A new phenotypic screen to map quantitative trait loci associated with rice tolerance for planthoppers

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There has been a long history of attempts to design screening tests to measure plant resistance to insects since the time Painter (1951) classified plant resistance into three mechanisms: nonpreference (antixenosis), antibiosis, and tolerance. As the most important insect pests of rice (*Oryza sativa* L.), brown planthopper *Nilaparvata lugens* (Stål) and whitebacked planthopper *Sogatella furcifera* (Horvath) demanded the attention of entomologists and breeders to develop easy and reliable screening techniques to screen a large number of germplasm and breeding materials to develop cultivars with improved resistance to planthoppers (Heinrichs et al 1985).

Tolerance is the most important component of resistance for breeding, but it has not been well used as the phenomenon of tolerance has not been fully understood, there is a lack of suitable techniques to identify and incorporate tolerance into an improved genetic background, and details of the genetics of tolerance have not been determined (Velusamy and Heinrichs 1986).

Panda and Heinrichs (1983) described some tolerance tests: functional plant loss index, tolerance index, antibiosis index, and plant dry weight loss per milligram of insect dry weight produced for BPH. Although various tests have been developed, the availability of tests that measure the nature of resistance precisely without the influence of another factor is still a concern and requires continuous exploration (Reese et al 1994). Plant resistance to planthoppers in rice has been a much studied subject since 1969 when varietal resistance was established for the first time at the International Rice Research Institute (Pathak et al 1969). However, studies on genetics of resistance have always relied on a standard seed box screening test, which can be effective only for qualitative resistance.

Current research has indicated that plant resistance to planthoppers is a quantitative trait, demanding insights into the complexities of signaling and interactions among host and pest genomes. This situation underscores the need for addressing the gap in our understanding of resistance phenotype at the molecular, cell, and whole-plant levels. Our work is one such attempt to design a screening test that can be a more sensitive phenotypic screen for genetic analysis of tolerance for planthoppers in rice.

We hypothesized that days-to-wilt, measured as the number of days after infestation required to kill the plants, can be a sensitive phenotypic screen to identify tolerance in rice for BPH. We evaluated a subset of 94 doubled haploid (DH) lines produced from a cross between IR64 and Azucena (Guiderdoni et al 1992), along with the parents, for days to- wilt after planthopper infestation.

A brief description of the screening procedure is given as follows. Days-to-wilt was measured at two plant age levels: 30 and 60 days after sowing (DW30 DAS and DW60 DAS, respectively) with an insect load of 50 first- and second- instar nymphs per plant. For DW30, 15-day-old seedlings were transplanted in 15-cm-diameter clay pots and placed inside a cylindrical mylar sheet cage (13 × 75 cm). For DW60, seedlings were transplanted in 30-cm-diameter clay pots and put in a 25 × 90-cm mylar cage. The nymphs were released on the plants and allowed to feed. The day the plant wilted completely was recorded. After BPH infestation, 30-day-old IR64 plants survived up to 12.7 days; the Azucena plants survived only up to 5.3 d. The 60-day-old IR64 plants survived up to 17.7 days, whereas the Azucena plants survived up to 11.3 days. Days-to-wilt of 30-day-old DH plants ranged from 6.3 to 13 days; those of 60-day-old DH plants ranged from 8.7 to 19.7 days.

Similarly, 30-day-old plants of IR64 survived up to 88 days after WBPH infestation, whereas the Azucena plants survived only up to 18.5 days. When 60-day-old plants were infested, the WBPH could not kill the IR64 plants, even beyond 90 days after infestation, but the Azucena plants wilted quickly (28 days). Days-to-wilt of 30-day-old DH lines ranged from 9 to 87.5 days, whereas those of 60-day-old plants ranged from 16 to 90 days (Table 1). The experiment was terminated 90 days after insect infestation.

Therefore, 90 days was considered as the days-to-wilt of plants that survived beyond 90 days after infestation. The frequency distribution of phenotypic values of DH lines for days-to-wilt after BPH and WBPH infestation clearly indicated the quantitative nature of resistance (Fig. 1). We did a QTL analysis to test the sensitivity of days-to-wilt with respect to the genetic mechanism behind tolerance for planthoppers using 175 marker data of an IR64/Azucena DH population through Mapmaker/ QTL (Lander et al 1987).

Putative BPH resistance QTLs were detected on chromosomes 6 and 7, with LOD scores of 2.5 and 3.1, when 30- and 60-day-old plants were infested with BPH nymphs, respectively (Soundararajan et al 2004). Similarly, days-to-wilt after WBPH infestation also indicated the presence of possible QTLs on chromosomes 1 and 6 (Table 2, Fig. 2). These QTLs were detected with threshold values of 1.6 and 1.8 for 30- and 60-day-old plants, respectively. The low number of replications might have affected the significance levels of these QTLs. Furthermore, the detected QTLs were specific to plant age 30 and 60 days old, supporting the observation that plant age influences resistance level. Genetic analysis at the appropriate growth stage is necessary. Based on these results, we propose that days-to-wilt after insect infestation could be a sensitive phenotypic screen to detect QTLs associated with resistance to planthoppers.

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Table 1. Phenotypic values of parents and DH lines of IR64/Azucena cross for resistance to planthoppers.

Trait	Parents[a]		DH lines		
	IR64	Azucena	Mean	SD	Range
Days-to-wilt (30 DAS) after BPH infestation	12.7	5.3	9	1.3	6.3–13.0
Days-to-wilt (60 DAS) after BPH infestation	17.7	11.3	14	2.2	8.7–19.7
Days-to-wilt (30 DAS) after WBPH infestation[b]	88.0	18.5	28.9	18.8	9–>90
Days-to-wilt (60 DAS) after WBPH infestation	>90	28.0	48.5	23.5	14–>90

[a]Mean of two replicates. [b]Days from infestation to complete wilting of plant; >90, not wilted on 90th day after infestation.

Table 2. Putative QTLs identified for various traits associated with resistance to planthoppers in IR64/Azucena DH populations of rice.

Trait[a]	Marker interval	Chromosome	LOD	Variance (%)	Additive[b]
Days-to-wilt after BPH infestation (30 DAS)	Pgi2–pRD10B	6	2.5	14.3	–0.5
Days-to-wilt after BPH infestation (60 DAS)	RG773–CDO59	7	3.1	17.6	1.1
Days-to-wilt after WBPH infestation (30 DAS)	W1–RG173	1	1.6	11.6	–7.9
Days-to-wilt after WBPH infestation (60 DAS)	RG172–Cat-1	6	1.8	8.8	–7.8

[a]Putative BPH resistance QTLs are presented here on the basis of the findings of Soundararajan et al (2004). [b]Effect of Azucena allele.

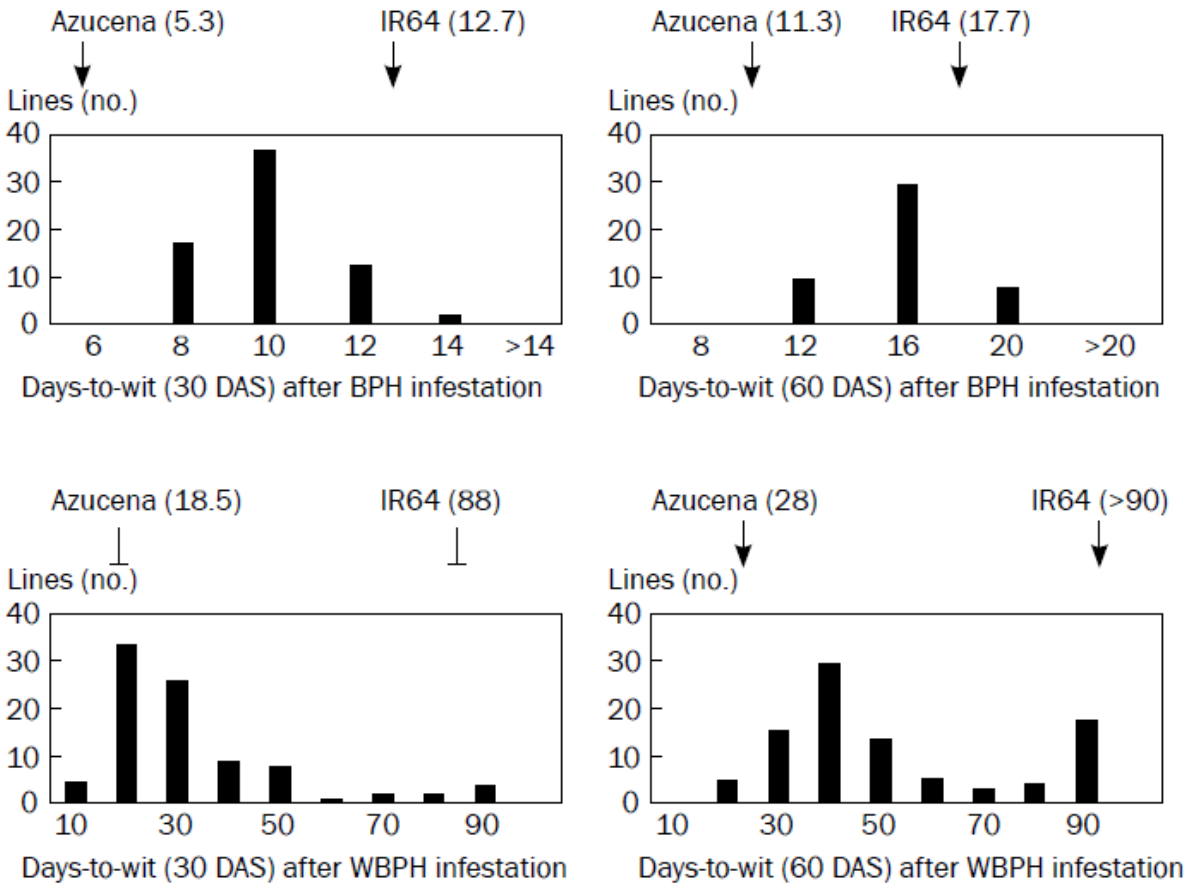

Fig. 1. Frequency distribution of DH lines of IR64/Azucena cross for days-to-wilt after planthopper infestation.

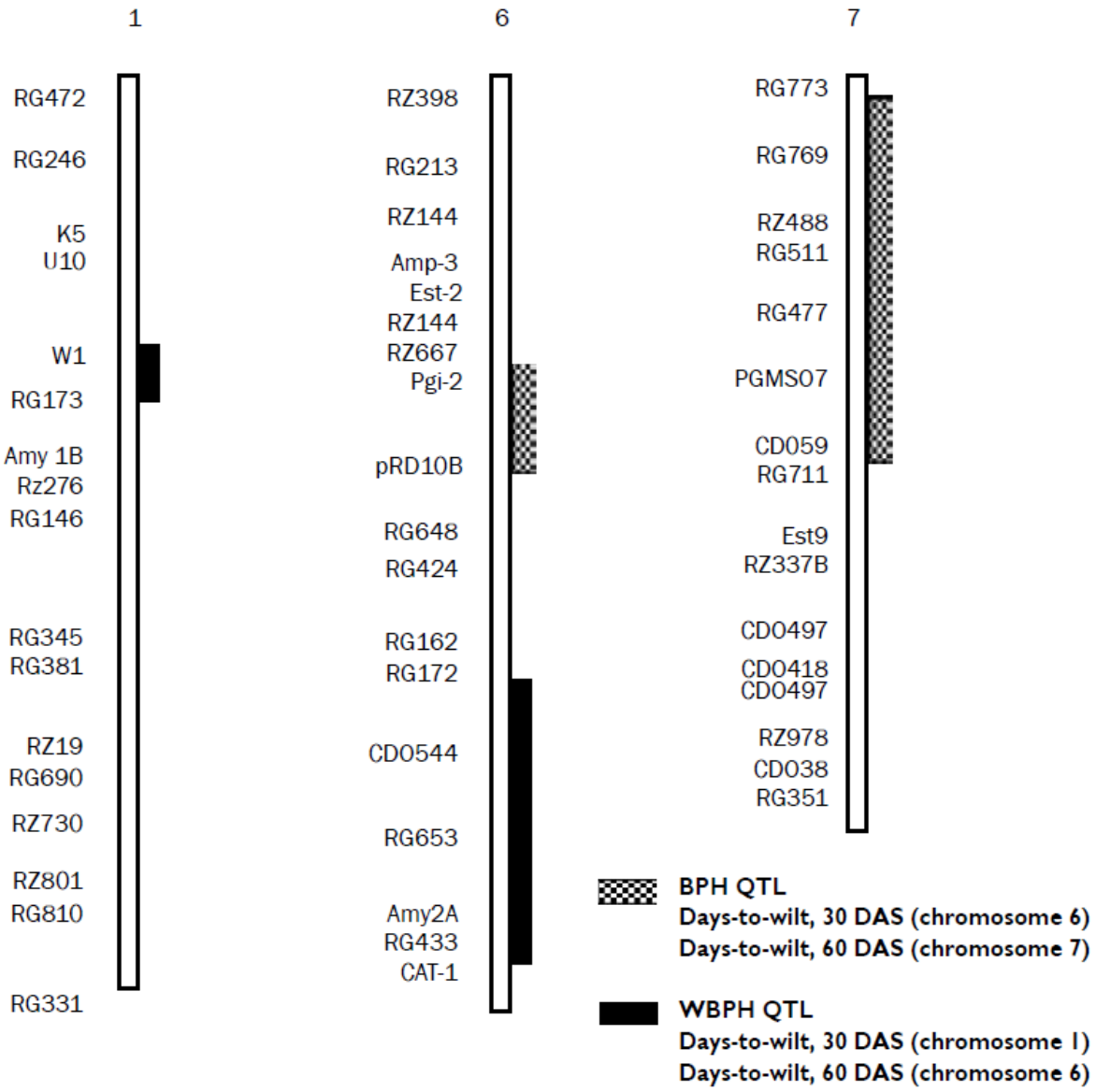

Fig. 2. Linkage map showing chromosomal locations of putative QTLs detected for tolerance for planthoppers.

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