromosome, leading to its degradation (Galizi et al., 2014, 2016; Simoni et al., 2020). Similarly, in Ceratitis capitata, CRISPR-Cas9-induced mutations in the transformer-2 (Cctra2) gene resulted in 100% female-to-male conversion (Aumann, Häcker & Schetelig, 2020). In Bactrocera dorsalis, miRNA-1-3p has been identified as a key regulator of male sexual determination. Microinjection of a mimic of this microRNA resulted in male-biased offspring, whereas its suppression led to a female-biased population, making it a potential target for sex ratio manipulation (Peng et al., 2020).

IMPAIRINGFEMALE FITNESS

Fitness, particularly flight ability, is crucial for survival, feeding, and reproduction in insects. Disrupting this ability can significantly impact population sustainability. In Aedes aegypti, mutations in the AeAct-4 and myo-fem genes produced flightless females, with AeAct-4 proving to be a viable candidate for gene-drive approaches due to its haploin sufficiency (O'Leary et al., 2020). In Bactrocera dorsalis, researchers have explored similar approaches. A study by Zheng et al. (2019) used CRISPR-Cas9 to modify the mew (multiple edematous wings) gene, leading to high levels of flight impairment in the first generation. However, in subsequent generations, the mutation did not significantly impact reproductive or foraging behaviors, suggesting limited long-term effectiveness. Another method to reduce fitness involves impairing the fly's olfactory system. Liu et al. (2020) demonstrated that RNA interference (RNAi)-mediated suppression of the BdorOBP2 gene reduced the insects' ability to detect odors, which could impact their ability to locate food and mates.

THE CRISPR-CAS9 SYSTEM

CRISPR-Cas9 represents a cutting-edge technology in genome editing (Khalil, 2020). Originally, it functions as an adaptive immune mechanism in bacteria, protecting against foreign genetic elements (Doudna et al., 2014; Khalil, 2020). The system consists of an endonuclease enzyme, Cas9, and a guide RNA (gRNA), which is a complex of two small RNA molecules: the CRISPR RNA (crRNA), which contains the target recognition sequence, and the trans-activating CRISPR RNA (tracrRNA), which plays a structural role (Doudna et al., 2014; Gratz et al., 2013; Hammond et al., 2016). Scientists design the gRNA sequence to be complementary to the target DNA, allowing it to guide Cas9 to the specific site. Cas9 induces a double-strand break at the target site after recognizing the Protospacer Adjacent Motif (PAM) sequence. Cellular repair mechanisms then take over,

either through the non-homologous end joining (NHEJ) pathway, which often results in small insertions or deletions, or the homology-directed repair (HDR) pathway, which can integrate donor DNA sequences (Gratz et al., 2013; Doudna et al., 2014; Khalil, 2020). The HDR pathway is particularly valuable for targeted gene modifications, enabling the precise insertion of desired genetic sequences (Figure 3). If no donor template is available, the NHEJ pathway repairs the break, often introducing mutations that can disrupt gene function. Conversely, if a donor template is present, the HDR pathway facilitates the integration of new genetic material, enabling gene corrections or insertions via homologous recombination.

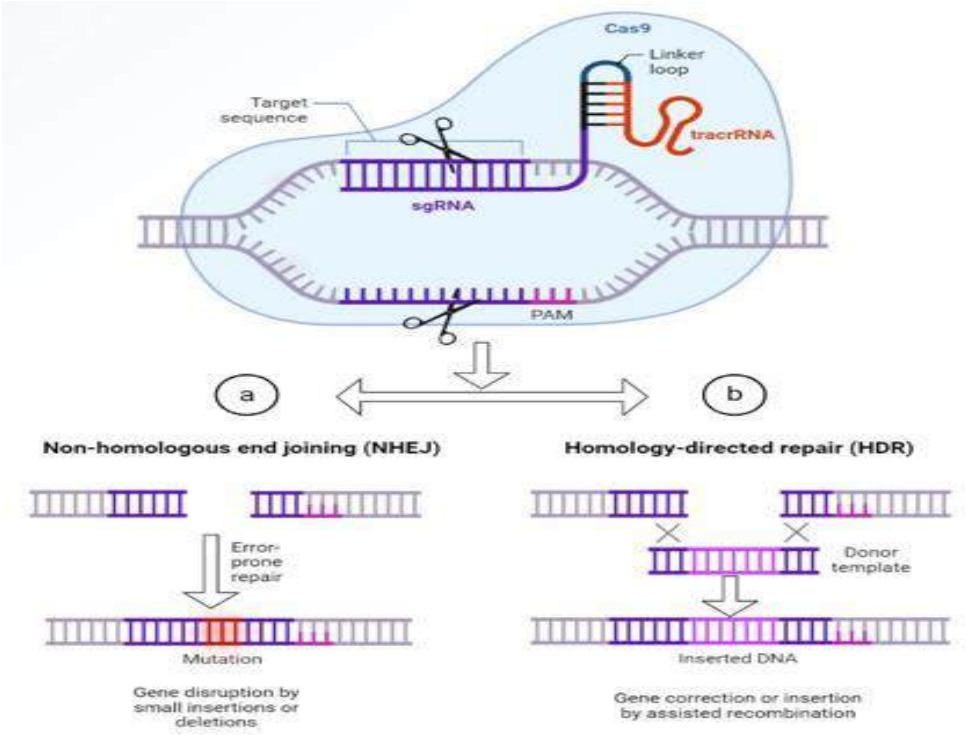

Figure 3: Mechanism of CRISPR-Cas9 (Adapted from BioRender.com)(BioRender.com, 2020). The gRNA attached to the Cas9 protein by the tracrRNA recognizes the target sequence by complementarity. The Cas9 protein cuts the target into double strands after recognizing the PAM sequence. Repair of the lesion results in mutations (deletion or insertion) due to repair errors caused by non-homologous end joining (NHEJ) repair (a) in the absence of a donor template, resulting in gene dysfunction. On the other hand, the presence of a donor template calls for Homology-directed repair (b), which allows. the integration of a fragment of interest to correct a gene or realize an insertion of a fragment by recombination

CRISPR-Cas9 is a powerful and highly specific genome editing tool, making it a valuable asset for genetic modifications and the development of genetic control systems in various insect species (Hammond et al., 2016). Researchers have successfully applied it in fruit flies like D.melanogaster (Terradas et al., 2021; Del Amo et al., 2022) and Ceratitis capitata (Meccariello et al., 2021), as well as mosquito species such as Anophelesgambiae (Galizi et al., 2016; Hammond et al., 2016, 2021; Kyrou et al., 2018) and Aedesaegypti (Li et al., 2020). In B. dorsalis, CRISPR-Cas9 has been successfully employed to study gene function (Baia et al., 2019; X. Chen et al., 2021; Peng et al., 2020; Wang et al., 2020; Zhao et al., 2019; Zheng et al., 2019). The versatility of this technology stems from the availability of different Cas9 variants derived from various bacterial sources (Khalil, 2020), as well as the flexibility of delivering Cas9 in different forms—plasmid DNA (Zheng et al., 2019), injected

mRNA (Peng et al., 2020; Wang et al., 2020), or direct protein injection (Baia et al., 2019). Given its adaptability, CRISPR-Cas9 is a significant advancement in genetic manipulation and pest control strategies for Bactrocera dorsalis.

RNA INTERFERENCE (RNAI)

RNA interference (RNAi) is a powerful gene-silencing technique that utilizes double-stranded RNA (dsRNA) to suppress specific gene expression (Xie, Y.F et al.,). This method has gained attention as a potential eco-friendly alternative for managing insect pests.

Previous studies have successfully silenced genes such as rpl19, v-ATPase-D, noa, and rab11 in adult Bactrocera dorsalis by feeding them corresponding dsRNA. Additionally, several genes associated with digestion and detoxification in the midgut have been identified as potential targets (Li, X.; Zhang and Dong, Y.-C et al.,). However, implementing RNAi for controlling the oriental fruit fly presents several challenges, particularly in the efficient delivery of dsRNA and mitigating unintended effects on non-target species. The full-scale application of dsRNA delivery methods remains underdeveloped, and concerns persist regarding its potential impact on beneficial insects and the host fruits and vegetables. Due to the sequence similarity of rpl19 among B. dorsalis and other beneficial insects, there is a risk of gene expression reduction in natural predators and pollinators. Therefore, refining RNAi approaches to minimize unintended consequences on non-target organisms is a critical focus in its development as a pest management strategy. In addressing insecticide resistance in B. dorsalis, a major global pest affecting diverse crops, a study investigated the role of UDP-glycosyltransferases (UGTs) in resistance mechanisms (Zhou, Y et al.,). These enzymes play a crucial role in metabolizing both plant-derived secondary metabolites and synthetic insecticides. The research identified 31 UGT genes in the B. dorsalis genome, with 12 of them exhibiting high expression in vital tissues such as the antennae, midgut, Malpighian tubules, and fat body. Furthermore, exposure to four different insecticides triggered a significant upregulation of 17 UGT genes. To examine their function, RNAi was employed to suppress five selected UGT genes, which led to a reduction in mortality rates of B. dorsalis when exposed to insecticides, ranging from 9.29% to 27.22% (Chen, M.-L et al.,).

EXPRESSION PROMOTERS FOR BACTROCERA DORSALIS GENOME EDITING

Expression promoters are crucial in transgenic experiments as they determine the spatial and temporal expression of a transgene. They also influence the gene's potential to spread within wild populations, which is particularly important for gene-drive technology (Quinn et al., 2020; Verkuijl et al., 2022). Gene-drive systems aim to modify genomes in a way that ensures the stable transmission of a gene of interest across natural populations. To achieve this, the genetic modification must be both heritable and minimally disruptive to the target species. At the molecular level, the effector system should function in germline cells, preferably before meiosis, to enhance the inheritance of the modified allele. This necessitates the use of carefully selected regulatory elements (Papathanos et al., 2009).

GERMLINE-SPECIFIC EXPRESSION PROMOTERS

A more efficient approach for genome editing is to create transgenic insect lines that express endonuclease genes specifically in germ cells. This is the preferred strategy for developing gene-drive organisms. Commonly used

germline-specific promoters include those of the vasa, nanos, actin, and tubulin genes (Huang et al., 2016). However, vasa and nanos promoters are particularly favored due to their effectiveness in various insect models such as Dmelanogaster (Bischof et al., 2007; Ejsmont et al., 2009; Port et al., 2014),Anophelesstephensi (Macias et al., 2017), Anopheles gambiae (Carballar-Lejarazú et al., 2020; Hammond et al., 2016; Papathanos et al., 2009), and Aedes aegypti (Reid et al., 2021).Although these promoters have not yet been applied to Bdorsalis transgenesis, recent studies have identified and characterized the Bdvasa and Bdnanos genes in this species. These genes are predominantly expressed in ovarian tissues and play essential roles in reproductive development, similar to their functions in other insects (Hou et al., 2021). Given their demonstrated effectiveness in other species, Bdvasa and Bdnanos promoters hold promise for driving transgene expression in B. dorsalis, but further experimental validation is needed.

FLUORESCENT MARKER EXPRESSION PROMOTERS

Fluorescent markers are commonly used in genome editing experiments to visualize genetic modifications. Two main categories of promoters drive their expression: eye-specific promoters and ubiquitous promoters (Schetelig et al., 2013). The synthetic 3xP3 promoter is the most widely used for eye-specific expression (Ejsmont et al., 2009). However, it has proven ineffective in some Tephritidae flies, including Ceratitiscapitata, Anastrephasuspensa, and Anastrephaludens (Schetelig et al., 2013). Further studies are needed to assess its functionality in Bactrocera dorsalis. For ubiquitous expression, the Poly-Ubiquitin (PUb) promoter is commonly employed (Hudson et al., 2014; Schetelig et al., 2013). In Bdorsalis, researchers have successfully isolated and used the BdU6 promoter to drive the expression of the DsRed fluorescent marker (Chen et al., 2011).

GENETIC TRANSFORMATION MARKERS FOR BACTROCERA DORSALIS

To establish and monitor transgenic lines over generations, appropriate transformation markers are essential (Chen et al., 2010a). These markers must enable easy and reliable identification of genetically modified individuals (Horn et al., 2002).

GREEN FLUORESCENT PROTEIN (GFP) MARKER

GFP originally derived from the jellyfish Aequorea Victoria, is a widely used fluorescent marker. However, wild-type GFP has limitations, including low solubility and UV-dependent excitation, which can be harmful to living organisms. The enhanced GFP (EGFP) variant addresses these issues with increased brightness and blue light excitation at 488nm.In Bactroceradorsalis, EGFP has been used successfully (Horn et al., 2002), but due to the species' natural autofluorescence under UV light, it is not the preferred marker. Instead, researchers favor the red fluorescent protein (RFP), DsRed (Chen et al., 2010b).

RED FLUORESCENT PROTEIN (RFP) MARKER (DSRED)

DsRed, derived from discosoma coral, emits stable red fluorescence and is highly resistant to photobleaching. It has been successfully used to identify transgenic B. dorsalis individuals across developmental stages (Chen et al., 2010b). Consequently, DsRed is now the preferred marker for genetic engineering studies in this species (Chen et al., 2011; Chang et al., 2016; Dai et al., 2021).

EMBRYO TREATMENT AND MICROINJECTION PROCEDURES FOR BACTROCERA DORSALIS

Microinjection is the primary technique for introducing genetic modifications in insects. Two key factors must be considered for its success. Firstly, selecting the most suitable embryonic stage for injection is critical (Huang, Liu, and Rong, 2016). Ideally, the pre-blastoderm stage is targeted, as nuclear morphology at this stage is uniform, indicating Synchronized cellular development—an essential aspect for accurately determining the timing of pre-blastodermic formation (Huang, Liu, and Rong, 2016). To confirm this stage, embryos are collected, stained with DAPI, and analyzed for developmental progression. Additionally, the primordial germ cells, which are the focus for heritable modifications, are found in the embryo's posterior region. The second important factor is an effective method for treating the chorionic layer of the embryo (Huang, Liu, and Rong, 2016). Standardized dichlorination techniques initially developed for Drosophila have been adapted successfully for Bactrocera dorsalis and other flies (S. Chen et al., 2010a; S. L. Chen et al., 2011; Handler et al., 2000). Newly laid embryos, less than three hours old, are collected and treated with sodium hypochlorite (NaOCl) to remove the chorion—typically using a 1% solution for 50 seconds to three minutes, or a 1.6% solution for 35 seconds (Baia et al., 2019; Choo et al., 2018; Handler et al., 2000; Wang et al., 2020; Zhao et al., 2019). The embryos are then thoroughly rinsed with 0.02% Triton-X100 followed by deionized water. After rinsing, the embryos are arranged on a slide covered with double-sided adhesive tape. They are then either air-dried or placed in a desiccation chamber for 8–10 minutes before being coated with Halocarbon 700 oil in preparation for microinjection (S. Chen et al., 2010a; S. L. Chen et al., 2011; Handler et al., 2000; C. Huang et al., 2016; Zhao et al., 2019).

Injections are carried out at the posterior region of the pre-blastoderm embryos using a microinjection system and a buffer solution with a pH of 6.8, composed of 5 mM KCl and 0.1 mM sodium phosphate (S. Chen et al., 2010a; S. L. Chen et al., 2011; Choo et al., 2018; Handler et al., 2000; C. Huang et al., 2016; Zhao et al., 2019). The process of aligning embryos and identifying the posterior pole is depicted in Figure 4. Following the injection, the embryos are thermally stimulated at 37°C for one hour before being incubated at 23–25°C under oxygenated and humidified conditions until they hatch (Chen et al., 2011; Huang, Dai, and Chang, 2016). Once the larvae emerge, they are transferred to an artificial diet and maintained under controlled rearing conditions.

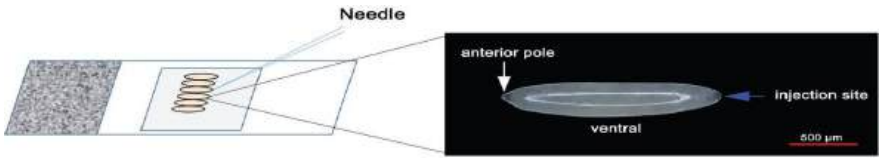

Figure 4: Schematic procedure for microinjection of embryos (Wang*et al.*, 2020). The diagram on the left shows dechorionated eggs coated with Halocarbon 700 oil and then aligned in the same direction on a slide for microinjection. The diagram on the right illustrates the direction of orientation of the embryo and the position

CONCLUSIONS AND FUTURE PERSPECTIVES

Bactrocera dorsalis remains a major threat to fruit production in India due to its adaptability, high reproductive rate, and growing insecticide resistance. While synthetic pesticides are widely used, their reduced efficacy necessitates sustainable alternatives. Genetic control strategies like gene drive technology, sterile insect technique (SIT), and RNA interference (RNAi) show promise but require a deeper understanding of pest biology and ecology. The recognition of B. dorsalis s.s., B. philippinensis, B. papayae, and B. invadens as a single species enables broader genetic control applications, while B. carambolae must also be considered. Challenges such as

polyandrous mating may require frequent sterile male releases or genetic modifications to improve control efficiency. Advancements in genetic engineering, including species-specific promoters (vasa, nanos), and effective markers like RFP, offer potential for targeted modifications. Further research should optimize gene drive systems, refine transgenic insect releases, and assess ecological safety. A sustainable pest management approach should integrate genetic control with biological methods and eco-friendly pesticides. Collaboration among researchers, policymakers, and farmers is essential to successfully implementing these strategies, ensuring long-term control of B. dorsalis, enhancing fruit production, and strengthening food security in India.

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