

C.; and for NL027: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 52° C. and 1 minute at 72° C., followed by 10 minutes at 72° C. The anti-sense T7 template was generated using specific forward and specific T7 reverse primers in a PCR reaction with the same conditions as described above. The sequences of the respective primers for amplifying the anti-sense template for each of the target genes are given in Table 4-NL. The resulting PCR products were analyzed on agarose gel and purified by PCR purification kit (Qiaquick PCR Purification Kit, Cat. Nr. 28106, Qiagen). The generated T7 forward and reverse templates were mixed to be transcribed and the resulting RNA strands were annealed, DNase and RNase treated, and purified by sodium acetate, following the manufacturer's instructions, but with the following modification: RNA prep is washed twice in 70% ethanol. The sense strand of the resulting dsRNA for each of the target genes is given in Table 8-NL.

[0393] The template DNA used for the PCR reactions with T7 primers on the green fluorescent protein (gfp) control was the plasmid pPD96.12 (the Fire Lab, <http://genome-www.stanford.edu/group/fire/>), which contains the wild-type gfp coding sequence interspersed by 3 synthetic introns. Double-stranded RNA was synthesized using the commercially available kit T7 RiboMAX™ Express RNAi System (Cat. N°. P1700, Promega). First two separate single 5' T7 RNA polymerase promoter templates were generated in two separate PCR reactions, each reaction containing the target sequence in a different orientation relative to the T7 promoter. For gfp, the sense T7 template was generated using the specific T7 FW primer oGAU183 and the specific RV primer oGAU182 (represented herein as SEQ ID NO: 236 and SEQ ID NO: 237, respectively) in a PCR reaction with the following conditions: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 55° C. and 1 minute at 72° C., followed by 10 minutes at 72° C. The anti-sense T7 template was generated using the specific FW primer oGAU181 and the specific T7 RV primer oGAU184 (represented herein as SEQ ID NO: 238 and SEQ ID NO: 239, respectively) in a PCR reaction with the same conditions as described above. The resulting PCR products were analyzed on agarose gel and purified (QIAquick® PCR Purification Kit; Cat. N°. 28106, Qiagen). The generated T7 FW and RV templates were mixed to be transcribed and the resulting RNA strands were annealed, DNase and RNase treated, and purified by precipitation with sodium acetate and isopropanol, following the manufacturer's protocol, but with the following modification: RNA prep is washed twice in 70% ethanol. The sense strands of the resulting dsRNA is herein represented by SEQ ID NO: 235.

D. Laboratory Trials to Screen dsRNA Targets Using Liquid Artificial Diet for Activity Against *Nilaparvata lugens*

[0394] Liquid artificial diet (MMD-1) for the rice brown planthopper, *Nilaparvata lugens*, was prepared as described by Koyama (1988) [Artificial rearing and nutritional physiology of the planthoppers and leafhoppers (Homoptera: *Delphacidae* and *Deltoccephalidae*) on a holidic diet. *JARQ* 22: 20-27], but with a modification in final concentration of diet component sucrose: 14.4% (weight over volume) was used. Diet components were prepared as separate concentrates: 10× mineral stock (stored at 4° C.), 2× amino acid stock (stored at -20° C.) and 10× vitamin stock (stored at -20° C.). The stock components were mixed immediately prior to the start of a

bioassay to ¼× concentration to allow dilution with the test dsRNA solution (4× concentration), pH adjusted to 6.5, and filter-sterilised into approximately 500 µA aliquots.

[0395] Rice brown planthopper (*Nilaparvata lugens*) was reared on two-to-three month old rice (*Oryza sativa* cv Taichung Native 1) plants in a controlled environment chamber: 27±2° C., 80% relative humidity, with a 16:8 hours light:dark photoperiod. A feeding chamber comprised 10 first or second instar nymphs placed in a small petri dish (with diameter 3 cm) covered with a single layer of thinly stretched parafilm M onto which 50 µA of diet was added. The chamber was sealed with a second layer of parafilm and incubated under the same conditions as the adult cultures but with no direct light exposure. Diet with dsRNA was refreshed every other day and the insects' survival assessed daily. Per treatment, 5 bioassay feeding chambers (replicates) were set up simultaneously. Test and control (gfp) dsRNA solutions were incorporated into the diet to a final concentration of 2 mg/ml. The feeding chambers were kept at 27±2° C., 80% relative humidity, with a 16:8 hours light:dark photoperiod. Insect survival data were analysed using the Kaplan-Meier survival curve model and the survival between groups were compared using the logrank test (Prism version 4.0).

[0396] Feeding liquid artificial diet supplemented with intact naked dsRNAs to *Nilaparvata lugens* in vitro using a feeding chamber resulted in significant increases in nymphal mortalities as shown in four separate bioassays (FIGS. 1(a)-(d)-NL; Tables 1a-d-NL). These results demonstrate that dsRNAs corresponding to different essential BPH genes showed significant toxicity towards the rice brown planthopper.

[0397] Effect of gfp dsRNA on BPH survival in these bioassays is not significantly different to survival on diet only.

[0398] Tables 10a-d-NL show a summary of the survival of *Nilaparvata lugens* on artificial diet supplemented with 2 mg/ml (final concentration) of the following targets; in Table 10(a)-NL: NL002, NL003, NL005, NL010; in Table 10(b)-NL NL009, NL016; in Table 10(c)-NL NL014, NL018; and in Table 10(d)-NL NL013, NL015, NL021. In the survival analysis column, the effect of RNAi is indicated as follows: +=significantly decreased survival compared to gfp dsRNA control (alpha <0.05); -=no significant difference in survival compared to gfp dsRNA control. Survival curves were compared (between diet only and diet supplemented with test dsRNA, gfp dsRNA and test dsRNA, and diet only and gfp dsRNA) using the logrank test.

E. Laboratory Trials to Screen dsRNAs at Different Concentrations Using Artificial Diet for Activity Against *Nilaparvata lugens*

[0399] Fifty µl of liquid artificial diet supplemented with different concentrations of target NL002 dsRNA, namely 1, 0.2, 0.08, and 0.04 mg/ml (final concentration), was applied to the brown planthopper feeding chambers. Diet with dsRNA was refreshed every other day and the insects' survival assessed daily. Per treatment, 5 bioassay feeding chambers (replicates) were set up simultaneously. The feeding chambers were kept at 27±2° C., 80% relative humidity, with a 16:8 hours light:dark photoperiod. Insect survival data were analysed using the Kaplan-Meier survival curve model and the survival between groups were compared using the logrank test (Prism version 4.0).

Feeding liquid artificial diet supplemented with intact naked dsRNAs of target NL002 at different concentrations resulted