Field Resistance Expressed when the Pi-ta Gene is Compromised by Magnaporthe oryzae

Fleet N. Lee, R.D. Cartwright, Yulin Jia and J.C. Correll

Abstract The Pi-ta gene provided 14 years of durable resistance to contemporary field population of Magnaporthe oryzae in southern USA rice production areas before being overcome during 2004 in 'Banks', a Pi-ta-based cultivar, by race IE-1k of the blast pathogen. Previously detected in production fields in 1994, the rarely recovered race IE-1k appeared to be poorly adapted to local conditions. Although stable molecular variations were defined in field isolates from 'Banks', virulence bioassays do not distinguish between isolates from Banks and the type race IE-1k isolate. After 2004, blast epidemics were noted in other 'Banks' production fields but have not been observed in other cultivars containing Pi-ta including 'Drew', 'Ahrent' and 'Cybonnet'. The Pi-ta allele in 'Banks' still confers resistance to all contemporary blast races except IE-1k and was determined to be molecularly identical to the Pi-ta allele in 'Katy'. 'Banks' does not contain the minor blast resistance genes Pi-kh or Pi-ks that are present in 'Drew', 'Ahrent' and 'Cybonnet'. An increase in leaf blast severity observed in moisture-stress tests using Pi-ta based cultivars suggests additional resistance genes, such as the Pi-kh and Pi-ks, enhance Pi-ta gene efficacy against race IE-1k under field conditions. The data suggest that the Pi-ta gene functions as a partial resistance gene in 'Katy', 'Ahrent', 'Cybonnet', 'Drew', 'Banks', 'Tetep' and 'Tadukan' in regard to the broadly virulent blast pathogen races IE-1k and IB-33.

Keywords Oryza sativa · Magnaporthe oryzae · Pi-ta · Pi-ks · Pi-kh · Blast · Field resistance · Partial resistance

1 Introduction

Record of per hectare production of rice, Oryza sativa L. has been achieved due to large-scale grower adoption of high yield potential cultivars and modern production techniques in the southern USA (Wilson and Branson 2005; Wilson and...
While many factors are responsible for the recent record-setting yield trends in southern rice, one very significant component is reliable control of rice blast disease caused by *Magnaporthe oryzae* ([*Magnaporthe grisea* (Herbert) Borr. (anamorph *Pyricularia oryzae* Cav.)]) (C.E. Wilson, personal communication 2008; Lee et al. 2006). Blast disease control strategies in Arkansas and other parts of the southern USA include the use of R genes and flood-irrigation-induced field resistance (Marchetti 1994; Lee 1994; Lee et al. 2006).

Major blast resistance genes, very desirable because of their ease in cultivar development and increased resistance via combination into a single cultivar, often are quickly lost to pathogen adaptation. However, the *Pi-ta* gene crossed into the ‘Katy’ cultivar from ‘Tetep’ (Moldenhauer et al. 1990) provided reliable resistance to the contemporary blast pathogen population for 14 years in Arkansas before being overcome in the new *Pi-ta* cultivar ‘Banks’ (Moldenhauer et al. 2007c) by race IE-1k (Lee et al. 2005b). This apparent failure of the *Pi-ta* gene is considered here.

2 Rice Blast in the USA

2.1 Contemporary *M. oryzae* Population

Marchetti (1994) defines a decade-long pathodeme driven pathogen adaptation when characterizing *M. oryzae* pathotypes from southern USA rice production areas. The *M. oryzae*-host relationship is further defined as being eight distinct MGR586 DNA fingerprint lineages with four distinct lineages (A, B, C, and D) predominating in the contemporary population. Multiple haplotypes occur within lineages but typically only one or two haplotypes prevail due to unknown fitness requirements. These investigations defined four distinct vegetative mating types, US-01, US-02, US-03 and US-04, in the contemporary USA blast pathogen population which has an exceptionally low level of genetic diversity relative to *M. oryzae* worldwide (Xia et al. 1993,2000; Correll et al. 2000a,b). Races IB-49 and IC-17 currently predominate in the southern US production fields with races IB-1, IE-1, IG-1, and IH-1 being recovered less frequently. Race IE-1k, rarely detected before 2004, has increased in ‘Banks’.

2.2 Partial Blast Resistance

Arkansas breeders utilize partial blast resistance (PBR) in high-yield rice cultivars by retaining and evaluating blast susceptible cultivars. PBR is evaluated using a combination of leaf lesion characteristics (Marchetti 1994) and panicle blast severity in drought stressed upland field nurseries (Bonman 1992; Lee et al. 2006). Following intense performance testing in nurseries and grower field plots, entries with higher yield and desirable agronomic characteristics are released as susceptible cultivars with the understanding that significant yield loss can occur if not properly managed with respect to blast disease.
2.3 Root Zone Induced Field Resistance

High PBR alone does not provide sufficient blast control. Long term PBR efficacy is determined by multiple environmental conditions, particularly those impacting the root zone (Bonman 1992; Lee et al. 2004, 2005a; Singh et al. 2004a,c). A highly effective and cumulative field resistance can be induced in PBR cultivars and mediated by anaerobic conditions (low dissolved oxygen (DO)) established in the root zone during continuous-flood-irrigation. (Lee et al. 2004; Singh et al. 2004a,c). Root zone DO mediates form and quantity of plant nutrients affecting disease susceptibility, production of ethylene and other hormones associated with disease-resistance, and specific morphological changes which enhance oxygen movement to rice roots and inhibits *M. oryzae* growth across vein tissue (Lee et al. 2004; Singh et al. 2004a,b,c). Arkansas rice growers routinely utilize flood-irrigation-induced field resistance as a primary rice blast control strategy (Lee et al. 2006).

3 *M. oryzae* Adaptation to the Pi-ta Gene

The Pi-ta gene in ‘Katy’ conferred complete resistance to all contemporary *M. oryzae* pathotypes in the US (Moldenhauer et al. 1990; Marchetti 1994; Correll et al. 2000a; Jia et al. 2004). ‘Katy’ was planted to 21 percent of Arkansas production in 1992 (Lee 1994). Higher yielding Pi-ta-based cultivars including ‘Kaybonnet’ (Gravois et al. 1995), Drew (Moldenhauer et al. 1998) and Ahrent (Moldenhauer et al. 2007d) were planted to a maximum of 35% of total state hectarage during 1998 (Lee et al. 2006). Thereafter, Pi-ta-based cultivar planting quickly declined (Wilson and Branson 2003, 2005; Lee et al. 2006) as growers changed to higher-yielding field-resistant cultivars including ‘Wells’ (Moldenhauer et al. 2007a), ‘LaGrue’ (Moldenhauer et al. 1994), and ‘Francis’ (Moldenhauer et al. 2007b).

Virulence adaptation to the Pi-ta gene was anticipated and monitored (Marchetti 1994; Correll et al. 2000a,b). All Pi-ta-based cultivars released until now are susceptible to laboratory isolates, races IB-33 and IE-1k, in greenhouse tests (Table 1). Collecting only rare random wild isolates, scientists concluded race IE-1k was poorly adapted to field conditions (Correll and Lee 2000, 2000a,b; Xia et al. 2000). Thus, the Pi-ta gene provided apparent durable blast resistance to contemporary races until 2004 when a seed production field of the newly released Pi-ta-containing cultivar ‘Banks’ was severely damaged by rice blast (Lee et al. 2005b).

4 Diagnosis of Apparent Pi-ta Failure

Research began immediately to determine why the race IE-1k epidemic occurred in ‘Banks’ during 2004. Investigations centered around a possibilities of a *M. oryzae* virulence adaptation, cultivar predisposal to blast by unusual environmental or cultural conditions, or genetic abnormalities within ‘Banks’.
Table 1 Cultivar blast reaction observed in inoculated greenhouse assays to plants growing upland
and in non-inoculated drought stressed production fields with race IE-1k present

<table>
<thead>
<tr>
<th>Cultivar Tested</th>
<th>Resistance genes&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Drought stressed upland field rating&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Cultivar susceptibility to &lt;i&gt;M. oryzae&lt;/i&gt; in inoculated greenhouse assays&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
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<tr>
<td>Katy</td>
<td>Pi-ta/ Pi-ta&lt;sup&gt;b&lt;/sup&gt;</td>
<td>R</td>
<td>Race IB-49 Race IS-17 Race IE-1k Isolates from Banks Race IB-33</td>
</tr>
<tr>
<td>Drew</td>
<td>Pi-ta/ Pi-ta&lt;sup&gt;b&lt;/sup&gt;</td>
<td>R</td>
<td>R</td>
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<tr>
<td>Ahrent</td>
<td>Pi-ta/ Pi-ta&lt;sup&gt;b&lt;/sup&gt;</td>
<td>R</td>
<td>R</td>
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<tr>
<td>Cybonnet</td>
<td>Pi-ta/ Pi-ta&lt;sup&gt;b&lt;/sup&gt;</td>
<td>R</td>
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<tr>
<td>Banks</td>
<td>Pi-ta/ Pi-ta&lt;sup&gt;b&lt;/sup&gt;</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Wells</td>
<td>Pi-ta/ Pi-ta&lt;sup&gt;b&lt;/sup&gt;</td>
<td>R</td>
<td>R</td>
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<tr>
<td>Cypress</td>
<td>Pi-ta/ Pi-ta&lt;sup&gt;b&lt;/sup&gt;</td>
<td>R</td>
<td>R</td>
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<tr>
<td>Francis</td>
<td>Pi-ta/ Pi-ta&lt;sup&gt;b&lt;/sup&gt;</td>
<td>R</td>
<td>R</td>
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<tr>
<td>LaGrue</td>
<td>Pi-ta/ Pi-ta&lt;sup&gt;b&lt;/sup&gt;</td>
<td>R</td>
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<sup>a</sup> Resistance gene presence as indicated by SSR marker analysis. NP = not present. Francis’ is known to contain the Pi-t gene. Cultivars test negative for the Pi-b and Pi-z genes.

<sup>b</sup> Cultivar blast susceptibility rating in moisture stressed production fields with race IE-1k present. VS = very susceptible, S = susceptible, MS = moderately susceptible and R = Resistant.

<sup>c</sup> Cultivar blast susceptibility rating for inoculated greenhouse plants grown under upland conditions. S = susceptible and R = Resistant.

4.1 Virulence Tests

Field isolates collected from diseased ‘Banks’ plants during 2004 were identified as being race IE-1k in DNA lineage group B, and Vegetative Compatibility Grouping US-02, negative for AVR-Pi-ta gene of the fungus and were compared with known race IE-1k isolates in standard greenhouse bioassays (Lee et al. 2005h). Isolates from ‘Banks’ samples caused susceptible type leaf lesions on Pi-ta-containing cultivars ‘Banks’, ‘Cybonnet’ (Gibbons et al. 2006), ‘Drew’, and ‘Katy’ and compared with those caused by type race IE-1k (Table 1). Differential cultivars were not found to distinguish between field isolates from ‘Banks’ and previously known isolates of IE-1k.

4.2 Environment

Field environmental conditions during 2004 were highly conducive for rice blast in seed fields and test plots of ‘Banks’. However, severe blast epidemics occurred in ‘Banks’ production fields during 2005–2007 when environmental conditions were much less conducive for the blast disease. In contrast, blast did not occur in fields of contemporary Pi-ta-based cultivars ‘Drew’, ‘Cybonnet’ or ‘Ahrent’ during 2004–2007.
4.3 Cultivar Predisposal and Field Resistance

Drought stress associated with sandy soils and poor flood-irrigation management were identified as primary predisposing conditions for blast in ‘Banks’. Samples of conducive soil types were collected for short-term greenhouse tests (Lee et al. 2007). An increased leaf blast severity in drought-stressed plants inoculated with isolate IE-1k occurred with all soil samples but the increase was not specific for soil samples collected from fields where ‘Banks’ blast epidemics had occurred (Fig. 1). In general, leaf blast was more severe in ‘Banks’ and compared with that observed in ‘Wells’. The flood-irrigation-response for test cultivars growing in the soil samples compared with that of ‘LaGrue’, and ‘Wells’ and Pi-ta-based ‘Katy’, ‘Kaybonnet’, ‘Drew’ and ‘Ahrent’ inoculated with race IE-1k (Lee et al. 2005a, 2007) and with that of Pi-ta-based cultivars ‘Tetep’ and ‘Tadukan’ (Jia et al. 2003) when inoculated with races IE-1k or IB-33 (Lee et al. 2005a).

4.4 Molecular Analysis

Zhou et al. (2007) defined a transposition mutation specific for isolate B2 from ‘Banks’ as not being clonal to additional Pi-ta-virulent wild type isolates from ‘Banks’ and concluded multiple molecular mechanisms may be operating to defeat the Pi-ta gene. The Pi-ta allele in Banks was determined to be molecularly identical to the Pi-ta allele in ‘Katy’ (Jia et al. 2007). DNA marker data indicated ‘Banks’ does not contain the blast resistance Pi-ks gene common to ‘Katy’, ‘Drew’ and ‘Ahrent’ or the Pi-ki gene contained in ‘Cybonnet’ (Fjellstrom et al. 2004; R.G. Fjellstrom, personal communication 2008; V.A. Boyett, personal communication 2008).

5 Discussion

Pi-ta-based cultivars provided immunity to rice blast in the USA for 14 years before blast epidemics occurred in ‘Banks’. This event initially suggested M. oryzae adaptation had defeated the Pi-ta gene. However, virulence specific isolates defining a new race shift were not recovered from field samples of ‘Banks’. Differential molecular variation observed with ‘Banks’ field isolate B2 was not associated with recognizable changes in isolate virulence. In the absence of differential cultivars to define a new virulence adaptation and the absence of field epidemics in contemporary Pi-ta-based cultivars ‘Ahrent’, ‘Cybonnet’ and ‘Drew’, there is little physical evidence for a M. oryzae race shift beyond that originally described over a decade ago for race IE-1k.

Although the Banks Pi-ta gene is molecularly identical to that in ‘Katy’ (Jia et al. 2007), the Pi-ta donor is less clear for ‘Banks’ which was developed through a
Fig. 1 Leaf blast severity observed in race IE-1k inoculated greenhouse tests to evaluate the impact of soil sample on rice blast severity. Soil samples were: an unknown sandy-loam (UA-PTES Sand-loam) from the University of Arkansas Pine Tree Experiment Station blast nursery (UA-PTES) near Colt, AR, USA; a De Witt silt-loam (UA-RREC Silt-loam) from the University of Arkansas Rice Research and Extension Center (UA-RREC), Stuttgart Arkansas, USA; a Bosket FSL soil (B-FSL 2005) from a 2005 Banks blast epidemic site; an unknown silt-loam (UA-PTES Silt-loam) from UA-PTES; a Bosket FSL soil (B-FSL 2004) from a 2004 Banks blast epidemic site. Cultivars growing in either upland-drought-stressed or continuous-flood-irrigation cultural treatments were: (A) Wells (B) Banks, (C) Cybonnet, (D) Ahrent, (E) Drew and (F) All cultivar summary.
backcross program which primarily retains characteristics of the recurrent parent, in this case ‘LaGrue’ (Moldenhauer et al. 2007c). The primary selection in the backcross was for race IE-1k blast resistance which has a potential for eliminating lesser resistance genes such as Pi-ks, Pi-kh, Ptr (t) gene and QTL’s (K.A.K. Moldenhauer, personal communication 2008; Jia and Martin 2008). Consequently, blast resistance genes Pi-kh or Pi-ks (Table 1) and possibly other resistance genes are not present in Banks. ‘Banks’ blast susceptibility in their absence suggests these genes served to sustain the Pi-ta gene in field situations by masking the inherent race IE-1k vulnerability in ‘Katy’, ‘Kaybonnet’, ‘Drew’, Ahrent’ and ‘Cybonnet’.

The specific role of Pi-ks or Pi-kh genes is unclear. The Pi-ks gene in Katy evidently provides resistance to race IB54 (Jia and Martin 2008). The Pi-kh gene is identified as a durable blast resistance gene (Sharma et al. 2005). ‘Wells’, which contains the Pi-kh gene but not the Pi-ta gene, rates susceptible or very susceptible to race IE-1k and other common blast races in inoculated greenhouse and drought-stressed field tests (Moldenhauer et al. 2007a; Lee et al. 2006). When properly managed to induce cumulative continuous-flood-irrigation resistance, however, ‘Wells’ exhibits field resistance equivalent to the Pi-ta cultivars and has been planted to over 30 percent of Arkansas rice production fields since 2001 (Lee et al. 2006).

The loss of flood-induced-field resistance inciting blast epidemics with drought stressed ‘Banks’ and the inherent vulnerability in greenhouse tests to race IE-1k strongly suggest that, relative to races IE-1k and IB-33, the Pi-ta gene in cultivars ‘Katy’, ‘Ahrent’, ‘Cybonnet’, ‘Drew’, ‘Banks’, and ‘Tetep’ function as a PBR gene which is being complimented by additional minor genes. Regardless, it is important to emphasize the Pi-ta gene derived from ‘Tetep’ as originally deployed in 1990, in conjunction with minor resistance genes, has served as a valuable source of practical immunity against the rice blast disease without being compromised during highly conducive field environments.

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**References**


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