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Chromosomal Location of a Recessive Red-Eye Mutant Gene in the Brown Planthopper *Nilaparvata lugens* (Stål) (Insecta: Hemiptera)

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Abstract

The color of compound eyes is an important biological characteristic of insects. A red eye color mutation is commonly found in the brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), a serious insect pest of rice in tropical and temperate Asia. The genetic inheritance and physiological effect of the eye color mutation in the BPH have been studied, but the location of a *red* gene controlling the red eye mutant phenotype on a chromosome has not been elucidated. In this study, simple sequence repeats (SSRs), together with bulked segregant analysis (BSA), was performed to identify and map the location of the *red* gene. A total of 387 SSR markers distributed throughout the BPH autosome were used to survey two bulked DNA samples. Samples were generated from 29 brown-eyed and 29 red-eyed individuals derived from an F_2 generation of a cross between brown-eyed wild type and red-eyed mutant colonies. The SSR marker BM20 was shown to be associated with the red eye mutant phenotype. Ninety-five offspring of the F_2 generation were then used to map the gene. The present study constitutes the discovery of the location of the *red* gene, which may lead to the acquisition of the genetic determinant of the compound eye color mutation in BPH.

Keywords

Brown Planthopper, Bulked Segregant Analysis, Linkage Map, Red-Eyed Mutant, Simple Sequence Repeat

1. Introduction

The brown planthopper (BPH) *Nilaparvata lugens* (Stål) (Arthropoda: Insecta), the most destructive phloem-sap-sucking insect pest of rice in Asia, belongs to the order Hemiptera, family Delphacidae. The BPH has a diploid chromosome number of 30 (28

autosomes combined with the sex determination system XY and XX in males and females, respectively) [1]. Among the few visible mutations in the BPH, eye color is one of the mutations that are regularly observed in laboratories [2] [3]. In Delphacidae, red-eyed mutants were reported in the small brown planthopper *Laodelphax striatellus* (Fallén) [4] and BPH [5]. Red-or orange-eyed mutant laboratory colonies of BPH have been established. Inheritance studies revealed that a single recessive gene on an autosome controlled the eye color mutations.

Compound eyes are the visual organs for the majority of insects. It is an important biological characteristic. The biological and biochemical characterizations of eye color in BPH mutants have been described. The mating behavior, egg production, developmental duration and mortality of nymphal stages were not significantly different between the normal-eyed and colored-eye mutant colonies [2] [3]. However, some phenotypes (*i.e.*, egg lethal effect, poor fecundity and survival ability) might be related to the eye color mutations [3] [5]. Genes having a stronger compensation ability for reproduction may also closely link to the red eye allele [6].

The color of BPH eyes is determined by ommochrome pigments. Xanthommatin (the pigment causing brown eye color) and pteridine (the pigment causing red eye color) are two primary forms of the pigments. Reduction of pigment granules and pigment concentrations is the biochemical basis of the red eye phenotype in the BPH [2]. It is believed that mutations in genes common to both pigments caused a reduction of both pigments [7] [8]. The causal mutation may occur in xanthommatin or pteridine biosynthesis, transportation of eye pigments, or in pigment granule formation [8].

Recent advances in BPH genomics facilitate the study of various phenotypes of the BPH. Numerous DNA markers were developed [9] [10] that made it possible to identify and map some genes in the BPH through linkage to existing DNA markers [10] [11]. In the present study, bulked segregant analysis (BSA) [12] with SSR markers was used to detect and locate the chromosomal location of the *red* gene controlling the red eye mutation in the BPH. The linked markers from this study will facilitate map-based cloning to identify the *red* gene in the BPH.

2. Materials and Methods

2.1. Insect Populations and Crosses

Two BPH populations collected in Thailand were used in this study: (1) Kalasin-2013 (KLS13) was collected in Kalasin province, northeastern Thailand in 2013 and (2) Krabi-2013 (KBI13) was collected in Krabi province, southern Thailand in 2013 (**Figure 1**). Both populations have been maintained in laboratory conditions for approximately 40 generations. A red-eyed mutant colony was established from a red-eyed female adult found in the KLS13 population. KBI13 was selected as a wild-type colony to cross with the red-eyed mutant colony to generate an F_2 mapping population. All BPH strains were reared on rice seedlings of cv. "TN1" and maintained in the laboratory at 26˚C ± 2˚C under a 14 L:10 D photoperiod. To generate the mapping population, a pair of insects from two different colonies was selected and crossed using single-pair mating. An F_2 population was generated from a cross of KLS13 (♀) × KBI13 (♂). The crosses were performed by placing a virgin female and a male from each colony on "TN1" seedlings

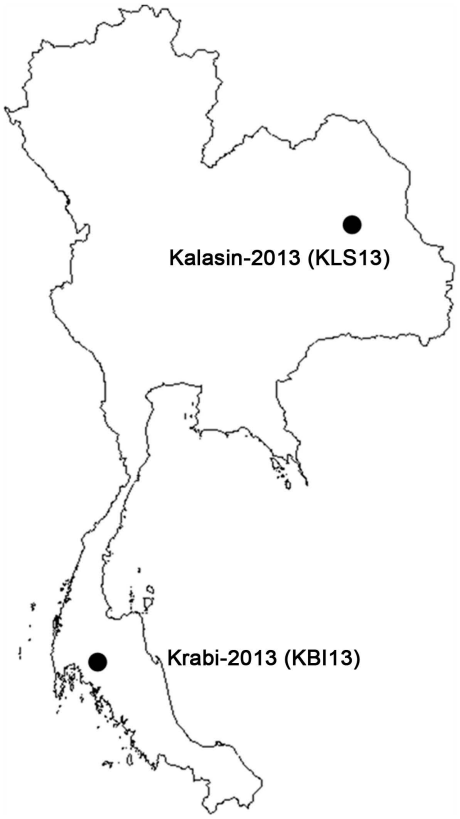

Figure 1. Locations of the collection sites of two brown planthopper, *Nilaparvata lugens* (Stål) populations in Thailand.

in a glass tube. After mating and oviposition, the parental insects were collected and stored in 95% ethanol. Seedlings with eggs were kept until the eggs hatched. Newly hatched nymphs of an F_1 progeny were transferred into a plastic 800 mL container containing 80 to 100 seedlings and reared to the fifth-in star nymph stage. An individual fifth-instar nymph of each sex was selected and reared in a separate glass tube to obtain unmated adults. The F_1 progenies were then allowed to inbreed to generate the F_2 generation. All offspring were then collected and stored in 95% ethanol.

2.2. Total DNA Extraction and Whole-Genome Amplification

Genomic DNA of the preserved samples was isolated individually using a modified version of the potassium acetate procedure of Dellaporta *et al.* [13]. To avoid viability problems that may occur at later stages, second- and third-instar nymph stages of F_2 offspring were collected for DNA isolation. The whole-genome amplification technique [14] was used to produce large amounts of DNA from individual insects for large-scale genotyping.

2.3. Tagging and Mapping of the Red-Eye Mutant Gene

BSA with SSR markers was used to tag a red-eye mutant locus. Based on the presence of eye color of the BPH, we generated two groups of 29 brown-eyed (B) and 29 red-eyed (R) progenies from the F_2 population. A total of 387 SSR markers from Jairin *et al.* [9] and Jing *et al.* [10] covering 14 chromosomes of the BPH were selected to identify the R

and B groups. The genetic linkage map was calculated by Join Map 4 [15] using genotype data from 95 F_2 individuals derived from crosses of KLS13/KBI13. Grouping of markers was performed using a minimum-independence LOD (logarithm of odds) threshold of 5.0. Groups were converted into linkage maps using the regression algorithm provided by Join Map with a recombination frequency of less than 0.45, and then Kosambi's mapping function [16] was used to calculate map distances. Linkage maps were drawn using the Map Chart software for Windows [17].

3. Results and Discussion

3.1. Establishment of the Red-Eyed Colony

A red-eyed female adult BPH was isolated from a field population collected from a farmer's rice field in KLS to establish a red-eyed mutant colony. The colony was used as genetic material for mapping the *red* gene. The mutant individuals could be easily discriminated from the brown-eyed individuals (**Figure 2**). Individuals in the mutant colony had the red eye phenotype at all developmental stages. We observed the red eye phenotype in both macropterous and brachypterous adults, as well as adults with both brown and black body colors. Except for eye color, no other external differences were observed between the colonies (**Figure 3**).

Figure 2. Eye colors of the brown-eyed wild type (a) and red-eyed mutant (b) brown planthopper, *Nilaparvata lugens* (Stål) female adults.

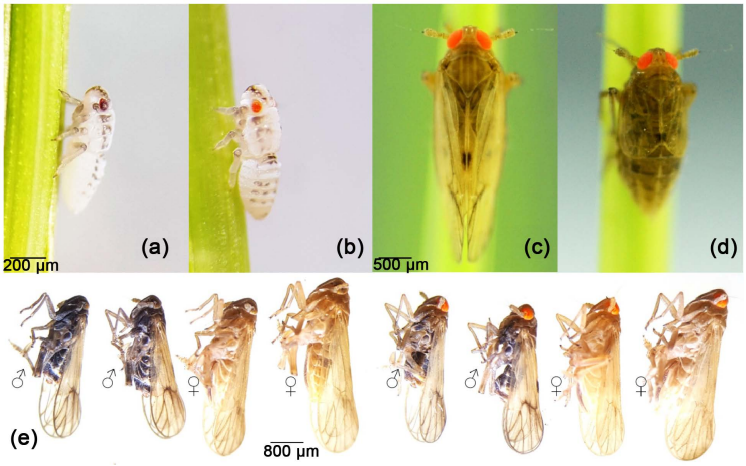

Figure 3. Wild type brown-eyed and red-eyed mutants of brown planthopper, *Nilaparvata lugens* (Stål) at different developmental stages and external characteristics. (a), (b): first in star nymphs, (c), (d): adult females with different wing forms, e: brown and black body colors of adult brown-eyed and red-eyed offspring.

3.2. Crossing Experiment

Cross mating between the homozygous normal brown-eyed KBI13 (♀) and red-eyed mutant KLS13 (♂) produced F_1 offspring with wild-type brown eye color. The F_1 offspring from the reciprocal crosses also showed only normal brown eye color. Inbreeding between heterozygous F_1 progenies resulted in F_2 offspring with brown eye color and red eye color at the expected ratio of 3:1 for a single recessive gene inheritance. The sex ratio in the offspring produced from most crosses was equal based on a chi-square test analysis (**Table 1**). These results suggest that the inheritance of the red eye mutant phenotype is controlled by a single autosomal recessive gene same as the previous findings [2] [3].

3.3. Mapping of the Red-Eye Mutant Gene

To detect the map position of the *red* locus, BSA was employed to identify SSR markers linked to the red eye mutant phenotype. Two bulks from F_2 offspring were generated, each consisting of 29 individual brown-eyed and red-eyed second and third in star nymphs. A total of 387 SSR markers distributed throughout 14 chromosomes were used to screen the pair of bulks. Only SSR markers on sex chromosomes were not selected for the analysis. The primer BM20 generated amplification products for heterozygous alleles in the B bulk and homozygous alleles in the R bulk. The brown-eyed F_2 offspring consisted of nymphs that were homozygous or heterozygous for the mutant allele, while the red-eyed group consisted of only nymphs that were homozygous at the locus. This result indicated that the *red* gene was linked to BM20 in the F_2 population.

We employed an additional 20 SSR markers surrounding the BM20 locus on chromosome 9 [10] to assay 95 F_2 offspring. To obtain accurate estimates of marker positions on the map, we only selected clearly polymorphic markers and carefully scored them to reduce errors. Unfortunately, many markers could not be scored, mostly due to banding patterns that were too difficult to interpret, including multiple bands and bands that did not show clear polymorphism on the 6% polyacrylamide gel. Of 20 SSR markers tested, only three markers showed clearly distinguishable polymorphisms and were easy to score. A genetic linkage map was constructed based on the segregation data. Finally, the *red* gene was mapped to a location between SSR markers BM20 and NLGS182 (**Figure 4**) on chromosome 9 according to Jing *et al.*'s map [10] or to the linkage group 2 according to our previous map [9]. This is the first study to successfully locate the *red* gene in the genome of this important insect pest of rice using marker-based genetic mapping. We have demonstrated that BSA coupled with SSR markers can

Table 1. Eye colors in F_2 offspring of crosses between brown-eyed wild-type (B) and red-eyed mutant (R) brown planthopper, *Nilaparvata lugens* (Stål).

Cross (♀ × ♂)	B	R	Total	$\chi^2_{3:1}$	*P*	Female		Male		B ♀:♂		R ♀:♂	
						B	R	B	R	$\chi^2_{1:1}$	*P*	$\chi^2_{1:1}$	*P*
R × B	48	19	67	0.40	0.53	25	12	23	7	0.08	0.77	0.32	0.25
B × R	34	10	44	0.12	0.73	19	4	15	6	0.47	0.49	0.40	0.53
B × R*	66	29	95	1.55	0.21	-	-	-	-	-	-	-	-

*F_2 offspring from this cross were used as a mapping population. All offspring were collected at the 2nd-3rd in star nymphal stages.

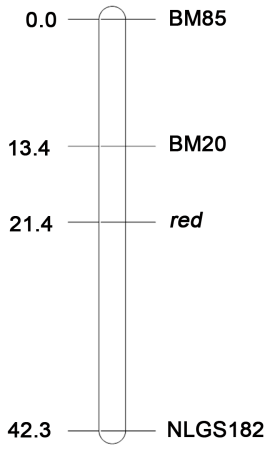

Figure 4. Genetic linkage maps indicating the positions of the red eye mutant gene (*red*) on chromosome 9 of brown planthopper, *Nilaparvata lugens* (Stål). Marker names are listed to the right of the chromosome. The distance between markers is measured in centi Morgans.

easily and rapidly identify DNA markers linked to the *red* gene. The results from genetic crosses and genetic inheritance from the present and previous studies [2] [3] revealed that the red eye color is inherited as a simple Mendelian autosomal recessive gene. The location of the *red* gene on BPH chromosome 9 confirms that the red eye mutation is located on an autosome.

The red-eyed mutant colony has the potential for utilization in BPH research as a visible genetic marker and can be applied in various studies of genetics and ecology at a molecular level (e.g., population dynamics and genetics, evolution of compound eyes, visual acuity, migration, mating behavior, transgenic and RNAi technologies [7]). A xanthommatin-related gene, karmoisin (*Nlka*), reported to be involved in eye mutations in some insect species [18], was expected to cause the red eye mutation phenotype in the BPH. Liu *et al*. [7] identified the karmoisin gene from the BPH and found that the red eye phenotype of the BPH has no close connection with this gene. To identify the gene related to the red eye mutation, map-based cloning is one of the most effective approaches for cloning the *red* gene. Our finding will facilitate fine-scale mapping experiments to isolate the genes responsible for the red-eye mutation in the BPH through genotyping-by-sequencing approaches.

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