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ORIGINAL PAPER

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Transgenic rice plants expressing the snowdrop lectin gene (*gna*) exhibit high-level resistance to the whitebacked planthopper (*Sogatella furcifera*)

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Abstract Transgenic rice plants, expressing snowdrop lectin [*Galanthus nivalis* agglutinin (GNA)], obtained by *Agrobacterium*-mediated genetic transformation, were evaluated for resistance against the insect, the whitebacked planthopper (WBPH). The transgene *gna* was driven by the phloem-specific, rice-sucrose synthase promoter RSs1, and the *bar* was driven by the CaMV 35S promoter. In our previous study, the transgenic status of these lines was confirmed by Southern, Northern and Western blot analyses. Both the transgenes, *gna* and *bar*, were stably inherited and co-segregated into progenies in T_1 to T_5 generations. Insect bioassays on transgenic plants revealed the potent entomotoxic effects of GNA on the WBPH. Also, significant decreases were observed in the survival, development and fecundity of the insects fed on transgenic plants. Furthermore, intact GNA was detected in the total proteins of WBPHs fed on these plants. Western blot analysis revealed stable and consistent expression of GNA throughout the growth and development of transgenic plants. Transgenic lines expressing GNA exhibited high-level resistance against the WBPH. As reported earlier, these transgenics also showed substantial resistance against the brown planthopper and green leafhopper .

Introduction

Rice (*Oryza sativa* L.), the dietary staple for about three billion people, is grown worldwide in diverse agroclimatic zones. However, its grain yield is affected by several biotic and abiotic factors. Among the biotic factors, insects belonging to the orders Homoptera and Lepidoptera cause extensive damage to rice production in major rice-growing countries. Sap-sucking homopteran pests, viz., the brown planthopper (BPH), the green leafhopper (GLH) and the whitebacked planthopper [(WBPH) *Sogatella furcifera* (Horvath.)], all three of which constitute nearly 35% of the insect pests of rice, not only cause severe physiological damage to the rice plant, but also act as vectors for major viral diseases. The WBPH is an important, major sap-sucking pest of the rice in tropics and subtropics of Asia (Heinrichs 1994). It attained major pest status after the intensive cultivation of short-statured, nitrogen-responsive and high-yielding varieties, where both the BPH and the WBPH generally have similar ecological niches (Heinrichs and Rapusas 1983). These insects are well known throughout southeastern and eastern Asia as the most destructive pests of rice (Cheng et al. 2001). The WBPH feeds by phloem abstraction and causes damage to the rice plant by hopper burn (Khan and Saxena 1985; Reissig et al. 1986).

The massive application of pesticides results in adverse effects on the beneficial organisms besides posing serious risks to human health and the environment. Indiscriminate usage of insecticides, especially during the early crop stages, caused outbreaks of sap-sucking insects (Hadfield 1993). Different major (as well as several minor) genes conferring resistance to sap-sucking insects have been identified in the rice germplasm, and as such, the progress has been slow in evolving resistant varieties. Both dominant and recessive genes responsible for WBPH resistance have been identified in different rice accessions (Angeles et al. 1981; Hernandez and Khush 1981; Nair et al. 1982; Saini et al. 1982; Sidhu et al. 1979; Wu and Khush 1983). Nonetheless, so far no significant resistance against the WBPH has been achieved in high-yielding rice cultivars.

Genetic-engineering approaches offer ingenious solutions for introducing alien pest-resistance genes into popular rice cultivars. Artificial-diet bioassays revealed

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that plant lectins are toxic to homopteran insects (Powell et al. 1993). *Galanthus nivalis* agglutinin (GNA), known as snowdrop lectin, is one such lectin found to be highly toxic to sap-sucking insects. It is a mannose-specific, tetrameric protein, consisting of identical subunits of approximately 12 kDa (Van Damme et al. 1987). GNA was found most toxic among the lectins tested, even at concentrations as low as 6 μM (Powell et al. 1995). It also proved toxic to major insect pests belonging to the orders Coleoptera and Lepidoptera (Gatehouse et al. 1995, 1997). Because of the potent entomotoxic properties exhibited by the GNA, the snowdrop lectin gene (*gna*) was introduced into a number of dicot and monocot plants (Bell et al. 2001; Gatehouse et al. 1997; Hilder et al. 1995; Rao et al. 1998; Stoger et al. 1999). However, it is worthwhile to note that GNA proved to be non-toxic to mammals (Pusztai 1991). Also, it was found to have negligible effects on the development, survival and fecundity of beneficial insects (Down et al. 2003)

In an earlier study, we reported that the cv. Chaitanya transgenic rice expressing GNA exhibited substantial resistance against both the BPH and the GLH (Nagadhara et al. 2003). Since the WBPH also feeds on the phloem sap of rice plants, we investigated the effectiveness of GNA against this insect. The present study—the first of its kind—clearly demonstrates that the GNA-expressing transgenic rice confers substantial protection against the WBPH. Furthermore, these transgenic lines constitute a rare genetic stock, showing significant resistance to all three major sap-sucking pests of rice.

Materials and methods

WBPH rearing

WBPHs were reared on the susceptible 30-day-old Taichung Native 1 (TN1) plants, under controlled conditions in the greenhouse, at the Directorate of Rice Research (DRR), Rajendranagar, Hyderabad, India. WBPH pre-mated, gravid females were allowed to ovi-posit on TN1 plants for 2 days, and freshly hatched nymphs or nymphs of desired age were utilized for various insect bioassay experiments.

Transgenic plants used in insect bioassays

Transgenic rice plants used in this investigation have been obtained by *Agrobacterium*-mediated transformation of *indica* rice cv. Chaitanya (Nagadhara et al. 2003). The Ti-plasmid pSB111-bar-gna harboured in the *Agrobacterium* strain LBA4404 contained the selectable marker gene, *bar*, driven by CaMV35S promoter, and the snowdrop lectin gene, *gna*, driven by phloem-specific rice sucrose synthase 1 (RSs1) promoter (Nagadhara et al. 2003). Calli derived from mature embryos were infected with the *Agrobacterium* culture, and putatively transformed calli were selected on the Murashige and Skoog (1962) medium containing phosphinothricin (8–10 mg/l). Plants were regenerated from the selected calli and grown to maturity in the glasshouse (Nagadhara et al. 2003). Southern blot analysis was carried out as described by Nagadhara et al. (2003).

Insect bioassays in T_1 generation

Selfed seed collected from 1T and 2T transgenic plants were germinated, and $1T_1$ and $2T_1$ progenies were subjected to the Basta test and WBPH bioassays under controlled conditions. Based on the Basta leaf-dip assay, selected homozygous lines of $1T_2$ and $2T_2$ were also subjected to WBPH bioassays. All the insect bioassays were conducted at the DRR. Untransformed plants of cv. Chaitanya var. TN1, susceptible to the WBPH, and var. MO1, resistant to the WBPH, were used as comparable controls in insect bioassays. Thirty-five-day-old progenies of $1T_1$ and $2T_1$ along with var. TN1 and var. MO1 were transplanted in seed-box trays for WBPH bioassays, using the glasshouse mass screening method (Kalode et al. 1975). Twenty WBPH nymphs of second-to-third instar stages were released per plant and allowed to feed for 10 days. After 10 days of infestation with the WBPH, the surviving plants were scored as resistant, while the dead were recorded as susceptible. The degree/level of resistance exhibited by transgenic plants was scored based on a scale of 0–9, as used in the International Rice Testing Programme.

Insect survival, development and fecundity assays

Thirty-five-day-old homozygous transgenic plants of $1T_2$ and $2T_2$ and untransformed control plants of cv. Chaitanya were used to assess the anti-metabolic effects of GNA on the WBPH. For insect survival and developmental assays, 20 first-instar WBPH nymphs were released on each plant and confined in a mylar cage (to prevent escape of the test insect and also to prevent predator insect attack) in 15 replications. Observations were recorded after every 3 days up to 21 days of bioassay period. For the fecundity assay, the male and female insects were confined together in a 1 male:1 female ratio to avoid differences in the nymph production based on the sex ratio. The numbers of nymphs produced from the eggs were counted until no new nymphs were found emerging. Data were analysed using the Sigma plot software, version 5.0, for Windows (SPSS, Richmond, Calif., USA). Differences between the mean values were subjected to unpaired *t*-tests or ANOVAs.

Quantitative assay of honeydew production

The extent of insect feeding on $1T_2$ and $2T_2$ homozygous plants was estimated by semi-quantitative assay of the liquid honeydew excreted by the sap-sucking insects (Pathak et al. 1980). Whatman No. 1 filter paper, dipped in Bromocresol Green (2 mg/ml in ethanol) solution, was used for honeydew estimation; the filter paper was placed at the base of each plant and covered with a plastic cup. On each of the transgenic and control plants, five pre-starved (2 h), adult WBPH females were released and allowed to feed for 48 h. Care was taken not to release gravid adult females. Honeydew, excreted by WBPHs, reacts with the Bromocresol Green in the filter paper, resulting in blue/white spots. Areas of spots developed on filter papers were measured using millimetre graph paper and expressed in units (1 unit = 1 mm^2).

Western blot analysis for the stable expression of GNA at different developmental stages

Total leaf proteins were isolated from the plants of the T_4 generation of 1T and 2T transformants at vegetative, flowering and seed-formation stages (Rao et al. 1998). Total leaf proteins of 5 μg each were subjected to SDS-PAGE (Laemmli 1970). After electrophoresis, the proteins were transferred onto a nitrocellulose membrane (Amersham) by electroblotting (Towbin et al. 1979). The nitrocellulose filter was soaked in 20% hydrogen peroxide for 20 min, rinsed and blocked by the blocking agent (Amersham) in phosphate buffer with 0.1% Tween 20 for 2 h at 37°C. The filter was probed

with polyclonal rabbit anti-GNA serum (1:10,000 dilution) and goat anti-rabbit IgG horseradish peroxidase conjugate as a secondary antibody (1:10,000). After thorough washing, the bound secondary antibodies were detected by enhanced chemiluminescence as per the manufacturer's protocol (Amersham). Total protein was estimated in leaf-extract samples, as described by Lowry et al. (1951).

Western blot analyses of WBPH total proteins and honeydew

Total insect protein was isolated from a sample of 20 fourth-to-fifth instar WBPH nymphs fed on control and transgenic plants independently as per the protocol described earlier. About 5 µg of insect protein was used for Western blot analysis. To collect insect honeydew, parafilm was cut into 10 cm × 5 cm and folded in the middle. Portions of the stems (5 cm) of transgenic and control plants was covered with the parafilm sachet and was dilated and pressed gently to the stem, sealing all the edges. Fifth-instar WBPH nymphs, pre-starved for 2 h, were released into parafilm sachet through a narrow opening and sealed immediately. After 24 h, honeydew was collected from the sachets. Western blot analysis was carried out using 20 µl (5 µg protein) of honeydew collected from WBPHs.

Results

Insect bioassays in T_1 generation

Bioassays for resistance to the WBPH were carried out *in planta*, and host resistance was assessed 10 days after infestation. Thirty-five-day-old T_1 progeny plants of 1T and 2T transformants, expressing GNA, showed significant resistance against WBPHs, with minimal plant damage (Fig. 1). With a 1–2 point score on 0–9 scale, the GNA-transgenic plants exhibited higher levels resistance to WBPHs when compared to the WBPH-resistant rice var. MO1. On the other hand, the susceptible var. TN1 with a score of 9 showed severe damage caused by WBPH infestation (Fig. 1b). Insect bioassays on segregating progenies of $1T_1$ and $2T_1$ showed good fit to the Mendelian ratio of three resistant:one susceptible to the WBPH (Table 1). Likewise, for bar a segregation ratio of three tolerant:one susceptible was observed in the T_1 progenies.

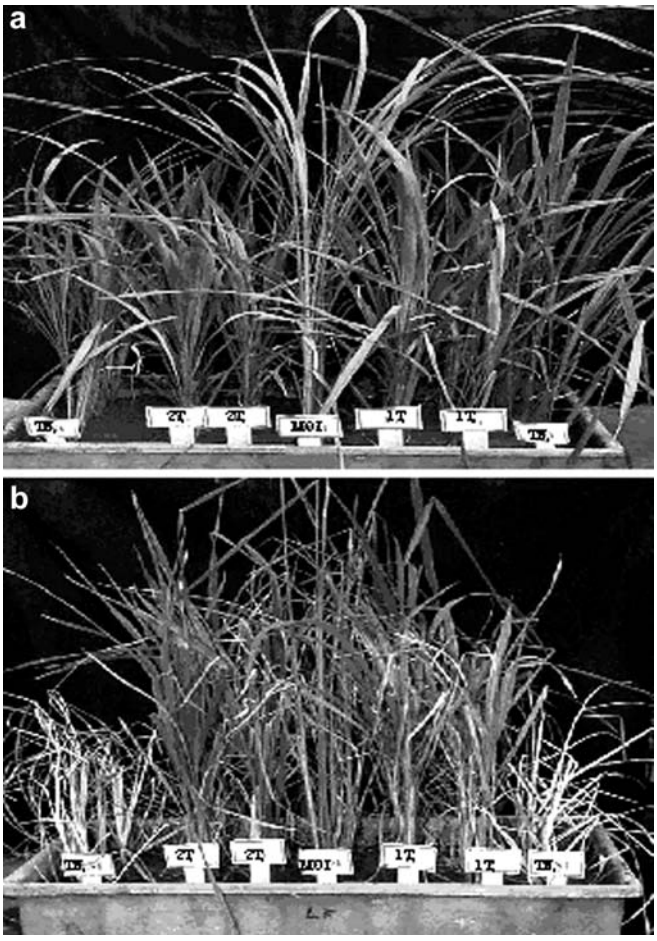

Fig. 1a, b Whitebacked planthopper (WBPH) bioassays on T_1 generation of transgenic rice plants. **a** 35-Days-old T_1 transgenic plants along with the respective controls prior to WBPH infestation. *Rows 1 and 7* TN1-susceptible control plants (14 plants in each row, total plants: 28), *rows 2 and 3* $2T_1$ progeny plants (14 plants in each row, total plants: 28), *row 4* MO1-resistant control plants (14 plants), *rows 5 and 6* $1T_1$ progeny plants (14 plants in each row, total plants: 28). **b** After 10 days of WBPH infestation. *Rows 1 and 7* TN1 plants showing complete damage, *rows 2 and 3* $2T_1$ progeny plants segregating for resistance (22 plants)/susceptibility (six plants), *row 4* MO1 showing resistance, *rows 5 and 6* $1T_1$ progeny plants segregating for resistance (20 plants)/susceptibility (eight plants)

Table 1 Whitebacked planthopper (WBPH) bioassays and inheritance pattern of transgenes in T_1 and T_2 generations of transgenic rice

Progenies of transformants[a]	Test/bioassay	No. of plants resistant	No. of plants susceptible	Segregation ratio	χ^2 [b]	*P*-value
$1T_1$	Basta	26	7	3:1	0.25	>0.50
$2T_1$	Basta	25	8	3:1	0.01	>0.80
$1T_1$	WBPH	20	8	3:1	0.189	>0.60
$2T_1$	WBPH	22	6	3:1	0.189	>0.60
$1T_2$ l	Basta	67	0	–	0.000	>0.99
$1T_2$ l	WBPH	15	0	–	0.000	>0.99
$2T_2$ 56	Basta	78	0	–	0.000	>0.99
$2T_2$ 56	WBPH	15	0	–	0.000	>0.99

[a] *$1T_1$* T_1 progeny of 1T primary transformant, *$2T_1$* T_1 progeny of 2T primary transformant, *$1T_2T_2$* progeny of 1T transformant homozygous line, *$2T_256$* T_2 progeny of 2T transformant homozygous line
[b] Significant at $P<0.05$

Effect of GNA on the survival of the WBPH

WBPH nymphs fed on transgenic plants showed significant decline in their survival from the third day onwards after infestation (Fig. 2). During the 21-day bioassay period, the survival of WBPHs on transgenic plants was significantly reduced to a mean of 1.0 insect/plant compared to 11.73 insects/plant observed on untransformed controls (Fig. 2). Overall, the survival of WBPH nymphs fed on transgenic plants was reduced by >90%, when compared to that of susceptible control plants.

Effect of GNA on the development and fecundity of the WBPH

First-instar WBPH nymphs were released onto the transgenic/control plants for survival assays and were monitored for developmental delays as well as fecundity of the insects. Insects fed on transgenic plants exhibited a delay of 5–7 days in reaching adulthood compared to the insects fed on control plants. Moreover, among the survivors, only 25% could reach the adult stage on transgenic plants when compared to 77% on control plants (Fig. 3a).

Effect of GNA on the fecundity of the WBPH was assessed by estimating the total number of nymphs produced by the insects on transgenic plants. A mean number of 12.60±1.48 nymphs/plant were recorded on transgenic plants compared to 139.20±9.96 nymphs/plant produced on susceptible control plants (Fig. 3b). Thus, the fecundity of WBPHs fed on transgenic plants was significantly (P<0.05) reduced by >90% compared to the insects fed on control plants (Fig. 3b).

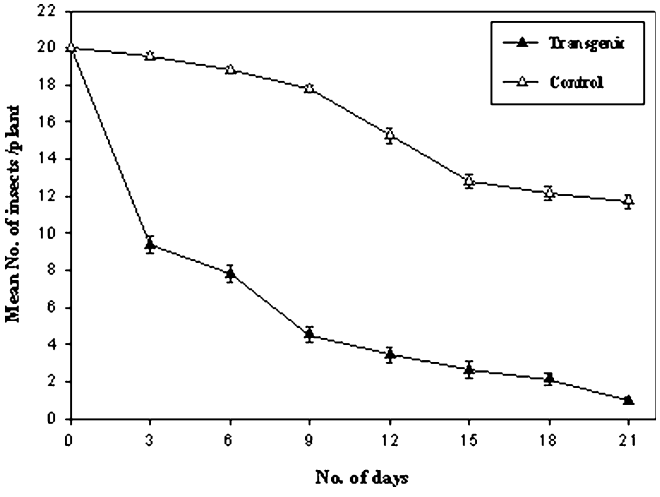

Fig. 2 WBPH bioassays on transgenic rice expressing snowdrop lectin [*Galanthus nivalis* agglutinin (GNA)]. Twenty first-instar WBPH nymphs were released separately on to each plant at day 0. Transgenic plants (data points represented by *black triangles*) were T_2 homozygous lines ($1T_2$l). Control plants (data points represented by *white triangles*) were untransformed cv. Chaitanya. Mean(±SE) survival of the insect per plant was plotted against time (days). Bioassays were carried out with 15 plants of each transgenic and control. Differences between control and transgenic were significant at P<0.005 after day 3 (ANOVA). Data are mean±SE

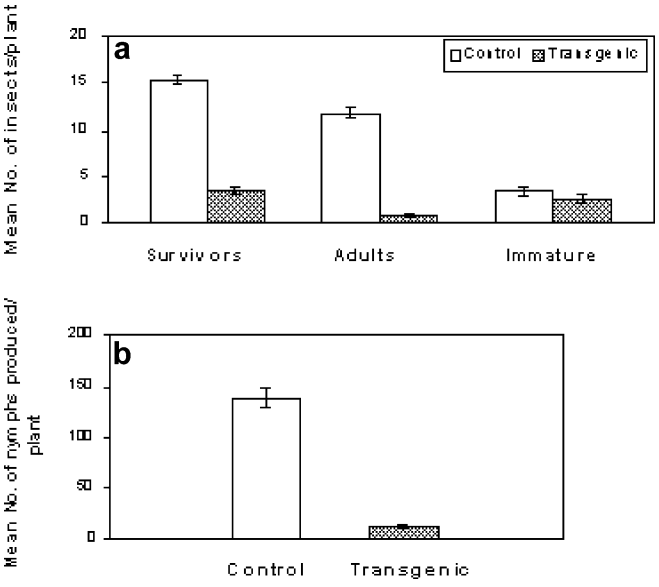

Fig. 3 **a** Effect of GNA on the development of the WBPH. Twenty first-instar WBPH nymphs were released separately onto transgenic ($1T_2$l) and control plants. The number of nymphs which reached adult stage and in immature stage on control (*white bars*) and transgenic (*grey cross-hatched bars*) plants were plotted on the graph. Bioassays were carried out with 15 plants each of transgenic and control. Differences between control and transgenic were significant at P<0.05. Data are mean±SE. **b** Effect of GNA on the fecundity of the WBPH. Total number of WBPH nymphs produced on transgenic ($1T_2$l) and control plants from five pairs of insects were plotted on the graph. Bioassays were carried out with 15 plants of each transgenic and control. Differences in nymph production were assessed by unpaired t-test (P<0.05). Data are mean±SE

Semi-quantitative honeydew assay and feeding behaviour of the WBPH

Effect of GNA on the feeding of the WBPH was assessed based on the amount of honeydew excreted by the insects. After a lapse of 48 h of feeding on untransformed control/transgenic plants, the number of blue spots (honeydew units) developed on the Bromocresol Green paper was counted to measure the phloem-feeding capacity of the insects. A mean number of 35.00±3.98 honeydew units/plant were excreted by WBPHs when fed on transgenic plants compared to 295.00±22.97 honeydew units/plant excreted by the insects fed on control plants (Fig. 4). Further, honeydew assay also revealed a significant (P<0.01) 88% reduction in the feeding of WBPHs on transgenic plants as compared to the susceptible control plants.

Western blot analysis of total WBPH proteins

Western blot analysis was carried out with the total insect proteins isolated from the nymphs that were fed on susceptible control/transgenic plants (Fig. 5a). This analysis showed the presence of a 12-kDa protein in the WBPH nymphs fed on transgenic plants, which corresponded to the GNA used as a positive control (Fig. 5a).

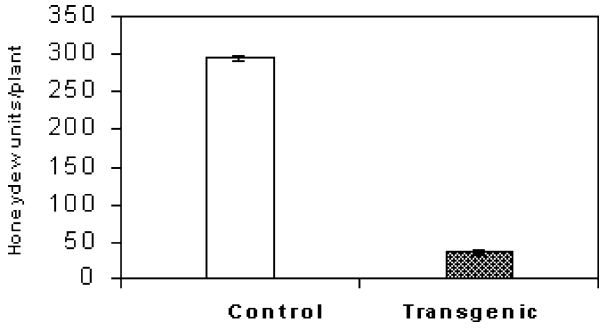

Fig. 4 Effect of GNA on the feeding of WBPH. Honeydew of WBPHs fed (48 h) on transgenic ($1T_2$l) and control plants was estimated semi-quantitatively. Each plant was inoculated with five adult female insects. The honeydew units produced by the insects per plant were plotted for transgenic (*grey cross-hatched bar*) and control (*white bar*). Assays were carried out with 15 plants of each transgenic and control. The differences between control and transgenic were significant at $P<0.01$. *Data points* are mean±SE

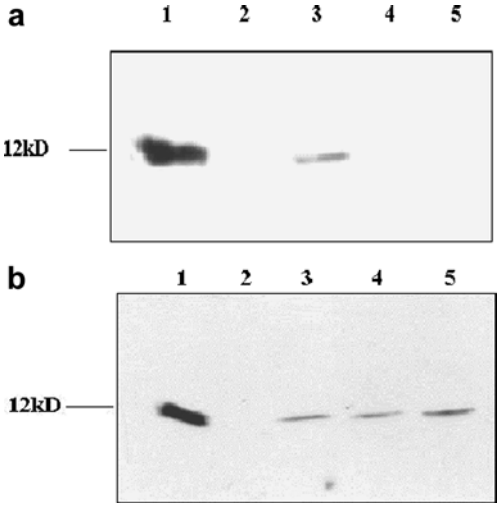

Fig. 5 **a** Detection of GNA in the total protein extract of the WBPH. *Lane1* GNA protein (+ve), *lane 2* total proteins of WBPH fed on control plants, *lane 3* total proteins of WBPH fed on $1T_2$l plants, *lane 4* honeydew of WBPH fed on control plants, *lane 5* honeydew of WBPH fed on $1T_2$l plants. **b** Expression profile of GNA at different developmental stages of transgenic rice (T_4 generation) under field conditions. *Lane 1* GNA protein (+ve), *lane 2* total leaf protein (5 μg) of untransformed control plants (80 days old), *lane 3* total leaf protein (5 μg) isolated at vegetative stage (40 days old) of transgenic plants, *lane 4* total leaf protein (5 μg) isolated at flowering stage (80 days old) of transgenic plants, *lane 5* total leaf protein (5 μg) isolated at seed maturation stage (100 days old) of transgenic plants

Conversely, no such band was observed in the WBPH nymphs fed on untransformed control plants. However, Western blot analysis of honeydew collected from the nymphs fed on transgenic/control plants failed to show the GNA polypeptide.

Western blot analysis of GNA-transgenic rice plants at different developmental stages

When Western blot analysis was carried out to assess the expression pattern of GNA protein at different developmental stages of transgenic rice plants, viz., vegetative (40 days old), flowering (80 days old) and seed maturation (100 days old), GNA was invariably present in the leaf proteins sampled at three growth stages (Fig. 5b).

Discussion

In the present investigation, we dealt with the potent insecticidal effects of GNA towards the WBPH when fed on GNA-expressing transgenic rice plants. The cv. Chaitanya transgenic lines evaluated in this study exhibited high-level resistance against this insect.

Southern blot analysis of 1T and 2T transformants revealed the stable integration of *gna* and *bar* genes in the genomes of these rice plants (Nagadhara et al. 2003). Segregation analyses of the transgenes *gna* and *bar* in T_1 progenies conformed to the monogenic ratio of 3:1 for both WBPH resistance and herbicide tolerance, signifying that these genes are stably integrated into the rice genome at a single location (Table 1; Fig. 1b). Furthermore, these transgenes also were stably inherited and transmitted to the progenies in subsequent generations.

The insect bioassays, employing no choice method, showed a highly significant (>90%) reduction in the nymphal survival of WBPH reared on transgenic plants as compared to untransformed control plants (Fig. 2). This decline in the nymphal survival is attributable to the selective expression of GNA in the phloem sap of transgenic plants, since *gna* is driven by the phloem-specific RSs1 promoter (Rao et al. 1998). The amount of GNA expressed in these two transformants (1T and 2T) was 0.3% of the total soluble proteins (Nagadhara et al. 2003). The cv. Chaitanya transgenic lines used in this assay, when tested against the BPH and the GLH, exhibited 55% and 49% reduction in their survival, respectively. (Nagadhara et al. 2003). In artificial diets, GNA fed at a 0.1% level was shown to be anti-metabolic to members of different homopteran insects (Powell et al. 1993). In the present study, the untransformed control plants with severe hopper burn failed to survive the 21-day bioassay period. In contrast, the GNA-transgenic plants could survive the infestation well and were grown to maturity with normal vigour and seed fertility. These results amply denote that the transgene *gna* confers high-level protection against the WBPH without interfering with the normal metabolism of rice plant.

GNA also decreased the development and fecundity of insects besides reducing their survival. A general delay of 5–7 days in the life cycle of the WBPH was observed on transgenic rice plants compared to the insects fed on untransformed control plants. Further, a significant ($P<0.05$) reduction in the number of nymphs reaching adulthood was observed on transgenic plants compared to that of susceptible control plants (Fig. 3a). These results clearly testify to the potent insecticidal and anti-metabolic effects of GNA on the WBPH. The number of WBPH nymphs produced on transgenic plants, as compared to

susceptible controls, was significantly reduced by >90%, amply indicating the adverse effects of GNA on the egg-laying capacity and on the nymphal emergence from the eggs (Fig. 3b). Thus, in this study, we have observed for the first time, substantial entomotoxic effects of GNA on the survival, development and fecundity of the WBPH.

To investigate the effect of GNA on the feeding behaviour of WBPH insects, pre-starved, early-adult females were used in honeydew assays. Feeding of the phloem sap by WBPH was measured in terms of area of blue spots developed on the Bromocresol Green paper. The frequency of blue spots reflects the phloem-feeding capacity of the insects, since the pH of phloem sap is alkaline. A marked decrease (88%) in the honeydew excretion of the WBPH was observed on transgenic plants when compared to that of untransformed controls (Fig. 4), thus confirming the high feeding deterrent effect of GNA against WBPH when GNA is expressed *in planta*. The honeydew excreted by the hoppers, in general, serves as a medium for mould growth in rice fields (Dale 1994). Such a drastic reduction in the honeydew produced by WBPH insects on GNA-transgenic rice might also retard the growth of moulds and minimize fungal attacks.

A significant reduction in the feeding, survival and fecundity of WBPH was observed when fed on GNA-expressing transgenic rice plants. Conceivably, these insecticidal effects in turn might cause a decline in the population build-up under field conditions. It was reported earlier that GNA could reduce the populations of the potato aphid (Down et al. 1996) and that of the peach-potato aphid (Sauvion et al. 1996). Furthermore, GNA also caused significant reduction in the growth of the peach-potato aphid besides decreasing female fecundity (Sauvion et al. 1996).

Since populations of the BPH and the WBPH co-exist on rice plants, the cv. Chaitanya transgenic lines expressing GNA invariably confer resistance to both the pests. In an earlier study, we demonstrated the significant impact of GNA against the BPH and the GLH in terms of their survival, feeding and fecundity when fed on cv. Chaitanya transgenic rice plants. As such, these transgenic lines have been found to bestow significant resistance against the BPH and the GLH (Nagadhara et al. 2003). In the current study, the cv. Chaitanya transgenic lines exhibited higher entomotoxic effects against the WBPH when compared to that of the BPH and the GLH.

Western blot analysis for the presence of GNA was carried out on WBPHs fed on the transgenic/untransformed control plants. GNA could not be detected from the WBPH proteins as well as from the honeydew excreta when insects were fed on untransformed control plants (Fig. 5a). Conversely, proteins sampled from WBPHs fed on transgenic plants revealed a distinct 12-kDa band corresponding to the GNA protein. However, no GNA was detected in the honeydew collected from these insects, suggesting that the ingested GNA might have bound to the mid-gut epithelial cells of the WBPH, leading to toxic effects. However, the precise mechanism of lectin toxicity towards insects is not clear. Physiological studies on GNA-fed BPHs revealed that GNA could bind to various midgut proteins, thereby disrupting the brush-border membrane as well as cellular functions (Fitches et al. 2001; Powell et al. 1998).

Western blot analysis of leaf proteins at three developmental stages of T_4 lines grown under field conditions suggested that the GNA expression is stable and consistent throughout the growth and development of transgenic rice plants (Fig. 5b). The transgenic rice plants expressing GNA did not show any significant variation in the seed yield (32.26±2.41 gm/plant) when compared to untransformed control (31.84±2.05 gm/plant) plants

The T_5 transgenic rice lines expressing GNA protein against major sap-sucking insects, under natural infestation conditions, are being evaluated in open field trials at three locations, viz., the Centre for Plant Molecular Biology, Osmania University, Hyderabad; the DRR, Hyderabad; and the Rice Research Station, Maruteru, West Godavari (A.P), India.

To sum up, the GNA-expressing transgenic lines, by virtue of their high anti-metabolic and anti-feedant effects, afforded high-level resistance against the WBPH. These transgenic lines, endowed with high-level exotic resistance to major sap-sucking insects, might serve as a novel genetic stock in recombination breeding, besides their use for direct commercial cultivation in hopper-prone areas.

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References

Angeles ER, Khush GS, Heinrichs EA (1981) New genes for resistance to whitebacked planthopper in rice. Crop Sci 21:47–50

Bell HA, Fitches EC, Down RE, Ford L, Marris GC, Edwards JP, Gatehouse JA, Gatehouse AMR (2001) Effect of dietary cowpea trypsin inhibitor (CpTI) on the growth and development of the tomato moth *Lacanobia oleracea* (Lepidoptera: Noctuidae) and on the success of the gregarious ectoparasitoid *Eulophus pennicornis* (Hymenoptera: Eulophidae). Pest Manage Sci 57:57–65

Cheng J, Zhao W, Lou Y, Zhu Z (2001) Intra- and inter-specific effects of the brown planthopper and whitebacked planthopper on their population performance J Asia Pac Entomol 4:85–92

Dale D (1994) Insect pests of the rice plant—their biology and ecology. In: Heinrichs EA (ed) Biology and management of rice insects. Wiley Eastern, New Delhi

Down RE, Gatehouse AMR, Hamilton WDO, Gatehouse JA (1996) Snowdrop lectin inhibits development and decreases fecundity of the glasshouse potato aphid (*Aulacorthum solani*) when administered in vitro and via transgenic plants both in laboratory and glasshouse trails. J Insect Physiol 42:1035–1045

Down RE, Ford L, Woodhouse SD, Majerus MEN, Gatehouse JA, Gatehouse AMR (2003) Tritrophic interactions between transgenic potato expressing snowdrop lectin. Transgenic Res 12:229–241

Fitches E, Woodhouse SD, Edwards JP, Gatehouse JA (2001) In vitro and in vivo binding of snowdrop lectin (*Galanthus nivalis* agglutinin; GNA) and jackbean (*Canavalia ensiformis*; Con A) lectins within tomato moth (*Lacanobia oleraceae*) larvae; mechanisms of insecticidal action. J Insect Physiol 47:777–787

Gatehouse AMR, Powell KS, Peumans WJ, van Damme EJM, Gatehouse JA (1995) Insecticidal properties of lectins: their potential in plant protection. In: Pusztai AJ, Barcdocz S (eds) Lectins: biomedical perspectives. Taylor and Francis, Hants, pp 35–57

Gatehouse AMR, Davison GM, Newell CA, Merryweather A, Hamilton WDO, Burgess EPJ, Gilbert RJC, Gatehouse JA (1997) Transgenic potato plants with enhanced resistance to the tomato moth (*Lacanobia oleraceae*) growth room trials. Mol Breed 3:49–63

Hadfield P (1993) Japanese aid may upset Cambodia's harvests. New Sci 13:5

Heinrichs EA (1994) Biology and management of rice insects. Wiley Eastern , New Delhi

Heinrichs EA, Rapusas HR (1983) Levels of resistance to the whitebacked planthopper, *Sogatella furcifera* (Homoptera: Delphacidae), in rice varieties with different resistance genes. Environ Entomol 12:1793–1797

Hernandez JE, Khush GS (1981) Genetics of resistance to whitebacked planthopper in some rice (*Oryza sativa* L.) varieties. Oryza 18:44–50

Hilder VA, Powell KS, Gatehouse AMR, Gatehouse JA, Gatehouse LN, Shi Y, Hamilton WDO, Merryweather A, Newell CA, Timans JC, Peumans WJ, Van Damme EJM, Boulter D (1995) Expression of snowdrop lectin in transgenic tobacco plants results in added protection against aphids. Transgenic Res 4:18–25

Kalode MB, Viswanathan PRK, Seshu DV (1975) Standard test to characterize host plant resistance to brown planthopper in rice. Indian J Plant Protein 3:204–206

Khan ZR, Saxena RC (1985) A selected bibliography of the whitebacked planthopper *Sogatella furcifera* (Horvath) (Homoptera:Delphacidae). Insect Sci Appl 2:115–134

Laemmli UK (1970) Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature 227:680–685

Lowry OH, Roseborough NJ, Farr AL, Randall JL (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Murashige T, Skoog FA (1962) Revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Nagadhara D, Ramesh S, Pasalu IC, Rao YK, Krishnaiah NV, Sarma NP, Bown DP, Gatehouse JA, Reddy VD, Rao KV (2003) Transgenic *indica* rice plants resistant to sap-sucking insects. Plant Biotech J 1:231–240

Nair RV, Masajo TM, Khush GS (1982) Genetic analysis of resistance to whitebacked planthopper in twenty-one varieties of rice (*Oryza sativa* L). Theor Appl Genet 61:19–22

Pathak PK, Heinrichs EA, Pathak MD (1980) Development of biotypes of *Nilaparvata lugens* (Stål). International Rice Research Institute, Los Banos, pp 1–13

Powell KS, Gatehouse AMR, Hilder VA, Gatehouse JA (1993) Antimetabolic effects of plant lectins and plant and fungal enzymes on nymphal stages of two important rice pests, *Nilaparvatha lugens* and *Nephotettix cinciteps*. Entomol Exp Appl 66:119–126

Powell KS, Gatehouse AMR, Hilder VA, van Damme EJM, Peumans WJ, Boonjawat I, Horsham K, Gatehouse JA (1995) Different antimetabolic effects of related lectins towards nymphal stages of *Nilaparvata lugens*. Entomol Exp Appl 75:61–65

Powell KS, Spence J, Bharathi M, Gatehouse JA, Gatehouse AMR (1998) Immunohistochemical and developmental studies to elucidate the mechanism of action of the snowdrop lectin on rice brown planthopper, *Nilaparvata lugens* (Stål). J Insect Physiol 44:529–539

Pusztai AJ (1991) Plant lectins. Cambridge University Press, Cambridge

Rao KV, Rathore KS, Hodges TK, Fu X, Stoger E, Sudhakar D, Williams S, Christou P, Bharathi M, Bown DP, Powell KS, Spence J, Gatehouse AMR, Gatehouse JA (1998) Expression of snowdrop lectin (GNA) in transgenic rice plants confers resistance to rice brown planthopper. Plant J 15:469–477

Reissig WH, Heinrichs EA, Litsinger JA, Moody K, Fiedler L, Mew TW, Barrion AT (1986) Illustrated guide to integrated pest management in rice in tropical Asia. International Rice Research Institute, Laguna

Saini RS, Khush GS, Heinrichs EA (1982) Genetic analysis of resistance to whitebacked planthopper, *Sogatella furcifera* (Horvath), in some rice varieties. Crop Prot 1:289–297

Sauvion N, Rahbe Y, Peumans WJ, van Damme EJM, Gatehouse JA, Gatehouse AMR (1996) Effects of GNA and other mannose binding lectins on development and fecundity of the peach-potato aphid *Myzus persicae*. Entomol Exp Appl 79:285–293

Sidhu GS, Khush GS, Medrano FG (1979) A dominant gene in rice for resistance to white-backed planthopper and its relationship to other plant characteristics. Euphytica 2:227–232

Stoger E, Williams S, Christan P, Down RE, Gatehouse JA (1999) Expression of the insecticidal lectin from snowdrop (*Galanthus nivalis* agglutimin; GNA) in transgenic wheat plants: effects on predation by the grain aphid *Sitobion avenae*. Mol Breed 5:65–73

Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76:4350–4354

Van Damme EJM, Allen AK, Peumans WJ (1987) Isolation and characterization of lectin with exclusive specificity towards mannose from snowdrop (*Galanthus nivalis*) bulbs. FEBS Lett 215:140–144

Wu CF, Khush GS (1983) A new dominant gene for resistance to whitebacked planthopper in rice. Crop Sci 25:505–509