Figure 7. BPH macropterous adult (IRRI property).

Figure 8. BPH brachypterous adults. The big one is a gravid female and the smalls ones, males.

Each male can mate with up to nine females for 24 hours and an individual female can copulate more then twice during its lifetime. BPH can have 2 to 8 generations during a single rice crop, depending of the location. For example, in south Japan there are 5 generations, in central China, 5 to 6 generations and in Java (Indonesia) 4 to 5 generation. Each BPH lives from 18 to 20 days (3-4 weeks) and the total life cycle is among 19 to 26 days (Mochida, 1977).

Both the nymphs and adults of BPH, insert their sucking mouthparts into the plant tissue to remove plant sap from phloem cells. During feeding, BPH secretes feeding sheaths into the plant tissue to form feeding tubes or feeding sheaths. The removal of plant sap and the blockage of vessels by the feeding tube sheaths causes the tillers to dry and turnbrown, a condition known as hopperburn (Figure 9).

Figure 9. Hopperburn in the field (IRRI property).

Because of the pronounced negative effects of insecticides on natural enemies of BPH and the corresponding outbreaks caused by insecticide usage in rice, new sustainable methods of BPH management are required. Since the 1970s, IRRI (International Rice Research Institute) has been developing rice varieties with resistance to the hopper. On resistant rice plants, hoppers have reduced feeding, decreased adult and nymphal survival, decreased fecundity and consequently a slower build-up of populations compared to susceptible varieties.

The first rice variety released with resistance to BPH was IR26. This variety contained the *bph1* gene for resistance to BPH. The variety was initially successful; however, within two years BPH populations had adapted to the gene. IRRI responded with a second series of resistant varities (IR36 + related) with the *bph2* gene for resistance. Again, in less than 5 years, widespread BPH adaptation had occurred to *bph2*. A further range of varieties developed at IRRI and containing the *bph3* gene were released in the 1980s. These varieties were not planted on a wide-scale and the gene still function is many regions. However, there is also some evidence that BPH in some regions has adapted to the *bph3* gene.

Recent physical mapping of planthopper resistance genes have positioned *bph3* gene on the short arm of chromosome 6 (Jairin et al, 2006) of the rice plant. Were different clusters of resistance genes occur at the different chromosome arms (Figure 10). Never was demostrated the meaning of the clusters and in this case, being that the *bph3* gene was inside of the arm of chromosome 6, was analyzed the properties of the genes in this location to observed whether the breakdown of *bph* 3 may affect the other related genes.

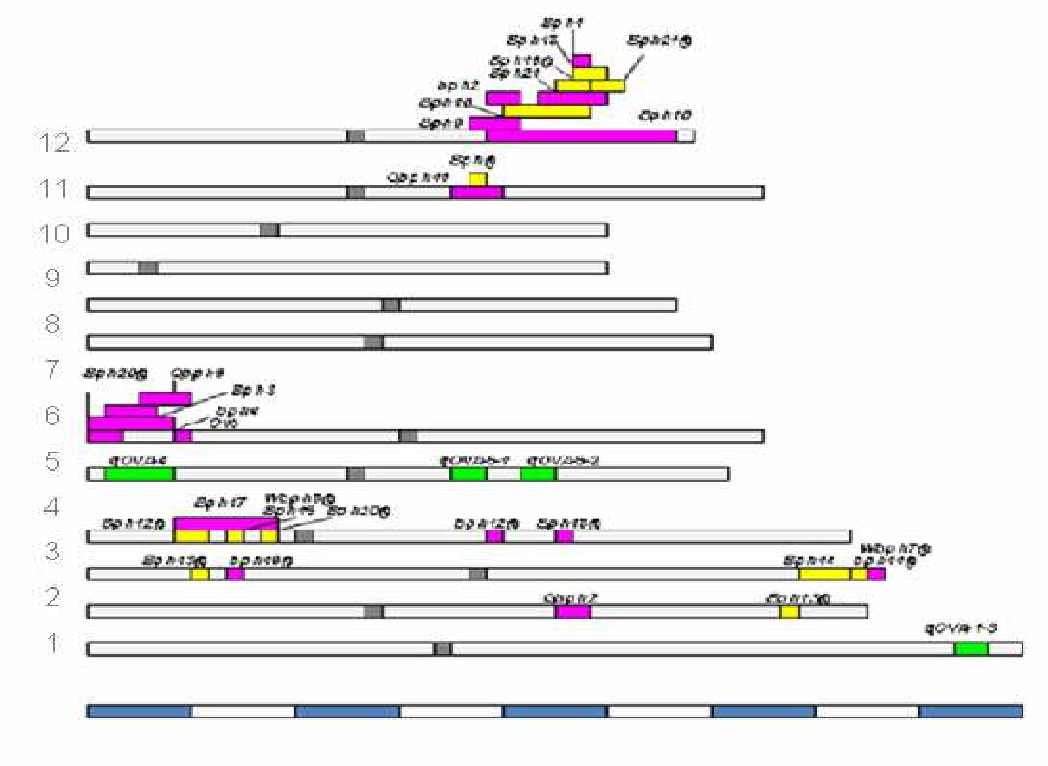

Figure 10. The location of the genes in the arms of chromosome.

In this thesis were examined the effects of the *bph3* gene in IR62 variety on BPH fitness and population build-up. Using colonies previously adapted to the IR62 variety, were examined the mechanisms of adaptation by the hopper. Furthermore, was examined whether adaptation to *bph3* gene in IR62 affects related varieties that derive their resistance from the same gene. Was also examined whether adaptation to *bph3* gene allows BPH to more successfully attack other varieties with difference genetic sources of resistance. In this latter experiment, the resistance genes chosen were those located in the same chromosomal region as *Bph3*.

To demonstrate adaptation and fitness of BPH on different varieties, were conducted a series of bioassays, including honeydew tests, oviposition tests, nymphal and adult survival tests and population build-up tests. Differences in BPH development times on different varieties were also recorded.

OBJETIVES

1) To know the effects of different concentrations of nitrogen applied as fertilizer, on a resistant and a susceptible variety of rice by measuring the honeydew produced, survivorship and development time for two colonies.

2) To examine the effects of adaptation to Bph3 gene (in IR62) by measuring the honeydew produced, the survivorship and development time of BPH from two colonies on resistant varieties with genes from the same region of the rice chromosome 6.

3) To examine the effects of gene breakdown on the resistance of related varieties by measuring the honeydew produced, survivorship and development time of BPH from two colonies.

MATERIAL AND METHODS

Rice varieties

Two varieties of rice were used for the experiments. TN1, is a standard susceptible variety that in all the experiments was used as a control. IR62 is a highly resistant variety containing the *bph3* gene. In the second experiment, were used PTB33 (with *bph 2* and *bph 3* genes), IR62 an introgressed line with BPH 20 (*bph 20*) and Babawee (*bph 4*).

In the third experiment, were used, IR60, IR62, IR66, IR68, IR70, IR72, IR74 and RH (Rathu-Heenati) varieties of rice (all of then with *bph3* gene).Rathu Heenati was included in the experiments because it was the donor plant for *bph3*. PTB33 is also suggested to contain the *bph3* gene, but has other resistance genes also, and was used a resistance check. The IR varieties were breed at IRRI and contain the *bph3* gene from RH.

Seed was acquired from the IRRI-genebank (International Rice Research Institute-genebank). However, BPH20 was acquired from the Plant Genetics Department of Kyushu University, Japan. The plants were grown in soil in a greenhouse and transplanted to 6.5cm of diameter pots (called #0 pots) after 7 to 10 days. Plants were grown under two nitrogen regimes: 0 added nitrogen (N1) and 150 kg/ha of nitrogen (N2) fertilizer.

BPH colonies

For this work a BPH colony was initiated with field collected hoppers from Laguna Province (The Philippines) in 2008. Founder populations (>500 individuals were placed in wire mesh cages of 120×60×60 cm (H×W×L) under greenhouse conditions (Temperatures ranged from 25 to 45°C). The colonies were continually fed with ≥30-day old rice plants. The colony was reared only with TN1 like host plant (which is a highly susceptible rice variety).

After about 40 generations, one sub-group of hoppers was separated from the colony and was placed in cages with IR62 plants. This colony was maintained during 13 generations without introgression of wild caught individuals, on IR62 to allow the population to adapt to feed on this resistant rice variety.

Honeydew test

The honeydew excretion is widely used as a measurement of feeding activity and consequently as an index for resistance and susceptibility of a crop variety to homopteran pests (Auclair, 1959; Liu et al., 1994). Many techniques exist developed to measure the feeding response of *N. lugens* on resistant and susceptible rice plants (Paguia et al, 1980; Pathak et al, 1982; Begum and Wilkins, 1998). The most important two, are the test of filter paper dipped in a solution of bromocresol green and the test of a parafilm sache. In these work the filter paper technique was used.
The plant was located through two holes of the cup (up and down of the cup (5×5:H×R)). The filter, was placed at the base and inside of the cup with a paper protecting it of the humidity of the soil.

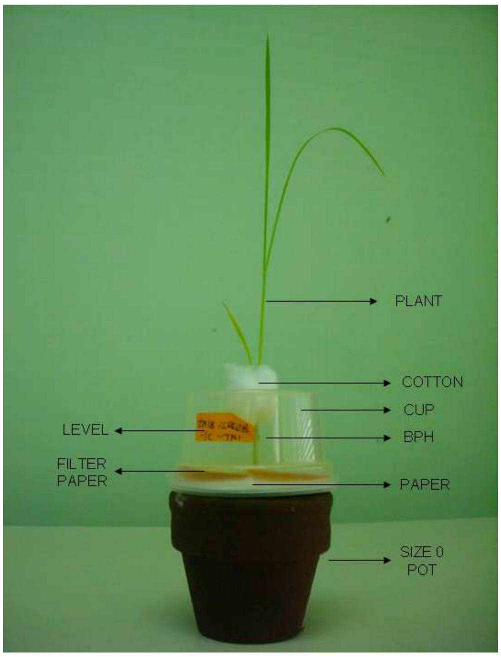

Figure 11. Honeydew test

While the preparation of the plant and filter, the gravid female hopper was at least, 2 hours starving. Then, the female hoppers were allowed to feed for 24 hours on the plant, after which the filter papers were collected.
Bromocresol green indicates phloem-based honeydew as blue-rimmed spots (indicate susceptible plants) and xylem-based honeydew as transparent (indicate resistant plants). The area of each spot on the bromocresol green-filter paper was measured using a digital scanner and "Image J" software.

Nymphal survival test

The nymphal survival test shows the differences of the survival of the nymphs in different varieties of rice plants.

For this, were placed ten newly hatched nymphs in a pot with two rice plant inside the mylar cages (45×5:H×R). The number of surviving nymphs was recorded every two days until they became adults (15 days). The survivors were then dried and weighed.

Figure 12. Nymphal survival test

Effects of nitrogen on resistance

To evaluate the effect of nitrogen fertilization of rice in the BPH, the next experiment was conducted 90 seeds per variety of rice (TN1 and IR62), were planted in 120 size #0 pots with 100 g of soil.

For each variety of rice there were 4 groups with 6 replicates that included the three factors: 1) rice variety, 2) adapted hopper colony and 3) nitrogen levels (N1 and N2) (Figure 13).

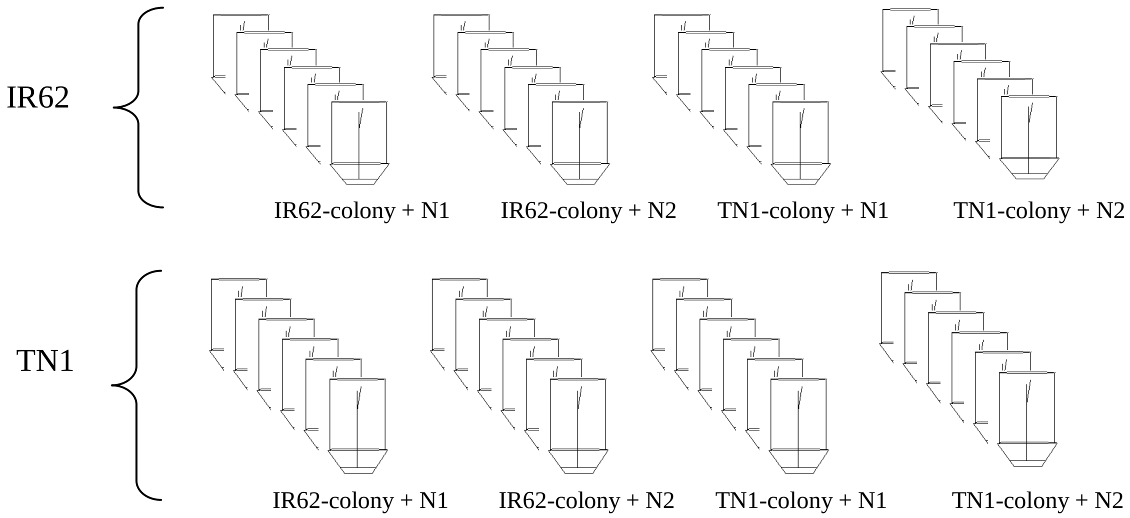

Figure 13. The distribution of the variety of rice, the colony and the nitrogen level in the experiment.

This lay-out was set-up twice, once for the honeydew test and a second time for the nymphal survival test. Per variety, there were 24 rice plants, because 2 varieties and 2 colonies were used, that is 48 seed for the first set. In the second set, two seeds were planted per pot, so were used 96 seed for the second set. Hence, the total is 144 seeds and were used 36 extra seed to ensure the germination of every plant that were need for the experiment (the 25% of the seeds).

After planting, seeds were left for 14 days for the development of the plants before infesting with the BPH. After 7 days, 30 plants per set were fertilized with 150 kg/ha. In tests, two gravid females were confined on the 14 day-old plants in small plastic containers (mylar cage), the base and top of each container having a hole through which the aspirator could pass. When in place, the hole was closed using a cotton plug.

After make the honeydew test, the females were maintained on the plants (without the filter paper) for 5 days, after which time they were removed. The plants were left for a further 20 days. At that time the final biomass of the plants and the population build-up (number of emerging hoppers and their developmental stage) and biomass of this BPH was recorded.

Effects of adaptation to *bph3* gene and related genes (*bph20* and *bph4*)

To evaluate the adaptation of BPH to different resistance rice genes, were used several varieties of rice. The resistant varieties that were used have different genes of resistance, but all of them occur in the same chromosome region (Figure 14).

14 day-old plants in 40 pots per colony and of varieties PTB33, IR62, Bph 20, Babawee and TN1were used. The first 20 pots were used in the honeydew test (as described above) and population build-up (as described above). Another 20 pots were used for the nymphal survival test (as described above). All bioassays were replicated ten times for each variety, with TN1 as a susceptible check, and conducted under the light and temperature regimes described above.

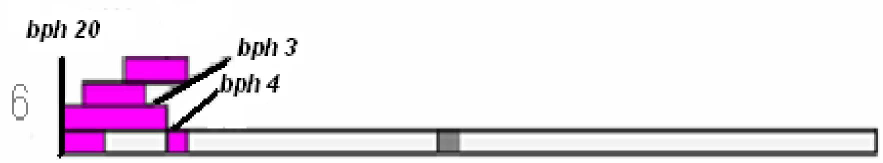

Figure 14. Schematic of the short arm of rice chromosome 6 indicating the position of three interested genes of planthopper resistance genes.

Effects of adaptation to *bph3* gene on related varieties (each containing the *bph3* gene)

To evaluate the effects of adaptation of BPH to *bph 3* gene, different resistant varieties (IR60, IR62, IR66, IR68, IR70, IR72, IR74 and RH) were used. The bioassays (honeydew, population build-up and survival) were conducted as described above. All bioassays were replicated ten times for each variety, with TN1 as a susceptible check, and conducted under the light and temperature regimes described above.

Statistical Analyses

To the statistical analyses, was used SPSS (Statistical Package for the Social Sciences), a useful software for statistical analyses in social sciences and research companies.

Planthopper feeding-responses to rice varieties were analysed using MANOVA with areas of phloem and xylem spots produced as dependent variables and colony, variety and nitrogen level as independent factors. Honeydew areas were log-square root transformed.

Planthopper population build-up and nymphal survival were each analysed as three separate tests: Hopper number per plant, proportion of surviving nymphs per plants and total planthopper biomass per plant with a biomass of plant as covariate. Were analysed using General Linear Models (GLM) with colony, variety and nitrogen level as independent factors. Development was analysed using MANOVA with ranked proportions of hoppers in each instar as dependent variables and colony, variety and nitrogen levels as the independent factors. Residuals were plotted following all analyses and were found to be normal and homogenous.

Only data from apparently healthy plants (the ones without signs of hopperburn) were included in the analyses; however, final plant biomass was also initially used as a covariate in relevant analyses, but was removed from the models where o effect was detected. Many of these plants had reduced biomass or had died, because the plants were highly stressed during population build up and nymphal survival bioassays (bioassay duration ≥15 days), hopper fitness parameters were affected.

RESULTS

Effects of nitrogen on resistance

Relative honeydew areas were affected by host-plant variety in phloem ($F=4.455$, $P \leq 0.05$) and in xylem ($F=56.743$, $P \leq 0.001$), but BPH colony was just affected in the phloem ($F=9.417$, $P \leq 0.005$). There were no significant interactions (Table 2). However, there was a near-significant variety × colony interaction ($F = 3.78$, $P = 0.059$) because non-adapted hoppers produced significantly smaller phloem spots on IR62 than on TN1 (Table 2).

Table 2. Statistical analyses of the bioassays (honeydew test, population build up and nymphal survival) of the effects of nitrogen on resistance.

	HONEYDEW TEST		POPULATION BUILD UP			NYMPHAL SURVIVAL		
	XYLEM	PHLOEM	POPULATION BUILD UP	Tests of Between-Subjects Effects	PBBIOMASS OF BPH	SURVIVAL	Tests of Between-Subjects Effects	SVBIOMASS OF BPH
Variety (V)	56,743***	4,455 *	2,393[a] NS	1st * 3rd *	8,479 *	6,633[a] ***	4th * Total adults ***	28,959 ***
BPH Colony (C)	1,018 NS+	9,417 **	2,611[a] *		0,002 NS+	47,686[a] ***	4th * 5th *** Total adults ***	17,729 ***
Nitrogen level (N)	0,323 NS+	0,042 NS+	0,992[a] NS+		6,141 *	6,214[a] **	4th * Total adults ***	1,709 NS+
V x C	1,061 NS+	3,78 NS	2,233[a] NS	1st *	0,863 NS+	4,463[a] **	4th * Total adults ***	6,144 *
V x N	1,133 NS+	0,686 NS+	0,467[a] NS+		0,498 NS+	1,064 NS+		3,299 NS
C x N	1,147 NS+	0,326 NS+	1,526[a] NS+	2nd *	2,373 NS+	4,800[a] *	Total adults ***	1,973 NS+
V x C x N	0,315 NS+	2,142 NS+	1,551[a] NS+		0,529 NS+	1,714[a] NS	5th **	0,210 NS+
Biomass plant					5,696 *			0,160 NS+

In the TN1 variety of rice, shows that, in both colonies excreted more phloem than xylem, actually, the most of the excretions were phloem. Also, when the IR62 variety was with IR62 colony, even if the xylem level is bigger than with the TN1 variety, the phloem excretion level is significantly bigger than in xylem. Instead of that, when the variety is IR62 and the colony TN1, the phloem levels reduced and the xylem increased (Figure 13).

In all the cases, when the treatment is with N2 (biggest nitrogen level), were showed more excretion of phloem, except in the treatment with IR62 variety and TN1 colony, that was with more xylem. All of them shows bigger production of honeydew (Figure 15).

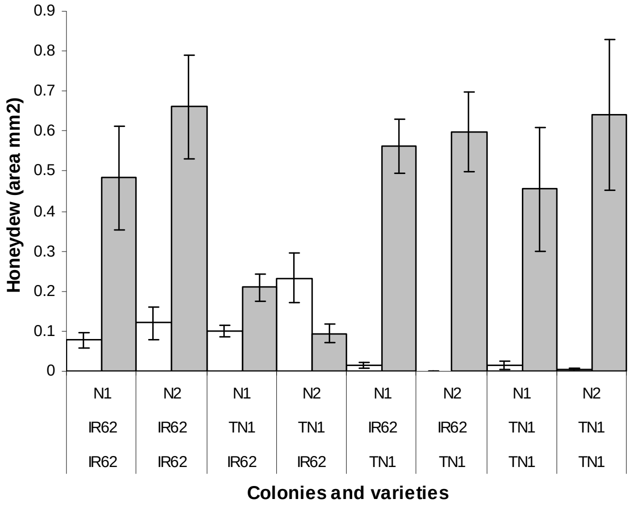

Figure 15. Honeydew test with variety, colony and nitrogen level (since down to up) in the X-axis. White columns are excretion of xylem and blues are phloem.

There were significant factor or interaction affects on nymphal survival, as a variety (F=6.633[a], P ≤ 0.001), colony (F=47.686[a], P ≤ 0.001) and nitrogen (F=6.214[a], P ≤ 0.005). Also was significant variety x colony interaction (F=4.463[a], P ≤ 0.005), because the survival hoppers were younger, when the variety was resistant and the colony susceptible. And the colony x nitrogen level effect (F=4.800[a], P ≤ 0.05), because survival of adapted hoppers decreased overall with increasing Nitrogen, whereas high nitrogen levels caused an increase in survival on non-adapted hoppers (Annex1). Patterns in total survivor biomass were similar to those described above for phloem spots (as in Figure 13): Hopper biomass on TN1 and the IR62-adapted colony was higher with a significant variety × colony interaction (F =6.144, P ≤ 0.005) because the biomass of non-adapted hoppers was lower on IR62 plants than on TN1 (Annex 1). Planthopper development was significantly more advanced on TN1 and for the adapted colony (Annex 1).

In the population build-up bioassay, many of the TN1 plants had symptoms of herbivore and other stresses (possibly heat) and hopperburn. Plant biomass was generally lower for TN1 and twice as many plants had died compared to IR62 at the end of the experiment. Biomass of both varieties increased with nitrogen fertilizer and was generally lower where adapted hoppers had fed. Therefore, results for dead plants of TN1 were eliminated from the analyses.
The numbers of hopper progeny from adapted females were higher on IR62 than from non-adapted females, therefore, colony factor was significant (F=2.611[a], P ≤ 0.05); however, differences were not significant (Number of hoppers: Nitrogen, (F=0.992[a], P = 0.428), variety (F=2.393[a], P = 0.074) and all interactions; total hopper biomass: Colony-type (F = 0.002, P = 0.924) and all interactions). The relative proportions of lifestages on IR62 were affected by colony type. However, unexpectedly, lifesatges of non-adapted hoppers were more advanced (Annex 2). Nitrogen had no effect on development, and the interaction was non-significant.

Effects of adaptation to *bph3* gene and related genes (*bph20* and *bph4*)

The relative amounts of phloem spots produced was affected by variety (F = 8.336, P ≤ 0.001), hopper colony (F = 0.660, P ≤ 0.005) and variety x colony (F = 5.182, P ≤ 0.001). However, the production of xylem spots was significantly higher for hoppers from the non-adapted colony, but was not affected by rice variety (F = 2.139, P = 0.084) or interactions (F = 0.893, P = 0.472).

Table 3. Statistical analyses of the bioassays (honeydew test, population build up and nymphal and adults survival) of the effects of adaptation to *bph3* gene and related genes (*bph20 and bph4*).

	HONEYDEW TEST		POPULATION BUILD UP		NYMPHAL SURVIVAL			ADULTS SURVIVAL	
	XYLEM	PHLOEM	POPULATION BUILD UP	PBBIOMASS OF BPH	SURVIVAL	Tests of Between-Subjects Effects	SVBIOMASS OF BPH	SURVIVAL ADULTS	Tests of Between-Subjects Effects
Variety (V)	2,139 NS	8,663 ***	0,821 NS+	4,869 **	6,209 ***	1st *** 2nd * 5th *** Adult ***	5,498 ***	2,177 *	
BPH Colony (C)	13,286 ***	10,370 **	0,691ª NS+	0,660 NS+	27,991ª ***	1st *** 4th *** 5th *** Adult ***	10,465 **	3,014ª *	SWMALE *
V x C	0,893 NS+	5,182 ***	0,761 NS+	1,306 NS+	5,956 ***	1st *** 2nd * 5th *** Adult ***	9,637 ***	3,179 ***	LWMALE * SWFEMALE ***
Biomass plant				0,243 NS+					

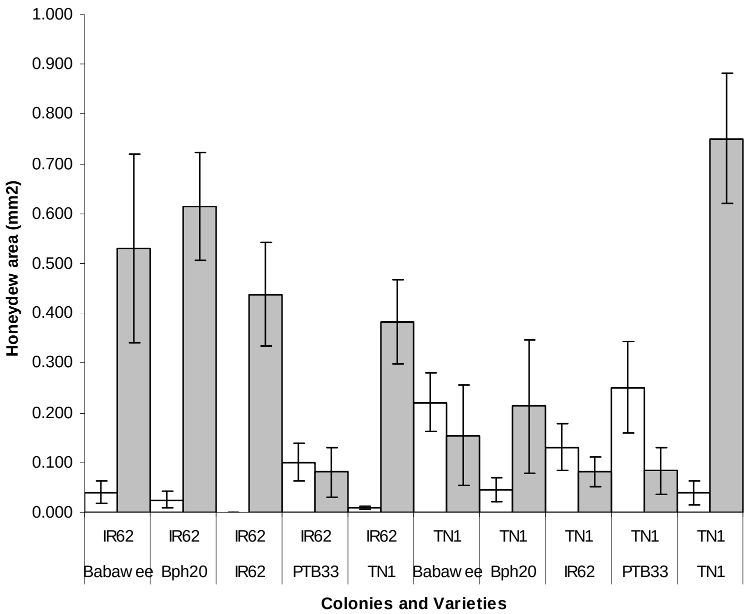

Figure 16. Honeydew test with variety and colony (since down to up) in the X-axis. White columns are excretion of xylem and blues are phloem.

The, largest xylem spots were produced on PTB33 with TN1 colony and the smallest on TN1 with IR62 colony and IR62 colony on IR62 plants. The largest phloem spots were associated with BPH20 with IR62 colony and TN1 colony on TN1 plants. The smallest phloem spots were produced PTB33 with both colonies. Phloem spots were larger for the adapted colony and the interaction was significant, because phloem spot production was high for the adapted colony on Babawee, BPH20 and IR62, but low for these plants for the non-adapted colony (Figure 14).

In the nymphal survival experiment, final plant biomass was affected in BPH20 and TN1 plants, because of hopper feeding, therefore were generally smaller. Their was also a significant interaction because IR62 and Babawee plants were smaller when feed-on by adapted hoppers only.

Nymphal survival was affected by variety ($F = 6.209$, $P \leq 0.001$), colony ($F = 27.991^{a}$, $P = 0.001$) and interactions ($F = 5.956$, $P \leq 0.001$). Final hopper biomass was affected by variety ($F = 5.498$, $P \leq 0.001$), colony ($F = 10.465$, $P = 0.005$) and variety x colony ($F = 9.637$, $P \leq 0.001$) (Table 3).

Development was significantly slower for TN1 colony on Babawee, IR62 and PTB33 than for the adapted colony (higher proportions of first instars, few fifth instars and adults (Annex 3).

High mortality of plants and a low biomass of survivors (especially BPh20 and TN1) at the end of the population build-up experiment, irrespective of colony, suggested that these varieties were heavily damaged by hopper feeding. Many of the surviving plants also showed signs of hopperburn. For this reason, the results from the experiment are not presented.

Effects of adaptation to *bph3* gene on related varieties (each containing the *bph3* gene)

The production of xylem spots was significantly higher for hoppers from TN1 colony (F= 48.16, P $\leq$ 0.001), but was not affected by rice variety (F = 0.809, P = 0.596) or interactions (F = 1.203, P = 0.302). In contrast, the production of phloem spots was higher by hoppers from IR62 colony (F = 31.546, P $\leq$ 0.001) and differed between host plants (F = 7.667, P $\leq$ 0.001): Significantly less phloem spots were produced when feeding on Rathu Heenati (with both colonies) and more phloem IR72 variety when TN1 colony was feeding compared to all other varieties (Figure 15).

Table 4. Statistical analyses of the bioassays (honeydew test, population build up and nymphal and adults survival) of the effects of adaptation to *bph3* gene on related varieties (each containing the *bph3* gene).

	HONEYDEW TEST		POPULATION BUILD UP			NYMPHAL SURVIVAL			
	XYLEM	PHLOEM	POPULATION BUILD UP	Tests of Between-Subjects Effects	PBBIOMASS OF BPH	SURVIVAL	Tests of Between-Subjects Effects	SVBIOMASS OF BPH	SURVIVAL ADULTS
Variety (V)	0,809 NS+	7,667 ***	3,558 ***	1st *** 4th * 5th ***	4,326 ***	4,626 ***	1st *** 2nd ** 4th ** 5th ***	9,232 ***	1,042 NS+
BPH Colony (C)	48,16 ***	31,546 ***	1,633[a] NS+	1st * 4th *	2,283 NS+	19,206 ***	1st *** 2nd *** 5th ***	0,300 NS+	2,625 NS+
V x C	1,203 NS+	0,818 NS+	1,241 NS+	1st *	0,990 NS+	3,368 ***	1st *** 2nd *** 5th *	3,975 ***	1,702 NS+
Biomass plant					3,714 NS			4,068 *	

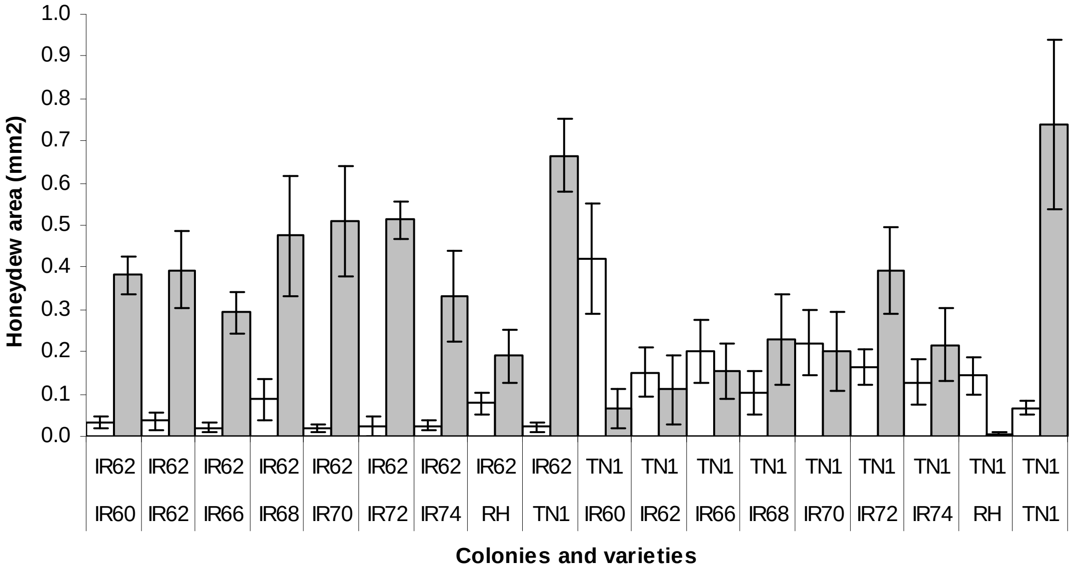

Figure 17. Honeydew test with variety and colony (since down to up) in the X-axis. White columns are excretion of xylem and blues are phloem.

Nymphal survival was affected by variety ($F = 4.626$, $P \leq 0.001$), colony ($F = 19.206$, $P \leq 0.001$) or their interaction ($F = 3.368$, $P \leq 0.001$); Variety ($F = 9.232$, $P \leq 0.001$) but not colony ($F = 0.300$, $P = 0.585$) affected the total hopper biomass; however, the covariate, final plant biomass, had a significant effect ($F_{1,151} = 4.068$, $P = 0.045$). The interaction term was non-significant. The relative proportions of hopper lifestages among the surviving nymphs was affected by variety, colony, and their interaction. A significant interaction occurred because of faster development of IR62 colony hoppers on some varieties (IR60, IR62, IR66 and Rathu Heenati), but not on others (IR68, IR70, IR72, IR74 and TN1) compared to the TN1 hoppers (Annex 4).

In the population build-up bioassay, plant biomass was affected by variety, colony and their interaction. IR62 and IR68 plants were significantly larger than all other varieties at the end of the experiment, after feeding by hoppers from both colonies. Plants tended to be smaller following feeding by IR62 hoppers. The numbers of hoppers on the plants was affected by variety ($F = 3.558$, $P \leq 0.001$), but was not affected by colony and their interaction (Table 4). Variety affected total hopper biomass ($F = 4.326$, $P \leq 0.001$), biomass was lowest on IR60 and highest on IR74. Was an interaction between the variety and colony, because of slower development of IR62 colony hoppers on IR60 and faster development on Rathu Heenati compared to the TN1 colony hoppers (Annex 5).

DISCUSSION

Ecological fitness characters of the BPH increased proportionally with increasing nitrogen content of the rice plants they were bred on (Lu et al, 2004). In nitrogen-rich host plants the hoppers survive better (Cheng, 1971) and lay more eggs (Sogawa, 1971) and as in the choice test of Lu and Heong (2004), the BPH adults would select nitrogen-rich plants over nitrogen-poor plants to feed and oviposit. The results of the study of the effects of nitrogen on resistance, indicated that the biomass of the plants was greater with more nitrogen even for the non-adapted colony and planthopper fitness increased on resistant and susceptible plants whether adapted or not, when the levels of nitrogen were increased.

In the honeydew bioassays of this study, it was confirmed the adaptation of the IR62 colony to the two varieties of rice, whereas fitness of the TN1 colony declined when fed on IR62 (the high amount of phloem spots indicated the adaptation of the IR62 colony to its host plant (IR62)). A high amount of xylem spots indicates that the colony was still likely affected by resistance factors of the variety. Nitrogen fertilization increased honeydew production, indicating that even the TN1 colony had improved survival on the resistant variety. Similar results were found with the survival bioassays, but in this case, the adaptation was detected only through the rate of development of the BPH. Accordingly, the most resistant treatment was IR62 with the TN1 colony, with most planthoppers still at 3rd instar or below whereas, in for the adapted colony many individuals had already reached to adult stage. Also, is was possible to detect the effect of the nitrogen on development rate: the development was faster under increased nitrogen levels, even where the plant was resistant to the colony.

Also, this work demostrated that the population and biomass of hoppers were higher when plants were applied with nitrogen fertilizer. Although, the development of the hoppers from the eggs was generally not faster under increased nitrogen levels in the population build up bioassays, development of the IR62-adapted colony bred on TN1 plants was accelerated.

The use of BPH-resistant host plants has been recognized as the most economic, effective measure for BPH management and the most environmentally friendly (Jairin et al, 2006). Rice varieties have been bred to carry *bph1*, *bph2* and *bph4* genes; however, many varieties have lost their ability to protect against BPH in most of the rice growing areas of Thailand and Indochina, whereas rice cultivars carrying *bph3* have shown a higher degree and broader-spectrum of resistance against the BPH (Jairin et al, 2006). In the study of the effects of adaptation to *bph3* gene and related genes (*bph20* and *bph4*), it was showed that *bph4* gene is still resistance against BPH from the Philippines because, phloem spots were generally small and xylem spots large with the TN1-colony when fed on Babawee (the *bph4* donor) rice variety. Also, when IR62-adapted planthoppers were fed on Babawee they produced large phloem spots, indicating that the IR62-colony had also adapted to *bph4* gene. This suggests that the *bph4* and *bph3* genes likely code for the same resistance mechanisms and that adaptation to one, results in breakdown of the other gene.

The rice variety PTB33 (containing *bph3* and *bph2* genes) demonstrated resistance to all BPH biotypes identified at IRRI and in some field populations in Asia, including India, Philippines, Vietnam, China, Bangladesh, Laos and Thailand (Angeles et al. 1986; Jairin et al. 2005; Khush 1984; Li et al. 2002; Soundararajan et al. 2004; Velusamy et al. 1995). In this study, resistance to BPH in this variety of rice was also demonstrated, but this resistance was not compared with Babawee or IR62 variety once planthoppers had adapted to the *bph3* gene (IR62-colony). However, PTB33 did show high resistance against the TN1 colony. The interaction between *bph3* and *bph2* genes in PTB33, would require further investigation, perhaps these genes work together to increase resistance even against *bph3* adapted hoppers and inspite of *bph2* gene not functioning against hoppers in either colony. However, it is also likely that PTB33 contains further genes or resistance QTLs that have so-far been undetected. Curiously, Ikeda and Kaneda (1981) demostrated that BPH resistance of the cultivar PTB21, was controlled by two sets of genes, either *bph1* and *bph4* or *bph2* and *bph3*. The mechanisms in these related rice varieties require further research attention.

Rahman et al. (2009) conducted QTL (Quantitative trait loci) analyses on the resistant variety ASD7 and determined two new BPH-resistance loci; These they designated as *bph20(t)* on chromosome 4 and *bph21(t)* on chromosome 12. Myint et al. (2008) demonstrated that the *bph20(t)* gene was resistant to different strains of BPH from across Asia. These authors have also shown that a resistance mechanism such as feeding inhibition is caused by these resistance gene, and similary affects nymphal and adults stages causing low adult survivorship, low nymphal survivorship, prolonged nymphal developmental period and lower adult biomass. In this study, plants with the *bph20(t)* gene showed resistance to the TN1-colony in the honeydew test compared to the IR62-colony. Nevertheless, all further bioassays indicated that, the TN1-colony was not affected by the *bph20(t)* gene that allegedly, was resistant. The results of this study therefore indicate that the *bph20(t)* gene does not affect planthoppers in the Philippines, and that adaptation to IR62 (*bph3*) likely further facilitated hopper feeding on varieties with this gene.

Kawaguchi et al. (2001) using two backcross mapping populations indicated that *bph3* and *bph4* are localized on the short arm of rice chromosome 6 and also, the newly identified resistance gene *bph20(t)* is also localized on chromosome 6 (Fujita et al, 2009). This study suggests that these genes may have similar characteristics and code for similar resistance affects, and because of this many of the varieties with these genes had no protection against the IR62-adapted colony. However, it should be noted that some of these genes had brokendown at different times since the TN1-colony was already adapted to *bph20(t)* before this study commenced.

In another study, 29 additional resistant varieties were analyzed genetically and two new genes, *bph3* and *bph4*, were identified (Lakshminarayana and Khush, 1977). These genes were incorporated into improved germplasm. In 1982, when a biotype capable of damaging IR36 appeared in small pockets in the Philippines and in Indonesia, IR56 and IR60 with the *bph3* gene for resistance were released (IRRI 1983). IR66 with *bph4* for resistance was released in 1987 (there is confusion as to the identity of the gene in IR66: Khush and Virk (2005) and Khush et al. (2007) indicate that IR66 contains the *bph3* gene and not *bph4*; however, recently Virk has indicated that the original study is likely correct and IR68, IR70, and IR72, all with *bph 3*, were released in 1988. These varieties were widely grown in tropical and subtropical rice-growing countries (Brar et al, 2009). The tightly linked markers to *bph3* gene will facilitate marker-assisted breeding to improve BPH resistance of rice cultivars as well. The resistant gene *bph3* has been used extensively in rice breeding programs in Asia since 1980 (Khush 1984). In this study (Effects of adaptation to *bph3* gene on related varieties (each containing the *bph3* gene**)** it has been demonstrated that the adaptation of the IR62-colony to IR62, permitted the planthoppers to feed freely on others varieties which contain the *bph3* or *bph4* gene. When planthoppers from the IR62-colony fed on IR60, IR62, IR66, IR68, IR70, IR72, IR74 and RH varieties, they produced a high amount of phloem and almost no xylem spots. Furthermore, the planthoppers from the TN1 colony produced high amounts of xylem spots comparing with the phloem spots, indicating that these planthoppers had not adapted to most of these varieties. However, on the IR72 variety the TN1-planthoppers did produce high amounts of phloem spots suggesting that the *bph3* derived resistance in this varietal background is weaker. The IR60 variety of rice was especially resistant to this colony, as indicated by a high production of xylem spots when planthoppers fed on the variety (this also occurred with the IR62-adapted colony), suggesting that IR60 contains further unidentified resistance. A low BPH biomass, higher mortality and slow development of planthoppers was often linked to the observed feeding trends as detected in the honeydew test and confirmed the generally high resistance of IR60 and weaker resistance of IR70, IR72 and IR74.

A Sri-Lankan *indica* rice (*Oryza sativa* L.) variety Rathu Heenati was found to be resistant to all four biotypes of the brown planthopper (Sun et al, 2005). The mechanism behind BG300 and BG379/2 resistant varieties was derived from Ratthu Heenati (*Bph3* gene) and Stevenson et al (1996) suggest that this is anti-feeding rather than toxic (Horgan, 2009). Rathu Heenati demonstrated resistance to all BPH biotypes identified at IRRI and in some field populations in Asia, including India, Philippines, Vietnam, China, Bangladesh, Laos and Thailand (Angeles et al. 1986; Jairin et al. 2005; Khush 1984; Li et al. 2002; Soundararajan et al. 2004; Velusamy et al. 1995). In this study, this variety remained resistant even to the IR62-adapted colony suggesting that Rathu Heenati has further resistance genes of QTLs besides the *bph3* gene. Adaptation of the IR62-colony to RH was the lowest compared with others varieties, but appeared higher along with IR60 when compared to the effects of these varieties on fitness measures of planthoppers from the TN1 colony.

The results of this study indicated, that the colonies of BPH feeding on plants with the same gene had almost the same behaviour. The IR62-colony produced high amounts of phloem spots and the TN1colony produced high amounts of xylem spots, indicating that the colony adapted to IR62 variety of rice, was adapted also to other varieties with *bph3* gene. Furthermore, most of the varieties were resistant to the TN1-colony which was not adapted to IR62 plants. This indicates that many rice varieties with the same resistance genes and without any interactions of other genes or QTLs, should breakdown at the same time.

CONCLUSION

In the three studies, adaptation of the BPH was tested under different conditions and it was demostrated that, with higher amounts of nitrogen, fitness of both adapted and non-adapted planthoppers was higher. Adaptation affected genes in similar chromosome locations differently (i.e., *bph4* and *bph20*(t)), and varieties bred to contain the same source of resistance, often showed similar affects on hoppers, such that adaptation to one variety affected the resistance of other, related varieties.

These results suggest that genes in the same location may code for the same effects or in some cases, might be the same genes but identified in different varieties. Plants with the same resistance gene will breakdown at the same time by the BPH, in a similar way that pesticides with the same active ingredient break down together -if insects adapt to one active ingredient, then they overcome all pesticides with this same active ingredient. Therefore, varieties with new resistance sources should be developed in the future.

The usual means to control the BPH is by spraying poisonous chemicals. This is costly in terms of labor, money and for the environment. The application of resistant cultivars has generally been considered to be the most economic and environmentally friendly strategy for pest management, however, recently, researchers have tended to look for new varieties with the same few gene. This is mainly because of the availability of markers that aid in selection. However, this strategy is not sustainable, unless new genes, and genes with different effects are employed in breeding programs.

Resistant host plants work to avoid the BPH attacks, but if farmers continue to use excessive fertilizers, the effects of the resistance decline, because the development is faster and the adaptation is higher with higher amounts of nitrogen. This is another reason to reduce the application of fertilizers in the field, in the same way that high pesticide applications must be stopped, since these results in low natural biological control. Intensive rice production areas with these characteristics, are more vulnerable to BPH outbreaks (Lu et al, 2004).

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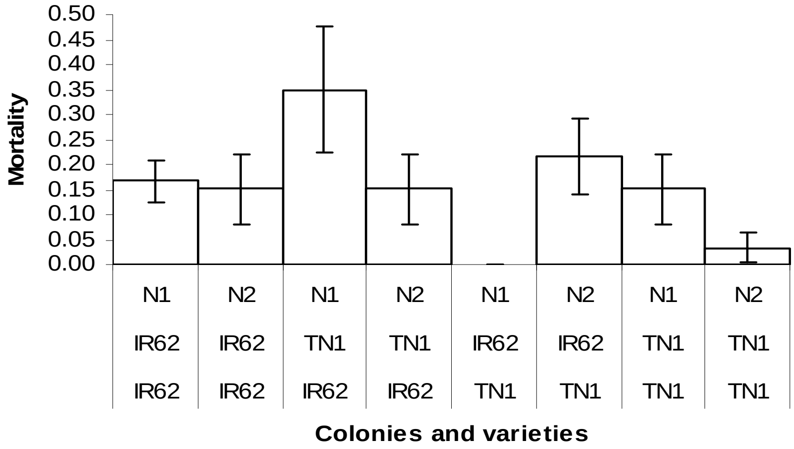
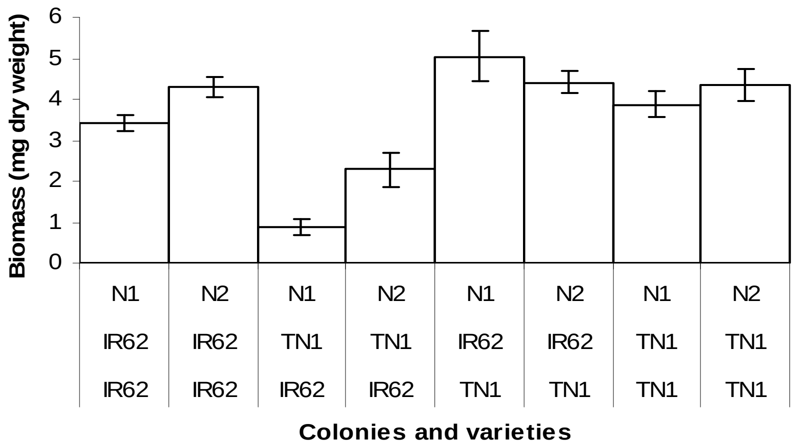
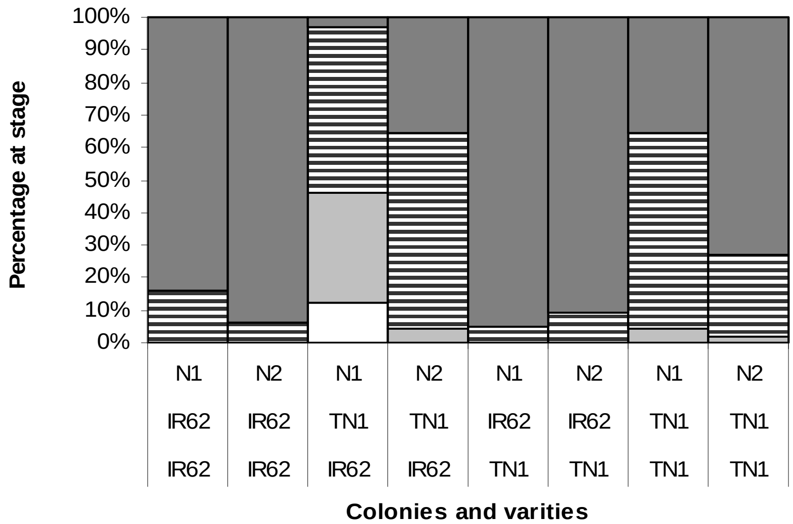
ANNEX 1: Survival bioassays graphics of effects of nitrogen on resistance (Mortality, biomass of BPH and percentage at stage).

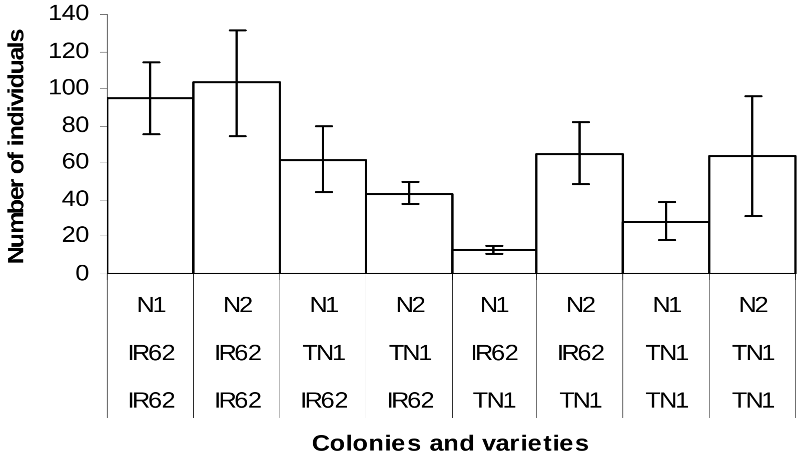


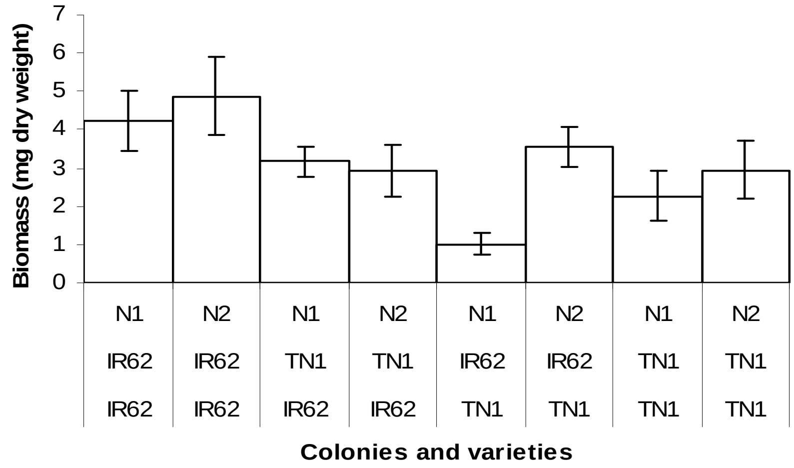


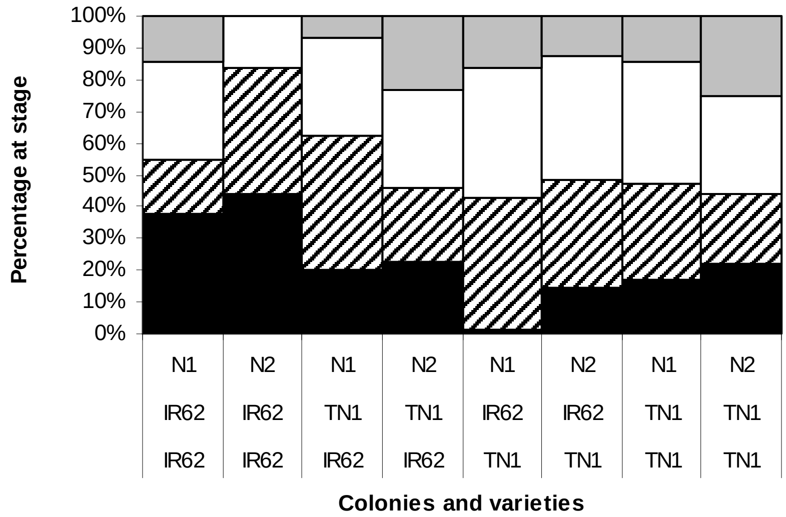


The lower stage is below that other stages (3rd, 4th, 5th and adults to up).

ANNEX 2: Population build up bioassays graphics of effects of nitrogen on resistance (Number of individuals, BPH biomass and percentage at stage graphics).

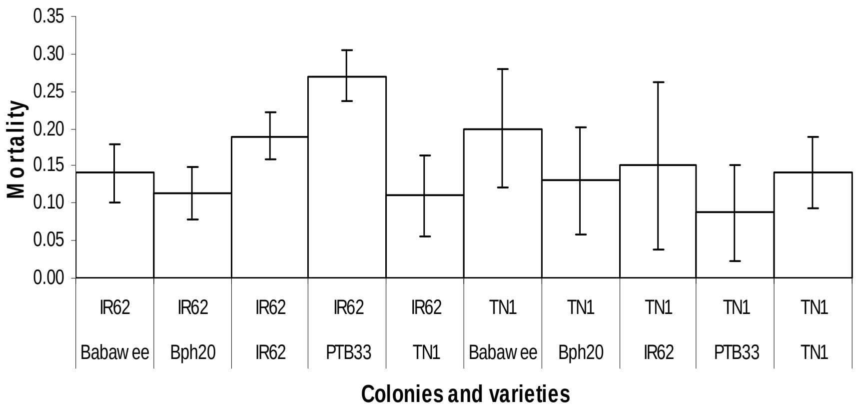


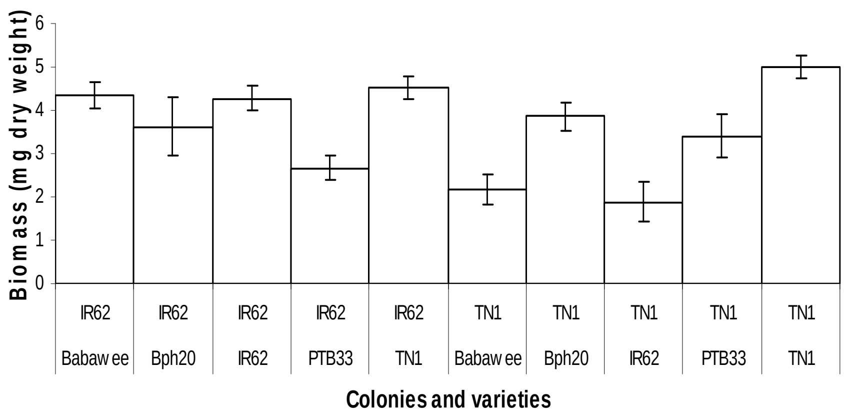


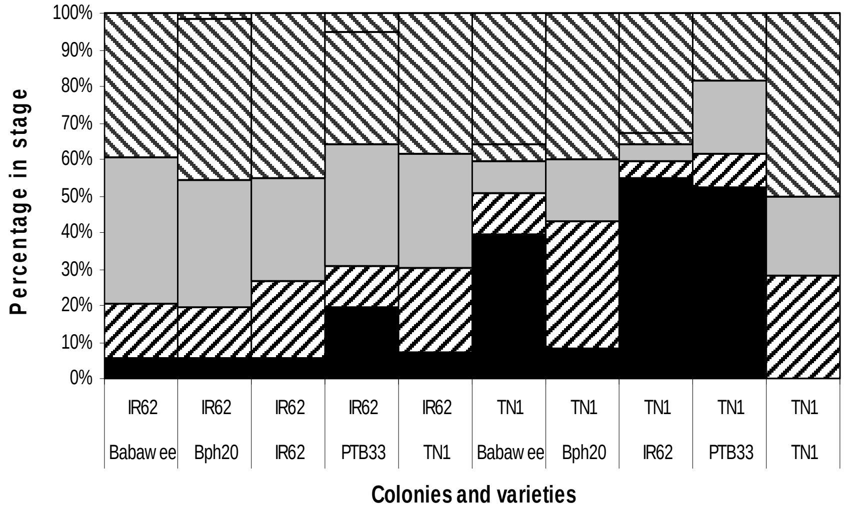


The lower stage is below that other stages (3rd, 4th, 5th and adults to up).

ANNEX 3: Survival bioassays graphics of effects of adaptation to *bph3* gene and related genes (*bph20* and *bph4*). (Mortality, biomass of BPH and percentage at stage).







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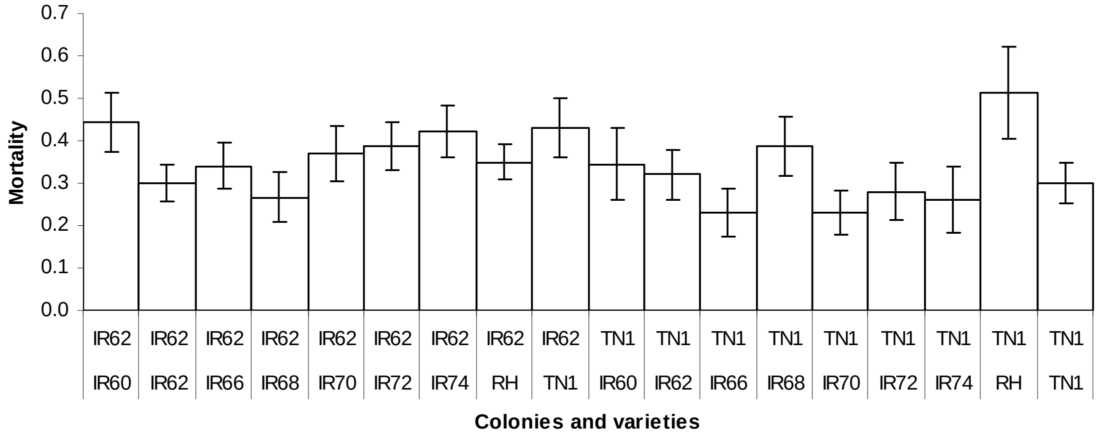
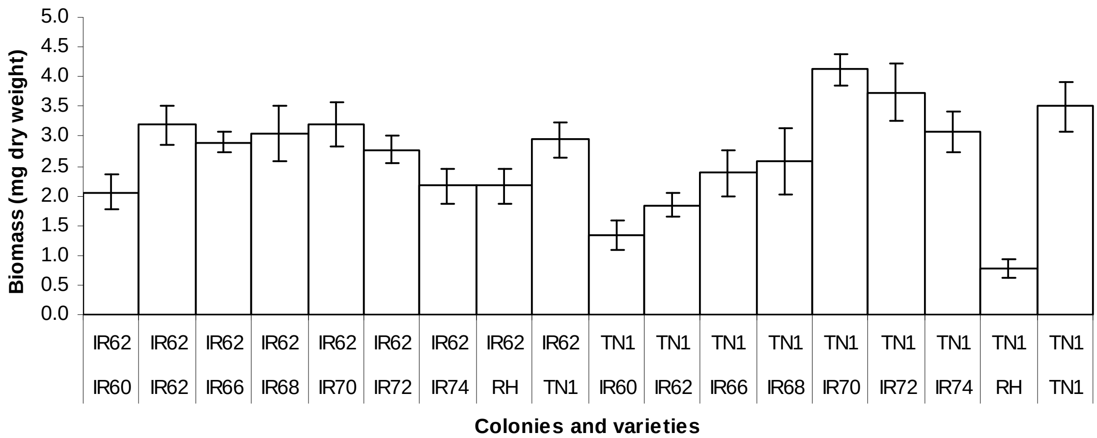
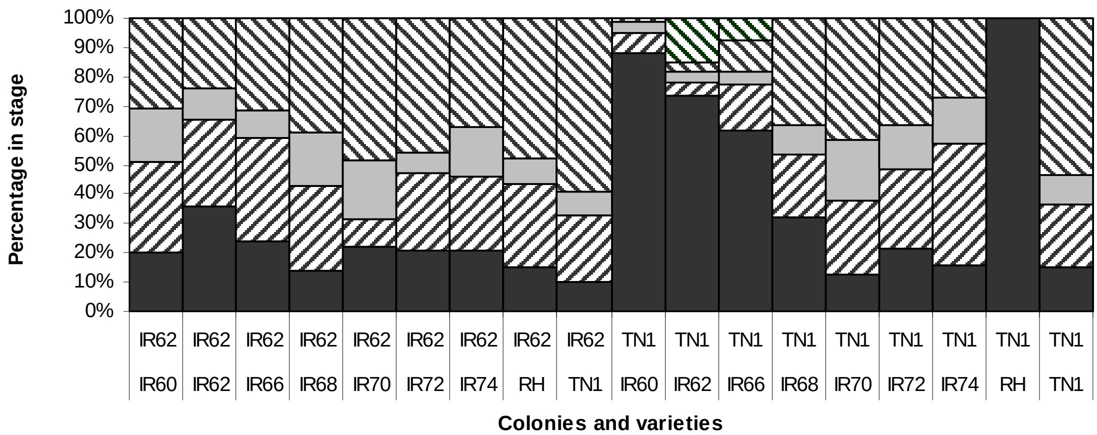
ANNEX 4: Survival bioassays graphics of effects of adaptation to *bph3* gene on related varieties (each containing the *bph3* gene). (Mortality, biomass of BPH and percentage at stage).

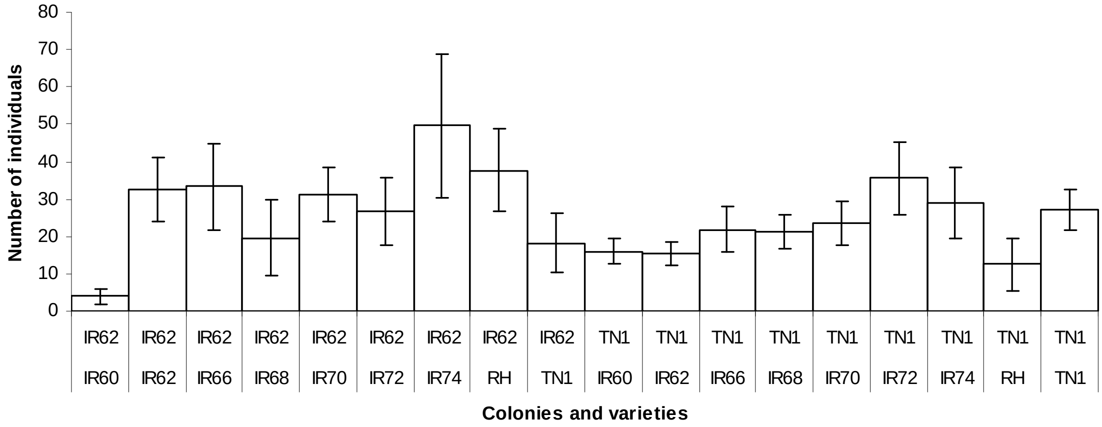


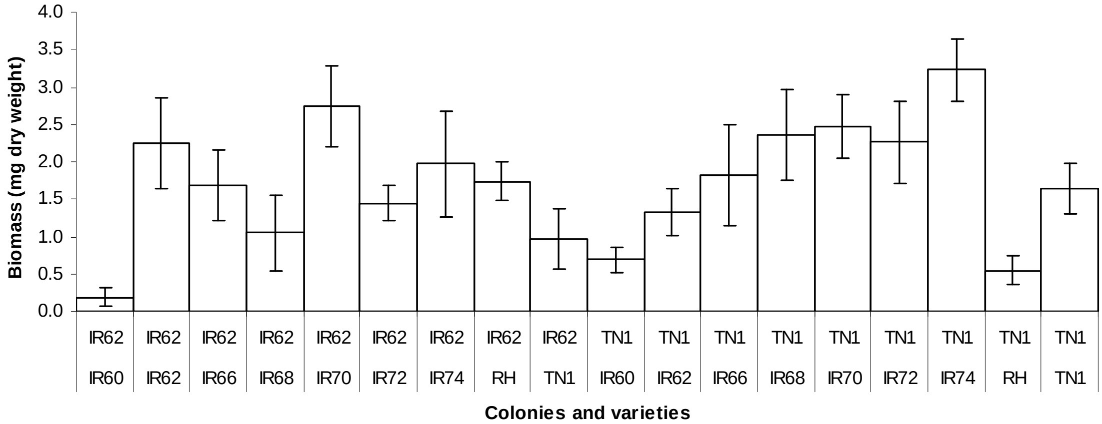


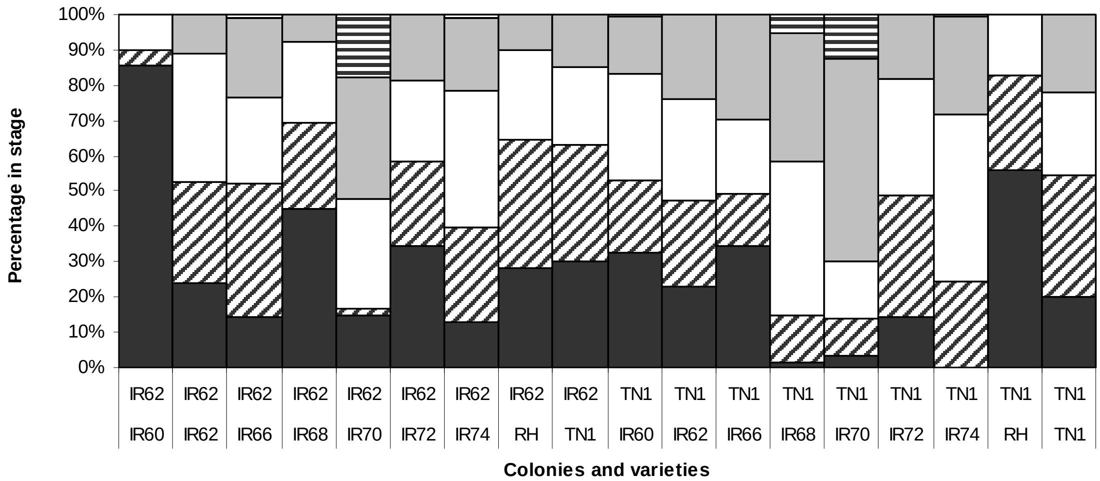


The lower stage is below that other stages (3rd, 4th, 5th and adults to up).

ANNEX 5: Population build up bioassays graphics of effects of adaptation to *bph3* gene on related varieties (each containing the *bph3* gene). (Number of individuals, BPH biomass and percentage at stage graphics).







The lower stage is below that other stages (2nd, 3rd, 4th, 5th and adults to up).