Rice Research: Open Access

Gao and Sun, J Rice Res 2013, 1:1
http://dx.doi.org/10.4172/jrr.1000104

Review Artcle Open Access

In vitro Screening and Molecular Characterization of a Bacterial Blight Resistance Gene in Rice

Dongying Gao* **and Lihua Sun**

Jiangsu Academy of Agricultural Sciences, China

Abstract

Bacterial blight (BB) is one of the most serious bacterial diseases of rice in the world. Since 1986, our laboratory has conducted *in vitro* screening and application of BB resistance somaclonal mutants for rice breeding. In this paper, we review our nearly 30-year research on BB resistance somaclonal variation, including the development of an *in vitro* selection system, discovery of BB resistance mutants, evaluation of BB resistance in a somaclonal mutant named HX-3, genetic analysis and molecular mapping of the new BB resistance gene Xa-25. In addition, we also used the new resistance gene to develop BB resistance cultivar and hybrid rice. Our long-term research contributed a novel resistance gene in rice, it also provided an example for new gene creation and discovery using somaclonal variation.

Keywords: Bacterial blight; Resistance; Somaclonal variation; Xa-25; Molecular mapping

Introduction

In 1884, bacterial blight (BB) in rice, caused by Xanthomonas oryzae pv. oryzae, was first reported in Fukuoka, Japan. Up to date, bacterial blight is considered to be one of the most destructive diseases of rice worldwide, especially in South and Southeast Asia [1,2]. In some cases, this disease can cause 30 to 50% yield loss [3], and has been a serious threat to sustainable rice production. In addition to its economically importance, Xanthomonas oryzae pv. oryzae has been used as a model organism for host-pathogen interaction studies, in particular after the full-length sequences of both rice genome [4,5] and bacterial blight genome [6,7] were available publicly.

In China, bacterial blight was initially discovered in 1900 in the Guangdong province. Since then, bacterial blight has become a serious problem in all rice growing areas, especially in the central, east and south China [8]. Due to a wide utilization of susceptible cultivars, overuse of chemical fertilizers and other factories, bacterial blight was introduced and spread to new regions. The use of resistant cultivars is the most economic and environmentally friendly strategy to control the disease. So far, at least 29 major resistance genes have been identified in different rice varieties, and 6 of them have been cloned [9]. However, some bacterial blight resistance genes, such as Xa-1 and Xa-2, don't confer resistance against BB strains that occur in China (see data below), and the resistant resources which can be used to control the disease are limited. Therefore, identification and application of new resistance genes has become an important and urgent task for rice breeders and geneticists in China [10].

Many plant cells have the ability and possibility to regenerate a whole plant under certain conditions. This phenomenon has been well known as plant totipotency. Based on the totipotency theory, cells of the organisms reproduce with almost exact fidelity and give rise to daughter cells of the same genotype. In general, plants derived from the same cultured cell exhibit identical or similar phenotypes. Based on this theory, *in vitro* culture technique has been developed and widely used to rapidly propagate plants with some valuable performances for commercial applications and scientific researches. However, not all regenerated plants from a given genotype display the same phenotype in every operation. Some plants may exhibit different characters from the wild type, and in some cases, the differences can be dramatic and obvious.

In early studies, this kind of change was misunderstood as pollen contamination or residual mutations. Neverthness, when more and more such variations were observed in a variety of plants, including many self-pollinated plants such as rice, wheat, and barley [11-13], Larkin and Scowcroft [14] termed variation among plants regenerated from *in vitro* culture as somaclonal variation. It provides a powerful and complementary tool to create novel variations that would be hard or impossible to generate by conventional plant breeding. Now somaclonal variation has been widely used in rice breeding programs, including development of rice varieties for disease resistance.

Somaclonal variation is unpredictable in nature and can be useful or useless for plant breeding programs, depending on the stability of the variation. In this respect, somaclonal variation is similar to that induced by chemical or physical mutagens [14-16]. Some mutants with variable characters such as fertility, flowering date, plant height or other morphology characters can be easily identified in the field. These variations are usually identified based on phenotypic changes of regenerated plants, a strategy known as *in vivo* selection. However, other somaclonal mutants, such as physical or biochemical mutations (mutations in enzyme activity, seed acid amino content, etc), cannot be detected easily based on phenotypic changes.

In addition, frequency of a specific desirable variation is usually fairly low. Therefore, it is difficult and time-consuming to identify chemical or physiological mutants from many regenerated plants. In order to resolve this problem, *in vitro* selection strategy was established. Based on this strategy, calli were cultured on the media containing certain stress factors (antimetabolites, toxin, salt, etc) at an early developmental stage. As a result, only the callus resistant or

***Corresponding author:** Dongying Gao, Center for Applied Genetic Technologies, University of Georgia, USA, E-mail: dgao@uga.edu

Received June 17, 2013; **Accepted** August 02, 2013; **Published** August 09, 2013

Citation: Gao D, Sun L (2013) *In vitro* Screening and Molecular Characterization of a Bacterial Blight Resistance Gene in Rice. J Rice Res 1: 104. doi: 10.4172/jrr.1000104

Copyright: © 2013 Gao D, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

tolerant to stress factors (potential mutation cells) were able to grow and propagate on the media.

Then the regenerated plants from these calli were evaluated and selected in subsequent generations. With this technology, mutants tolerant or resistant to biotic or abiotic stresses can be isolated within a shorter period [16]. Also, this strategy can efficiently eliminate those useless mutants at an early stage. However, it is still unclear whether the stress factors per se can enhance the mutation frequency or act as the mutagens during tissue culture.

In 1986 our laboratory started a project which focused on development of rice mutants resistant to bacterial blight [10]. During more than two decades we established an *in vitro* selection system and obtained many resistance mutants via this system. From a somaclonal mutant HX-3, a new bacterial blight resistance gene, Xa-25, has been identified. Xa-25 showed broad spectrum resistance to Chinese BB strains and has been used to develop resistance cultivar in China. Below is the report of our research.

Establishment of *in vitro* Selection System for Bacterial Blight Resistance Mutants

The idea of *in vitro* selection for disease resistance mutants is that there is a genetic correlation between tolerance to toxins at the cell level and resistance to pathogens at the plant level [17]. So far, a variety of disease resistance mutants have been generated from susceptible cells using toxins of different pathogens as selection pressures [10,18,19]. However, difficulties in extraction and purification of certain toxins, as well as the instability of some toxins, have become the major obstacles for development of novel resistance resources via *in vitro* selection.

Since 1986, our laboratory successfully established an *in vitro* selection system for the isolation of bacterial blight-resistance mutants (Figure 1). In this system, mature, immature embryo or other explants of susceptible varieties were used to induce callus, then the calli were subcultured to generate more cells. Induced callus was then co-cultured with BB live strain. Although most of the calli arrested in development or died after 40-50 days, sometimes a few calli could survive on the medium with BB strains.

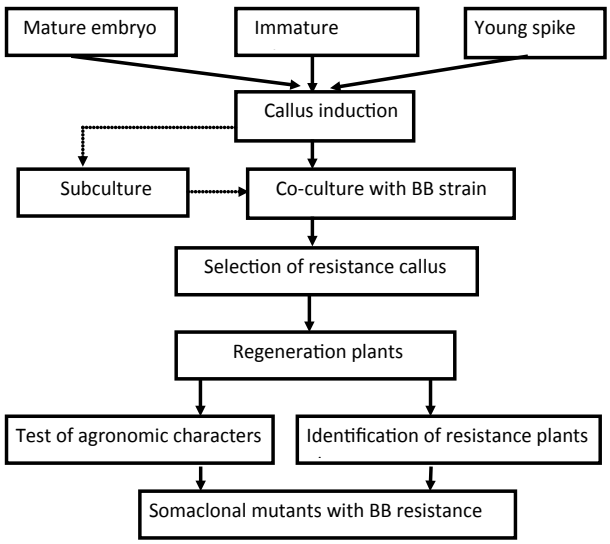

Figure 1: *In vitro* selection system for bacterial blight resistance somaclonal mutants.

These calli were transfered to another medium for plant regeneration. Finally, all regenerated plants were tested at adult stage for their BB resistance. Using this system, we obtained 44 regenerated plants with BB resistance from the susceptible variety Nangeng 34. Further tests showed that BB resistance in their progenies was stably and heritable [10]. In comparison with other systems which use toxins as selection pressures, our modified system eliminated the procedure of extraction and purification of toxins, and allowed us to conduct *in vitro* selection without any prior information about the toxins. Considering the limited resources for many rice breeders and geneticists, our system is inexpensive and easy to perform. Also, this system can be used for *in vitro* selection of plant mutants resistant to other bacterial pathogens.

Identification and Molecular Mapping of a New Bacterial Blight Resistance Gene

Development of a bacterial blight resistance mutant HX-3

We chose Minghui 63 as the material to develop bacterial blight resistance mutant.

This is because: 1) Minghui 63 is the restorer line of Shanyou 63, the most popular and competitive elite rice hybrid in China. The major fault of Shanyou 63 is its high susceptibility to bacterial blight, which usually causes heavy yield losses. 2) Minghui 63 is still commercially competitive in China. It carries many valuable traits of agronomic importance such as grain quality and yield, plant architecture, and environmental adaptability. It is possible to modify one or several traits in a short breeding period without changing its agronomic potential; 3) Although it is likely to improve BB resistance in Minhui 63 via conventional breeding methods, in many cases they were too slow to keep pace with pathogen mutation and host adaptation. Alternatively, our *in vitro* selection system provided us with an efficient approach to develop BB resistant mutants from Minghui 63, a susceptible yet economically important variety in China.

The mature embryos of Minghui 63 were used as explants to induce callus, and all the embryogenic calli were subcultured. A total of 30,000 calli were collected and incubated with the Chinese BB strain Zhe173 for 40-50 days on the reduction medium. Among all these calli, 66 calli survived and were transferred onto the regeneration medium. As a result, we obtained 44 regenerated plants. To evaluate the resistance and agronomic characters of these regenerated plants, they were grown in a greenhouse at Jiangsu Academy of Agricultural Sciences, China. Among these plants, a mutant named HX-3, which exhibited high BB resistance and good agronomic characters, was chosen for our further research (Figure 2a and 2b).

Evaluations of bacterial blight resistance in HX-3

The successful use of somaclonal variation is very much dependent on its genetic stability in the subsequent sexual generations [14]. Not all somaclonal variations are heritable, some of them are potentially reversible in later generations. Such phenominon has been observed frequently in the procedure of *in vitro* selection. For example, Larkin and Scowcroft [20] have found that some clones identified for tolerance to eyespot toxin were either reversible or unstable. *In vitro* selection for salt tolerance, potential salt tolerant cell lines on high NaCl concentration media commonly lost their salt tolerance in subsequent generations.

The possible reason is the salt tolerance of cells was caused by temporary adaptation to salt stress [14]. The epigenetic factors such as DNA methylation might be another reason for reversible somaclonal

mutantions [14,21,22]. In order to test whether the resistance in HX-3 was inherited, we evaluated BB resistance in HX-3 for 15 consecutive generations during 1992–2005 [23,24], Bacterial blight strain Zhe173 (Chinese pathotype 4), a popular strain in the lower regions of Yangtze River valley, was used to examine the resistance of HX-3 at adult stage using the leaf clipping method [23,24].

The wild type Minghui 63 was highly susceptible to bacterial blight strain Zhe173 in all experimental years, demonstrating a strong pathogenicity of the Zhe173 strain. In contrast, HX-3 showed high resistance in 15 generations (R_1 to R_{15}; Table 1). These results indicated that the resistance of HX-3 to bacterial blight was transmitted to the subsequent sexual generations, and the resistance in HX-3 was heritable and consistent. In addition, our data showed that the resistance in HX-3 did not segregate in all generations, indicating that the resistance was derived from a homologous somaclonal variation.

Evaluations of bacterial blight resistance in HX-3 at different growth stages

Disease resistance in rice can be divided into two types based on the developmental stage expressing resistance: adult resistance and all growth stages resistance. Plants with adult plant resistance only show disease resistance at adult stage, not at seedling stage. To test which type of resistance HX-3 had, we evaluated the resistance of HX-3 to the Zhe173 strain at seedling (the sixth leaf stage), the maximum tillering and adult stages. At the same time, we examined the resistance of other 19 rice varieties to the same BB strain. They included 19 near-isogenic lines (NILs) developed by the International Rice Research Institute (IRRI), which carried different BB resistance genes in the genetic background of IR24 (indica type).

In contrast to Minghui 63 and Jingang 30, two susceptible controls which showed high susceptibility to Zhe173 at all the stages tested, HX-3 was highly resistant to Zhe173 at all three stages, indicating that BB resistance in HX-3 could be categorized as all growth stage resistance (Table 2). Among the 19 NILs tested, only IRBB21 showed BB resistance at the adult stage, but not at seedling and tilling stages; almost all of the other NILs, which contained different resistance genes, including Xa-1, Xa-2, Xa-3, xa-8, Xa-10, Xa-11, Xa-12, Xa-14, Xa-16 and Xa-18, were susceptible to BB strain Zhe173. Our results proved that these resistance genes could not be used as resistance resources to control Chinese BB strain Zhe173 [24].

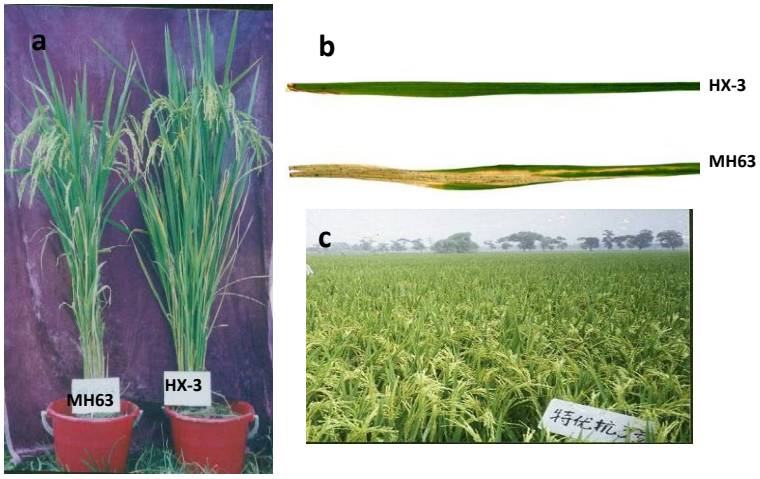

Figure 2: a) somaclonal resistance mutants HX-3 and its wildtype Minghui 63; b) Leaves of HX-3 and MH63 plants after inoculation with BB bacteria; c) A BB resistance hybrid rice Teyoukang No.3 which was developed using HX-3 as a restorer line.

Years	Generations	HX-3		Minghui 63 (CK)	
		Lesion length (cm)	Reaction	Lesion length(cm)	Reaction
1992	R1	2.1 ± 0.3	R	14.0 ± 1.1	S
1993	R2	2.0 ± 0.4	R	17.0 ± 1.8	S
1994	R4	2.1 ± 0.4	R	16.3 ± 1.6	S
1995	R5	2.7 ± 0.5	R	20.6 ± 2.3	S
1996	R6	2.8 ± 0.4	R	20.0 ± 2.7	S
1997	R7	2.0 ± 0.5	R	18.6 ± 1.5	S
1998	R8	2.4 ± 0.7	R	19.5 ± 2.7	S
1999	R9	2.5 ± 0.8	R	21.1 ± 2.6	S
2000	R10	2.7 ± 0.5	R	20.6 ± 2.5	S
2001	R11	1.5 ± 0.6	R	17.5 ± 3.6	S
2002	R12	2.8 ± 0.6	R	12.9 ± 1.7	S
2003	R13	1.1 ± 0.5	R	14.0 ± 1.1	S
2004	R14	1.7 ± 0.6	R	15.9 ± 2.2	S
2005	R15	1.3 ± 0.6	R	19.1 ± 3.3	S

Note: No data for R3 generation. R=Resistance S=Susceptible

Table 1: Resistance reaction of HX-3 to bacterial blight strain ZJ173 in different generations.

Genetic analysis of BB resistance in HX-3

In order to explore the genetic basis of BB resistance in HX-3, we crossed HX-3 with three susceptible rice varieties: Minghui 63, Yuetai A and Longtepu A. As a result, the F1 progenies of all the crosses were resistant to bacterial blight strain Zhe 173, and the resistant and susceptible plants in F2 families segregated in approximately 3:1 ratios for all the crosses (510:145, 241:88 and 324:94, respectively). The backcrossing progenies from crosses of HX-3 and Minghui63 segregated at a ratio of 1R:1S (Table 3). All these results suggested that the resistance of HX-3 to bacterial blight was controlled by a single dominant gene.

Resistance spectrum of HX-3 in comparison with cultivars with other BB resistance genes

22 BB resistance genes have been identified in difference rice varieties [25-28], which include 7 recessive gene xa-5, xa-8, xa-13, xa-15, xa-19, xa-20 and xa-24 [25,28]. As an initial examination, to determine if HX-3 had a novel BB resistance gene, we compared the resistance spectrum of HX-3 with other rice lines containing different dominant BB resistance genes. During 1999 to 2000, we collected 36 BB strains from China, Philippines and Japan, and then used them to test the resistance spectrums of HX-3 and 13 rice cultivars with different dominant BB resistance genes [24].

Among all the 36 BB strains tested, HX-3 was highly resistant to 32 of them, and was only susceptible to two Chinese BB strains (OS172, 83-69) and two Philippine strains (Pxo86, Pxo124). The other resistance genes Xa-1, Xa-2, Xa-10, Xa-11, Xa-12, Xa-14, Xa-16 and Xa-18 were highly susceptible to almost all Chinese BB strains tested; Xa-3, Xa-4, Xa-7, Xa-12, Xa-17 and Xa-21 were resistant to 18, 17, 30, 23, 27 and 27 tested strains, respectively. Interestingly, HX-3 showed different resistance reactions to BB strains, compared with other resistance varieties tested. Also, based on our results, not a single gene we tested exhibited resistance to all the BB strains, therefore, it is a good strategy for durable BB resistance to pyramid several resistance genes into a single host genotype (Table 4).

Allelic tests of the resistance gene in HX-3

To further determine whether the resistance gene in HX-3 is different from other BB resistance genes already reported, we performed allelic tests by crossing HX-3 with four lines (IRBB4, IRBB7, CBB12 and IRBB21), each of them containing a different dominant BB resistance gene with resistance spectrums similar to HX-3.

If the resistance gene in HX-3 is different from those in other cultivars, segregation of resistance and susceptibility should be observed in F2 populations; otherwise, no segregation would happen in the F2 population. Consequently, the segregation ratios of resistant to susceptible plants in the three F2 populations were 181:15, 195:16 and 241:15 for crosses between HX-3 IRBB4 (containing BB resistance gene Xa-4), IRBB7 (containing Xa-7) and IRBB21(containing Xa-21), respectively, all fitting to a ratio of 15R:1S (Table 5).

These results suggested that the resistance gene in HX-3 is different with Xa-4, Xa-7 and Xa-21; its chromosome location was also different

Materials	Genes	Seedling stage		Tillering stage		Adult stage	
		Lesion length (cm)	Reaction	Lesion length (cm)	Reaction	Lesion length(cm)	Reaction
Minghui 63		6.8 ± 0.8	S	13.8 ± 1.1	S	20.6 ± 2.5	S
Jingang 30		10.6 ± 0.8	S	17.8 ± 1.8	S	20.5 ± 1.9	S
IR24		9.0 ± 1.0	S	14.2 ± 1.4	S	15.6 ± 3.5	S
IRBB1	Xa-1	6.8 ± 1.3	S	15.9 ± 1.7	S	20.2 ± 2.7	S
IRBB2	Xa-2	8.3 ± 0.8	S	14.5 ± 2.9	S	22.6 ± 3.4	S
IRBB3	Xa-3	7.9 ± 1.2	S	14.1 ± 1.8	S	21.1 ± 4.3	S
IRBB4	Xa-4	2.1 ± 0.8	R	6.1 ± 0.6	R	6.3 ± 0.6	R
IRBB5	xa-5	1.6 ± 0.1	R	1.3 ± 0.9	R	1.9 ± 1.1	R
IRBB7	Xa-7	0.8 ± 0.6	R	0.2 ± 0.2	R	1.5 ± 0.6	R
IRBB8	xa-8	6.8 ± 1.3	S	13.5 ± 2.6	S	13.2 ± 2.8	S
IRBB10	Xa-10	9.1 ± 1.4	S	14.3 ± 2.6	S	18.5 ± 4.1	S
IRBB11	Xa-11	8.7 ± 1.1	S	19.9 ± 3.0	S	18.4 ± 2.4	S
IRBB13	xa-13	2.1 ± 1.1	R	3.8 ± 0.9	R	15.2 ± 4.1	R
IRBB14	Xa-14	8.1 ± 1.1	S	15.1 ± 0.7	S	13.6 ± 4.1	S
Tetep	Xa-16	11.6 ± 0.9	S	17.0 ± 4.2	S	25.1 ± 4.5	S
Asominori	Xa-17	1.0 ± 0.5	R	3.4 ± 1.1	R	1.4 ± 1.0	R
Toyashiki	Xa-18	7.3 ± 0.6	S	14.8 ± 2.8	S	25.5 ± 2.8	S
IRBB21	Xa-21	8.1 ± 1.0	S	17.0 ± 4.0	S	2.7 ± 1.0	R
CBB12	Xa-12	8.1 ± 1.3	S	11.9 ± 3.3	S	10.1 ± 1.9	S
HX-3	Xa-?	1.9 ± 0.3	R	2.5 ± 0.7	R	2.7 ± 0.5	R

Table 2: Resistance reactions of HX-3 to the BB strain Zhe 173 at various growing stages.

Crosses	Reaction	Reaction					
		R	S	Total	Exp	X2	P Value
Minghui63/HX-3	R	510	145	655	3:1	2.71	0.1
Minghui63/HX3//Minghui63		59	43	102	1:1	2.21	0.1~0.5
HX3/Minghui63//Minghui63		41	35	76	1:1	0.32	0.5~0.9
YuetaiA/HX-3	R	241	88	329	3:1	0.45	0.5
LongtepuA/HX-3	R	324	94	418	3:1	1.28	0.1~0.5

Table 3: Resistance reaction of F2 and backcross progeny to bacterial blight strain Zhe173 from crosses of HX-3 and three susceptible varieties.

Cultivars	IR24	IRBB1	IRBB2	IRBB3	IRBB4	IRBB7	IRBB10	IRBB11	CBB12	IRBB14	Tetep	Asominori	Toyoshiki	IRBB21	HX-3
Resistance gene	-	Xa-1	Xa-2	Xa-3	Xa-4	Xa-7	Xa-10	Xa-11	Xa-12	Xa-14	Xa-16	Xa-17	Xa-18	Xa-21	Xa-?
BJ84-3	S	S	R	R	R	R	S	R	R	R	R	R	S	S	R
KW-2	S	R	R	R	S	R	S	S	R	R	S	R	S	R	R
4	S	S	S	R	R	R	S	S	R	S	S	S	S	S	R
Ks-6-6	S	S	S	R	R	R	S	S	R	S	S	R	S	R	R
ZJ173	S	S	S	S	R	R	S	S	S	S	S	R	S	R	R
OS28	S	S	R	S	S	R	S	S	R	S	S	S	S	S	R
OS93	S	S	S	S	R	R	S	S	S	S	S	R	S	S	R
ZJ16	S	S	S	S	S	R	S	S	S	S	S	R	S	R	R
OS54	S	S	S	S	R	R	S	S	R	S	S	S	S	S	R
OS44	S	S	S	R	R	R	S	S	R	S	S	R	S	R	R
OS218	S	S	S	R	S	R	S	S	R	S	S	S	S	R	R
RB209	S	S	S	R	R	S	S	S	R	S	S	R	S	R	R
ZJ26	S	S	S	S	R	R	S	S	S	R	S	R	S	R	R
RBFJ21	S	R	S	R	S	R	S	S	R	S	R	R	R	R	R
GD-32	S	S	S	S	R	R	S	S	S	S	S	R	R	R	R
OS37	S	S	S	S	R	R	S	S	S	S	R	R	S	R	R
X-S-2	S	S	S	S	R	S	S	S	R	S	S	R	S	R	R
ZJ25	S	S	S	S	R	R	S	S	S	S	S	R	S	S	R
OS172	S	S	R	R	S	R	S	S	R	R	R	R	S	R	S
HB8417	S	S	S	R	S	R	S	S	R	S	S	R	S	R	R
GX-09	S	S	S	R	S	R	S	S	R	S	S	S	S	R	R
Hen114	S	S	S	R	S	S	S	S	R	S	S	R	S	R	R
ZJ28	S	S	S	S	S	R	S	S	R	S	S	R	S	R	R
JS158-2	S	S	S	S	S	R	S	S	S	S	S	R	S	R	R
OS35	S	S	S	S	S	R	S	S	S	S	S	S	S	R	R
OS171	S	S	S	R	S	R	S	S	R	S	S	S	S	S	R
83-69	S	S	S	S	S	R	S	S	R	S	S	S	S	S	S
OS225	S	S	S	S	R	R	S	S	S	S	S	R	S	R	R
#2	S	S	S	S	S	R	S	S	S	S	S	R	S	S	R
Pxo61	S	S	S	R	R	S	S	S	R	S	S	S	S	R	R
Pxo71	S	S	S	R	R	S	S	S	R	S	S	R	S	R	R
Pxo79	S	S	S	R	S	R	S	S	R	S	S	R	S	R	R
Pxo86	S	S	S	R	S	S	S	S	R	S	S	R	S	R	S
Pxo124	S	S	S	S	S	R	S	S	S	S	S	R	S	R	S
T3	S	S	S	R	R	R	S	S	R	S	S	R	S	R	R
T4	S	S	S	S	S	R	S	S	S	S	S	R	S	R	R

Table 4: Resistance reactions of HX-3 and 14 rice varieties to different bacterial blight strains.

Crosses	Reaction	Resistance/susceptibility segregation in F2 population					
		R	S	Total	Exp	X^2	P Value
IRBB4/HX-3	R	181	15	196	15:1	0.44	0.5~0.9
CBB12/HX-3	R	292	96	388	3:1	0.03	0.5~0.9
IRBB7/HX-3	R	195	16	211	15:1	0.43	0.5~0.9
IRBB21/HX-3	R	241	15	256	15:1	0.02	0.9

Table 5: Allelic tests of HX-3 to the bacterial blight strain Zhe173.

with the other three genes. Furthermore, it should be noted that the cross between HX-3 and CBB12, which was susceptible to Zhe173, resulted in a segregation ratio of 3:1 in the F2 population, therefore confirming that the resistance in HX-3 was controlled by a dominant gene.

Based on our results of resistance spectrum and allelic tests between in HX-3 and other 13 lines with different dominant resistance genes, the resistance gene in HX-3 was different with these 13 genes. Recently, two new dominant resistance genes Xa-22(t) and Xa-23(t) were identified from the Yunnan rice variety ZCL [26] and *O. rufipogon* [27], respectively. ZCL is resistant to Pxo86 but susceptible to ZJ173; WBB1 showed resistance to all 9 Philippines races of BB. In comparison, HX-3 was resistant to ZJ173 and susceptible to Pxo86 and Pxo124. This result suggests that the resistance reaction of HX-3 is different from both ZCl and WBB1. All these results proved that there was a new dominant resistance gene in HX-3. We have designated the new gene as Xa-25.

Molecular mapping of resistance gene Xa-25

Development of a doubled haploid (DH) population and its resistance evaluation: The reasons we chose 02428 to develop a doubled haploid (DH) population were as follows: 1) Hybridization between 02428 (japonica) and HX-3 (Indica) might result in plenty of molecular polymorphisms in the hybrids, given the fact that these two varieties are different subspecies of oryza genus; 2) 02428 has proved to be highly compatible and was able to produce fertile pollens when crossed to indica rice varieties; 3) As a variety with a high regeneration ability in

another culture, 02428 will help us to regenerate more doubled haploid plants; 4) 02428 was susceptible to Chinese bacterial blight strains; 5) The DH population is a permanent mapping population because there will be no segregation in further generations. A remarkable advantage of using DH lines is that they can be tested repeatedly and be shared with other researchers.

During 2000-2001, a total of 11,048 anthers were incubated on the medium to induce callus, and 129 diploid plants were regenerated from 1146 calli. These regenerated plants were grown in soil in the greenhouse at the Jiangsu Academy of Agricultural Sciences or planted in the experimental field in Hainan island (China) in winter. All these plants produced enough seeds for further studies.

Resistance evaluation of the DH population: The bacterial blight strain Zhe173 was used to test the resistance of 02428, HX-3 and the DH population. 02428 was high susceptible to ZJ173 (with the mean lesion length of 10.2 ± 1.2 cm), while HX-3 showed a high level of resistance to the same BB strain (with the mean lesion length of 2.1 ± 0.4 cm). Among all the DH lines tested, 65 were susceptible and 64 were resistant to ZJ173, respectively. Thus, the 1:1 segregation ratio for the resistant to susceptible genotypes further confirmed the existence of a single dominant resistance gene in HX-3.

It has been reported that gametic selection in another culture may lead to distorted segregation ratios, and the deviation is generally biased toward the male or female parent alleles in the DH population [29]. Since the segregation ratio of resistant and susceptible lines was not distorted in our DH population, this population provided desirable materials for molecular mapping of the new resistance gene Xa-25.

Selection of polymorphic SSR markers and genetic mapping of Xa-25: To identify the polymorphic SSR markers linked to Xa-25, a total of 274 SSR primer pairs covering 12 rice chromosomes [30] were used for polymorphism survey of 02428 and HX-3. Among these primers, 67 showed polymorphism between the parents, with a polymorphic rate of 24.5%. Using these 67 polymorphic SSR markers, we performed bulked segregant analysis on the DH population. As a result, the SSR marker RM252 on chromosome 4 was found to link to Xa-25.

Based on a recent rice genetic map [31], we selected 26 pairs of of SSR primers near RM252 for parental polymorphism survey, and from them we chose 6 polymorphic markers for the following linkage analysis. Data were analyzed using Mapmaker 3.0 program at a LOD score threshold of 3.0, and all map distances (cM) were reported in Kosambi units. Linkage analysis revealed that all these 6 markers were linked to Xa-25, and the map distance between Xa-25 and the two nearest SSR markers RM6748 and RM5506 was 9.3 cM and 4.2 cM, respectively. Therefore, Xa-25 was located at the terminal region of the long arm of chromosome 4.

So far, at least 17 of 29 bacterial blight resistance genes have been localized on chromosome 4, 5, 6, 7, 8 or 11 [25-27,32-41], with most of these resistance genes located on chromosome 4 or 11. This fact indicates a cluster architecture for these bacterial blight resistance genes. For instance, 7 resistance genes, Xa-3 [33], Xa-4 [33], Xa-10 [25], Xa-21 [38], Xa-22 [26], Xa-23 [27] and Xa-26(t) [39], were located on chromosome 11; five BB resistance genes, Xa-1 [17], Xa-2 [17], Xa-12 [25], Xa-14 [37] and Xa-25 [41], were located on chromosome 4. Interestingly, although Xa-25 was localized on the same chromosome as 4 other BB resistance genes, the resistance spectrums of Xa-25 were different with them. Also, allelic testing between HX-3 and CBB12 indicated that Xa-25 was different with Xa-12. Once again, molecular mapping of Xa-25 also confirmed our former conclusion that Xa-25 was a novel bacterial blight resistance gene.

With the final aim to fine map and positionally clone Xa-25, we have constructed two large F2 mapping populations. The first F2 population derived from a cross between 02428 (japonica) and HX-3 (indica). So far 11,372 individuals have been tested for their BB resistance, and 1569 highly susceptible individuals have been chosen for further analysis. Another F2 population was developed from the cross between the susceptible Jingang 30 (indica) and HX-3 (indica). 10960 individuals have been tested for resistance and 1100 highly susceptible individuals have been chosen for DNA isolation. Fine mapping and cloning of Xa-25 is currently underway.

Application of Xa-25 to rice improvement

HX-3 is not only highly resistant to bacterial blight; it also retained most of the good agronomic characters of its wild type Minghui 63, including a grain yield comparable to Minghui 63. In addition, HX-3 has a restoring property, which can help us develop hybrid crosses. To date, the hybrid rice Teyoukang No. 3 has been developed from Long A (sterile line) x HX-3 (Figure 2c). This hybrid is not only resistant to bacterial blight; it also has good agronomic characteristics such as high yield, middle growth duration, fine grain quality and wide adaptability.

Conclusion

After over two decades' effort, our laboratory has established an *in vitro* selection system for BB resistance somaslonal mutants, and a numbers of resistant somaclonal mutants derived from susceptible cultivars have been developed though this system. Among them, a particularly valuable resistance mutant, HX-3, was obtained from the susceptible variety Minghui 63, the restorer line of the former competitive elite rice hybrid Shanyou 63 in China. To identify the resistance gene in HX-3, we carried out genetic analysis, resistance spectrum evaluation and molecular mapping studies.

Our results proved that the resistance of HX-3 to BB was stable and heritable, and was controlled by a major dominant gene. Further genetic analysis confirmed that HX-3 has a new BB resistance gene, Xa-25, and it was localized at the terminal region of the long arm of chromosome 4. Xa-25 showed a wide-spectrum of resistance throughout all growth stages to most Chinese BB strains, and has also been used to develop resistant hybrid rice cultivars. Our research proved that somaclonal variation can provide a valuable resource for gene discovery. Our next research will focus on fine-mapping and cloning of the new resistance gene Xa-25, with the long-term aim of using the new resistance gene for the development of competitive commercial rice cultivars or hybrids.

References

1. Mew TW (1987) Current status and future prospects of research on bacterial blight of rice. Annu Rev Phytopathol 25: 359-382.
2. Adhikari TB, Cruz C, Zhang Q, Nelson RJ, Skinner DZ, et al. (1995) Genetic Diversity of Xanthomonas oryzae pv. oryzae in Asia. Appl Environ Microbiol 61: 966-971.
3. Reddy APK (1989) Proceeding of the International Workshop on Bacterial Blight of Rice. International Rice Research Institute, Manila, the Philippines.
4. Yu J, Hu S, Wang J, Wong GK, Li S, et al. (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. indica). Science 296: 79-92.
5. Goff SA, Ricke D, Lan TH, Presting G, Wang R, et al. (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. japonica). Science 296: 92-100.
6. Ochiai H, Inoue Y, Takeya M, Sasaki A, Kaku, H (2005) Genome sequence of Xanthomonas oryzae pv. oryzae suggests contribution of large numbers of effector genes and insertion sequences to its race diversity. JARQ 39: 275-287.

7. Lee BM, Park YJ, Park DS, Kang HW, Kim JG, et al. (2005) The genome sequence of Xanthomonas oryzae pathovar oryzae KACC10331, the bacterial blight pathogen of rice. Nucleic Acids Res 33: 577-586.

8. Wu SZ (1983) Bacterial blight of rice and its controlling, Shanghai Science and Technology Publishers, Shanghai, China.

9. Niño-Liu DO, Ronald PC, Bogdanove AJ (2006) Xanthomonas oryzae pathovars: model pathogens of a model crop. Mol Plant Pathol 7: 303-324.

10. Sun LH, She JM, Lu XF (1986) *In vitro* selection of Xanthomonas oryzae-resistent mutants in rice. I. Induction of resistant cetins and screening regenerated plants. Acta Genet Sin 13: 188-193.

11. Oono K (1978) Test tube breeding of rice tissue culture. Trop Agric Res Series 11: 109-124.

12. Ryan SA, Larkin PJ, Ellison FW (1987) Somaclonal variation in some agronomic and quality characters in wheat. Theor Appl Genet 74: 77-82.

13. Baillie AMR, Kartha KK, Rossnagel BG (1993) Evaluation of 10 Canadian barley (*Hordeum vulgare* L.) cultivars for tissue culture response. Can J Plant Sci 73: 171-174.

14. Larkin PJ, Scocroft WR (1981) Somaclonal variation: a novel source of variability from cell cultures for plant improvement. Theor Appl Genet 60: 197-214.

15. Evans DA, Sharp WR, Medina-Filho HP (1984) Somaclonal and gametoclonal variation. Amer J Bot 71: 759-774.

16. Jain SM (2001) Tissue culture-derived variation in crop improvement. Euphytica 118: 153-166.

17. Carlson PS (1973) Methionine sulfoximine--resistant mutants of tobacco. Science 180: 1366-1368.

18. Ling DH, Vidhyaseharan P, Borromeo ES, Zapata FJ (1985) In vitro screening of rice germplasm for resistance to brown spot disease using phytotoxin. Theor Appl Genet 71: 133-135.

19. Toyoda H, Shimizu K, Chatani K, Kita N, MatsudaY (1989) Selection of bacterial wilt resistant tomato through tissue culture. Plant Cell Report 8: 317-320.

20. Larkin PJ, Scowcroft WR (1983) Somacional variation and eyespot toxin tolerance in sugarcane. Plant Cell Tissue Organ Culture 2: 111-121.

21. Kubis SE, Castilho AM, Vershinin AV, Heslop-Harrison JS (2003) Retroelements, transposons and methylation status in the genome of oil palm (Elaeis guineensis) and the relationship to somaclonal variation. Plant Mol Biol 52: 69-79.

22. Muller E, Brown PTH, Harke S, Lorz H (1990) DNA variation in tissue-culture-derived rice plants.Theor Appl Genet 80: 673-679.

23. Kauffman HE, Reddy APK, Hsien SPY, Merca SD (1973) An improved technique for evaluating resistance of rice varieties to Xanthomonas oryzae. Plant Dis Rep 57: 537- 541.

24. Gao DY, Xu ZG, Chen ZY, Sun LH, Sun QM, et al. (2002) [Identification of a resistance gene to bacterial blight (Xanthomonas oryzae pv. oryzae) in a somaclonal mutant HX-3 of indica rice]. Yi Chuan Xue Bao 29: 138-143.

25. Kinoshita T (1995) Report of committee on gene symbolization, nomenclature and linkage groups. Rice Genet Newslett 12: 9-115.

26. Lin XH, Zhang DP, Xie YF, Gao HP, Zhang Q (1996) Identifying and Mapping a New Gene for Bacterial Blight Resistance in Rice Based on RFLP Markers. Phytopathology 86: 1156-1159.

27. Zhang Q, Lin SC, Zhao BY, Wang CL, Yang WC, et al. (1998) Identification and tagging a new gene for resistance to bacterial blight (Xanthomonas oryzae pv. oryzae) from O. rufipogon. Rice Genet Newslett 15: 138-142.

28. Khush GS, Angeles ER (1999) A new gene for resistance to race 6 of bacterial blight in rice, *Oryza sativa* L. Rice Genet Newslett 16: 92-93.

29. Guiderdoni E (1991) Gametic selection in anther culture of rice (*Oryza sativa* L.) Theor Appl Genet 81: 406-441.

30. Temnykh S, Park WD, Ayres N, Cartinhour S, Hauck N, et al. (2000). Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L) 100: 697-712.

31. McCouch SR, Teytelman L, Xu Y, Lobos KB, Clare K, et al. 2002, Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Research 9: 199-207.

32. Yamada T (1984) Multiple alleles at the Xa-l and Xa-kg loci for resistance to bacterial leaf blight. Rice Genet Newslett 1: 97-98.

33. Yoshimura SA, Yoshimura A, Saito N, Kishimoto M, Kawase M, et al. (1992) RFLP analysis of introgressed chromosomal segments in three near-isogenic lines of rice for bacterial blight resistance genes, Xa-1, Xa-3, and Xa-4. Jpn J Genet 67: 29-37.

34. Blair MW, McCouch SR (1997) Microsatellite and sequence-tagged site markers diagnostic for the rice bacterial leaf blight resistance gene xa-5. Theor Appl Genet 95: 174-184.

35. Singh K, Vikal Y, Singh S, Leung H, Dhaliwal HS, et al. (2002) Mapping of bacterial blight resistance gene xa8 using microsatellite markers. Rice Genet Newslett 19: 94-96.

36. Zhang G, Angeles ER, Abenes ML, Khush GS, HuangN (1996) RAPD and RFLP mapping for the bacterial blight resistance gene xa-13 in rice. Theor Appl Genet 93: 65-70.

37. Tan ZB, Zhang Q, Zhu LH, Wang CL (1998) RFLP mapping of a rice bacterial blight resistance gene Xa14. Hereditas 20: 30-33.

38. Ronald PC, Albano B, Tabien R, Abenes L, Wu KS, et al. (1992) Genetic and physical analysis of the rice bacterial blight disease resistance locus, Xa21. Mol Gen Genet 236: 113-120.

39. Yang Z, Sun X, Wang S, Zhang Q (2003) Genetic and physical mapping of a new gene for bacterial blight resistance in rice. Theor Appl Genet 106: 1467-1472.

40. Gu K, Tian D, Yang F, Wu L, Sreekala C, et al. (2004) High-resolution genetic mapping of Xa27(t), a new bacterial blight resistance gene in rice, *Oryza sativa* L. Theor Appl Genet 108: 800-807.

41. Gao DY, Liu AM, Zhou YH, Cheng YJ, Xiang YH, et al. (2005) [Molecular mapping of a bacterial blight resistance gene Xa-25 in rice]. Yi Chuan Xue Bao 32: 183-188.

Submit your next manuscript and get advantages of OMICS Group submissions

Unique features:

- User friendly/feasible website-translation of your paper to 50 world's leading languages
- Audio Version of published paper
- Digital articles to share and explore

Special features:

- 200 Open Access Journals
- 15,000 editorial team
- 21 days rapid review process
- Quality and quick editorial, review and publication processing
- Indexing at PubMed (partial), Scopus, EBSCO, Index Copernicus and Google Scholar etc
- Sharing Option: Social Networking Enabled
- Authors, Reviewers and Editors rewarded with online Scientific Credits
- Better discount for your subsequent articles

Submit your manuscript at: http://www.omicsonline.org/submission/

Citation: Gao D, Sun L (2013) *In vitro* Screening and Molecular Characterization of a Bacterial Blight Resistance Gene in Rice. J Rice Res 1: 104. doi: 10.4172/jrr.1000104