

# Gene Combinations for Durable Rice Blast Resistance in Colombia

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## Introduction

Rice blast caused by *Magnaporthe grisea* is the main limiting factor of rice production in Colombia. Breakdown of resistance normally occurs in one to three years after cultivar release, with the exception of the commercial cultivar Oryzica Llanos 5 released in 1989 and the cultivar Fedearroz 50 released in 1998 (Table 1). Understanding the genetic structure and avirulence genes frequency in the pathogen has been the focus of the Rice Project at CIAT for identifying suitable resistance gene combinations and developing breeding strategies for durable blast resistance.

## Materials and Methods

- Cultivars with known resistance genes were obtained from IRRI, CIRAD, or developed at CIAT.
- MGR-DNA and rep-PCR fingerprinting techniques were performed according to Levy *et al.* and George *et al.*, respectively. Microsatellite markers were used as described by McCouch *et al.*, with modifications made at CIAT and CIRAD.
- A total of 283 recombinant inbred lines from the cross C101 LAC (Pi-1, Pi-33) x C 101 A51 (Pi-2) were developed for the identification of microsatellites associated with the three resistance genes and development of near isogenic lines with different gene combinations.
- Field evaluations are performed under "hot spot" conditions using a spreader row technique to maintain a high and diverse pathogen population representing the genetic and virulence diversity found in our long-term studies.

## Results

Four out of seven lineages predominate (SRL-2, SRL-4, SRL-5, and SRL-6). Resistance genes in rice effective against all members of a lineage were detected (Table 2). Isolates exhibit compatibility with most resistance genes but none against all of them (Table 2). Breeders at CIAT developed the durable blast resistant cultivar Oryzica Llanos 5 whose parents exhibit susceptibility to different genetic lineages of the pathogen (Table 1, Figure 1). These parents should carry complementary genes that in combination confer durable resistance to the cultivar. The combination of the resistance genes Pi-1, Pi-2, and Pi-33 seem to be the most relevant genes for breeding durable blast resistance in Colombia (Table 2). No isolate defeats the three genes. The combination of the three resistance genes (Pi-1, Pi-2, Pi-33) in a single near isogenic line confers complete blast resistance when tested in the field (Figure 2). In addition, the resistance genes Pi-k<sup>h</sup>, Pi-sh, and Pi-z seem to be potentially useful (Table 2). The presence of different resistance genes in Colombian commercial cultivars has been studied based on greenhouse inoculations with blast isolates carrying corresponding avirulence genes (Table 3). We have initiated a backcrossing program to introduce the resistance genes Pi-1, Pi-2, and Pi-33 in several commercial rice cultivars from Latin America. Incorporation of the resistance genes is being followed through the use of microsatellite and scar molecular markers, as well as greenhouse and field screening (Figure 3). Breeding populations with the three resistance genes will be distributed to different Latin American countries for field evaluation and selection of appropriate resistant rice lines.

**Table 1. Rice Cultivars Released in Colombia and Breakdown of the Resistance to *Pyricularia Grisea***

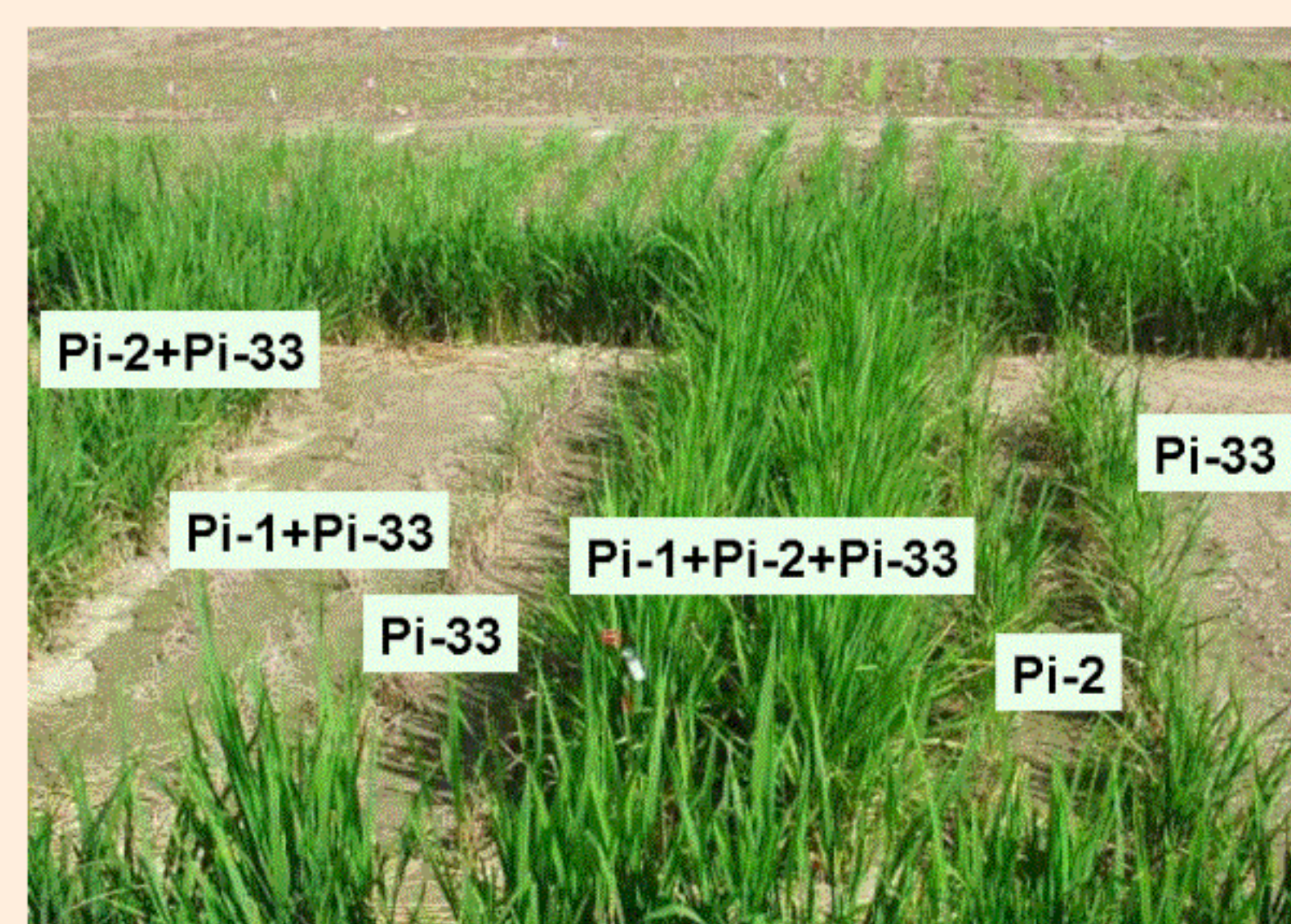
| CULTIVAR     | SOURCE OF RESISTANCE              | YEAR OF RELEASE | RESISTANCE BREAKDOWN | YEARS OF RESISTANCE |
|--------------|-----------------------------------|-----------------|----------------------|---------------------|
| CICA 4       | PETA                              | 1971            | 1972                 | 1                   |
| CICA 6       | IR-822-432                        | 1974            | 1975                 | 1                   |
| CICA 7       | COLOMBIA 1                        | 1976            | 1978                 | 2                   |
| CICA 9       | C46-15                            | 1976            | 1977                 | 1                   |
| CICA 8       | TETEP                             | 1978            | 1980                 | 2                   |
| METICA 1     | COLOMBIA 1                        | 1981            | 1982                 | 1                   |
| ORYZICA 1    | C46-15, COLOMBIA 1, TETEP         | 1982            | 1985                 | 3                   |
| ORYZICA 3    | COLOMBIA 1, TETEP                 | 1984            | 1985                 | 1                   |
| LINEA 2      | ORYZICA 1                         | 1988            | 1989                 | 1                   |
| O. LLANOS 5  | IR36, 5685, COLOMBIA1, CICA9      | 1989            | ?                    | > 10                |
| O. CARIBE 8  | TETEP, IR 665, COLOMBIA 1, CICA 9 | 1993            | 1995                 | 2                   |
| FEDEARROZ 50 | IR 665, 5685, COLOMBIA 1, CICA 9  | 1998            | ?                    | >3                  |

## Discussion

- Avirulence gene frequency studies using rice isogenic lines with known resistance genes are very useful for the identification of appropriate combinations of blast resistance genes.
- The blast pathogen in Colombia exhibits virulence to most known resistance genes. Therefore, durable resistance should be developed through the combination of resistance genes.
- The combination of the resistance genes Pi-1, Pi-2, and Pi-33 confer resistance to blast populations from Colombia. Microsatellite markers and greenhouse inoculations are very useful for the introgression of these resistance genes in breeding populations.

**Table 2. Avirulence Genes Present in Colombian Populations of *Pyricularia Grisea***

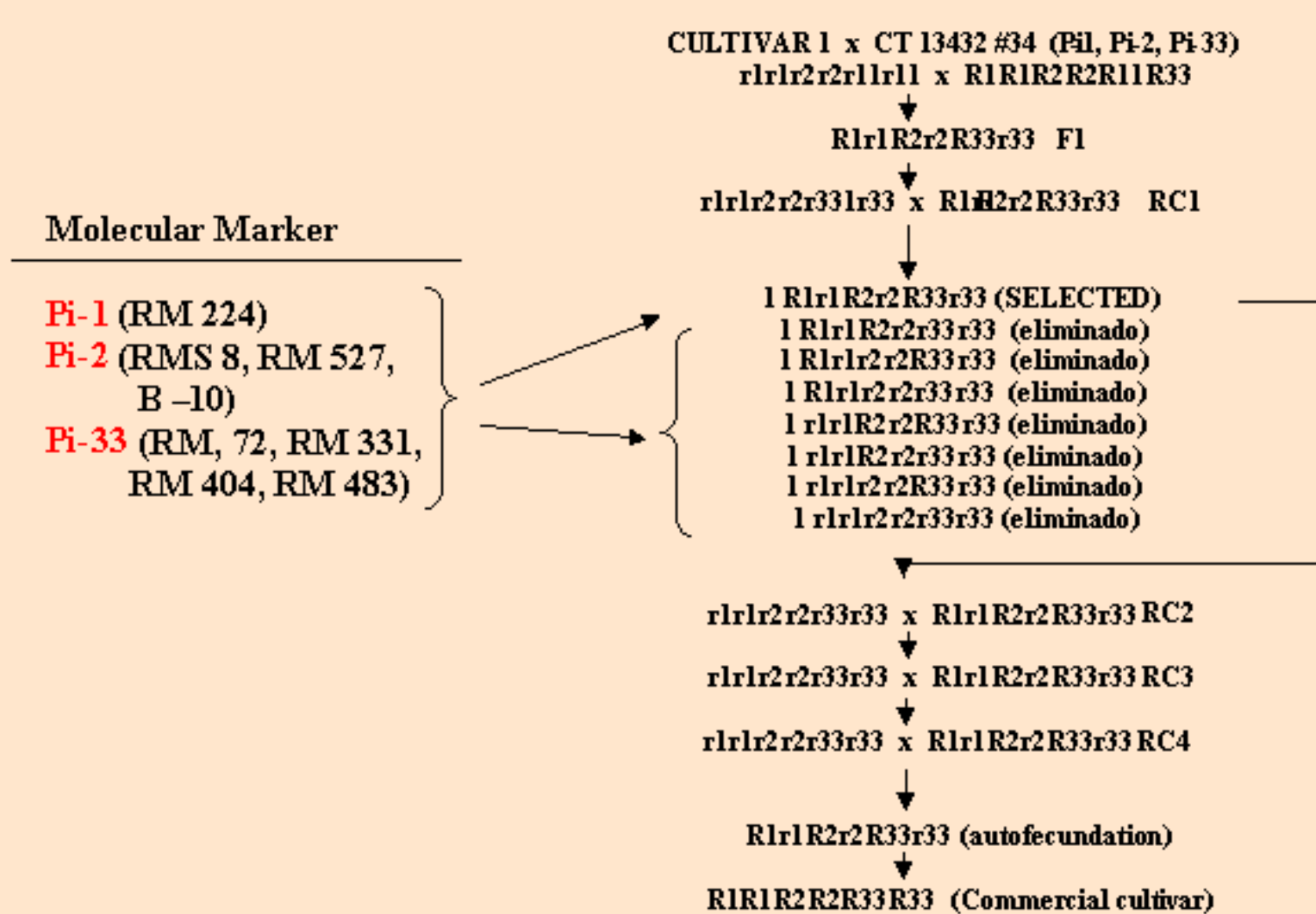
| Cultivar       | Resistance Gene    | Isolate / Genetic Lineage (SRL) |   |   |   |   |   |   |   |   |
|----------------|--------------------|---------------------------------|---|---|---|---|---|---|---|---|
|                |                    | 1                               | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| CT 13432-68    | Pi-1               | +                               | + | R | R | R | R | R | R | + |
| CT 13432-54    | Pi-2               | +                               | + | + | + | + | + | + | + | R |
| CT 13432-55    | Pi-33              | R                               | R | R | + | + | + | + | + | + |
| C104 PKT       | Pi-3               | +                               | + | + | + | + | + | + | + | + |
| C 101 PKT      | Pi-4a              | +                               | + | + | + | + | + | + | + | + |
| C 105 TTP4-L23 | Pi-4b              | +                               | + | + | + | + | + | + | + | + |
| F 124-1        | Pi-ta              | +                               | + | + | + | + | + | + | + | + |
| F 128-1        | Pi-ta <sup>2</sup> | +                               | + | R | + | + | R | R | + | + |
| F 80-1         | Pi-k               | +                               | + | R | + | + | + | + | + | + |
| F 98-7         | Pi-k <sup>h</sup>  | +                               | + | + | + | + | + | + | + | + |
| F 129-1        | Pi-k <sup>h</sup>  | +                               | + | R | + | + | + | + | + | + |
| F 145-2        | Pi-b               | +                               | + | R | + | R | + | + | + | + |
| AISHI ASAHI    | Pi-a               | +                               | + | R | + | + | + | + | + | + |
| K3             | Pi-k <sup>h</sup>  | +                               | + | R | R | R | R | R | R | + |
| K 59           | Pi-t               | +                               | + | + | + | + | + | + | + | + |
| RICO 1         | Pi-k <sup>h</sup>  | +                               | + | + | + | + | + | + | + | + |
| NIPPONBARE     | Pi-sh              | +                               | + | R | + | + | + | + | + | R |
| NATO           | Pi-i               | +                               | + | + | + | + | + | + | + | + |
| OU 244         | Pi-z               | R                               | R | R | + | + | + | + | R | R |
| TORIDE         | Pi-z <sup>2</sup>  | +                               | R | R | + | + | + | + | + | R |
| CT 13432-34    | Pi-1, Pi-2, Pi-33  | R                               | R | R | R | R | R | R | R | R |



**Figure 2. Complete Blast Resistance of Near Isogenic Line CT 13432-34 Conferred by the Combination of Resistance Genes Pi-1+Pi-2+Pi-33.**

**Table 3. Possible Resistance Genes Present in Colombian Commercial Rice Cultivars**

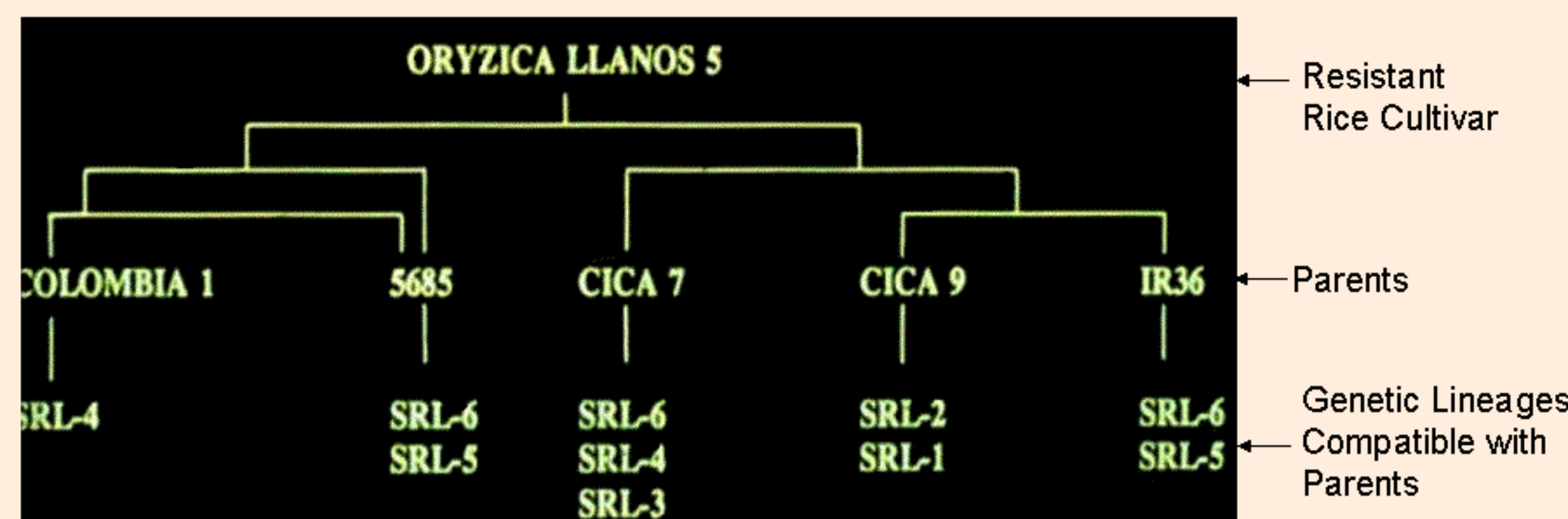
| Cultivar         | Resistance Gene |      |       |      |                   |                    |       |                   |      |      |
|------------------|-----------------|------|-------|------|-------------------|--------------------|-------|-------------------|------|------|
|                  | Pi-1            | Pi-2 | Pi-33 | Pi-z | Pi-z <sup>2</sup> | Pi-ta <sup>2</sup> | Pi-sh | Pi-k <sup>h</sup> | Pi-k | Pi-b |
| Oryzica 2        | X               | X    |       |      |                   | X                  | X     | X                 | X    | X    |
| Oryzica 3        |                 |      |       |      |                   | X                  |       | X                 |      |      |
| Cica 8           | X               |      |       |      |                   | X                  |       |                   | X    | X    |
| Cica 9           |                 | X    |       |      |                   | X                  |       |                   |      |      |
| IR 22            |                 |      |       |      |                   | X                  | X     |                   | X    |      |
| Linea 2          |                 | X    |       |      |                   |                    |       |                   |      |      |
| Oryzica Llanos 4 |                 | X    |       |      |                   |                    | X     |                   | X    |      |
| Oryzica Caribe 8 |                 | X    |       |      |                   |                    | X     |                   | X    |      |
| Oryzica Yacu 9   |                 | X    |       |      |                   |                    |       |                   |      |      |
| Oryzica Llanos 5 | X               | X    | X     | X    | X                 | X                  | X     |                   | X    | X    |
| Fedearroz 50     | X               | X    | X     | X    | X                 | X                  | X     |                   | X    | X    |



**Figure 3. Backcross procedure for the introgression of the resistance genes Pi-1, Pi-2, Pi-33**

## References

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**Figure 1. Durable Blast Resistant Cultivar Oryzica Llanos 5 and Lineage Susceptibility of its Parents**