Transgenic Rice with Hypersensitive Resistance to Rice Hoja Blanca Virus (RHBV) in the Field

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Introduction

Rice hoja blanca virus (RHBV) is one of the major diseases affecting rice in tropical Americas (Fig. 1A). RHBV disease was first reported in 1935 and since then, major outbreaks of the disease has caused up to 80 % yield losses (Morales & Niessen, 1983). The resistance to this virus is conferred by one or two genes (most varieties have resistance source from Colombia 1), but plants carrying this source of resistance are susceptible at ages younger than 25 days after planting. The uncertainty of epidemics induces farmers to spray insecticides to control sogata, the plant-hopper vector *Tagosodes orizicolus* (Muir)(Fig. 1B).

RHBV is a member of the tenuivirus group. The genome of RHVB consists of four ssRNA species (Ramirez et al., 1992 and 1993)(Fig. 1C). The nucleotide sequences of RHBV RNA3 and RNA4 are known and the genome encodes two genes in an ambisense manner (Ramirez et al., 1993). The nucleoprotein (N) (refers as coat protein in other type of viruses) gene is encoded by the viral complementary (vc) RNA3 strand (Fig. 1D). The expression of the nucleoprotein (coat protein) in transgenic plants has been demonstrated with many viruses to produce viral resistant plants (Beachy et al., 1987). The coat protein mediated cross protection method has been successful for the tenuivirus rice stripe virus, RStv (Hayakawa et al., 1992).

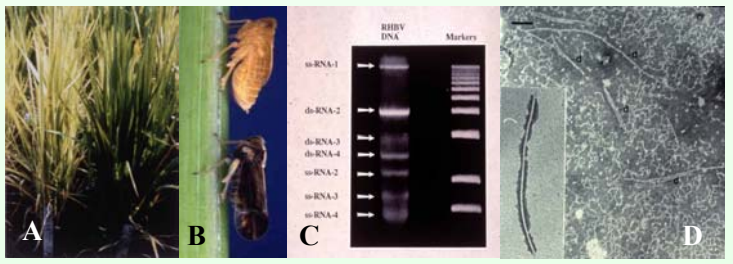

Figure 1.- (A) RHBV disease symptoms. (B) RHBV vector *T. orizicolus*. (C) RHBV genome. *(D)* RHBV threadlike nucleoprotein-virus particles.

Objective

The main goal for this project is to provide new source(s) of resistance to complement the single source of resistance present in most of the commercial varieties currently grown in Latin America.

Methodology

Preparation of plasmid and rice transformation with RHBV-N constructs.

The plasmid pVR3 used for introgression of the nucleoprotein gen (N-gene) of RHBV into rice is a combination of two genes. The *hph*-gene that confers hygromycin resistance used as a selection marker, and the RHBV-N gene encoding for the nucleopreotein gene. The genes were flanked by the CaMV 35S constitutive promoter and the Nos Poly (A) terminal sequences. The direct delivery of genes into immature panicle derived callus was conducted using DNA-coated gold particles accelerated by the PDS-1000 He system particle gun. The commercial variety Cica 8 with high yield and high quality, but susceptible to RHBV throughout the whole life cycle was used as target.

Screening to identify RHBV resistant transgenic rice plants.

First assessment of resistance was conducted using viruliferous insects under biosafety greenhouse conditions. A selection of a total of 421 transgenic lines representing T2 to T5 generations, and F2 populations derived from crosses between the selected transgenic lines with other commercial varieties including Fedearroz 50, Oryzyca 1, Iniap 12 were planted in the field on November 2000 after approval by the Colombian Biosafety Council. Plants were inoculated in the field 15 days after planting using insect colonies of at least 80% of virulence (Fig. 2A), and with an average of two insects per plant (Fig. 2B). The level of resistance conferred by the viral transgene was evaluated by scoring the percentage of leaf area diseased, severity of symptoms, and vigor. Weekly evaluations were initiated 15 days after the insect innoculation until 90 days of age. Non-transgenic Cica 8 plants, and a set of 13 commercial varieties with known RHBV reaction were used as controls.

Molecular analyses of the transgenic rice plants.

Transgenic plants were assayed by Southern blot to confirm gene integration into the plant genome, and by Northern, Western and ELISA analyses to determine expression of the RHBV transgene.

Results and Discussion

After the complete plant regeneration process on hygromycin 50 mg/l, a total of 187 Cica 8 plants were obtained from the RHBV-N circular plasmid bombardments. Sixty plants recovered contained the N gene. Inherited analyses indicated Mendelian segregation for some lines, and skewed segregation for other lines at the T1 generation. Plants derived from line A3-49 showed a significant reduction in disease progression and severity, and increased performance for various agronomic traits compared to the non transgenic control plants infected with RHBV in the greenhouse, (Fig. 3A and 3B). These transgenic lines showed a yield potential of 5 to 6 fold higher than the non-transgenic control and some of the transgenic plants had a yield similar to the non-RHBV infected Cica 8 control, suggesting that the N gene appears to protect plants from RHBV beginning at early stage of development. Similar results were seen in the field. Reactions to inoculation with RHBV ranged from susceptible to completely resistant plants (immunity). The most frequent reaction was characterized by local necrotic lesions (hypersensitive reaction) (Fig. 3C) followed by the production of new leaves without symptoms. In contrast, the Cica 8 non-transgenic control is highly susceptible throughout the whole life cycle, showing severe disease development and most plants die at 60 days after inoculation (Fig. 3B). Transgenic lines of advanced T5 generation with stable RHBV resistance in the field were identified (Fig. 3D). Some of the transgenic lines were as resistant as the variety Fedearroz 2000 which seems to have antibiosis to sogata, and several transgenic lines were more resistant than Fedearroz 50, the currently most widely commercially grown variety in Colombia (Fig. 4). The transgenic resistance to the virus was also inherited in a stable manner in the F2 population. Transgenic trial was just harvested and yield data of these plants is being analyzed. Results suggest that the resistance conferred by the *RHBV-N* gene is expressed independently of the genotype background, and that the transgene could be used to complement the natural resistance source.

The RHBV resistant transgenic rice lines expressed the *N* gene RNA at low levels that could only be detected using RT-PRC analysis (Fig. 5). The nucleoprotein could not be detected in any of the transgenic plants either by Western or ELISA tests. These results suggest that resistance conferred by the *N* gene is RNA mediated. These transgenic rice lines could become a new genetic resource in developing RHBV resistant cultivars.

Figure 2.- (A) Maintenance of viruliferous proven insect vectors. (B) Release of viruliferous insects in the field.

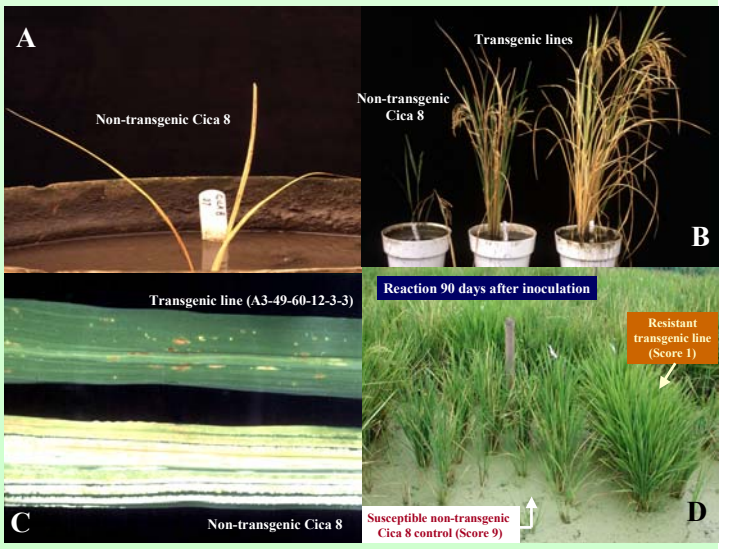

Figure 3.- (A) RHBV diseased Cica 8 non-transgenic control 20 days after inoculation. (B) Inoculated and diseased non-transgenic Cica 8, left; Inoculated and healthy transgenic plants (center and right) at maturity. (C) RHBV typical disease symptoms in Cica 8; hypersensitive resistance reaction in transgenic line. (D) Field evaluation of transgenic RHBV resistant rice.

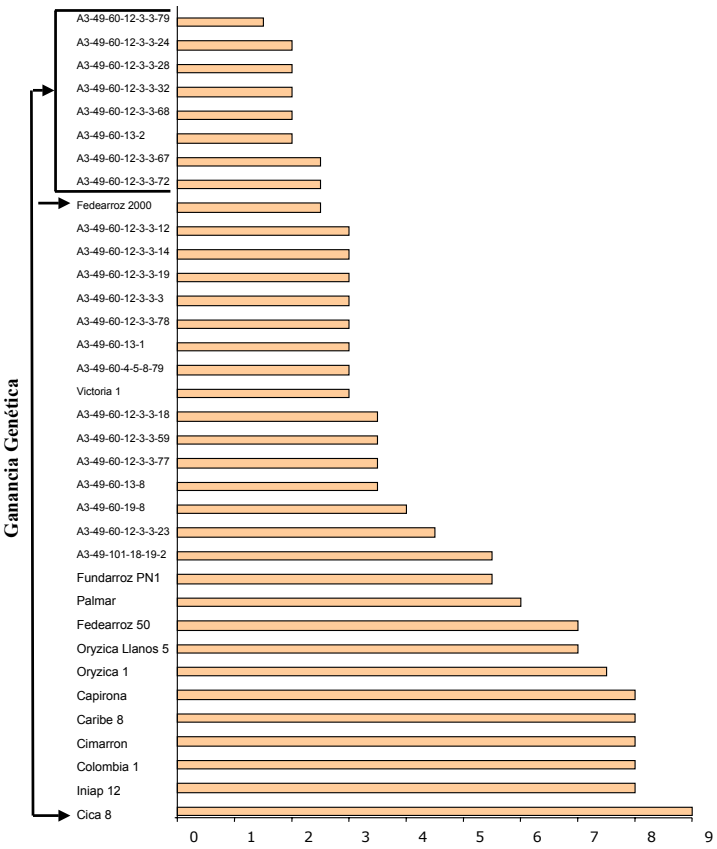

Figure 4.- Percentage of plants with disease reaction within each transgenic line and commercial rice varieties in the field, when inoculated at 15 days after planting. Resistant (score 0-1). Intermediate (score 3-5). Susceptible (score 7-9).

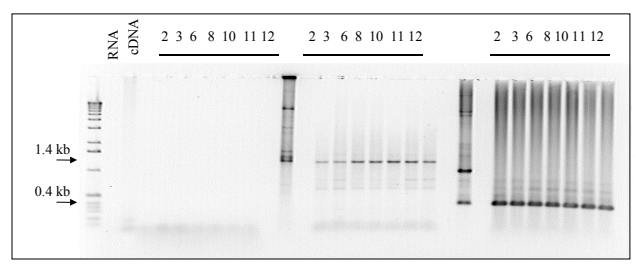

Figure 5.- RT-PCR of total RNA from T5 progeny plants derived from transgenic RHBV resistant A3-49-60-12-3-3

Conclusions

The protection conferred by the N gene was noted as a significant reduction in disease progression and severity with respect to inoculated Cica 8 controls. Several resistant reactions were observed including the production of local lesions resembling hypersensitive resistance reaction. Preliminary results indicate that RHBV-N resistance could be used to complement the natural source of resistance to the virus. Transgenic F1s were significantly more resistant to RHBV than the corresponding F1 non-transgenic cross when inoculated at 10-day-old plants. These results suggest that the protection conferred by the RHBV-N transgene is expressed independently of the plant age, the genotype background, and that the transgene could be used to complement the natural resistance source. Current work includes the evaluation of the resistance segregation in F2-backcross population to determine the inheritance and stability of this trait through breeding, and the initiation of a marker assisted selection scheme for the introgression of the RHBV-N transgene into various commercial varieties.

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