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## Pyramiding of *xa -5*, *xa -13*, *Xa -21* genes for the development of bacterial blight resistant maintainer and restorer in Hybrid Rice



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## Introduction

- Rice is a staple food crop of India providing 43 percent of calorie requirement for more than 70 percent of Indian population
- Hybrid rice can meet the demands of increasing Indian population
- Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* is the major production constraint in India
- Available hybrids do not have required level of resistance against bacterial blight

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- Disease is prevalent in almost all the rice growing states of the country and yield losses range between 74 to 81 per cent in susceptible cultivars ( Veena *et al.* 1996)
- Host plant resistance is the only environmentally safe, economical and effective method of control for this disease ( Khush *et al.*1989)
- Gene pyramiding is the best approach for development of durable and multi race resistant genotypes
- Pyramiding of *xa -5*, *xa -13*, *Xa -21* genes in maintainers and restorers will give durable BB resistant hybrids

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## Bacterial blight infestation



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## Materials and methods

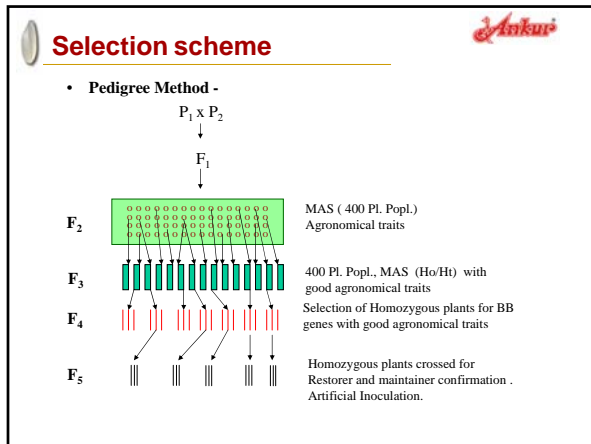
### Germplasm used in the experiment

- **AR-9** : Short-slender, aromatic, mid-early duration, high yielding genotype with maintainer background
- **AR-15** : Short-slender, medium duration, high yielding genotype with good cooking quality and maintainer background
- **ARR 84** : Long- slender, medium duration, strong restorer
- **ARR-513** : Medium-slender, mid-early duration, strong restorer
- **ARB-137** : Long-slender, medium duration genotype with *xa5*, *xa13* and *Xa21* genes

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## Crosses

- **Maintainer development**
  - AR-9 X ARBN-137
  - AR-15 X ARBN-137
- **Restorer development**
  - ARR-84 X ARBN-137
  - ARR-513 X ARBN-137



- ### Marker aided selection
- Ankur*
- DNA isolation and PCR analysis as per Zheng *et al.* 1995
  - STS markers (Huang *et al.* 1997)
    - RG 556 : xa-5
    - RG 136 : xa-13
    - pTA 248 : Xa-21

### Marker used for BB Resistance genes

**xa5, xa13, Xa21**

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Linked Marker	Gene	Chromosome	Enzyme	Primers (5'--3')	Marker Type
RG556	xa5	5	TaqI	F TAG CTG CTG CCG TGC TGT GC R AAT ATT TCA GTG TGC ATC TC	PCR-RFLP
RG136	xa13	8	HinfI	F TCC CAG AAA GCT ACT ACA GC R GCA GAC TCC AGT TTG ACT TC	PCR-RFLP
pTA248	Xa21	11		F AGA CGC GGA AGG GTG GTT CCC GGA R AGA CCG GTA ATC GAA AGA TGA AA	ALP

- ### Screening for BB resistance
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- Collected eight Xoo isolates viz. Xoo-1 to Xoo-8 from different geographical locations in India (MS, CG, OR, KA, RJ, PB, AP.)
  - Isolated and maintained on Hayward's Medium
  - Clip inoculated 40-day plants with bacterial suspension of  $10^9$  cfu/ml (Kaufmann *et al.* 1973)
  - Observations recorded 15 days after inoculation
  - 0-4 cm mean lesion length classified as Resistance and > 4 cm mean lesion length classified as Susceptible (Shanti *et al.* 2001)



- ### Evaluation of pyramided lines:
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- Restorer and maintainer status
  - Yield and yield contributing characters
  - Quality traits

## Results and Discussion



- Parents revealed absence of *xa5*, *xa13* and *Xa21* genes in molecular analysis showing susceptible reaction against eight Xoo isolates tested
- Donor parent ARBN-137 had all the three BB resistance genes in homozygous condition and showed resistant reaction
- The check TN-1 showed highly susceptible reaction against the eight Xoo isolates

## Xa genes & Markers

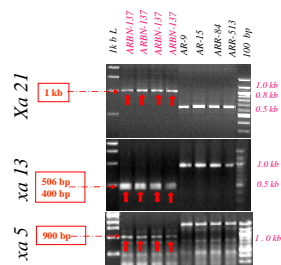


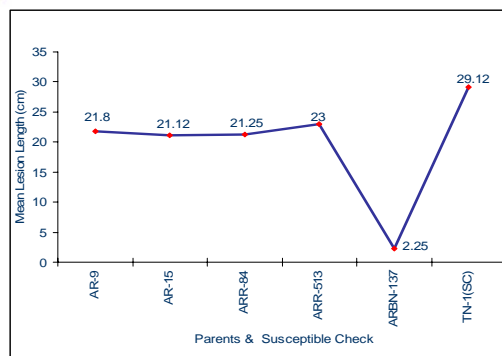
Table-1: Evaluation of Parents for BB resistance genes *xa5*, *xa13* and *Xa21* genes using MAS and artificial inoculation



S N	Breeding Lines	BB resistance genes			Isolates / Lesion Length (cm)								Mean Lesion Length (cm)
		<i>xa5</i>	<i>xa13</i>	<i>Xa21</i>	Xoo1	Xoo2	Xoo3	Xoo4	Xoo5	Xoo6	Xoo7	Xoo8	
1	AR-9	A	A	A	18	14	29	26	27	15	28	18	21.8
2	AR-15	A	A	A	16	13	28	24	26	14	29	19	21.12
3	ARR-84	A	A	A	17	12	26	24	27	16	29	19	21.25
4	ARR-513	A	A	A	16	14	28	27	29	19	30	21	23.00
5	ARBN-137	P	P	P	0.5	0.5	4.0	3.0	3.5	1.0	4.0	1.5	2.25
6	TN-1(SC)	A	A	A	24	23	35	33	32	25	36	25	29.12

A-Absent, P- Present, SC- Susceptible Check

## Evaluation of parents for BB resistance



- $F_1$ 's were heterozygous for all the three resistant genes

- Four  $F_2$  populations revealed 55 plants with *xa5*, *xa13* and *Xa21* genes and desirable agronomic traits. These plants in homozygous or heterozygous conditions were advanced to  $F_4$  generation through pedigree selection

- Homozygous plants with superior agronomic traits were selected in  $F_4$  generation

- In  $F_5$  generation 58 triple positive homozygous plants (32 from maintainer development programme and 26 from restorer development programme) were selected

- 58 homozygous positive plants were crossed with IR-58025A to assess the status of restoration

- Out of 32, three crosses showed highest sterility (>99 per cent), while out of 26 crosses, five crosses showed more than 88 % fertility in 1 % I-KI Stain.

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**Table-3: Restoration confirmation of pyramided lines**

SN	Pyramided Lines	Pollen Fertility (%)	Seed set (%)	Remark
1	IR-58025A X ARPB-1	0.18	0.50	Maintainer
2	IR-58025A X ARPB-2	0.27	0.12	Maintainer
3	IR-58025A X ARPB-3	0.11	0.00	Maintainer
4	IR-58025A X ARPR-1	90.25	92.60	Restorer
5	IR-58025A X ARPR-2	92.20	90.80	Restorer
6	IR-58025A X ARPR-3	89.60	91.00	Restorer
7	IR-58025A X ARPR-4	91.35	93.20	Restorer
8	IR-58025A X ARPR-5	88.15	92.40	Restorer

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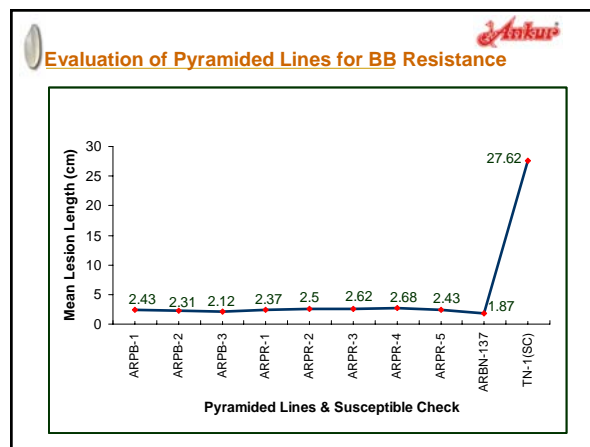
- ARPB-1, ARPB-2, and ARPB-3 were identified as good maintainers while ARPR-1, ARPR-2, ARPR-3, ARPR-4, and ARPR-5 were identified as restorers
- These pyramided maintainers and restorers showed resistant reaction against all the isolates tested exhibiting less than 4 cm mean lesion length

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**Table-2: Evaluation of Pyramided lines for BB resistance genes xa5, xa13 and Xa21 genes using MAS and artificial inoculation**

SN	Pyramided Lines	BB resistance genes			Isolates / Lesion Length (cm)								Mean Lesion Length (cm)
		xa5	xa13	Xa21	Xoo1	Xoo2	Xoo3	Xoo4	Xoo5	Xoo6	Xoo7	Xoo8	
1	ARPB-1	P	P	P	1.5	1.5	3.5	3.0	3.0	1.0	4.0	2.0	2.43
2	ARPB-2	P	P	P	1.0	2.0	4.0	3.0	3.0	1.5	3.0	1.0	2.31
3	ARPB-3	P	P	P	0.5	0.5	3.5	3.0	3.5	1.5	3.0	1.5	2.12
4	ARPR-1	P	P	P	1.5	1.0	3.0	3.0	3.0	2.0	3.5	2.0	2.37
5	ARPR-2	P	P	P	1.0	1.5	4.0	4.0	3.0	2.0	3.0	1.5	2.50
6	ARPR-3	P	P	P	1.0	2.0	4.0	3.5	3.0	1.5	4.0	2.0	2.62
7	ARPR-4	P	P	P	1.5	1.5	4.0	3.5	3.0	2.0	4.0	2.0	2.68
8	ARPR-5	P	P	P	1.5	1.5	3.0	3.5	3.5	1.5	3.0	2.0	2.43
9	ARB-137	P	P	P	0.5	0.5	3.0	3.0	3.0	1.5	4.0	1.0	1.87
10	TN-1(SC)	A	A	A	22	21	34	31	33	23	35	22	27.62

P-Present, A-Absent, SC- Susceptible check



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- The pyramided maintainers and restorers were evaluated for agronomical and quality traits
- Most promising maintainer, ARPB-3 with desirable agronomical and quality traits, was subjected to CMS conversion
- Restorers will be utilized as pollen parents for developing new bacterial blight resistant hybrid combinations

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**Table- 4: Agronomic traits of selected pyramided lines**

SN	Pyramided lines	Yield per plant (g)	Days to 50% Flowering	Maturity days	Plant height (cm)	Panicle Length (cm)	Grains/ panicle	No of tillers/ plant
1	ARPB-1	32.0	79	116	98	29.3	276	18
2	ARPB-2	30.5	76	115	100	28.5	270	15
3	ARPB-3	33.5	101	135	93	30.0	285	19
4	ARPR-1	33.8	95	130	108	29.5	250	17
5	ARPR-2	38.0	108	138	120	31.5	274	24
6	ARPR-3	36.5	92	122	110	29.4	298	20
7	ARPR-4	37.5	108	133	118	30.0	268	23
8	ARPR-5	34.0	88	120	112	28.5	265	18

Black color indicates Maintainers & Red colour indicates Restorers

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**Table- 5: Quality traits of selected pyramided lines**

S N	Pyramid ed lines	Test weight (g)	Milling %	Head rice recovery %	Kernel length (mm)	Kernel Breadth (mm)	L/B ratio	Grain type	Grain chalkiness	A S V	Amylose content %	GC
1	ARPB-1	14.8	72.0	65.5	5.64	1.69	3.34	SS	VOC	5	21.8	65.0
2	ARPB-2	15.1	71.6	66.3	5.29	1.58	3.35	SS	A	5	22.5	60.0
3	ARPB-3	14.2	73.0	68.4	5.73	1.61	3.56	SS	A	6	23.6	58.0
4	ARPR-1	18.7	69.6	65.0	6.25	1.85	3.38	LS	VOC	5	23.1	57.4
5	ARPR-2	20.7	72.3	66.4	6.87	2.07	3.32	LS	VOC	6	22.6	66.0
6	ARPR-3	19.9	74.3	64.6	5.59	2.21	2.53	MS	A	6	22.2	65.5
7	ARPR-4	20.5	71.5	70.8	5.58	2.16	2.58	MS	A	5	21.6	52.0
8	ARPR-5	19.8	73.5	69.2	5.90	2.13	2.77	MS	VOC	6	21.5	61.0

Black color indicates Maintainers & Red colour indicates Restorers

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### Conclusion

- These newly developed maintainers and restorers would be useful to develop bacterial blight resistant, high yielding and good quality hybrids
- The pyramided lines with desirable yield and quality parameters could be used as pre-breeding donor lines for transfer of resistance in rice germplasm
- Marker assisted selection can be efficiently used for the pyramiding of BB resistance genes in Maintainer and Restorer background

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### Acknowledgement

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