Identification, cloning and characterization of the metabolic enzyme genes from three rice planthoppers, *Nilaparvata lugens* (Stål), *Laodelphax striatellus* (Fallén) and *Sogatella furcifera* (Horváth) (Hemiptera: Delphacidae)

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Insect cytochrome P450-dependent monooxygenases (P450, CYP) and Glutathione S-transferases (GST) are closely related to the living and adaptation of insects for their roles in the metabolism of xenobiotics and endogenous compounds [1]. As the most important rice pests across Asian countries, the small brown planthopper (SBPH) *Laodelphax striatellus* (Fallén), the brown planthopper (BPH), *Nilaparvata lugens* (Stål) and the white-backed planthopper (WBPH) *Sogatella furcifera* (Horváth) cause serious damage mainly to rice by feeding the rice plants and serving as vectors for many plant viruses [2]. One of the most urgent problems faced by researchers and farmers for these pests is the rapid adaptation to both insecticides and resistant rice cultivars [3]. Thus, a comprehensive profiling of the P450 and GST superfamilie genes for these pests can contribute to the resolving of the key problems like insecticide resistance and pest evolution to resistant plants.

Based on the establishment of the transcriptomes of these rice planthoppers, we profiled the P450 and GST superfamilies from them. Specifically, we identified and cloned 57 P450 and 9 GST expressed sequence tags (ESTs) for BPH, 50 and 9 for SBPH, 50 and 12 for WBPH. Most of these ESTs cover at least one third of the whole gene length, and 56 of them appear to have complete Open Reading Frames (ORF). Significant nucleotide sequence similarity of the orthologenes exist among the three planthoppers, and conservation can be found in the distribution of gene types within the same family or subfamily, which indicate an analoguous evolution pattern of the metabolic enzymes from these insects under a homologous niche in the rice field.

The expression analysis of 20 P450 genes of BPH showed that most P450 genes are expressed in the midgut of adult insect; particularly those in the families of CYP4 and CYP6, which is consistent with the roles of P450 as metabolic enzymes. The same phenomenom has been discovered in the constitutive transcription of GSTs in SBPH. About 6 and 4 GSTs show a higher expression level (>1.5-fold) in the midgut and malpighian tubules of SBPH compared to antenna. As to the life stage transcription, the egg and first instar nymph each has 7 P450 genes expressed, much higher than the number of fourth instar and male adult. It is very interesting to see that 7 P450 genes expressed in the egg are obviously different from those in the first instar, even from the adult. This may indicate a special metabolic network that BPH eggs need to survive. Most of the 9 GSTs in SBPH also show a higher expression in the egg and nymph than the macropterous adult. On the contrary, the 8 GSTs in the BPH haven't got such a significant difference.

In order to explore the possible function of these GSTs in the adaptation of the three planthoppers, we carried out further studies to exam the influence of insecticides and rice cultivars on the expression of them. We selected a variety of six different insecticides (DDT, Chlorpyrifos, Fipronil, Imidacloprid, Buprofezin and Beta-Cypermethrin) with the rice-stem dipping method. Gene2 from SBPH show a relative higher expression after the insect being expsoed to all these six insecticides, especially in about 6 hours and 24 hours. The expression of Gene4 also appears to be stimulated by three insecticides in about 6 hours. On the contrary, Gene8 show a higher expression in 24 or 48 hours. These SBPH GST genes above may be related to the detoxification of insecticides. In addition, we selected Pei Liangyou 14 (with resistance gene *bph14*) and Liangyou 15 (with resistance gene *bph15*) as

the resistant rice cultivars [4], and Taichung Native 1 is thought to be suitable for the growing of BPH. Compared to the control group Shanyou 63, which we applied for keeping the population of BPH for more than 10 generations in our lab, most of the BPH GSTs show a relative higher expression in 24h of feeding the cultivar TN1, while show a decrease of expression on the two resistance cultivars in about 48h. It is possible that the BPH has got a relative integrated detoxification systems for TN1 in which most of the GSTs can perform certain function. On the contrary, since the Pei Liangyou 14 and Liangyou 15 are not suitable for the growth of BPH, the decrease of GST expression may have been caused by the abnormal physiological state.

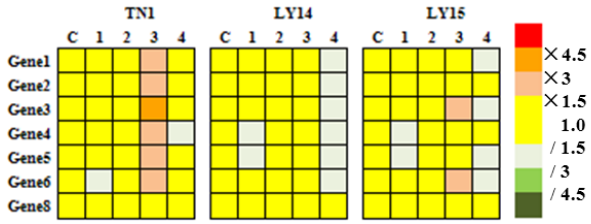

Fig 1, Transcription profiles of GSTs in BPH 3th nymph exposed from 6 to 48h to 3 rice cultivars. Control (C), 6h (1), 12h (2), 24h (3), 48h (4), Taichung Native 1 (TN1), Pei Liangyou 14 (LY14), Pei Liangyou 15 (LY15) . For each rice cultivar, transcription levels are expressed as mean fold transcription relative to controls (unexposed larvae). Red and green indicate significant over- and under-transcription respectively (ratio >1.5-fold in either direction and Mann–Whitney test *P*-value < 0.05). Yellow indicates no significant transcription variations.

As the three important rice pests in Asia, *N. lugens*, *S. furcifera* and *L. striatellus* cause a serious decrease of rice production every year. Our research for the first time profiled the P450 and GST gene families in these pests from different aspects. We hope our data can provide a base for the further study in these pests, not only in the areas of insect control, but also in the basic understanding of the role that P450 and GST play in the physiology of these pests.

References

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