

## GENETIC ANALYSIS OF RESISTANCE TO BACTERIAL BLIGHT IN RICE (*Oryza sativa* L.)

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### ABSTRACT

Twenty eight resistant rice cultivars were studied for the inheritance of resistance to bacterial blight using Philippines races of *Xanthomonas oryzae* pv. *oryzae*. Results revealed that twenty six cultivars have two recessive genes for resistance. One of these governs resistance to race 1 (PXO 61) while the other gene confers resistance to race 6 (PXO 99). One cultivar Pankiraj has three recessive genes for resistance, two of that convey resistance to race 1 and while third gene conveys resistance to race 6. Another cultivar Kalonchi has one dominant and one recessive gene for resistance to race 1 and another recessive gene for resistance to Race 6. Allelism test with *Xa4* and *xa5* showed that the dominant and recessive gene of Kalonchi that confer resistance to race 1 are allelic to *Xa4* and *xa5* respectively. Similarly one of the recessive gene of Pankiraj and single recessive genes conferring resistance to race 1 in 22 cultivars are allelic to *xa5*. Whereas the single recessive gene for resistance to race 1 in cultivar Bazail 408, Kaun Kaek, Phcar Tien P65 and Aus 364 are different and segregate independently of *xa5*. Similarly allele test with *xa13* and *xa24* showed that single recessive gene conferring resistance to race 6 in Aus 364 is allelic to *xa13*. All the others 27 cultivars have single recessive gene for resistance to race 6 are non allelic to *xa13* and *xa24* and hence are new genes. Molecular analysis of the cultivars using STS marker for *xa5* and *xa13* confirmed most of the results (presence of new genes) obtained from genetic analysis.

**Key Words:** Inheritance; Resistance; Allelism; Cultivars; Rice.

### INTRODUCTION

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama, 1922) is major and the most destructive disease of rice growing countries. It is wide spread throughout Asia but also has been reported to occur in Australia, the United States and several rice growing countries of Latin America and Africa. The incidence of BB increased due to the introduction of improved varieties that utilize intensive agronomic practices such as high dosages of nitrogenous fertilizers, close plant-spacing, and continuous cropping.

Bacterial blight is most prevalent in irrigated and rain-fed lowland environments in the tropics and in temperate countries, especially during the rainy months. The disease causes not only yield reduction but also lowers the grain quality. Yield losses due to this disease range from 20 to 30% (Ou, 1985) though in severely infected fields the losses may reach up to 80% (Singh *et al.*, 1977). Generally, plant-bacterial diseases are difficult to control by chemicals. Although a few chemicals were reported effective, their use is limited due to the pollution they produce and the increase in production costs. Moreover, the bacterium can develop resistance to the chemicals over a period of time. The use of varietal resistance is considered the best strategy and the most economical method of controlling bacterial blight.

Breeding for resistance has therefore become an integral part of rice crop improvement in many Asian countries (Mew, 1987). So far, twenty-two major genes for resistance to bacterial blight have been identified (Khush and Angeles, 1999; Kihupi, 2001). Several of these genes have already been incorporated into improved rice varieties that are now widely grown in many rice growing countries. However, new races of the pathogen that can overcome the resistance of these major genes continue to evolve (Mew, 1987; 1989). Thus, the present study was undertaken to identify new genes for resistance to *Xoo*. For this purpose twenty-eight cultivars having resistance to six races *Xoo* were genetically analysed and the results were further confirmed by molecular techniques. The availability of diverse sources of resistance will ensure successful breeding program for BB resistance in rice.

### MATERIALS AND METHODS

Twenty-eight BB resistant cultivars from four rice-growing countries were used in the study (Table 1). According to Ogawa *et al.* (1991) and Kihupi *et al.* (2001), rice cultivars belonging to this group carry gene(s) for resistance to race 6 (*xa13* or *xa24* or possibly new genes) in addition to another one or two genes which convey resistance to races 1, 2, 3, 4 and 5.

**Table 1.** Rice cultivars used in the study and their reactions to the six Philippine races of *Xanthomonas oryzae* PV. *Oryzae* (Xoo).

VARIETY NAME	IRRI ACC. NO.	COUNTRY OF ORIGIN	REACTIONS TO XOO RACES <sup>1/</sup>					
			1	2	3	4	5	6
Bazail 408	6540	Bangladesh	R	R	R	R	R	R
Laki 659	6622	Bangladesh	R	R	R	R	R	R
ARC 10025	20653	India	R	R	R	R	R	R
Kaun Kaek	22894	Cambodia	R	MR	MR	R	MR	R
Phcar Tien P 65	23174	Cambodia	MR	MR	MR	R	R	R
Terabali	25926	Bangladesh	R	R	R	R	MR	MR
Kaika	26361	Bangladesh	R	R	R	R	R	R
Lakhsmi Digha	26390	Bangladesh	R	R	R	R	R	R
Below Dhan	26430	Bangladesh	R	MR	MR	R	MR	R
Hida	26471	Bangladesh	R	R	R	R	R	R
Bhaturi	27510	Bangladesh	R	R	R	R	R	R
Hanpa	27547	Bangladesh	R	R	R	R	R	R
Loroi	27567	Bangladesh	R	MR	R	R	MR	MR
Pankiraj	27575	Bangladesh	R	R	R	R	MR	MR
Aus 133	28986	Bangladesh	R	R	R	R	MR	MR
Aus 154	28997	Bangladesh	R	R	R	R	R	R
Aus 176	29008	Bangladesh	R	R	R	R	R	R
Aus 190	29014	Bangladesh	R	R	R	R	R	R
Aus 364	29151	Bangladesh	R	R	R	R	R	R
Bolium	31572	Bangladesh	R	R	R	R	MR	MR
Korchamuri	31594	Bangladesh	R	R	R	R	R	R
Noroi	31611	Bangladesh	R	R	R	R	MR	MR
Saita	31618	Bangladesh	R	R	R	R	MR	MR
Molla Diga	31674	Bangladesh	MR	MR	MR	R	R	R
Baila Borki	31699	Bangladesh	R	R	R	R	MR	MR
Baturi	31716	Bangladesh	R	R	R	R	R	R
Kalonchi	31836	Bangladesh	R	R	R	R	MR	R
Ngasein Kalagyi	33498	Myanmar	R	R	R	R	R	R

Two races of *Xoo*, race 1(PXO61) and race 6 (PXO 99) were employed in genetic analysis. Test varieties were crossed to a bacterial blight susceptible variety TN1 for investigating the inheritance of resistance. They were also crossed to BB resistant isogenic lines IRBB4, IRBB5, IRBB13 and a cultivar DV 85 for the allele tests. IRBB4 has *Xa4* which confers resistance to races 1 and 5 of *Xoo* while IRBB5 has *xa 5* that gives resistance to races 1,2,3 and 5 and moderate level of resistance to race 4. IRBB13 on the other hand, has *xa13* and DV85 has *xa 24* both genes conveys resistance to race 6 of *Xoo*. F1 and F2 progenies from the crosses of the test varieties with TN1 and the four testers were evaluated for *Xoo* reaction by inoculation method developed by Kauffman *et al.* (1973). Pure cultures of the bacterial strains were maintained in the Bacterial Leaf Blight Laboratory. Bacterial suspension or Inoculum was prepared by mixing bacterial cultures in distilled water. Inoculum was adjusted to a concentration of about  $10^8$  cells per ml using a spectrophotometer

Plants were inoculated with bacterial inoculum at maximum tillering or pre booting stage and were scored for reaction 14 days after inoculation. Progenies of crosses with TN1 were evaluated using races 1 and 6 while progenies of crosses for allele tests with *Xa4* and *xa5* were evaluated using only race 1. Progenies of crosses for allelic test with *xa13*, and *xa24* were evaluated using race 6.

## RESULTS AND DISCUSSION

**Inheritance of resistance:** The reactions to race 1 of F1 hybrids and F2 progenies from the crosses of test cultivars with TN1 are presented in table 2. F1 hybrids from crosses involving 26 cultivars were susceptible and their F2 progenies segregated into 1 resistant: 3 susceptible. These results indicate that a single recessive gene governs resistance to race 1 in these cultivars. On the other hand, F1 hybrid of one cultivar Kalonchi was resistant and its F2 progeny segregated into 13 resistant: 3 susceptible ratios. These results show that this

**Table 2. Reactions of F<sub>1</sub> hybrids and F<sub>2</sub> populaions from the crosses of resistant rice cultivars with TN1 to races 1 of *Xanthomonas oryzae pv. oryzae* (Xoo).**

Variety Name	Reaction					
	F <sub>1</sub> Hybrid	R	S	F <sub>2</sub> Population Seg. Ratio	$\chi^2$	P Value
TN1/Bazail 408	S	114	294	1 : 3	1.88	.10 - .25
TN1/Laki 659	S	110	370	1 : 3	1.11	.25 - .50
TN1/ARC10025	S	109	275	1 : 3	2.35	.10 - .25
TN1/kaun Kaek	S	110	370	1 : 3	1.11	.25 - .50
TN1/Phcar Tien P 65	S	108	276	1 : 3	2.00	.10 - .25
TN1/Terabali	S	98	334	1 : 3	1.23	.25 - .50
TN1/Kaika	S	120	320	1 : 3	1.21	.25 - .50
TN1/Lakhsmi Digha	S	115	325	1 : 3	0.30	.50 - .75
TN1/Below Dhan	S	106	376	1 : 3	2.33	.10 - .25
TN1/Hida	S	115	285	1 : 3	3.00	.05 - .10
TN1/Bhatari	S	120	300	1 : 3	2.86	.05 - .10
TN1/Hanpa	S	115	333	1 : 3	0.11	.75 - .90
TN1/Loroi	S	90	330	1 : 3	2.86	.05 - .10
TN1/Pankiraj	S	165	235	7 : 9	1.02	.25 - .50
TN1/Aus 133	S	124	320	1 : 3	2.03	.10 - .25
TN1/Aus 154	S	112	320	1 : 3	0.20	.50 - .75
TN1/Aus 176	S	110	290	1 : 3	1.33	.25 - .50
TN1/Aus 190	S	95	305	1 : 3	0.33	.50 - .75
TN1/Aus 364	S	120	324	1 : 3	0.97	.25 - .50
TN1/Bolium	S	123	327	1 : 3	1.31	.25 - .50
TN1/Korchamuri	S	94	230	1 : 3	2.78	.05 - .10
TN1/Noroi	S	120	300	1 : 3	2.86	.05 - .10
TN1/Saita	S	110	274	1 : 3	2.72	.05 - .10
TN1/Molla Diga	MS	80	220	1 : 3	0.44	.50 - .75
TN1/Baila Borki	S	130	350	1 : 3	1.11	.25 - .50
TN1/Baturi	MS	109	315	1 : 3	0.11	.75 - .90
TN1/Kalonchi	R	239	61	13 : 3	0.49	.25 - .50
TN1/Ngasein Kalagyi	S	110	290	1 : 3	1.33	.25 - .50

<sup>1/</sup>R= Resistant; MS = Moderately Susceptible; S = Susceptible.

cultivar possess one dominant and one recessive gene for resistance to race 1. Although F<sub>1</sub> hybrid of cultivar Pankiraj was susceptible but its F<sub>2</sub> progeny segregated in a ratio of 7 resistant: 9 susceptible showing that this cultivar posses two recessive genes for resistant to race 1. The reaction to race 6 of progenies of these crosses are

shown in Table 3. F<sub>1</sub> hybrids from all the crosses were susceptible and F<sub>2</sub> progenies segregated in the ratio of 1 resistant: 3 susceptible. These results indicate that resistance to race 6 in all cultivars is controlled by single recessive gene.

**Table 3.** Reactions of F<sub>1</sub> hybrids and F<sub>2</sub> populations from the crosses of resistant rice cultivars with TN1 to race 6 of *Xanthomonas oryzae* pv. *oryzae* (Xoo).

Variety Name	Reaction/1					
	F <sub>1</sub>	F <sub>2</sub> Population				
	Hybrid	R	S	Seg. Ratio	$\chi^2$	P Value
TN1/Bazail 408	S	112	296	1 : 3	1.31	.25 - .50
TN1/Laki 659	S	115	365	1 : 3	0.28	.50 - .75
TN1/ARC10025	S	109	275	1 : 3	2.35	.10 - .25
TN1/kaun Kaek	S	115	365	1 : 3	0.28	.50 - .75
TN1/Phcar Tien P 65	S	105	279	1 : 3	1.13	.25 - .50
TN1/Terabali	S	97	335	1 : 3	1.49	.10 - .25
TN1/Kaika	S	104	338	1 : 3	0.51	.25 - .50
TN1/Lakhsmi Digha	S	105	335	1 : 3	0.30	.50 - .75
TN1/Below Dhan	S	112	370	1 : 3	0.80	.25 - .50
TN1/Hida	S	108	292	1 : 3	0.85	.25 - .50
TN1/Bhaturi	S	111	309	1 : 3	0.46	.25 - .50
TN1/Hanpa	S	119	329	1 : 3	0.58	.25 - .50
TN1/Loroi	S	102	318	1 : 3	0.11	.75 - .90
TN1/Pankiraj	S	97	303	1 : 3	0.12	.75 - .90
TN1/Aus 133	S	115	329	1 : 3	0.19	.50 - .75
TN1/Aus 154	S	100	332	1 : 3	0.79	.25 - .50
TN1/Aus 176	S	90	300	1 : 3	0.77	.25 - .50
TN1/Aus 190	S	90	310	1 : 3	1.33	.25 - .50
TN1/Aus 364	S	104	340	1 : 3	0.59	.25 - .50
TN1/Bolium	S	107	343	1 : 3	0.36	.50 - .75
TN1/Korchamuri	S	90	234	1 : 3	1.33	.25 - .50
TN1/Noroi	S	108	312	1 : 3	0.11	.75 - .90
TN1/Saita	S	100	284	1 : 3	0.22	.50 - .75
TN1/Molla Diga	S	77	223	1 : 3	0.07	.75 - .90
TN1/Baila Borki	S	125	355	1 : 3	0.28	.50 - .75
TN1/Baturi	S	110	314	1 : 3	0.20	.50 - .75
TN1/Kalonchi	S	80	220	1 : 3	0.44	.50 - .75
TN1/Ngasein Kalagyi	S	109	291	1 : 3	1.08	.25 - .50

<sup>1/</sup>R= Resistant; S = Susceptible.

**Allele Tests:** Of the 28 cultivars, only Kalonchi carries a dominant gene for resistance to race 1 in addition to another independent recessive gene. The F<sub>1</sub> and F<sub>2</sub> progenies from the cross of this cultivar with IRBB4 ( an IR24 isogenic line homozygous for *Xa4*) were tested for their reactions to race 1 to determine the allelic relationship of its dominant gene with *Xa4*. As expected, the F<sub>1</sub> progenies of the cross were resistant. The F<sub>2</sub> population, however, showed no segregation. These

reactions of the F<sub>1</sub> and F<sub>2</sub> progenies indicate that the dominant gene of Kalonchi for resistance to race 1 is allelic to *Xa4*.

The reaction to race 1 of F<sub>1</sub> hybrids from the crosses of the resistant cultivars with IRBB5 carrying *xa5* gene are shown in Table 4. F<sub>1</sub> progenies from the crosses of twenty four cultivars were resistant and no segregation was observed in the F<sub>2</sub> populations of these crosses, thus, also

confirming that the recessive genes in these cultivars including Kalonchi are allelic to *xa5* gene.

**Table 4.** Reaction of F<sub>1</sub> hybrids and F<sub>2</sub> populations from the crosses of resistant rice cultivars with IRBB5 to race 1 of *Xanthomonas oryzae pv. oryzae* (Xoo).

Variety Name	Reaction Chi sq.					
	F <sub>1</sub>	F <sub>2</sub> Population				
	Hybrid	R	S	Seg. Ratio	$\chi^2$	P Value
IRBB5/Bazail 408	MS	194	238	7: 9	0.10	.50 - .75
IRBB5/Laki 659	R	480	0	-	-	-
IRBB5/ARC 10025	R	480	0	-	-	-
IRBB5/Kaun Kaek	S	204	240	7: 9	0.87	.25 - .50
IRBB5/Phcar Tien P 65	S	196	228	7: 9	1.06	.25 - .50
IRBB5/Terabali	R	468	0	-	-	-
IRBB5/Kaika	R	420	0	-	-	-
IRBB5/Lakhsmi Digha	R	468	0	-	-	-
IRBB5/Below Dhan	R	480	0	-	-	-
IRBB5/Hida	R	408	0	-	-	-
IRBB5/Bhaturi	R	428	0	-	-	-
IRBB5/Hanpa	R	444	0	-	-	-
IRBB5/Loroi	R	468	0	-	-	-
IRBB5/Pankiraj	R	480	0	-	-	-
IRBB5/Aus 133	R	480	0	-	-	-
IRBB5/Aus 154	R	468	0	-	-	-
IRBB5/Aus 176	R	492	0	-	-	-
IRBB5/Aus 190	R	480	0	-	-	-
IRBB5/Aus 364	S	204	246	7: 9	0.46	.50 - .75
IRBB5/Bolium	R	468	0	-	-	-
IRBB5/Korchamuri	R	480	0	-	-	-
IRBB5/Noroi	R	480	0	-	-	-
IRBB5/Saita	R	468	0	-	-	-
IRBB5/Molla Diga	R	480	0	-	-	-
IRBB5/Baila Borki	R	480	0	-	-	-
IRBB5/Baturi	R	468	0	-	-	-
IRBB5/Kalonchi	R	420	0	-	-	-
IRBB5/Ngasein Kalagyi	R	480	0	-	-	-

<sup>1/</sup>R= Resistant; MS = Moderately Susceptible; S = Susceptible.

The F<sub>1</sub> progenies from the crosses involving Bazail 408, Kaun Kaek, Phcar Tien P65 and Aus 364 were susceptible suggesting that the recessive gene governing resistance to race 1 in these cultivars are nonallelic to *xa5* gene and F<sub>2</sub> progenies of these crosses segregated into 7 resistant: 9 susceptible. These data confirmed that

the recessive genes governing resistance to race 1 in these cultivars are different and segregate independently of *xa5*. The reaction to race 6 of F<sub>1</sub> hybrids and F<sub>2</sub> progenies from the crosses of twenty eight cultivars with IRBB13 carrying *xa13*

**Table 5. Reaction of F<sub>1</sub> hybrids and F<sub>2</sub> populations from the crosses of resistant rice cultivars with IRBB13 to race 6 of *Xanthomonas oryzae* pv. *oryzae* (Xoo).**

Variety Name	Reaction Chi sq.					
	F <sub>1</sub> Hybrid	R	S	F <sub>2</sub> Population Seg. Ratio	$\chi^2$	P Value
IRBB13/Bazail 408	S	194	230	7: 9	0.69	.25 - .50
IRBB13/Laki 659	S	201	241	7: 9	0.53	.25 - .50
IRBB13/ARC 10025	S	200	250	7: 9	0.09	.75 - .90
IRBB13/Kaun Kaek	S	190	254	7: 9	0.17	.50 - .75
IRBB13/Phcar Tien P6	S	208	260	7: 9	0.09	.75 - .90
IRBB13/Terabali	S	191	229	7: 9	0.51	.25 - .50
IRBB13/Kaika	S	208	260	7: 9	0.09	.75 - .90
IRBB13/Lakhsmi Digha	S	169	227	7: 9	0.19	.50 - .75
IRBB13/Below Dhan	MS	220	260	7: 9	0.85	.25 - .50
IRBB13/Hida	S	177	219	7: 9	0.14	.75 - .90
IRBB13/Bhaturi	S	200	280	7: 9	0.85	.50 - .75
IRBB13/Hanpa	S	227	253	7: 9	2.45	.10 - .25
IRBB13/Loroi	S	204	240	7: 9	0.87	.25 - .50
IRBB13/Pankiraj	S	205	287	7: 9	0.87	.25 - .50
IRBB13/Aus 133	S	224	244	7: 9	3.22	.05 - .10
IRBB13/Aus 154	S	214	254	7: 9	0.74	.25 - .50
IRBB13/Aus 176	S	190	278	7: 9	1.89	.10 - .25
IRBB13/Aus 190	S	210	258	7: 9	0.24	.50 - .75
IRBB13/Aus 364	R	480	0	-	-	-
IRBB13/Bolium	MS	198	258	7: 9	0.02	.75 - .90
IRBB13/Korchamuri	S	202	248	7: 9	0.24	.50 - .75
IRBB13/Noroi	S	190	266	7: 9	0.80	.25 - .50
IRBB13/Saita	S	195	292	7: 9	2.72	.05 - .10
IRBB13/Molla Diga	MS	219	257	7: 9	0.99	.25 - .50
IRBB13/Baila Borki	S	190	254	7: 9	0.17	.50 - .75
IRBB13/Baturi	S	198	270	7: 9	0.40	.50 - .75
IRBB13/Kalonchi	S	175	233	7: 9	0.12	.75 - .90
IRBB13/Ngasein Kalagy	S	185	223	7: 9	0.42	.50 - .75

<sup>1/</sup>R= Resistant; MS = Moderately Susceptible; S = Susceptible.

gene are shown in Table 5. The F<sub>1</sub> hybrid as well as F<sub>2</sub> progeny from the cross IRBB13/Aus 364 were resistant indicating that the recessive gene governing resistance to race 6 in Aus 364 is allelic to *xa13*. F<sub>1</sub> progenies from the rest of the crosses were either moderately susceptible or susceptible while the F<sub>2</sub> progenies from these crosses showed segregation that fitted the ratio of 7 resistant: 9 susceptible. These results indicate that the recessive

genes conveying resistance to race 6 in these cultivars are nonallelic and segregate independently of *xa13* F<sub>1</sub> hybrids from the crosses of the resistant cultivars with DV85, another race 6 resistant cultivar carrying *xa24* gene. F<sub>1</sub> progenies from all these crosses were susceptible indicating that the recessive genes governing resistance to race 6 in all the cultivars are also nonallelic to *xa24* (Table 6).

**Table 6.** Reactions of F<sub>1</sub> hybrids from the crosses of resistant rice cultivars with DV85 to race 6 of *Xanthomonas oryzae* pv. *oryzae* (Xoo).

Variety Name	Lesion Length <sup>1/</sup> (cm)	Reaction <sup>2/</sup>
DV 85/Bazail 408	30.0 ± 0.00	S
DV 85/Laki 659	16.9 ± 2.26	S
DV 85/ARC 10025	29.8 ± 0.67	S
DV 85/Kaun Kaek	22.4 ± 3.32	S
DV 85/Phcar Tien P 65	26.7 ± 4.39	S
DV 85/Terabali	17.7 ± 3.04	S
DV 85/Kaika	22.8 ± 2.59	S
DV 85/Lakhsmi Digha	18.1 ± 3.10	S
DV 85/Below Dhan	21.1 ± 2.03	S
DV 85/Hida	24.3 ± 3.46	S
DV 85/Bhatari	21.8 ± 4.63	S
DV 85/Hanpa	21.8 ± 1.86	S
DV 85/Loroi	30.0 ± 0.00	S
DV 85/Pankiraj	24.4 ± 4.88	S
DV 85/Aus 133	22.8 ± 3.42	S
DV 85/Aus 154	30.0 ± 0.00	S
DV 85/Aus 176	27.4 ± 3.91	S
DV 85/Aus 190	27.6 ± 3.81	S
DV 85/Aus 364	22.2 ± 0.97	S
DV 85/Bolium	30.0 ± 0.00	S
DV 85/Korchamuri	23.0 ± 5.10	S
DV 85/Noroi	26.6 ± 4.77	S
DV 85/Saita	30.0 ± 0.00	S
DV 85/Molla Diga	28.0 ± 3.16	S
DV 85/Baila Borki	30.0 ± 0.00	S
DV 85/Baturi	29.7 ± 0.71	S
DV 85/Kalonchi	20.4 ± 2.30	S
DV 85/Ngasein Kalagyi	30.0 ± 0.00	S

<sup>1/</sup>Average lesion length (3 leaves x 4 plants) 14 days after inoculation (DAI).

<sup>2/</sup>S = Susceptible

**Molecular Analysis:** In addition to conventional genetic analysis, the presence of resistance genes in the twenty-eight BB resistant cultivars was determined through PCR analysis. The results of PCR analysis on BB resistant cultivars using STS marker derived from RFLP marker RG556 showed that all BB resistant cultivars found to possess *xa5* through genetic analysis showed the DNA

band for *xa5* except the cultivars Below Dhan and Molla Diga. Similarly, the results of PCR analysis using STS marker derived from *xa13* RFLP marker RG136 showed that three cultivars Aus 364, Molla Diga and Baturi have DNA band for *xa13*. However, only Aus 364 was identified to possess *xa13* from the genetic analysis while

all other cultivars have new single recessive gene for resistance to race 6.

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