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Comparative fitness assessment of engineered transgenic mosquitoes expressing blood meal induced multiple-miR-shRNA to block dengue virus transmission in *Aedes aegypti*

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Genetic transformation of mosquitoes using transposon-mediated insertion allows scientists to study genetics of mosquito vectors and to develop genetic-based strategies to reduce vector-borne diseases. RNA interference (RNAi) is one of such strategies that can be used to block viral RNA replication. In our previous study, a transgenic *Aedes aegypti* was produced by integrating miR-shRNAs under control of *Aedes aegypti* carboxy peptidase promoter, a DsRed fluorescent reporter gene into a mosquito genome, using transposase encoded by *PiggyBac* transposable element to be effective for blocking dengue virus replication. In this study, the fitness of transgenic *Ae. aegypti* mosquitoes was measured. *Ae. aegypti* wild and transgenic mosquitoes were maintained at Arthropod Containment Level 2 conditions at 28°C temperature, 72-80% relative humidity, under a 14-15 h light and 9-10h dark cycle. WHO recommended larval food and 10% glucose solution were used to feed larvae and adult mosquitoes respectively during the fitness study. Insectary procedures were standardized to ensure that all mosquitoes were treated similarly to minimize biases and for fitness trait measurements. In fitness analyses of transgenic (TR) and wild type (WT) siblings the following results were observed; female oviposition of 42.8 ± 2.0 eggs/mosquito is significantly lower than 96.6 ± 2.4 eggs/mosquito (Unpaired t test: $p < 10^{-4}$) and the average eggs hatchability of 0.46 ± 0.02 for TR is significantly lower than 0.80 ± 0.02 for WT (Unpaired t test: $p < 10^{-4}$). The average longevity of larvae of 8.01 ± 0.06 days for TR is significantly higher than 6.77 ± 0.05 days for WT (Unpaired t test: $p < 10^{-4}$) and the average adult longevity of 26.57 ± 0.43 days for TR is significantly lower than 28.94 ± 0.50 days for WT (Log-rank (Mantel-Cox) test: $p < 10^{-4}$, Gehan-Breslow-Wilcoxon test: $p < 10^{-4}$). The adult female average longitudinal length of 3.84 ± 0.02 mm for TR is significantly higher than 3.64 ± 0.01 mm for WT (Unpaired t test: $p < 10^{-4}$). Transgenic *Ae. aegypti* mosquitoes' fitness results showed the reduction of fitness compared to wild mosquitoes, common to the transgenic siblings. Currently dengue challenging assays are underway to ascertain the effectiveness of the designed effector molecule in blocking dengue virus transmission in transgenic *Ae. aegypti* and recent results showed that transgenic mosquitoes are resistant to dengue serotype 2 and 4 of Sri Lanka.

Keywords: *PiggyBac*, Transgenic Mosquito, miR-shRNA

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