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The *Rxo1*/*Rba1* locus of maize controls resistance reactions to pathogenic and non-host bacteria

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Abstract Infiltration of different maize lines with a variety of bacterial pathogens of maize, rice and sorghum identified qualitative differences in resistant reactions. Isolates from two bacterial species induced rapid hypersensitive reactions (HR) in some maize lines, but not others. All isolates of the non-host pathogen *Xanthomonas oryzae* pv. *oryzicola* (bacterial leaf streak disease of rice) and some isolates of the pathogenic bacterium *Burkholderia andropogonis* induced HR when infiltrated into maize line B73, but not Mo17. Genetic control of the HR to both bacteria segregated as a single dominant gene. Surprisingly, both phenotypes mapped to the same locus, indicating they are either tightly linked or controlled by the same gene. The locus maps on the short arm of maize chromosome six near several other disease-resistance genes. Results indicate the same type of genes may contribute to both non-host resistance and resistance to pathogens.

Introduction

Resistance genes are widely used in plant disease management. In traditional crop-breeding programs, resistance genes are identified by screening numerous accessions of the crop species or closely related species. Host resistance genes (R genes) that control complete or partial resistance to specific races or isolates of phytopathogens are transferred to elite plant cultivars through classic breeding approaches. Most identified R genes appear to encode receptor components that recognize the presence of specific avirulence genes (*avr* gene) carried by the phytopathogens (Baker et al. 1997). The specific recognition between the R and *avr* genes, as predicted by a gene-for-gene model (Flor 1971), triggers a series of defense responses that are typically associated with a hypersensitive reaction (HR). Disease susceptibility results when the plant does not carry a R gene that can recognize an *avr* gene from the pathogen. Numerous plant R genes and pathogen *avr* genes have been isolated and characterized (Leach and White 1997; Laugé and DeWit 1999; Hulbert et al. 2001). Recent evidence indicates that at least some of the *avr* gene products are important pathogenicity factors (White et al. 2000).

Resistance genes available in any one crop species are limited, since they are often effective against only specific strains of pathogens and tend to lose their effectiveness, due to shifts in the pathogen population (McDonald and Linde 2002). One strategy to identify additional sources of resistance is screening non-host resistance resources, based on the observation that most plants are resistant to most phytopathogens (Dangl and Jones 2001), including some pathogens of closely related plant species. Non-host resistance is typically broad-spectrum, since entire plant species are commonly resistant to all races or isolates of a non-host parasite or pathogen (Heath 1991). Non-host resistance is also durable, since reports of pathogens acquiring new hosts are rare (Kamoun 2001; Thordal-Christensen 2003). Non-host resistance, especially if simply inherited, could be extremely useful if it could be transferred between species by either interspecific crossing or recombinant DNA techniques. It is not clear whether non-host resistance is controlled by the same types of gene that condition gene-for-gene resistance. Some, but not all, forms of non-host resistance are associated with a HR, which is also the hallmark of most incompatible interactions initiated by typical host resistance genes (Hammond-Kosack and Jones 1997; Heath 2000; Kamoun 2001). One indicator that non-host resistance may be controlled by typical R genes is the observation that plants carry genes that interact with *avr* genes from bacterial pathogens of other species (Whalen et al. 1988, 1991; Keen et al. 1991; Dangl et al. 1992; Fillingham et al. 1992; Swarup et al.

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1992; Innes et al. 1993; Wood et al. 1994). Genetic differences between fungal pathovars that control host range may also be inherited as one or a few genes (Matsumura and Tosa 2000; Murakami et al. 2003). This is expected if they interact with one or a few host genes in a gene-for-gene interaction. Similarly, *Phytophthora infestans* strains engineered to not express the elicitin INF1 can cause disease lesions on *Nicotiana benthamiana* (Kamoun et al. 1998), indicating that the resistance in this tobacco species is mediated by recognition of this peptide. A gene-for-gene interaction was demonstrated to account for at least part of the resistance of wheat (*Triticum aestivum*) to an oat (*Avena sativa*) pathovar of *Magnaporthe grisea* (Takabayashi et al. 2002).

Rice bacterial leaf streak (BLS), caused by *Xanthomonas oryzae* pv. *oryzicola* is emerging as a serious disease in areas of Asia where hybrid rice is widely grown. No major resistance genes for BLS have been identified from rice germplasm (Zhang Qi, personal communication). *Burkholderia andropogonis* (formerly *Pseudomonas andropogonis*) and *X. campestris* pv. *holcicola* are two major bacterial diseases on sorghum (Claflin et al. 1992; Muriithi and Claflin 1997; Claflin 2000). Some strains of *B. andropogonis* also infect sugar cane, sweet corn and a few dent corn lines (Ullstrup 1960; Vidaver and Carlson 1978). *Sorghum* species are the only known host of *X. c.* pv. *holcicola* (Claflin 2000). In this study, maize germplasm was examined for the ability to mount defense reactions against these bacterial pathogens. We report the identification and characterization of a locus in maize (*Rxo1*/*Rba1*) that controls resistance reactions to *X. o.* pv. *oryzicola* and *B. andropogonis*.

Materials and methods

Plant materials

Fifteen maize inbred lines, including A188, A619, B73, CM37, FRN28, H95, H99, Mo17, Mo20W, N6, OH28, Pa405, T232, W23 and *Wsm1*-Oh28, were evaluated for their reactions to the bacterial pathogens. Oh28 and *Wsm1*-Oh28 are near-isogenic lines which differ in that the *Wsm1* wheat streak virus resistance gene is present in the Oh28 genetic background (courtesy Mark Jones and Ray Louie, USDA ARS, Wooster, Ohio). Maize seed were sown in flats in a 10-cm soil:peat:perlite mix (2:1:1). Seedlings were grown either in growth chambers with 12 h light at 30 °C and 12 h dark at 25 °C or in greenhouses. Two-week-old seedlings with two or three fully expanded leaves were used for infiltration. Recombinant inbred (RI) lines derived from a cross between B73 and Mo17 were used for mapping two genes controlling resistance reactions against *X. o.* pv. *oryzicola* and *B. andropogonis*. Other mapping populations used included an F_2 population made from a B73 × Mo17 cross and a set of intercross RI lines derived from a B73 × Mo17 cross that included four generations of random mating before inbreeding the individual lines (Lee et al. 2003; Sharopova et al. 2003).

Bacterial strains and culture media

Several isolates each of *X. o.* pv. *oryzicola*, *X. o.* pv. *oryzae*, *X. c.* pv. *holcicola* and *B. andropogonis* were inoculated into maize lines to determine whether they induced a defense reaction. Xanthomomads were grown in either peptone/sucrose broth (PSA; Tsuchiya et al. 1982) or nutrient broth at 28–30 °C. For growth on solid medium, 1.5% agar was added. Strains of *B. andropogonis* were grown overnight in nutrient broth at 28–30 °C [ca. 1×10^9 colony-forming units (cfu)/ml]. Bacterial cultures were centrifuged (2,700 *g* for 6 min) and the pellets resuspended in distilled water. The cell density was then adjusted to 30–50 Klett units (Summerson photoelectric colorimeter, New York, N.Y.), which is equivalent to approximately 5×10^7 cfu/ml.

The second and third fully expanded leaves of greenhouse-grown maize seedlings were inoculated by infiltration of bacterial suspensions, using a needle-less syringe (Reimers and Leach 1991). Plants were examined for induction of HR at 2 days and 3 days after infiltration. For bacterial growth curves, the same infiltration procedure was used but the bacterial suspensions were diluted to 10^5 cfu/ml. Leaf disks from the infiltrated leaves were collected at 0, 1, 2, 3 days after infiltration, using a cork borer (0.5 cm diam.). Six leaf disks from three different infiltrated seedlings were pooled together as one sample, with three samples taken for each plant genotype at each time-point. The pooled leaf disks from each sample were ground with sand in 1 ml of ddH_2O and the supernatant was serial-diluted and plated on PSA plates containing cycloheximide (75 μg/ml). Colonies were counted after the plates were incubated at 28 °C for 3 days. Averaged bacterial colony numbers were transformed to $\log_{10}$ cfu/cm^2 and plotted using MS-Excell.

PCR and gel blot analysis

PCR amplification for the microsatellite markers was performed essentially as described by Xu et al. (1999). DNA amplifications were performed in a standard reaction mix containing 100 ng of genomic DNA, 10 mM Tris-HCl (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$, 0.3 mM dNTPs, 0.5 pmol of each primer and 0.5 units of *Taq* DNA polymerase (Amersham Pharmacia, Freiburg, Germany). After an initial denaturation step at 94 °C for 2 min, the template DNA was amplified using 35 cycles with the following conditions: 1 min at 94 °C, 2 min at 55 °C and 2 min at 72 °C. The final extension step was conducted at 72 °C for 2 min. PCR products were resolved on 3% metaphor (FMC, Rockland, Me.) agarose gels (1× Tris-borate EDTA buffer), stained with ethidium bromide and visualized under UV light.

Total RNA was isolated from freshly harvested leaf tissue using Trizol reagent (Life Technologies, Rockville, Md.), following the manufacturer's recommendations. A maize DNA fragment of a PR5-coding gene (Morris et al. 1998) was labeled with [α-^{32}P] dATP by random priming (Feinberg and Vogelstein 1983). RNA gel blotting procedures and Northern analyses were performed as described by Webb et al. (2002). Northern hybridizations were performed at 42 °C and washed at 55 °C with 0.5× SSC, 0.1% SDS.

Results

Maize lines respond differentially to some non-host bacterial pathogens

A collection of maize lines was tested with several isolates of *X. o.* pv. *oryzicola*, *X. o.* pv. *oryzae*, *X. c.* pv. *holcicola* and *B. andropogonis* that were isolated mainly from other cereal species. Suspensions of bacteria were infiltrated into seedling leaves and then observed for disease symptoms, HR or other visible responses. Four strains of *X. o.* pv. *oryzae*, representing four different Philippine races, showed no visible reaction on any tested maize line (Table 1). Three *X. c.* pv. *holcicola* strains caused small water-soaked lesions on most maize lines, suggesting that

Table 1 Reactions of different maize lines to bacterial pathogens. *Host-plant species* Plant species from which the strain was originally cultured. Reaction of maize lines: +++ very strong hypersensitive response (HR), ++ moderate HR and necrosis, + weak HR and necrosis, *Ws* water-soaked lesion, – no necrosis or water-soaking, *P* both necrosis and water-soaking occur, but it is not clear whether HR occurs

Bacterial strain	Maize inbred lines															
	B73	Mo17	A188	A619	CM37	FRN28	H95	H99	Mo20W	N6	OH28	Pa405	T232	W23	Wsm1Oh28	Host-plant species
Xanthomonas oryzae pv. *oryzicola*																
BLS101	+++	–	+++	+++	+++	+++	–	+++	+++	+++	+++	–	+	+	–	Rice
BLS179	+++	–	+++	+++	++	+++	–	+++	++	+++	+++	–	+	++	–	Rice
BLS222	+++	–	+++	+++	+++	+++	–	+++	+++	+++	+++	–	++	+++	–	Rice
BLS256	+++	–	+++	+++	+++	+++	–	+++	+++	+++	+++	–	++	+++	–	Rice
BLS295	+++	–	+++	+++	+++	+++	–	+++	+++	+++	+++	–	++	++	–	Rice
BLS303	+++	–	+++	+++	+++	+++	–	+++	+++	+++	+++	–	+++	+++	–	Rice
Xoo44	+++	–	+++	+++	+++	+	–	+++	++	+++	+++	–	+	+	–	Rice
XooB-911	+++	–	+++	+++	+++	+++	–	+++	+++	+++	+++	–	++	++	–	Rice
X. campestris pv. *holcicola*																
Xch86	–	–	Ws	Ws	Ws	–	Ws	Ws	Ws	P	Ws	++	Ws	Ws	Ws	Sorghum
Xch93	Ws	P	Ws	Ws	P	–	Ws	Ws	–	++	Ws	++	Ws	Ws	Ws	Sorghum
Xch112	Ws	P	Ws	Ws	P	–	Ws	Ws	–	P	+++	Ws	Ws	Ws	Ws	Sorghum
X. o. pv. *oryzae*																
PXO61	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Rice
PXO79	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Rice
PXO86	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Rice
PXO99	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Rice
Burkholderia andropogonis																
Pa3342	+++	–	Ws		+++		–	+++	–	–	–	–	–	–	–	Stizolobium
Pa3390	P	Ws	Ws	P	P	P	Ws	Ws	P	P	P	P	P	P	P	Sorghum
Pa3394	+++	–	+++	++	+++	–	–	+++	++	–	+++		+	+		Sorghum
Pa3397	P	+	Ws	–	–	–	–	P	–	–	–				–	Sorghum
Pa3459	+++	–	+++	P	–	P	–	P	P	P	P	P	P	P	P	Sorghum
Pa3544	++	–	++	+	+++	–	–	+++	+++	+++	++	–	++		–	Sorghum
Pa3549	+++	–	++	+++	+++	++	–	+++	+++	+++	+++	–	+	+++	Ws	Sugarcane
Pa3558	P	Ws	P	–	–		Ws	–	–	–	–	–	P	P	–	Sorghum
Pa3317	+++	P	–		+++		–	–								/
Pa3319	+++	–	+++		+		–	+++								/
Pa3395	+++	–	+		–		–	+++								Sorghum
Pa3399	P	Ws	Ws		–		P	P								Sorghum
Pa3561	+++	–	+++		–		–	+++								/
Pa3562	+++	Ws	+++		–		–	+++								Sorghum
Pa3564	+++	Ws	+		–		–	+++								Sorghum
Pa3585	+++	Ws	+++		–		Ws	+++								Statice

these sorghum isolates can cause limited disease symptoms on maize when infiltrated at a high concentration. Each of the three strains also elicited a HR on at least one of the maize lines; and the maize lines were able to differentiate the three *X. c.* pv. *holcicola* strains. Some of the reactions were difficult to interpret, however, showing both water soaking and necrosis. Sixteen *B. andropoganis* strains (collected from sorghum, sugarcane, *Stizolobium*, and statice) induced varied reactions on the maize lines. As with the *X. c.* pv. *holcicola* strains, most were capable of causing some water-soaked disease symptoms when infiltrated with 5×10^7 cfu/ml bacterial suspensions and most strains could be differentiated by their reactions on the different maize lines. Eight strains of *X. o.* pv. *oryzicola* incited a strong HR on most maize inbred lines. Only four maize lines showed no reactions to these strains. The differences between the maize lines in their reactions to the *X. o.* pv. *oryzicola* strains appeared more qualitative than the differences between the maize lines in their reactions to the other bacteria. The maize lines either responded to the *X. o.* pv. *oryzicola* strains with a rapid HR that was usually visible 24 h after infiltration, or no reaction was observed. No differences were observed among the *X. o.* pv. *oryzae* strains: all showed the same reactions on different maize lines.

To obtain a preliminary view of the inheritance of the reactions to the four bacterial pathogens, 15 RI lines derived from a Mo17 × B73 cross were also screened with the same collection of bacterial strains. Some bacterial strains showed clear differential reactions on the 15 RI lines, while others gave either no noticeable differences (e.g. *X. o.* pv. *oryzae* isolates), or less discrete differences (Table 2). Reactions to the eight *X. o.* pv. *oryzicola* strains clearly segregated in a qualitative fashion, with five of the

Table 2 Reactions of RILs of Mo17 × B73 maize lines to some bacterial pathogens. All codes for bacterial strains and reactions of maize lines are the same as in Table 1

Bacterial strain	Maize inbred lines														
	L1	L12	L14	L30	L32	L36	L41	L43	L49	L56	L57	L60	L64	L66	L87
X. o. pv. *oryzicola*															
BLS101	–[b]	++	–	+++	–	–	–	–	–	–	–	+++	+++	–	+++
BLS179	–	++	–	+++	–	–	–	–	–	–	–	+++	+++	–	+++
BLS222	–	++	–	+++	–	–	–	–	–	–	–	+++	+++	–	+++
BLS256	–	++	–	+++	–	–	–	–	–	–	–	+++	+++	–	+++
BLS295	–	+++	–	+++	–	–	–	–	–	–	–	+++	+++	–	+++
BLS303	–	+++	–	+++	–	–	–	–	–	–	–	+++	+++	–	+++
Xoo44	–	+++	–	+++	–	–	–	–	–	–	–	+++	+++	–	+++
XooB-911	–	+++	–	+++	–	–	–	–	–	–	–	+++	+++	–	+++
X. c. pv. *holcicola*															
Xch86	–	–	–	–	Ws	–	–	–	–	–	–	–	–	–	–
Xch93	Ws	–	Ws	–	Ws	–	–	–	–	–	–	–	–	–	–
Xch112	Ws	–	Ws	–	Ws	–	–	–	Ws	–	–	Ws	–	–	Ws
X. o. pv. *oryzae*															
PXO61	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PXO79	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PXO86	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PXO99	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B. andropogonis															
Pa3342	–	–	P	–	–	–	–	–	–	–	–	–	–	–	–
Pa3390	P	P	P	–	P	–	–	–	P	P	P	P	–	P	P
Pa3394	–	+++	–	+++	–	–	–	–	–	–	–	+++	+++	–	+++
Pa3397	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pa3459	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Pa3544	–	+++	–	+++	–	–	–	–	–	–	–	+++	+++	–	+++
Pa3549	–	+++	–	+++	–	–	–	–	–	–	–	+++	+++	–	+++
Pa3558	P		–	–	–	–	–	–	P	P	–	–	–	P	P
Pa3317	P	++	–	+	P	P	–	–	P	–	–	–	P	P	P
Pa3319	–	+++	–	+++	–	–	–	–	–	–	–	+++	+++	–	+++
Pa3395	–	+++	–	+++	–	–	–	–	–	–	–	+++	+++	–	+++
Pa3399	P	–	–	P	–	–	–	–	P	–	–	–	–	–	–
Pa3561	–	+++	–	+++	–	–	–	–	–	–	–	+++	+++	–	+++
Pa3562	–	+++	–	+++	–	–	–	–	–	–	–	+++	+++	–	+++
Pa3564	–	+++	–	+++	–	–	–	–	–	–	–	+++	+++	–	+++
Pa3585	–	+++	–	+++	–	–	–	–	–	–	–	+++	+++	–	+++

lines showing a strong necrotic reaction and ten of the lines showing no reaction. Surprisingly, nine *B. andropogonis* strains caused identical reactions on the same set of RI lines. That is, the same five maize lines that showed a HR to *X. o.* pv. *oryzicola* also showed a HR to the nine *B. andropogonis* strains, while the other ten maize lines showed no HR to either type of bacterium.

A single locus controls the HR to both *X. o.* pv. *oryzicola* and *B. andropogonis*

To further investigate the inheritance of maize genes controlling reactions to strains of *X. o.* pv. *oryzicola* and *B. andropogonis*, more RI lines (Mo17 × B73) were screened with *X. o.* pv. *oryzicola* strain BLS256 and *B. andropognis* strain Pa3549. Of 150 lines tested with BLS256, 74 showed the strong necrotic reaction of the B73 parent, 75 showed the minimal response of the Mo17 parent and one line segregated for the reaction. When tested with Pa3549, the exact same lines showed the necrotic and minimal reactions (Fig. 1). Taken together, this indicates that the reactions to both pathogens are controlled by single genes and that the two genes are tightly linked. The genes were designated *Rxo1* (reaction to *X. o.* pv. *oryzicola*) and *Rba1* (reaction to *B. andropogonis*).

To further confirm inheritance of *Rxo1*/*Rba1*, two additional populations from Mo17 × B73 crosses were infiltrated with strains BLS256 and Pa3549. An F_2 population of 625 individuals segregated as expected if a single dominant gene controlled the resistance reactions to both bacteria (Table 3). A third population, a series of 219 intercross RI lines, was examined (Lee et al. 2003; Sharopova et al. 2003). This population also segregated as expected if a single gene controlled both resistances (Table 3). The perfect cosegregation of the two genes in 625 F_2 individuals, 150 RI lines and 220 intercross RI lines indicated the two genes are very closely linked, if not identical. The resistant F_2 individuals are not very informative for estimating linkage, but the 150 susceptible individuals and the 150 RI lines are roughly the equivalent of examining 600 meioses for recombination between two closely linked markers (Burr et al. 1988). Furthermore, the intercross RI population exhibits roughly three times the recombination observed with traditional RI lines (Sharopova et al. 2003), so this population should be very efficient at detecting recombinants between linked markers. Therefore, recombinant individuals should be expected unless the two genes are within approximately 0.2 cM.

Fig. 1 Maize lines B73 (*left*) and Mo17 (*right*) infiltrated with a bacterial suspension of *Xanthomonas oryzae* pv. *oryzicola*. Hypersensitive reaction was observed in B73 (*Rxo1*/*Rxo1*) but not in Mo17 (*rxo1*/*rox1*). The picture was taken at 48 h after infiltration

In addition to the *Rxo1* and *Rpa1* genes being tightly linked, they appear to be in linkage disequilibrium in the maize germplasm examined. The *B. andropogonis* isolate Pa3549 induced necrotic reactions on the same maize lines as the *X. o.* pv. *oryzicola* isolates: they induced a HR when inoculated on 11 of the maize lines, but did not induce a HR on the remaining four (Table 1). Examination of an additional 12 maize lines showed the same result (data not shown). Although this is a small sample of the cultivated maize germplasm, it raises the possibility that the two genes are in disequilibrium, supporting the idea that they may actually be the same gene.

The *Rxo1*-mediated HR inhibits the growth of *X. o.* pv. *oryzicola*

X. o. pv. *oryzicola* is not pathogenic on maize. Therefore, it is not clear whether the necrotic reaction mediated by the *Rxo1* gene should be considered a resistant reaction. To shed more light on this interaction, the growth of *X. o.* pv. *oryzicola* populations in *Rxo1* and *rxo1* plants was examined. As shown in Fig. 2, when bacteria were infiltrated at concentrations of 10^5 cfu/ml into B73 leaves, their population decreased rapidly within 24 h and bacteria were barely recoverable at 48 h after inoculation. This is consistent with the observation that, by 24 h, there was noticeable necrosis in the region infiltrated and the entire region was typically totally necrotic by 48 h. Taken together, these results suggest that the response is a defense response associated with a HR (Hammond-Kosack and Jones 1997). Bacteria initially multiplied following infiltration of maize line Mo17, but multiplication ceased 48 h after infiltration and no disease symptoms were observed. At most, a mild chlorosis was observed at the site of inoculation (Fig. 1). This response was expected as these bacteria are not pathogens of maize.

Table 3 *Rxo1*/*Rba1* segregates as a single dominant gene in families derived from B73 × Mo17 crosses

B73 × Mo17 populations	HR+	HR−	HR+/− segregating	Total number of plants	Segregation ratio	Chi square
F_2	475	150	/	625	3:1	0.33
Recombinant inbred lines	74	75	1	150	1:1	0.01
Intercross recombinant inbred lines	113	106	0	219	1:1	0.22

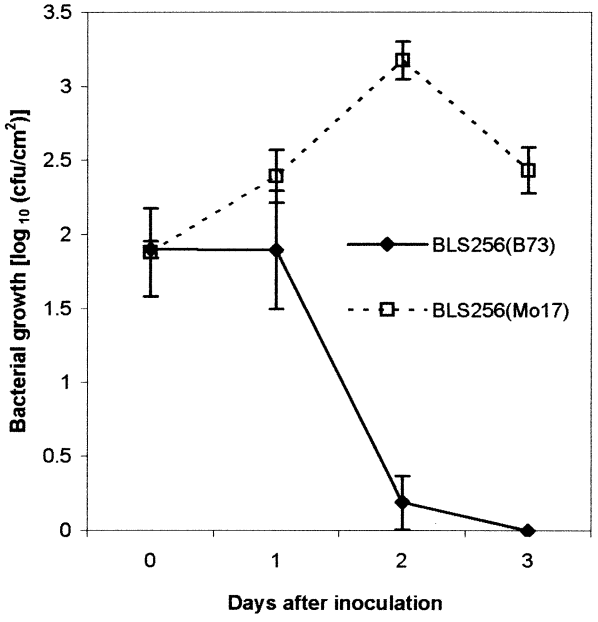

Fig. 2 Multiplication of *X. o.* pv. *oryzicola* (BLS256) in maize lines Mo17 and B73. Bacterial suspensions (10^5 cfu/ml) were infiltrated into seedling leaves and plants incubated at 28 °C. Leaves were sampled at 0, 1, 2 and 3 days after inoculation and tissue extracts plated onto plates of amended peptone/sucrose broth

Active defense in maize is involved in non-host resistance to *X. o.* pv. *oryzicola*

To investigate the defense gene induction in maize lines challenged with *X. o.* pv. *oryzicola*, expression of the maize *PR5* gene, which is commonly induced in defense reactions (Morris et al. 1998), was examined. The PR5 transcript was strongly induced in B73 (*Rxo1*/*Rxo1*) at 12 h after inoculation and was concurrent with the development of the rapid HR (within 24 h) observed in this line (Fig. 3). However, Mo17 (*rxo1*/*rxo1*) also showed increased PR5 transcript by 24 h after inoculation. This indicates that the resistance to *X. o.* pv. *oryzicola* in maize lines that do not carry *Rxo1* also involves an active defense mechanism. PR5 induction appears much stronger at the 12 h time-point in B73 than Mo17; and it is roughly equivalent to the amount of expression at 24 h in Mo17. PR5 expression in B73 was highest at the 12 h time-point and decreased at 24 h. This is probably because the interaction is essentially complete by 24 h in the *Rxo1*-mediated reaction, as most of the affected cells and the bacteria have died.

Response to *B. andropogonis* in the field

Two near-isogenic lines that differ in the presence/absence of the *Wsm1* wheat streak virus resistance gene differed in their reactions to both BLS256 and Pa3549. Oh28 gave necrotic reactions to both isolates, while *Wsm1*-OH28 did not react to BLS256 and inoculation with Pa3549 resulted in lesions that became water-soaked and extended up the leaf (Table 1). The *Rxo1* and *Rba1* genes in Oh28 were therefore probably replaced by their recessive alleles in the introgressed region carrying the *Wsm1* gene. The maize line Pa405, from which the *Wsm1* gene originated, does not exhibit the necrotic HR to either bacterial pathogen (*rxo1*, *rba1*), supporting the idea that *Wsm1* is linked in repulsion to the *Rxo1* and *Rba1* genes in this germplasm.

The Oh28 and *Wsm1*-Oh28 lines also differed in their susceptibility to *B. andropogonis* under natural infection in nurseries at the Rocky Ford Experimental Field (Manhattan, Kan.) in the summers of 1996 and 1997. The *Wsm1*-Oh28 line was unique because natural infection by *B. andropogonis* resulted in a high incidence and severity of disease on this line. Numerous lesions were observed on every plant in this line and these lesions often coalesced on the lower leaves by the time of flowering. Many of these plants failed to produce seed because of the severity of the disease. The Oh28 line, as all other genotypes in the nursery, was not noticeably affected by the pathogen. In 1997, an F_2 family from a *Wms1*-

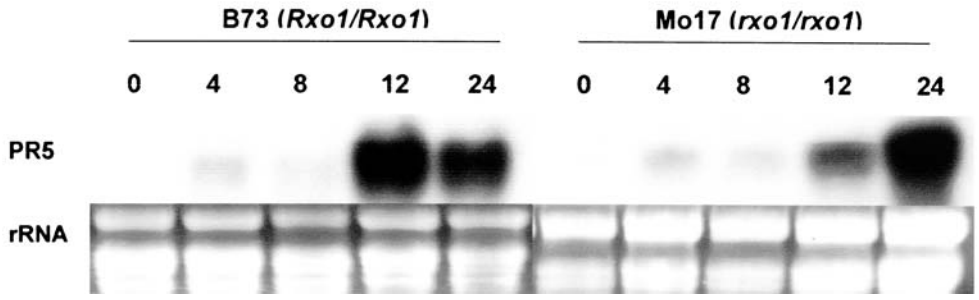

Fig. 3 *PR5* gene expression in maize seedling leaves after infiltration with *X. o.* pv. *oryzicola*. RNA samples were collected from B73 and Mo17 leaves at 0, 4, 8, 12 and 24 h after infiltration with *X. oryzae* pv. *oryzicola* BLS256. RNA blots were probed a maize PR-5 cDNA clone

Oh28 × Oh28 cross was planted in the nursery. Of 240 F_2 plants, 61 became severely infected with *B. andropogonis* and 139 were scored as resistant, showing few if any lesions. The remaining 40 plants were scored as intermediate, showing variable numbers of lesions but being less severely infected than the plants scored as susceptible. When resistant and intermediate classes were pooled into a single class, i.e. all considered resistant, the data fit a 3:1 ratio for resistant:susceptible (179:61; χ^2=0.02, P>0.90). To test the hypothesis that the *Rba1* gene controls this resistance, progeny from a sample of the F_2 family were tested against *B. andropogonis* strain Pa3549 in the greenhouse. Self-fertilized progeny were obtained from 39 of the F_2 plants, including 25 resistant plants, six susceptible plants and eight scored as intermediate. Fifteen seeds from each of the 39 F_3 families were planted and, 2 weeks later, the leaves were inoculated with Pa3549. Families from the six plants scored as susceptible in the field were homozygous for the *rba1* allele, as all seedlings showed susceptible water-soaked reactions. All remaining F_3 families, from resistant or intermediate F_2 plants, either segregated, or all seedlings showed the necrotic resistance reaction. The correlation between the greenhouse seedling and field resistance scores and the apparent linkage to the *Wsm1* gene indicates the *Rba1* gene controls resistance against *B. andropogonis* in the Oh28 background under field conditions.

No other maize lines in the summer nurseries showed noticeable symptoms of infection with *B. andropogonis*. The nurseries included at least two rows of Mo17 and H95 in each of the two summers, neither of which showed symptoms. Neither of these maize lines carries the *Rba1* gene, since they do not exhibit a necrotic HR to infiltration with *B. andropogonis*. These maize lines apparently possess another gene (or genes) that confers resistance to *B. andropogonis* but fails to result in a strong HR following seedling infection. The Oh28 line is devoid of other resistance factor(s).

Mapping the *Rxo1*/*Rba1* locus on the maize chromosome

Analysis of *Rxo1* segregation with the molecular markers already mapped in the B73 × Mo17 RI population indicated the gene mapped approximately one map unit from RFLP marker *umc85*, on the short arm of chromosome six. This agrees with the apparent linkage to the *Wsm1* gene, as indicated by the different resistance of the OH28 and WSM1-OH28 lines. Several other resistance genes map to the genomic area around *umc85* (McMullen and Simcox 1995), including genes for resistance against both viral and fungal diseases. The *rxo1* locus is distinct from most of these other R gene loci, however, since it maps distally (towards the telomere) from the *umc85* marker, while *mdm1* and *rhm* map proximally (McMullen and Louie 1989; Zaitlin et al. 1993; Simcox et al. 1995).

To identify more molecular markers around the *Rxo1*/*Rba1* region, 46 microsatellite (short sequence repeat; SSR) markers from this area of maize chromosome six (bin 6.00–6.01; http://www.maizegdb.org/ssr.php) were examined. Of 46 primer pairs examined, 24 pairs (52%) showed a clear polymorphism between B73 and Mo17 when the PCR products were compared on 3% agarose gels. Segregation analysis of the markers on 149 RI lines revealed markers flanking the locus. The closest marker identified on the centromere-proximal side of *Rxo1*/*Rba1* was the RFLP marker *umc85*. Three lines were derived from recombination events between the resistance locus and *umc85*, corresponding to a map distance of about 1 cM. On the distal side of the region, the closest markers were much farther from the locus. The SSR markers bnlg238 and phi126 identified 19 and 18 recombinants among the RI lines, respectively. These recombination frequencies correspond to distances of 6.9 cM and 7.3 cM. The SSR markers bnlg1538 and bnlg161 mapped closely to these SSRs, but none of the distal markers were within a few map units of the *Rxo1*/*Rba1* locus. The *umc85* RFLP marker was therefore the closest genetic marker to the *Rxo1*/*Rba1* locus.

Discussion

We identified a maize gene, *Rxo1*, which conditions a strong HR to the non-host bacterial pathogen *X. o.* pv. *oryzicola*. The same locus carries a gene (designated *Rba1*) controlling resistance to the maize and sorghum bacterial stripe pathogen *B. andropogonis*. It was surprising that the same locus controlled resistance to two of only four bacterial pathovars tested. This suggests that this locus may condition defense reactions to other bacterial pathogens.

The *Rxo1* locus maps to the same general area of the maize genome as several other resistance genes; for example, it is located within a few map units of genes conferring resistance to fungal and viral pathogens (McMullen and Simcox 1995). No genetic recombinants were identified that separated the resistance reactions to the two bacteria. Thus, the genes controlling the reactions are either tightly linked or identical genes. This raises the interesting possibility that the same gene or gene family confers resistance to both non-host (*X. o.* pv. *oryzicola*) and pathogenic (*B. andropogonis*) bacteria.

Non-host resistance is typically thought to be controlled by multiple genes with general (non-specific) effects on microbial pathogens (Heath 1991, 2000). Such resistance factors may include the production of preformed antimicrobial compounds, physical barriers, the lack of essential metabolites, or signaling compounds required for the pathogenesis. Engineering such resistance in heterologous species may be a complicated endeavor. There is, however, growing evidence that single loci can contribute significant effects towards non-host resistance. Examples include genetic defects in genes controlling the production of antimicrobial compounds (Papadopoulou et al. 1999) and enzymes that can detoxify microbial toxins (Multani et al. 1998). Some genetic defects that affect active defense

processes, like HR, also affect the host's response to non-host pathogens. For example, silencing of the tobacco *SGT1* gene abolished the HR incited by both the host pathogen and some non-host pathogens (Peart et al. 2002). The *SGT1* gene encodes a putative protein associated with the ubiquitin-like ligase, which may have a role in the protein degradation pathway (Austin et al. 2002; Azevedo et al. 2002). Another example is the *NHO1* gene that was identified in a mutagenesis screen in *Arabidopsis*. *NHO1* encodes a putative glycerol kinase (Kang et al. 2003) whose function in resistance is not clear, but lines homozygous for the mutant allele allowed significantly more growth of several non-host bacteria. Like the *SGT1* defect, *NHO1* mutants were also found to interfere with R gene-mediated defense against pathogens (Lu et al. 2001). Alternatively, defects in SNARE proteins affected the frequency with which powdery mildews penetrated cells of non-hosts but were not required for R gene-mediated resistance (Collins et al. 2003).

The *Rxo1* and *Rba1* genes are clearly involved in active defense responses that culminate in a rapid HR. They also control a rapid reduction in the size of infiltrated bacterial populations. Unlike many of the above-mentioned genetic defects demonstrated to affect non-host resistance, they do not appear to affect normal metabolic processes in healthy plants. Thus, with their dominant inheritance, control of HR and lack of phenotype in the absence of a pathogen, *Rba1* and *Rxo1* behave like typical resistance genes. The *Rba1* gene (and possibly genes like it in other species) may restrict the host-range of *B. andropogonis*, a species with a broad host-range. Resistance of maize to *X. o.* pv. *oryzicola* better fits the definition of non-host resistance (Heath 2000), since all accessions that were tested were resistant to all *X. o.* pv. *oryzicola* strains tested.

The *Rxo1* locus is just one component of non-host resistance. *X. o.* pv. *oryzicola* initially multiplied almost ten-fold in *rxo1*/*rxo1* plants after infiltration, but populations decreased 3 days after infiltration. The most severe symptoms observed in this line were a very mild chlorosis, confined to the area of infiltration. This resistance is likely associated with an active defense response, as indicated by the induction of PR5 transcripts. While the maize lines we examined were polymorphic for the *Rxo1*/*Rba1* locus, no obvious polymorphism was observed for the other component(s) of resistance, since all four *rxo1*/*rxo1* lines tested were resistant to *X. o.* pv. *oryzicola*.

It is not too surprising that polymorphism exists for the *Rxo1* gene in cultivated germplasm, since maize is quite polymorphic for disease resistance traits. For example, maize germplasm is polymorphic for resistance to *Cochliobolus carbonum* race 1, where susceptibility is caused by defects in both copies of a duplicated gene (*Hm1*, *Hm2*) coding for enzymes able to detoxify a toxin (HC toxin) produced by this fungus (Johal and Briggs 1992; Multani et al. 1998). The genes are thought to be conserved throughout the grasses, but polymorphic in maize. In the absence of *C. carbonum* race 1, the defective alleles have no apparent phenotype.

Molecular characterization of the maize *Rxo1* and *Rba1* resistance gene(s) will help us understand the mechanism of resistance and their role in non-host resistance. This will shed light on the relationship between host and non-host resistance. This will also be the next step in determining their potential for transfer between cereal species to control diseases in heterologous hosts.

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