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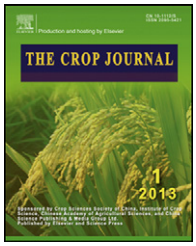

Identification and fine mapping of two blast resistance genes in rice cultivar 93-11

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ABSTRACT

Rice blast, caused by *Magnaporthe oryzae*, is a major disease of rice almost worldwide. The Chinese *indica* cultivar 93-11 is resistant to numerous isolates of the blast fungus in China, and can be used as broad-spectrum resistance resource, particularly in *japonica* rice breeding programs. In this study, we identified and mapped two blast resistance genes, *Pi60*(t) and *Pi61*(t), in cv. 93-11 using F_2 and F_3 populations derived from a cross between the susceptible cv. Lijiangxintuanheigu (LTH) and resistant cv. 93-11 and inoculated with *M. oryzae* isolates from different geographic origins. *Pi60*(t) was delimited to a 274 kb region on the short arm of chromosome 11, flanked by InDel markers K1-4 and E12 and cosegregated with InDel markers B1 and Y10. *Pi61*(t) was mapped to a 200 kb region on the short arm (near the centromere) of chromosome 12, flanked by InDel markers M2 and S29 and cosegregating with InDel marker M9. In the 274 kb region of *Pi60*(t), 93-11 contains six NBS-LRR genes including the two *Pia*/*PiCO39* alleles (*BGIOSGA034263* and *BGIOSGA035032*) which are quite close to the two *Pia*/*PiCO39* alleles (*SasRGA4 and SasRGA5*) in Sasanishiki and CO39, with only nine amino acids differing in the protein sequences of BGIOSGA035032 and SasRGA5. In the 200 kb region of *Pi61*(t), 93-11 contains four NBS-LRR genes, all of which show high identities in protein sequence with their corresponding NBS-LRR alleles in susceptible cv. Nipponbare. Comparison of the response spectra and physical positions between the target genes and other R genes in the same chromosome regions indicated that *Pi60*(t) could be *Pia*/*PiCO39* or its allele, whereas

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Pi61(t) appears to be different from *Pita*, *Pita-2*, *Pi19*(t), *Pi39*(t) and *Pi42*(t) in the same R gene cluster. DNA markers tightly linked to *Pi60*(t) and *Pi61*(t) will enable marker-assisted breeding and map-based cloning.

1. Introduction

Rice blast, caused by the fungus *Magnaporthe oryzae*, is an important disease in most rice production regions of the world because of its devastating effects on yield. In this pathosystem, pathotype- or race-specific resistance follows the gene-for-gene relationship [1]. *M. oryzae* is highly variable, and loss of resistance in varieties is quite common [2,3], especially when resistance is based on a single resistance (*R*) gene [4–6]. Nevertheless, the utilization of *R* genes is still considered to be the most effective and economical method to control the disease. A major strategy to develop more durable resistance is to combine multiple *R* genes that confer overlapping resistance spectra to multiple isolates/races of *M. oryzae* in a single variety [7]. In this regard, continued identification of new *R* genes in genetic resource materials is essential.

Genetic studies on blast resistance began as early as the 1960s and were intensified with the availability of genome sequences of the two subspecies of cultivated rice, *Oryza sativa* (ssp. *japonica* cultivar (cv.) Nipponbare and spp. *indica* cv. 93-11) and abundant genetic markers [8–10]. To date, more than 70 *R* genes and some quantitative trait loci (QTL) have been identified and mapped on rice chromosomes [11–13]. These *R* genes are largely clustered on chromosomes 6, 11 and 12, and involve different specificities [11–15]. Development of DNA markers closely linked to the *R* genes not only sets the stage for marker-assisted selection (MAS) in rice breeding programs, but also facilitates map-based cloning. Some blast *R* genes have been finely mapped [11,12,15–21], and among them *Pib* [22], *Pita* [23], *Pi9* [24], *Piz-t* and *Pi2* [25], *Pid2* [26], *Pi36* [27], *Pi37* [28], *Pikm* [29], *Pi5* [30], *Pid3*/*Pi25* [31,32], *Pit* [33], *Pish* [34], *pi21* [35], *Pb1* [36], *Pia*/*PiCO39* [37,38], *Pi-kh*/*Pi54* [39], *Pik* [40], *Pik-p* [41] and *Pi1* [21] have been isolated. Markers tightly linked to the *R* genes, and more recently, markers derived from cloned *R* genes should greatly facilitate pyramiding of the *R* genes into cultivars by MAS; for example, markers developed from *Pita* [42,43] and *Pib* [18].

The sequenced *indica* cv. 93-11 is a widely grown blast resistant cultivar and hybrid rice restorer in China [9,44–47]. It is resistant to *M. oryzae* races ZA49, ZE3 and ZG1 from Jiangsu, China [44], and to 80% of 45 *M. oryzae* isolates (22 from *japonica* and 23 from *indica*) from other provinces in China [48]. Although blast *R* gene *Pi41* was previously reported in cv. 93-11 [47], additional *R* genes must also be present [26,31,49–53]. The objectives of the present study were to evaluate blast resistance in cv. 93-11 using a wide range of Chinese *M. oryzae* isolates, and to identify and map *R* genes additional to *Pi41*.

2. Materials and methods

2.1. Rice genotypes and culture

Five rice cultivars, one near-isogenic line (NIL) and ten monogenic lines (MLs) were used in this study (Table 1). *Indica* cv. 93-11 (resistant, male parent) and *japonica* cv. Lijiangxintuanheigu (LTH, susceptible, female parent) were evaluated for reaction to *M. oryzae* isolates, and crossed to develop F_2 and F_3 populations for genetic analysis and gene mapping. Cultivars CO39, Aichi Asahi and IR64 together with 11 NILs/MLs, each carrying a single *R* gene (Table 1), were used as reference lines to differentiate the genes mapped in 93-11 from previously reported *R* genes. 93-11, LTH, Aichi Asahi, IR64 and F-128-1 are maintained at the

Table 1 – Rice genotypes evaluated for blast resistance.

ID	Designation	R gene	Remark	Reference
1	93-11	*Pi41*(t), ?	Chinese elite *indica* cultivar, *R* gene donor	[47]
2	LTH	None	Lijiangxintuanheigu, *japonica* cultivar with no blast *R* genes	[54,55]
3	IRBLa-A	*Pia*/*PiCO39*	LTH monogenic line	[38,56]
4	IRBLa-C	*Pia*/*PiCO39*	LTH monogenic line	[38,56]
5	Aichi Asahi	*Pia*/*PiCO39*, *Pi19*(t)	*Japonica* variety	[38,56,57]
6	CO39	*Pia*/*PiCO39*	*Indica* cultivar	[38,56]
7	IR64 [a]	*Pi30*(t)	*Indica* cultivar	[58,59]
8	IRBLta-K1	*Pita*	LTH monogenic line	[56]
9	IRBLta-CT2	*Pita*	LTH monogenic line	[56]
10	IRBLta-CP1	*Pita*	LTH monogenic line	[56]
11	IRBL12-M	*Pi12*(t)	LTH monogenic line	[56]
12	IRBL19-A	*Pi19*(t)	LTH monogenic line	[56]
13	IRBL20-IR24	*Pi20*(t)	LTH monogenic line	[56]
14	IRBLta2-Pi	*Pita-2*	LTH monogenic line	[56]
15	IRBLta2-Re	*Pita-2*	LTH monogenic line	[56]
16	F-128-1	*Pita-2*	LTH near-isogenic line	[54,55]

[a] Broad-spectrum blast resistant cultivar, harboring six *R* genes, viz. *Pi20*(t), *Pita*, *Pib*, *Pik*-s, *Piz*-t, and one unknown gene [58], or six *R* genes, *Pi25*(t), *Pi27*(t), *Pi29*(t), *Pi30*(t), *Pi31*(t) and *Pi32*(t) [59].