INTROGRESION OF *Xa7* AND *Xa21* FOR RESISTANCE TO BACTERIAL BLIGHT IN RESTORER LINES FOR DEVELOPMENT OF THREE-LINE HYBRID RICE

Truong Quoc Anh[1)], Ly Hau Giang[1)] and Duong Thanh Tai[2)]

[1)] Institute of Agricultural Sciences for Southern Vietnam

[2)] Southern Seed Company – Hochiminh City

INTRODUCTION

Bacterial blight (BB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is considered the most serious bacterial disease in rice-growing countries worldwide, especially in the area of hybrid rice cultivation. Chemical control for BB is not effective. Therefore, host plant resistance offers the most, economical and environmentally safe option for management of BB (Khush et al., 1989). Globally, more than twenty eight BB resistance genes have been identified from diverse sources (Chu et al., 2006). A number of these resistance genes have been tagged by closely linked molecular marker. A few of these genes like Xa4, xa5, xa13 have been incorporated widely in many high yielding varieties through conventional breeding (Khush et al. 1989). However, widespread cultivation of varieties has led to predominance of Xoo races that can overcome these genes. The deployment of rice cultivars that have multiple BB resistance genes is expected to lead to more durable resistance.

The objective of this study to develop the restorer lines containing *Xa7* and *Xa21* by a combination of phenotype against *Xoo* diagnostic strains and marker-assisted selection. The use of markers allowed us to combine resistance genes despite their epistatic interactions.

MATERIALS AND METHODS

Materials consist of 2 near isogenic lines of IRBB7 (*Xa7*), IRBB21 (*Xa21*) with genetic background of IR24. IR76912-26-6 for *Xa7* and *Xa21* was used as donor. PK8-38-3-2-1, MK6-3-2-1-1, and Que1-1-1 are restorer lines. The segregating population of BC_3F_1 and BC_2F_1 were produced. F_1 plants were backcrossed to recurrent parents.

MK63 (*Xa21*)= IR76912-26-6 /*6 MK63 (MK63=IR30/Gui630)
Que99 (*Xa21*)= IR76912-26-6 /*6 Que 99 (Que 99=Longye5-//IR661/IR206)
PK838 (*Xa21*)= IR76912-26-6 /*6 (PK838=226/MK63).

Figure 1. Rice field was affected by bacterial blight of rice

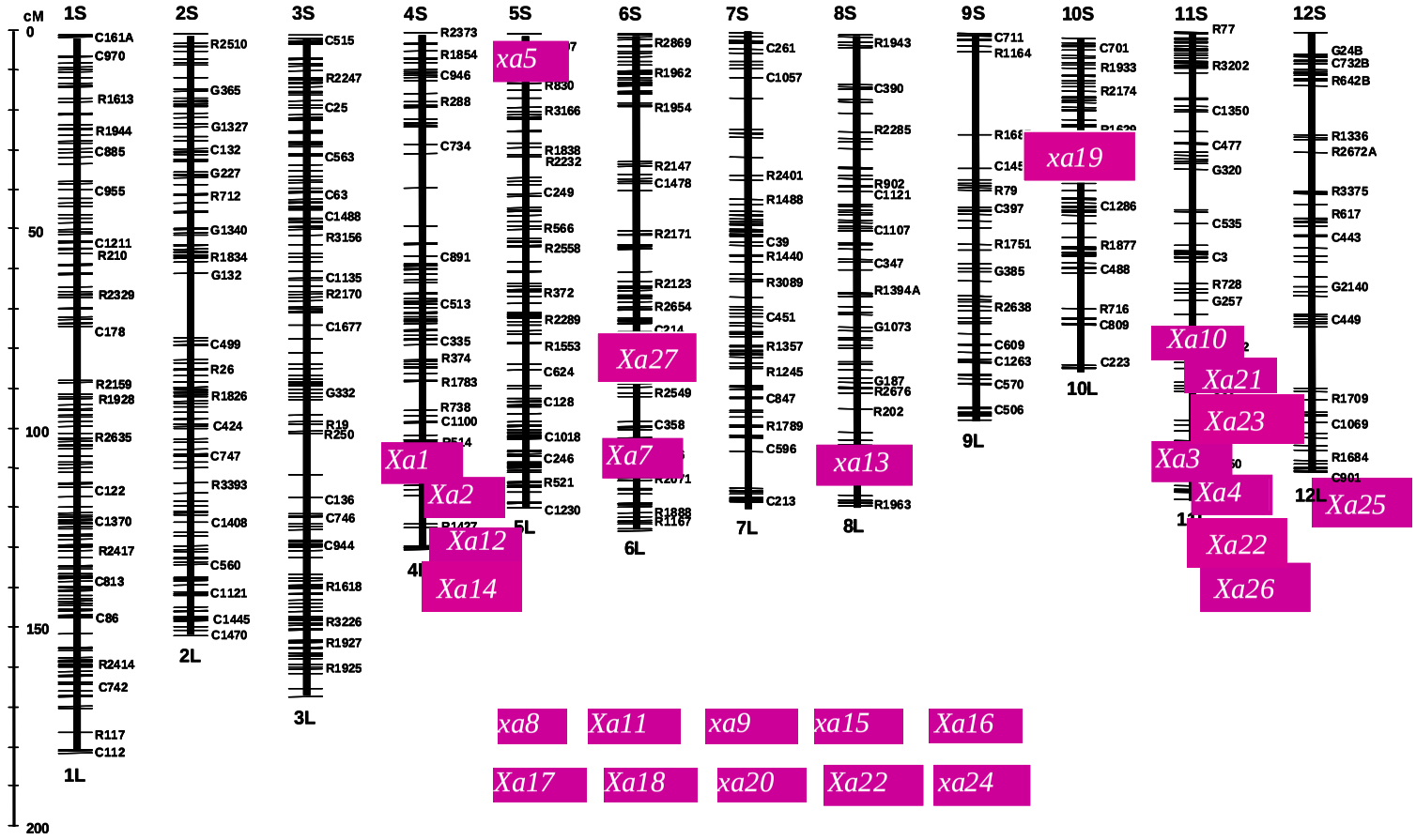


Fig.2. BB resistance genes were detected and distributed on chromosomes.

For inoculation preparation, the bacteria were transferred to the slant of potato semi-synthetic agar medium and incubated at 30^{o}C for 3 days. Inoculums was prepared by suspending the bacterial mass with sterilized water at a concentration of about 10^{9}cells/ml. Rice leaves were inoculated by the leaf-clipping method (Kauffman et al. 1973) at booting stage of rice growing. Disease reaction was assessed at 18 days after inoculation by lesion length measurement and classified into two categories, resistance and susceptible (Fig. 3).

- 2-days old culture on Wakimoto's medium was suspended in sterile distilled water.
- Bacterial concentration was adjusted to 10^{8}-10^{9} cfu /ml.
- Clipping method (Kauffman et al. 1973) – Uppermost fully developed leaves.

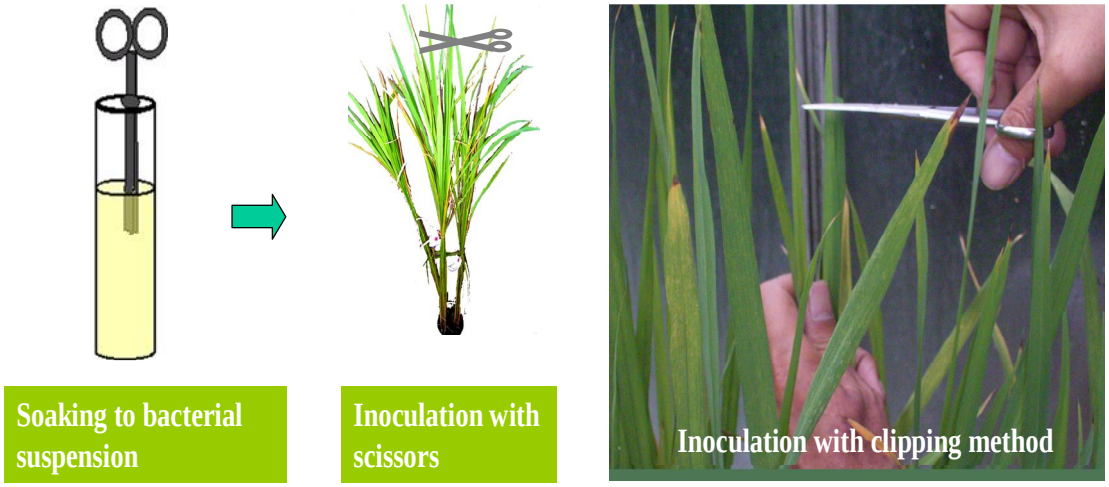


Fig.3. Inoculum's preparation and inoculation

DNA was extracted from fresh rice leaves using the CTAB (cetyltrimethylammonium bromide) method (Rogers and Bendich, 1988), or potassium acetate method (Dellaporta et al. 1983).

RESULT AND DISCUSSION

Linkage markers with resistance loci of *Xa7* and *Xa21* were designed and selected in experiment shown in table 1. Information of linkage markers with *Xa7* and *Xa21* were collected from (Shen Chen et al. 2008) and www.gramene.org.

Table 1. List of the markers and sequence primers were used in experiment

Marker	Forward primers	Reverse primers	Gene detection
GDSSR02	TGCCCACCGTCGAACTCGTGG	AGCTAGCAATTCGCATGATTGC	*Xa7*
RM5349	AGGGCATGCTTACATCCAAC	CATTTGCTTCTATGCCCCAG	*Xa21*
RM21	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG	*Xa21*
RM286	GGCTTCATCTTTGGCGAC	CCGGATTCACGAGATAAACTC	*Xa21*
RM473E	TATCCTCGTCTCCATCGCTC	AAGGATGTGGCGGTAGAATG	*Xa21*
M1Xa21	GGTGTTTTCTGCTCTACACTG	CGAATCCTGTTTGTGTTCATTG	*Xa21*
RM20580	CGTCACTTCACCAGCCTGTAGCC	GTCCATCAATGCCCATCCATCC	*Xa7*
RM20573	GGCTATTCCTTTCCTCCTCTCC	AATCTTCACGTGTGCGTAACTAGC	*Xa7*
RM20612	TGTCTCTCGATACCTCCCATACC	GCCCACCTCTCTTGTCCTATCC	*Xa7*
RM20590	TTCGATGAGCACCTTTCCTTGTCC	GCCTCGCCGATTCACTTATGC	*Xa7*
RM20591	CGTCTGCGCGAATATTTAGAGAGG	ATCTGCATCGGAGTCAGCAACG	*Xa7*

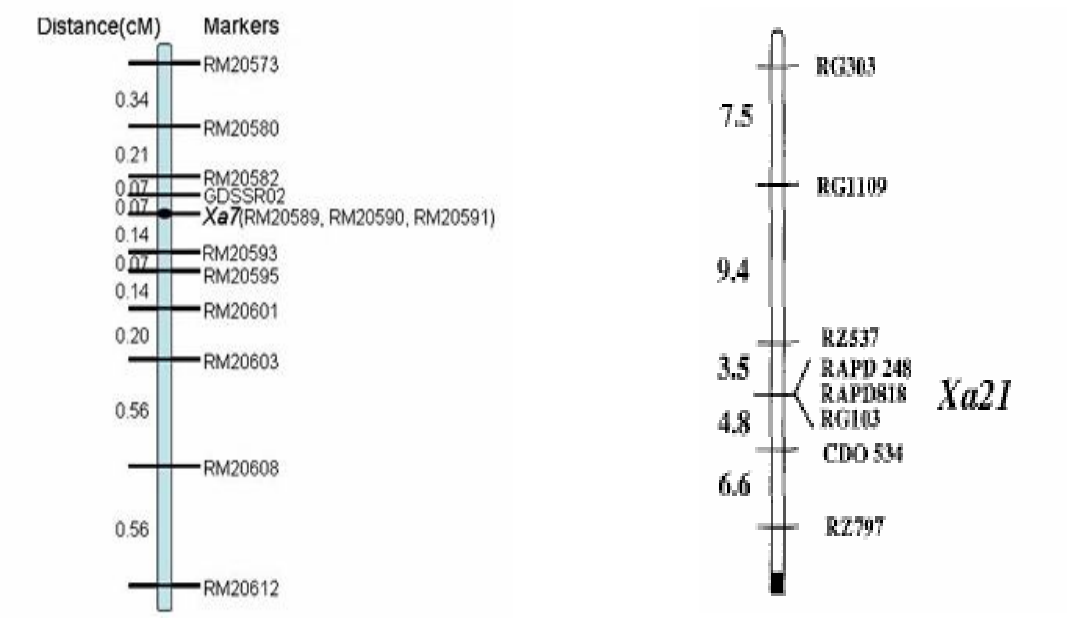
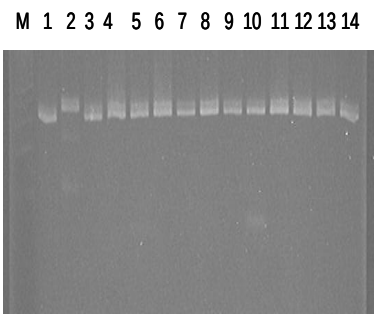
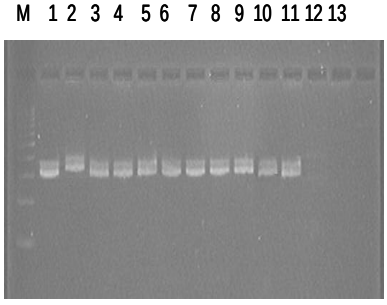


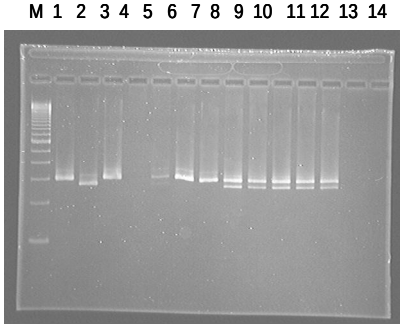
Fig 4. Genetic linkage map of *Xa7*, *Xa21* resistance genes in chromosome 6 and 11, respectively



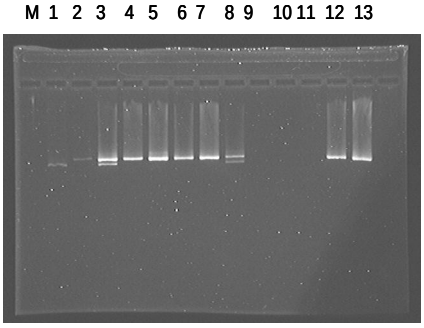
Electrophoresis result of PCR product using marker RM20590. Note, M: Marker ladder, 1: Que99, 2: IR24, 3 to 14 BC_3F_1 population Que99(Xa21)3*/IRBB7



Electrophoresis result of PCR product using marker RM20590 Note, M: Marker ladder, 1:MK63, 2: IR24, 3 to 13 BC_3F_1 population MK63 (Xa21)3*/IRBB7



Eloctrophoresis result of PCR product using marker M1Xa21 To detect *Xa21* gene, Note M: Marker ladder, 1: Que99, 2: IR 24 , 3 to 14 : BC_3F_1 population



Electrophoresis result of PCR using marker M1Xa21 To identify *Xa21* gene. Note M: Marker, 1: IR24 , 2: Pk63 , 3 to 13 : BC_3F_1 population

Phenotypic results indicated that the genes in combinations were more effective against the pathogen than a single gene (data not shown).

CONCLUSIONS

- *Xa7* and *Xa21* genes exhibited their resistance to bacterial blight strains, which collected in South Vietnam.
- The combination between *Xa7* and *Xa21* resistance genes exhibited more effective against the pathogen than a single gene.
- RM20590 marker linked to *Xa7* showed polymorphism between resistant and susceptible alleles. It can be used in marker-assisted selection breeding to detect promising progenies.
- Molecular marker linked to *Xa21* resistance gene was confirmed as M1Xa21, which was used for marker-assisted selection.

References

Kauffman et al. 1973
Rogers and Bendich, 1988
Shen Chen et al, 2008