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NEKAZARITZAKO INGENIARIEN GOI MAILAKO ESKOLA TEKNIKOA

BROWN PLANTHOPPER (*Nilaparvata lugens* Stal.) (DELPHACIEDAE: FAMILY) ADAPTATION TO RICE RESISTANCE GENES

presentado por

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INGENIERO AGRÓNOMO
NEKAZARITZA INGENIARITZA

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ABSTRACT

The Brown Planthopper (BPH), *Nilaparvata lugens* Stål, has re-emerged as a serious threat to rice production in Asia. Currently some 30 resistance genes have been identified and proposed for resistance breeding against BPH. However, to date only three resistance genes have been incorporated into IRRI (International Rice Research Institute) rice varieties. Two of these, *bph1* and *bph2*, are now ineffective against BPH at a regional scale and the third, *bph3*, is still effective in the Philippines. In the present study, we determined the effects of adaptation to IR62 (containing the *bph3* gene) on resistance of, related IRRI rice varieties, the resistance-donor variety (Rathu Heenati) and differential varieties with resistance genes (*bph4*, *bph20 (t)*) that are located on the same rice chromosome 6 at *bph3* gene. The non-adapted colonies that were used in the honeydew test, nymphal survival and population build up test, were reduced on IR62 compared to the susceptible standard TN1. However, nitrogen application increased the susceptibility of IR62. After 13 generations without introgression of wild caught individuals, BPH reared on IR62 had similar feeding rates and survival on both IR62 and TN1, and on related varieties (IR60, IR66, IR68, IR70, IR72 and IR74). Rates were generally faster than for non-adapted BPH. Furthermore, feeding and development rates on Babawee (*bph4*) and BPH20 (*Bph20(t)*) were also significantly higher for IR62-adapted BPH. Although Rathu Heenati and PTB33 remained relatively resistance against IR62-adapted BPH, resistance was compromised by adaptation. These results indicate that adaptation to varieties with *bph3* resistance gene can lead to virulence against varieties with the same gene, as well as virulence against varieties with different, but possibly related genes. The implications of these results are discussed the current strategies for resistance management.

RESUMEN

Hoy día el Brown Planthopper (BPH), *Nilaparvata lugens Stål*, se ha convertido en una seria amenaza para la producción de arroz asiática. Recientemente, se han identificado 30 genes de resistencia en contra del BPH, sin embargo, únicamente se introdujeron 3 de ellos en las variedades de arroz de IRRI (International Rice Research Institute). Los genes *bph1* y *bph2* son actualmente ineficientes contra el BPH, pero *bph3* es todavía eficaz en Filipinas. En el presente estudio, se determino los efectos de la adaptación a IR62 (con el gen *bph3*), de variedades de arroz creadas por IRRI, de la variedad donante de la resistencia (Rathu Heenati) y de diferentes variedades de arroz con otros genes de resistencia (*bph4*, *bph20 (t)*) que al igual que el gen *bph3*, están situados en el cromosoma 6 del arroz. La colonia no adaptada que se utilizo en los bioensayos como test de melaza, supervivencia de las ninfas y desarrollo de las poblaciones de BPH, se vio reducida en IR62 comparando con la variedad susceptible TN1. Sin embargo, la aplicación de nitrógeno incremento la susceptibilidad de IR62. Después de 13 generaciones, las colonias se criaron en la variedad IR62 y se obtuvieron una tasa de alimentación y supervivencia similar en ambas variedades (IR62 y TN1) e incluso en las variedades relativas (IR60, IR66, IR68, IR70, IR72 y IR74). Las tasas fueron más rápidas en colonias adaptadas. Además, la tasa de alimentación y desarrollo de la colonia IR62 adaptada, eran significativamente mayores en las variedades de arroz, Babawee (*bph4*) y BPH20 (*Bph20(t)*). Aunque Rathu Heenati y PTB33 continuaban siendo resistentes en contra de la colonia IR62, el resultado se vio comprometido por la adaptación. Estos resultados indican que la adaptación de las variedades con el gen de resistencia *bph3* puede conducir a la virulencia en contra de variedades con el mismo gen e incluso, contra variedades con diferentes genes pero que podrían estar relacionados. Las implicaciones de estos resultados discuten las estrategias actuales de manejo de resistencias.

LABURPENA

Gaur egun, Brown Planthopper (BPH), *Nilaparvata lugens Stål*-a asiako arrozaren produkzioarentzako mehatxu garrantzitsuan bihurtu egin da. Berriki, BPH-aren kontrako 30 erresistentzia gen aurkitu dira eta bakarrik hoietako hiru (*bph1*, *bph2* eta *bph3*), IRRI-ko (International Rice Research Institute) arroz barietatetan sartu egin dira. Gaur egun, *bph1* eta *bph2* genak ez dira erangileak BPH-aren kontra, baina *bph3*-a Filipinasetan eraginkortasunarekin jarraitzen du. Ikerketa honetan, IR62-ren (*bph3* gena daukan arroz barietatea) moldaketaren efektuak zehaztu egin dira hiru modu desberdinetan, IRRI-k sortutako arroz barietateekin, erresistentzia genaren jatorriaren barietatearekin (Rathu Heenati) eta beste erresistentzia genak (*bph4*, *bph20 (t*)) dauzkaten arroz barietateekin, *bph3* bezala, arrozaren seigarren kromosoman daudelarik. Egindako bioensayoen ondorioz (ezti-hondarraren proba, ninfen biziraupena proba eta BPH populazioaren garapenaren proba), adaptatu gabe zeuden koloniak, TN1 minberakorrarekin konparatuz, IR62 landareetan txikitzen zirela frogatu egin zen. Hala eta guztiz ere, nitrogenoaren aplikazioak IR62 barietatearen minbera handitu egin zuen. 13 belaunaldi ta gero, koloniak IR62 barietatean hazi egin ziren eta ikerketa honetan gehien erabilitako barietateak (IR62 eta TN1) eta barietate erlatiboak (IR60, IR66, IR68, IR70, IR72 y IR74), elikagai eta biziraupen tasa antzekoak dauzkatela egiaztatu egin zen. Tasa hauek, moldatuak zeuden koloniekin azkarragoak ziren. Bestalde, IR62 kolonia adaptatuaren elikagai eta garapen tasak, Babawee (*bph4*) eta BPH20 (*Bph20(t)*) barietatetan, handiagoak zituen. Rathu Heenati eta PTB33, IR62 koloniari erresistenteak izaten jarraitzen zuten arren, ikerketaren emaitzak adaptazioagatik arriskuan jarri zen. Emaitza hauek, *bph3* gena daukaten barietateen adaptazioa, gene berdina daukaten barietateen eta barietateak gene desberdinekin eta haien artean erlazionaturik daudenen birulentzia ekar dezaketela, erakutsi egin da. Emaitza honen ondorioak, gaur egungo erresistentzien erabilpenak eztabaidatzen ditu.

INTRODUCTION

The Brown Planthopper

The brown planthopper (BPH, *Nilaparvata lugens* Stål) was the major rice pest of the green revolution in the 1970s and 1980s. However, since the early 2000s, BPH has attracted increasing attention from farmers, scientists, local government organizations, non-governmental organizations (NGOs), and national institutes, as BPH populations and associated losses to Asian rice production have intensified and contributed to regional rice shortages.

Previous to the green revolution, periodic outbreaks of BPH occurred in Japan and Korea. These outbreaks have been documented in records for more than 400 years (Mochida et al, 1977). In tropical Asia outbreaks were sporadic and restricted to small areas. However, BPH has become very serious in many tropical countries within the last 50 years and since the green revolution. In the 1970s, BPH caused extensive damage to the rice crop in Asia (Dyck and Thomas, 1979), mainly, because of the unpredictability of infestations and the dramatically severe damage caused. BPH populations can increase dramatically after insecticide applications (Shepard et al, 1995), because the widespread misuse of insecticides kills the important BPH natural enemies in rice (Kenmore et al, 1984). Moreover, the excessive use of chemical fertilizer increases the fecundity and survival of BPH, further increasing populations.

Outbreaks in northern regions (Korea, Japan, and Northern China) are mainly linked to migrations by the insect. BPH has two phases of migration, one northward migration in spring and early summer, from tropical regions and Southern China to Korea, Japan and Central China, (1000 km more or less) and a second, southward migration in autumn, from the north of Asia to tropical regions (Mochida et al, 1977).

Planthoppers are phloem-feeding invertebrates, constituting 14 families in the superfamily Fulgoroidea, suborder Homoptera, order Hemiptera. The most economically important family is the Delphaciedae, because of the mechanical damage caused to rice and the transmission of rice viral diseases (Table 1).

Table 1. Checklist of delphacid planthoppers and the virus diseases they transmit.

Virus disease	Delphacid planthopper vector	Distribution
Grassy stunt Ragged stunt	*Nilaparvata lugens*	Philippines and Sri Lanka
Stripe virus	*Laodelphax striatellus* *Unkanodes sapporonues* *Terthron albifascia*	Korea and Japan
Black streak dwarf	*Laodelphax striatellus* *Unkanodes sapporonues* *Terthron albifascia*	Japan

(Dupo and Barrion, 2009)

Distribution and outbreak of BPH

BPH is the most economically important planthopper in Asia. It occurs throughout South (India, Sri-Lanka, Bangladesh) and South East Asia (South China, Indochina, Indonesia, and The Philippines). It also migrates annually from southern Asia to northern regions in China, Korea and Japan (Figure 1).

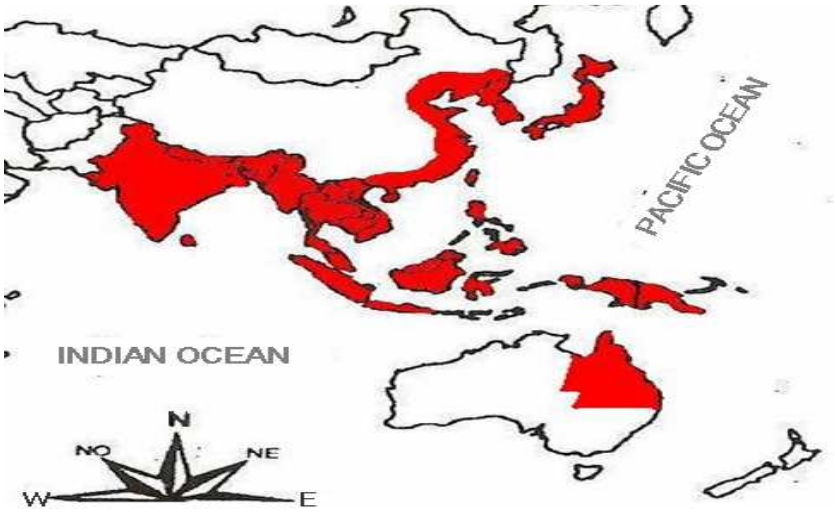

Figure 1. BPH is mainly distributed in Asia, Australasia and the Pacific Islands. In Asia, it is found in Bangladesh, Brunei, Burma (Myanmar), China, Hong Kong, India, Indonesia, Japan, Cambodia, Korea, Laos, Malaysia including Sarawak, Nepal Pakistan, The Philippines, Singapore, Sri Lanka, Taiwan, Thailand, and Vietnam. In Australia it occurs in Queensland. It also occurs on Pacific Islands: it is found on the Caroline Islands, Fiji, Mariana Islands, Papua New Guinea, and Solomon Islands (Mochida et al, 1977).

The Brown Planthopper, is mainly a pest of irrigated rice, but it can also be abundant in rainfed environments. It is rare in upland rice (Reissing et al, 1986). The population fluctuations of the hoppers in rainy season is depend of the rainfall and the humidity and in dry season, also depend of the temperature (Win et al, 2010).

BPH damages the rice plant directly by feeding and also indirectly by transmitting the grassy stunt and the ragged stunt diseases (Reissing et al, 1986). Adults and nymphs suck the sap from the plant and adults oviposit in the plant tissues causing plant wilting, called hopper burn (Figure 2), and resulting in dramatic yield reductions on susceptible rice varieties. Yield is reduced mainly because of reductions in leaf area that affects the photosynthetic rate. This in turn, affects leaf and stem nitrogen concentrations, chlorophyll contents, and organic dry weight of the rice plants (Cagampang et al, 1974).

Figure 2. BPH damages in the fields, called hopperburn.

Host range

BPH is monophagous, feeding only on rice (*Oriza sativa* L.). However, in no-choice conditions, BPH will feed or oviposit on *Eleusine coracana* G. (finger millet), *Saccharum officinarum* L. (sugarcane), *Leersia hexandra* Sw. (rice cut-grass), *Zea mays* L. (maize), *Echinochloa colonum* L. (echinochloa colona), *Cyperus rotundus* L. (coco grass), *Sorghum vulgare* L. (sorghum), and *Triticum aestivum* L. (wheat). Whether the species feeds on these species in the wild is unknown (Misra, 1980).

BPH Life-cycle

The major salient feature in the biology of planthopper species, like BPH, is their ontogenetic development. This consists of development through sequential stages, from the immediate product of reproduction (the eggs), through nymphal instars, until the formation of mature dimorphic adults (macropterous and brachypterous) of both sexes. The planthoppers has hemimetabolous or incomplete development. The duration of each stage depends on ambient temperature and host cultivars.

The eggs are usually laid as egg groups in the tissue of the lower part of the rice plant, mainly, in sheaths and partly in leafblades. The size of egg groups and oviposition sites, however, depend upon the stages of the rice plants. When the population of adults is high, eggs are found in the upper part of the plants (Mochida, 1964). The egg stage is about 7 to 10 days in the tropics. The nymphal stage is 10 to 15 days, with 2 to 3 days for each instar. Once the adults have mated, the preoviposition period is 3-4 days for brachypterous and 3-8 days for macopterous females (Figure 3). All development times are dependent mainly on temperature. The adults and nymphs usually stay on the lower part of rice plants. However, when the population is very high (more than 500 BPH per mylar cage), they are occasionally observed to swarm even on flag leaves, the uppermost internodes of panicles, and also on panicle axes.

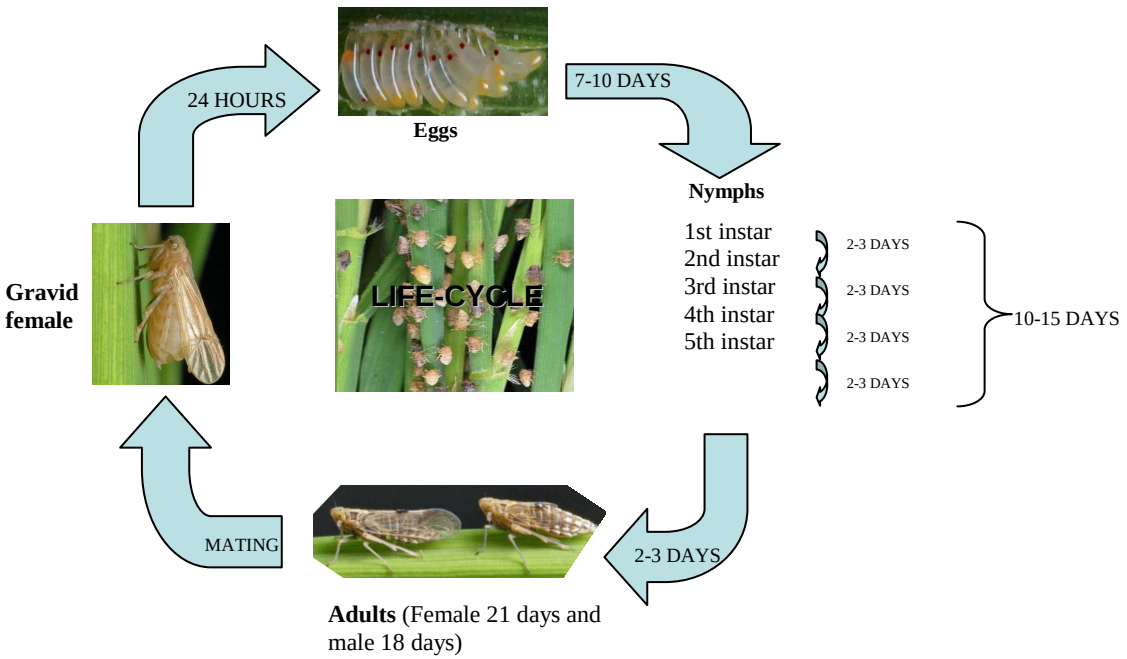

Figure 3. Schematic of the life-cycle of BPH.

Life stages

Figure 4. Unviable or young eggs (IRRI property).

The eggs are crescent-shaped, less pointed egg plug than "*Sogatella furcifera*" (Dupo and Barrion, 2009) and on average 0.99 mm long (ranging from 0.91 to 1.07 mm) (Misra, 1980). Newly laid eggs are whitish (Figure 4) and they turn darker when about to hatch. The egg consists of the chorion, vittelling membrane, protoplasm, nucleus, yolk, and mycetocyte. Before egg hatch, two distinct spots appear, representing the eyes of the developing nymph. Some of the eggs are united near the base of the flat egg cap and others remain free (Catinding and Heong, 2009). (Figure 5)

Figure 5. Viable eggs with red spots.

The female can lay from 100 to 500 eggs depending on the stage of growth of the rice plant (Van Der Laan, 1981). Maximum hatchability and survival of eggs occurs at around 25ºC. The eggs stage is about 7 to 11 days in the tropics and the number of eggs laid by female delphacids during their life-span ranges between 0 and 1474. The number of eggs laid is correlated to the life-span and ovipositional period.

In most cases, the eggs are thrust in a straight line, generally on the lower part of the host plant along the mid-region of the leaf sheath, though sometimes eggs are laid in clusters of 4-10 in longitudinal rows within the leaf midribs (Dupo and Barrion, 2009).

There are 5 nymphal instars that all feed on the host plant's phloem sap until the adult stage. The different instars can be distinguished based on the shape of the mesonotum and body size (Dupo and Barrion, 2009). The minimum growth of nymphs is at 28-30ºC of temperature.

The length of the nymphal period depends of the food conditions, density during development and environmental factors. Usually, the nymphal period is from 10 to 18 days (mainly in tropical regions) and is shorter for the brachypterous form (short-winged) than macropterous form (long winged).

The nymphs have a triangular head with a narrow vertex. The body is creamy white with a pale brown tinge. Mature nymphs are about 2.99mm long. The nymphs have a prominent median line from the base of the vertex to the end of the metathorax where it is the widest. This line crosses at a right angle to the partition line between the prothorax and mesothorax (Catinding and Heong, 2009).

Newly hatched (First instar)

These are cottony white and turn purple-brown within an hour. The body length ranges from 0.88 to 1.11 with an average length of 0.97 mm. Demarcation between the thorax and abdomen is clear. The junction is narrow, with brown pigmentation at the vertex, at the junction of pro and mesothorax and in the first two abdominal segments (Misra, 1980).

Second instar

Range in length from 1.07 to 1.37 and with an average of 1.29 mm. Their colour is brownish white, with eyes slightly red. The abdomen is uniformly brownish white, but the thoracic segments are more brownish (Misra, 1980).

Third instar

Range in length from 1.30 to 1.56 with an average of 1.42 mm. They have a brownish colour with red eyes (Misra, 1980). There is a longitudinal midline from base of vertex to the tip of the metathorax and also, the wing rudiments make their appearance.

Fourth instar

The body length is between 1.63 and 2.60 with an average of 1.99 mm. In this case, the nymphs are brown with red eyes. The longitudinal midline extends from the base of the vertex to the end of the metathorax with the rudiment wings covering the first two abdominal segments and about to cover the third (Misra, 1980) (Figure 6).

Figure 6. Nymphs in 5th and 4th instars, respectively.

Fifth instar

The body length ranges from 2.02 to 3.12 with an average of 2.69 mm. The body colour is brownish black, with grayish blue eyes. Also, the midline appears as a prominent median line from the base of the vertex to the end of the metathorax, where it is the widest, this crosses at right-angles to the partition line between the pro and mesothorax (Misra, 1980) (Figure 6). At this stage, the developing wings cover the first three abdominal segments.

Usually, **the adult** female measures from 4.2 to 4.5 mm and the male 3.80 to 4.12 mm including the tegumin. The body length of macropterous adults (Figure 7) ranges from 3.7 to 5.0 mm and that of brachypterous adults (Figure 8) from 2.4 to 3.3 mm (Mochida, 1977).

The adults range in colour from brownish black to yellowish brown. There is a distinct white band on the mesonotum with dark brown on the outer sides. Macropterous or long-winged adults have normal front and hind wings, whereas brachypterous or short-winged forms have stunted hind wings (Catinding and Heong, 2009). The wings are also normally shorter in males than in females. A prominent tibial spur, present on the third leg, also distinguishes this species.

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.

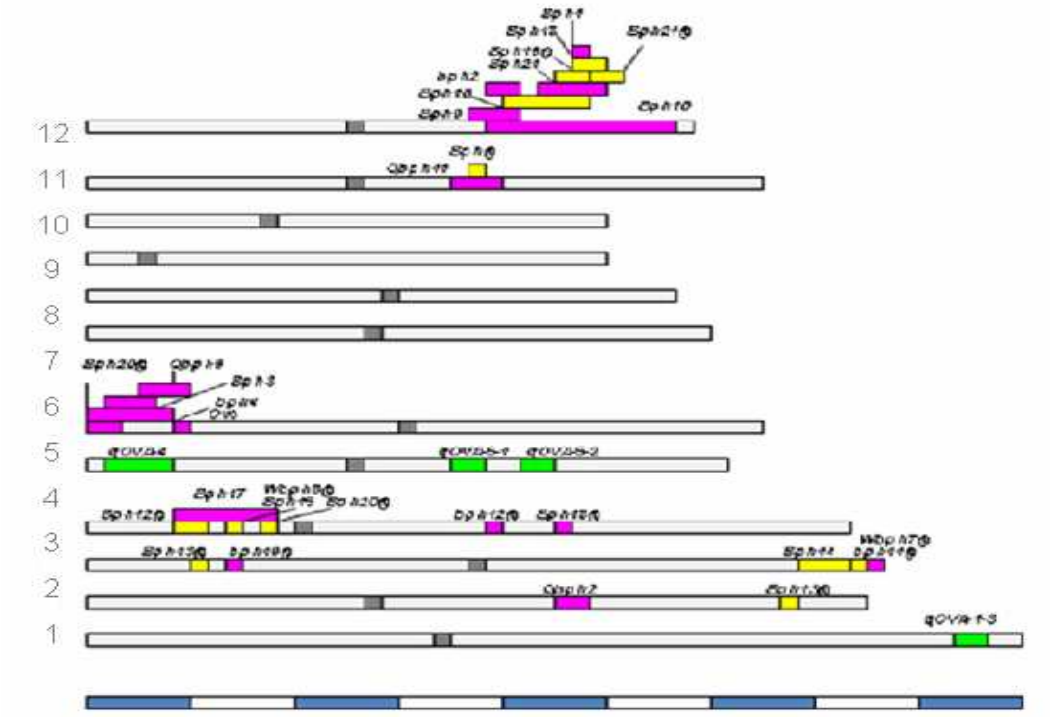

Figure 10. The location of the genes in the arms of chromosome.

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.

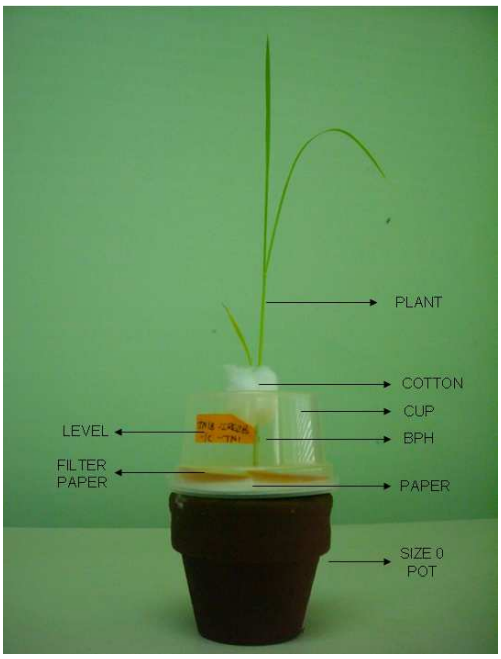

Figure 11. Honeydew test

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).

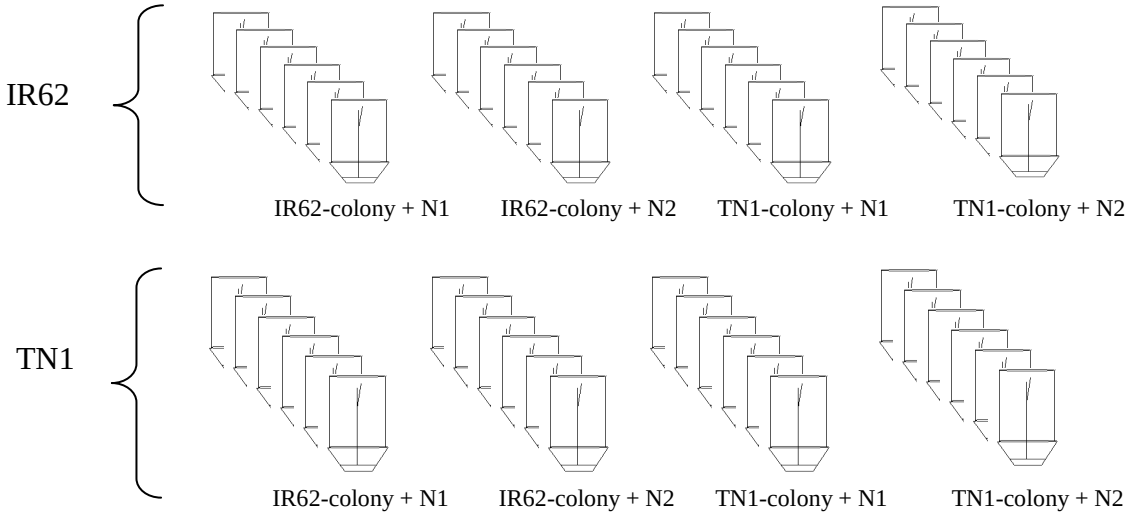

Figure 13. The distribution of the variety of rice, the colony and the nitrogen level in the experiment.

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 ≤ 0.05) and in xylem (F=56.743, P≤ 0.001), but BPH colony was just affected in the phloem (F=9.417, P ≤ 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).

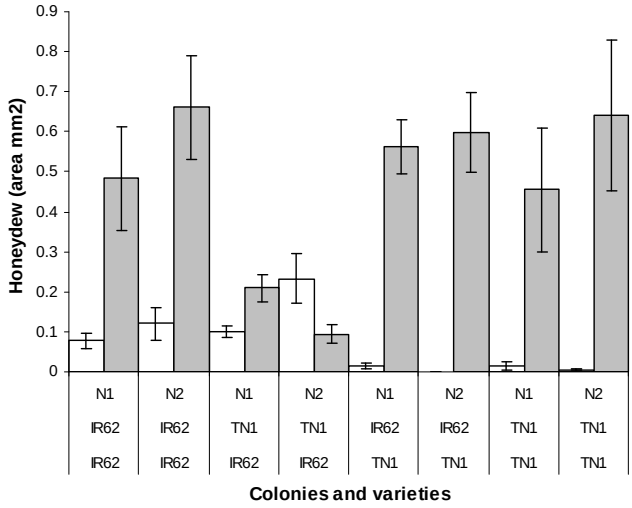

Figure 15. Honeydew test with variety, colony and nitrogen level (since down to up) in the X-axis. White columns are excretion of xylem and blues are phloem.

There were significant factor or interaction affects on nymphal survival, as a variety ($F=6.633^a$, $P \leq 0.001$), colony ($F=47.686^a$, $P \leq 0.001$) and nitrogen ($F=6.214^a$, $P \leq 0.005$). Also was significant variety x colony interaction ($F=4.463^a$, $P \leq 0.005$), because the survival hoppers were younger, when the variety was resistant and the colony susceptible. And the colony x nitrogen level effect ($F=4.800^a$, $P \leq 0.05$), 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 ($F =6.144$, $P \leq 0.005$) 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 ($F=2.611^a$, $P \leq 0.05$); 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 ($F = 8.336$, $P \leq 0.001$), hopper colony ($F = 0.660$, $P \leq 0.005$) and variety x colony ($F = 5.182$, $P \leq 0.001$). 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 **	0,691[a] NS+	0,660 NS+	27,991[a] ***	1st *** 4th *** 5th *** Adult ***	10,465 **	3,014[a] *	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+					

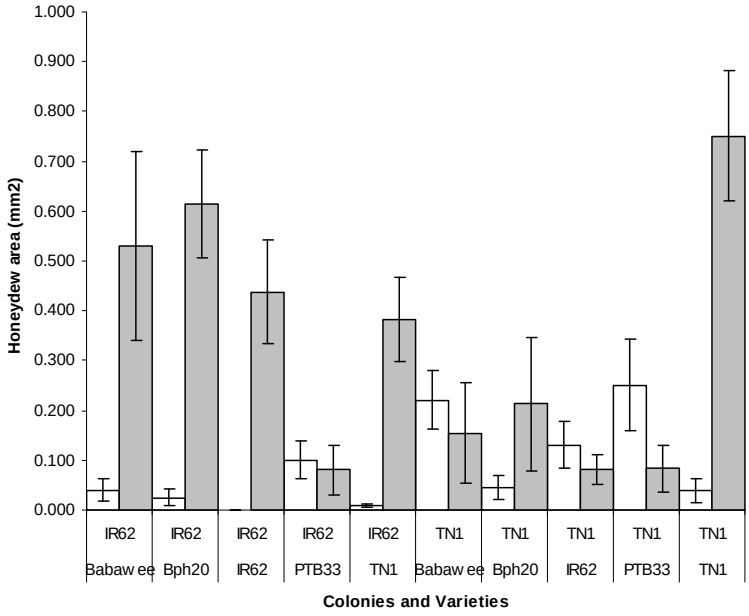

Figure 16. Honeydew test with variety and colony (since down to up) in the X-axis. White columns are excretion of xylem and blues are phloem.

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 ≤ 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 ≤ 0.001) and differed between host plants (F = 7.667, P ≤ 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 *	

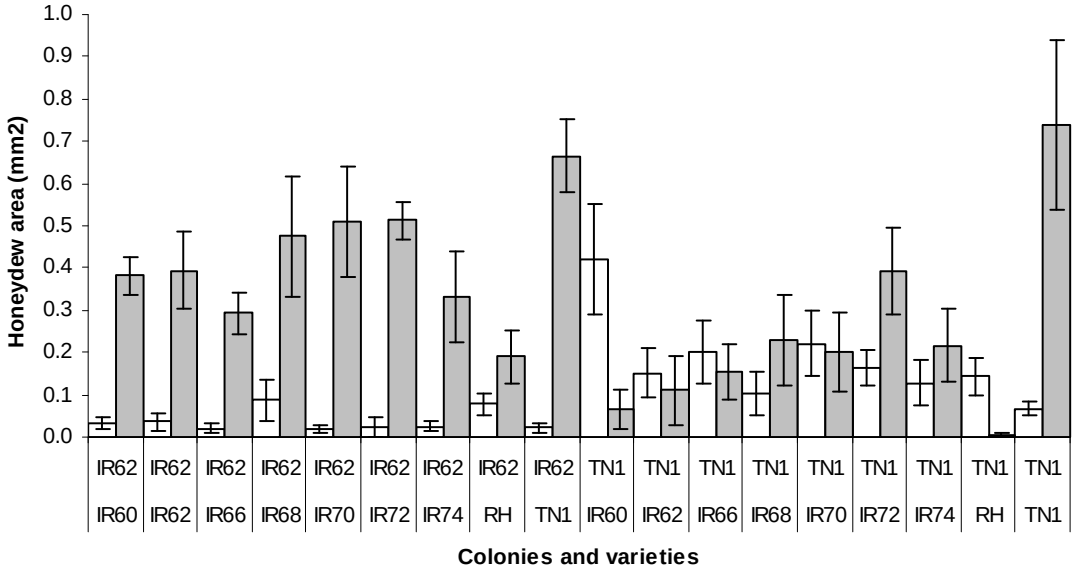

Figure 17. Honeydew test with variety and colony (since down to up) in the X-axis. White columns are excretion of xylem and blues are phloem.

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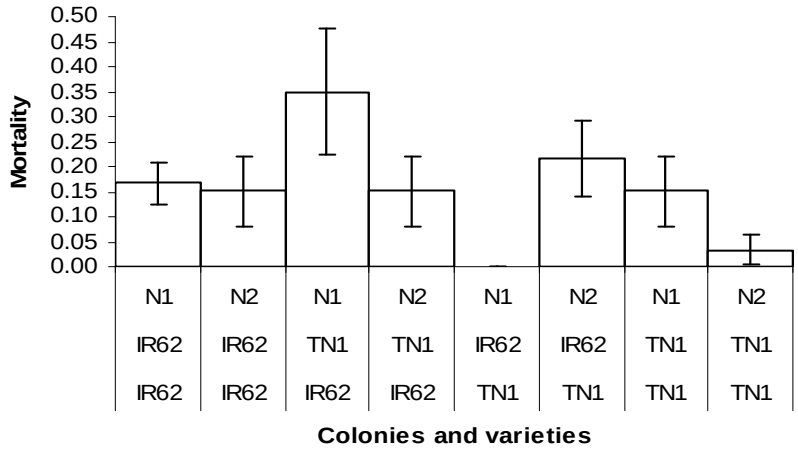
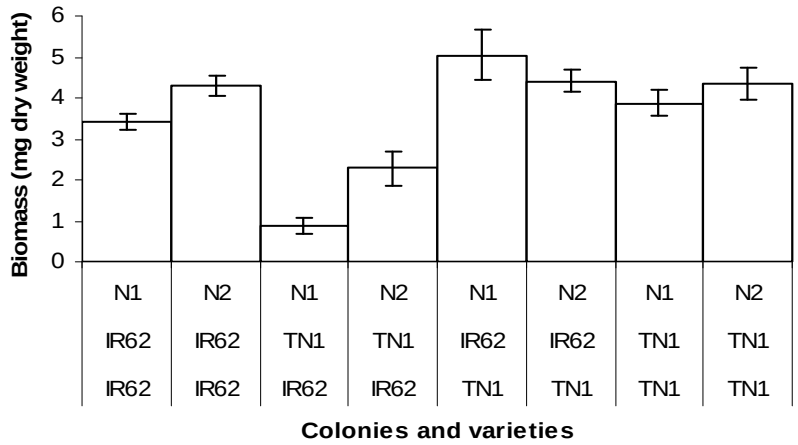
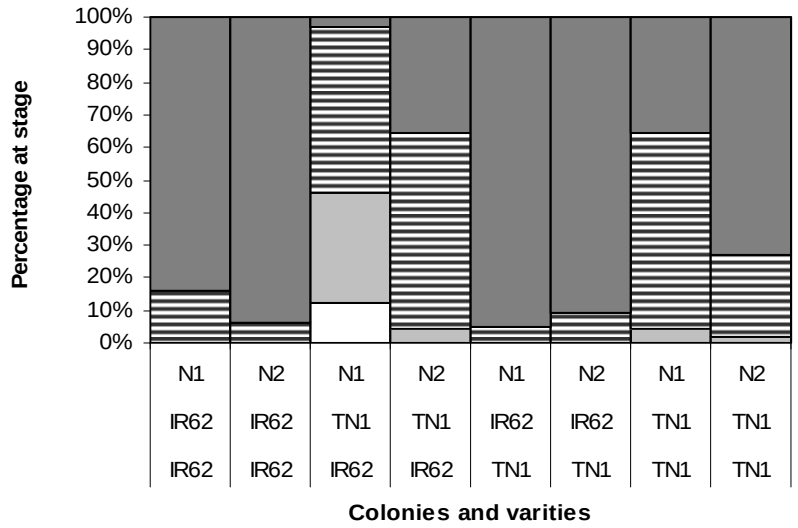
ANNEX 1: Survival bioassays graphics of effects of nitrogen on resistance (Mortality, biomass of BPH and percentage at stage).

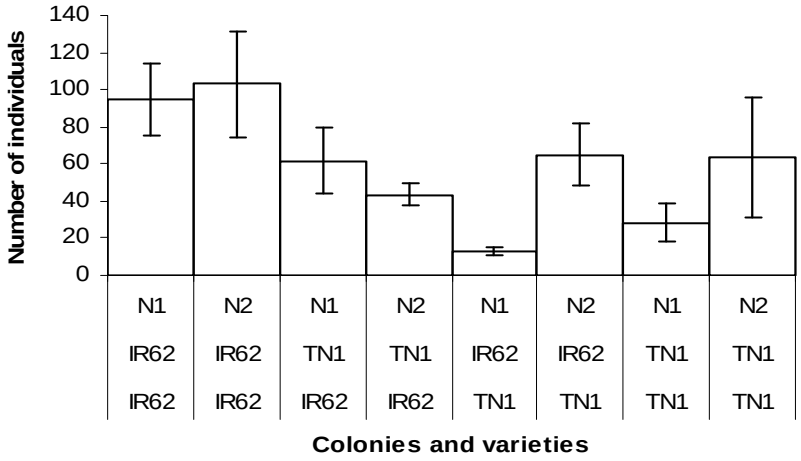


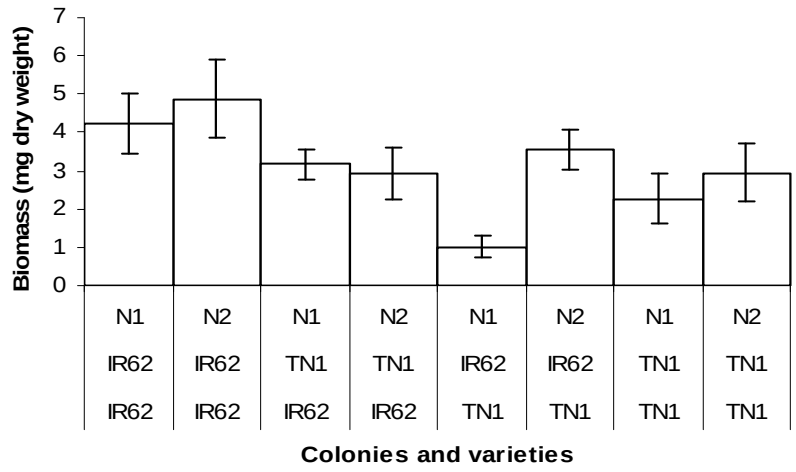


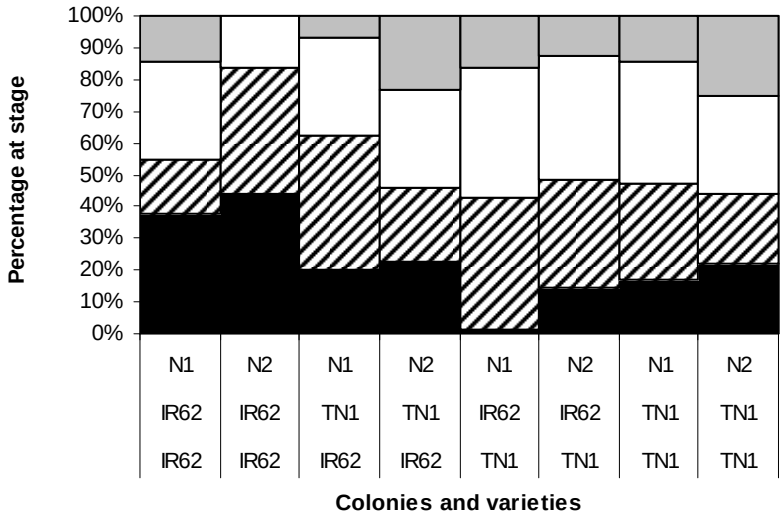


The lower stage is below that other stages (3rd, 4th, 5th and adults to up).

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

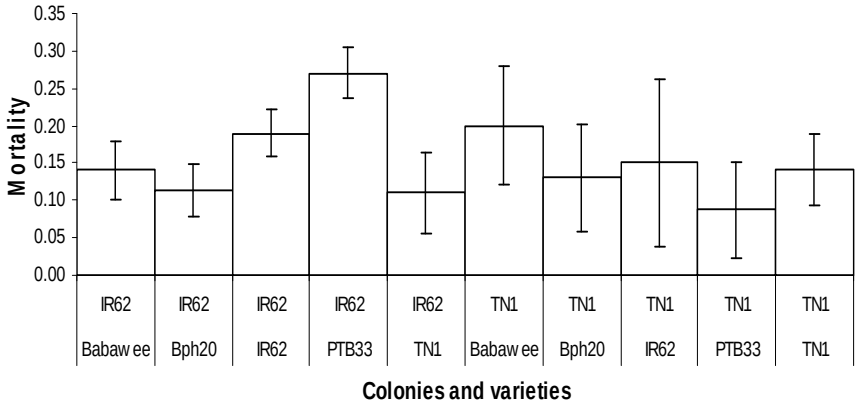


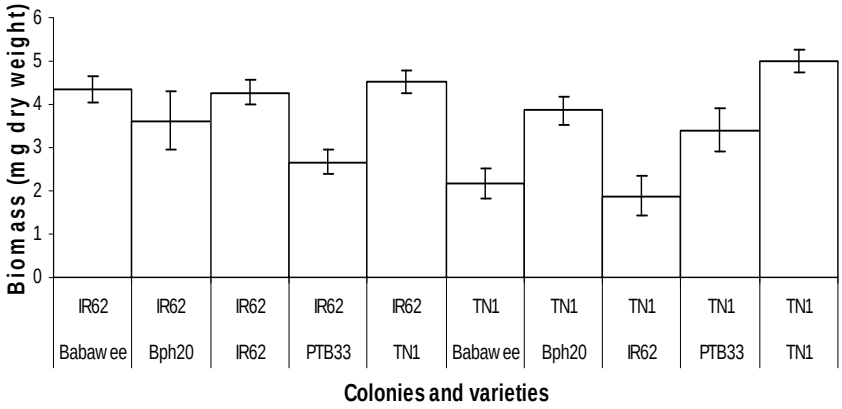


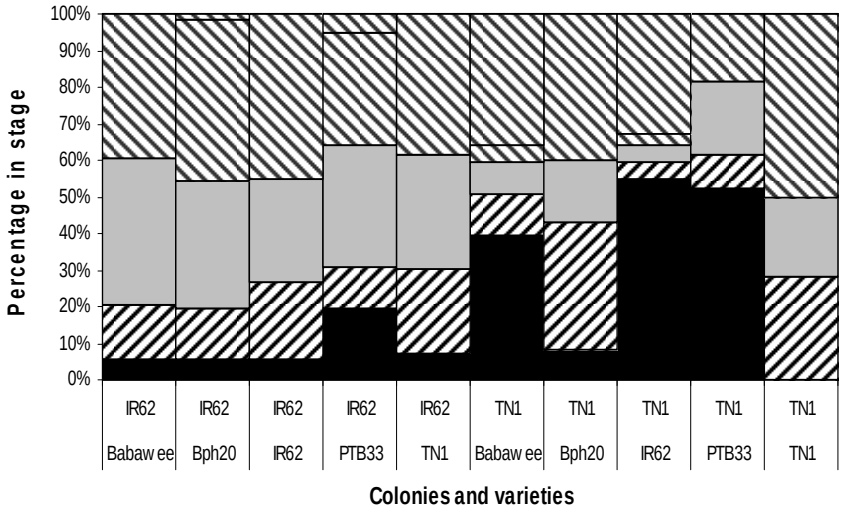


The lower stage is below that other stages (3rd, 4th, 5th and adults to up).

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).

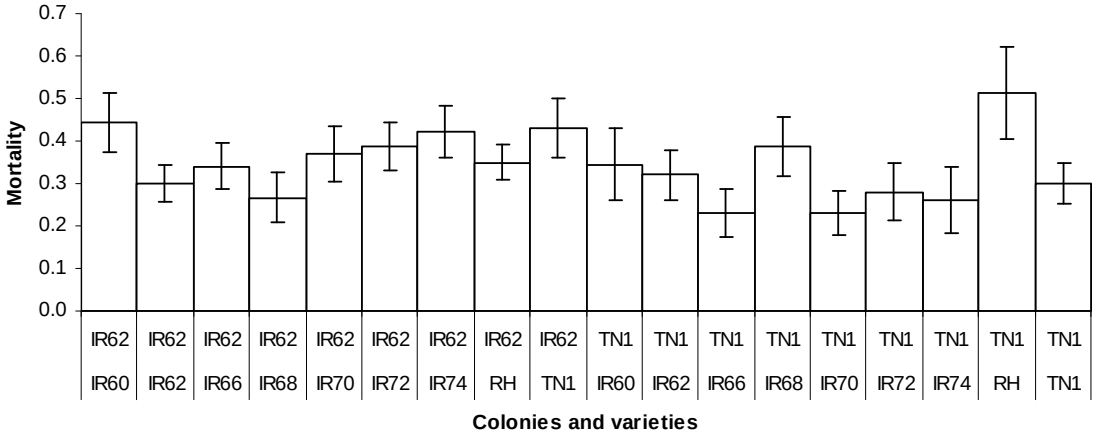


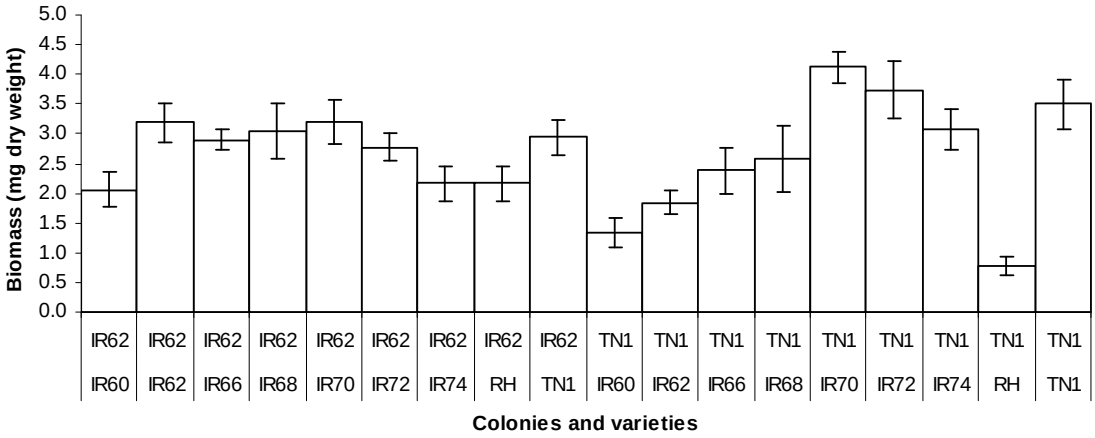


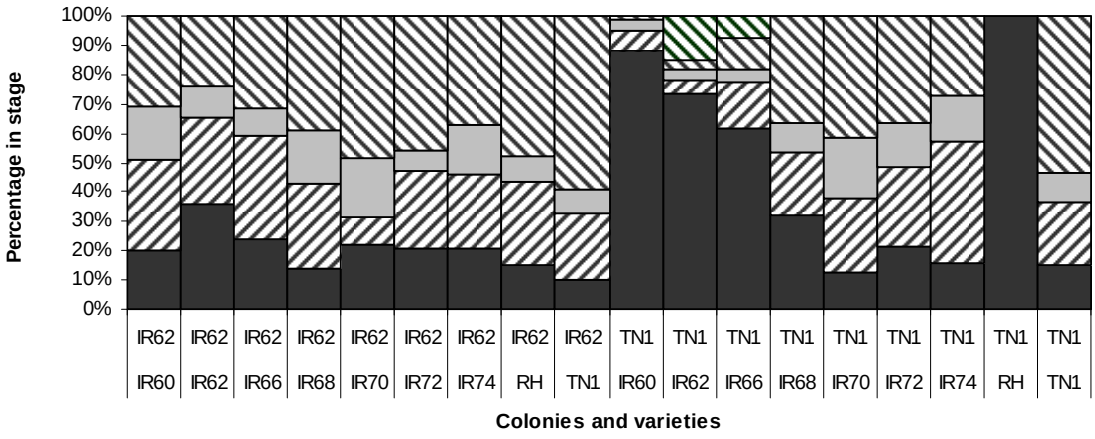


The lower stage is below that other stages (2^{nd}, 3^{rd}, 4^{th}, 5^{th} and adults to up).

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).

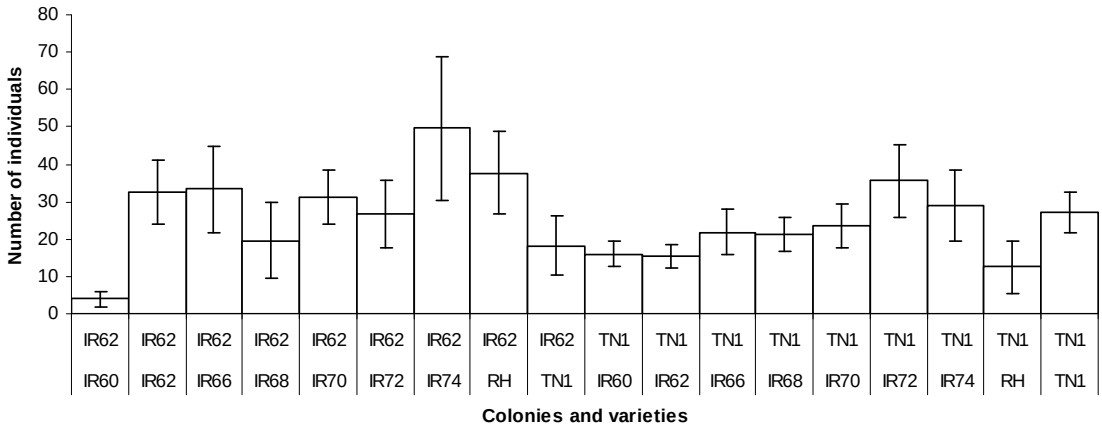


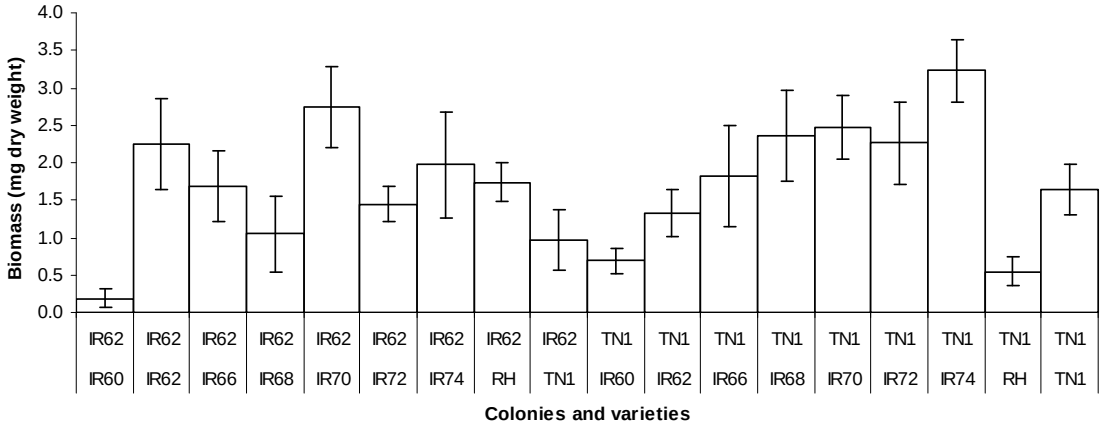


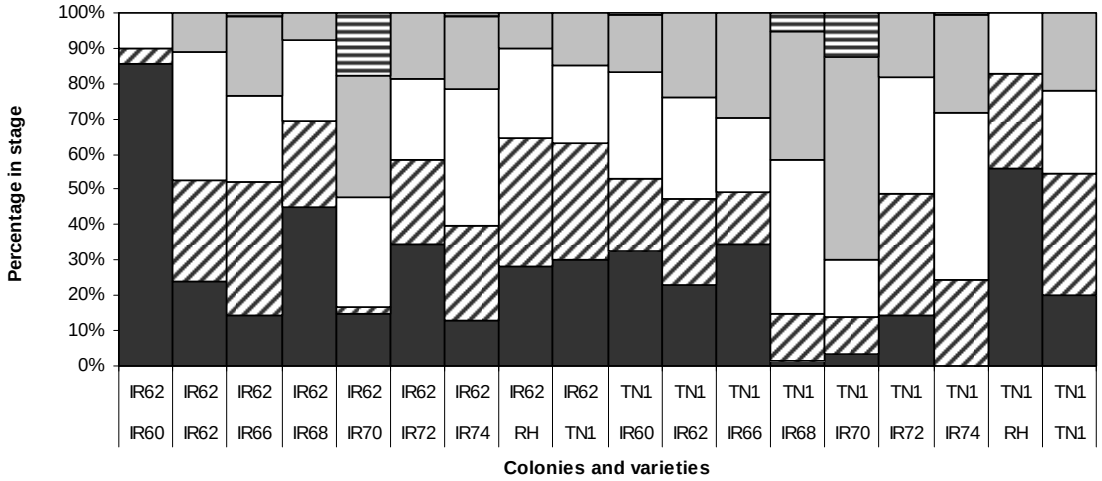


The lower stage is below that other stages (3rd, 4th, 5th and adults to up).

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 (2^{nd}, 3^{rd}, 4^{th}, 5^{th} and adults to up).