

*Pia-1 (RGA4)*を用いた系統解析実習

Pia

イネ (*Oryza sativa* L.) で同定されたイネいもち病抵抗性遺伝子
イネの11番染色体に座乗
*Pia*遺伝子座は *RGA4*と *RGA5*の2つの遺伝子で構成される

イネいもち病

糸状菌: *Pyricularia oryzae* Cavara (syn. *Magnaporthe oryzae* Couch and Kohn)
イネの幅広い部位に感染…葉、葉節、穂首、穂軸、枝梗、籾
イネの最重要病害…食料生産に甚大な被害をもたらす
病原性の異なる菌系統がある…病原性の分化、race分化

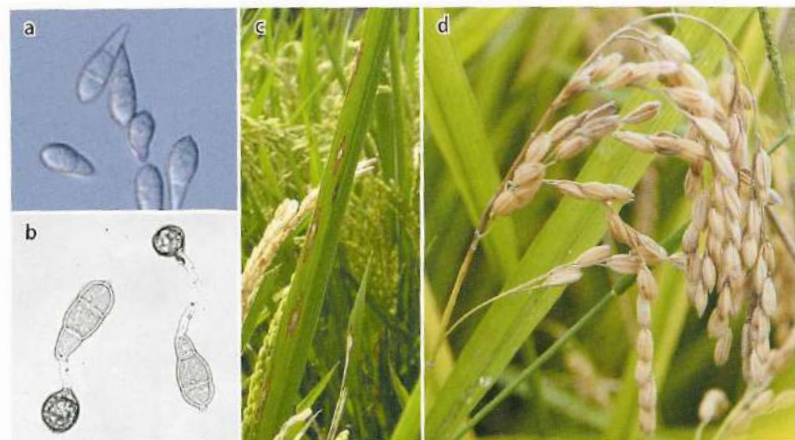


図 2-44 *Pyricularia oryzae* によるイネいもち病および菌

a: *Pyricularia oryzae* の分生子, b: 分生子から延びた発芽管およびその先端に形成されたメラニン化した付着器, c: イネ葉上に形成された病斑 (葉いもち), d: 穂いもち, (写真提供: 中馬いづみ氏 (a, c, d), 池田健一氏 (b))

眞山滋志 土佐幸雄 編, 2020
『植物病理学 第2版』 文英堂出版

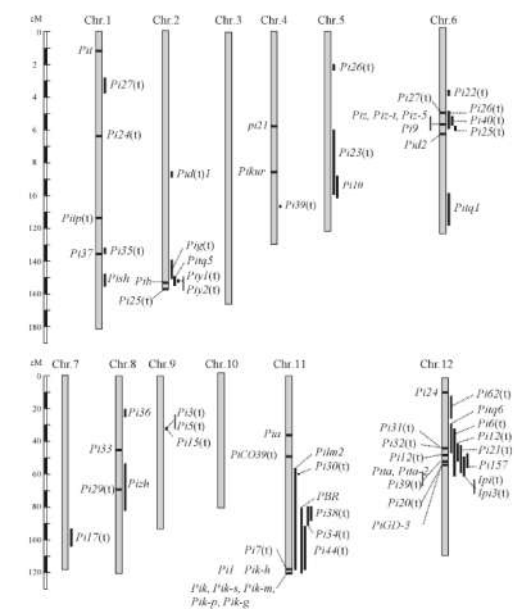


Fig. 1. Putative location of the blast resistance genes reported by 2008
The genetic location of each gene is based on the public databases (Oryzabase and Gramene) and the references for each gene (see Table 1).

Koide et al. 2009

Resistance Genes and Selection DNA Markers for Blast Disease in Rice (*Oryza sativa* L.) JARQ, Volume 43, Issue 4, Pages 255-280
<https://doi.org/10.6090/jarq.43.255>

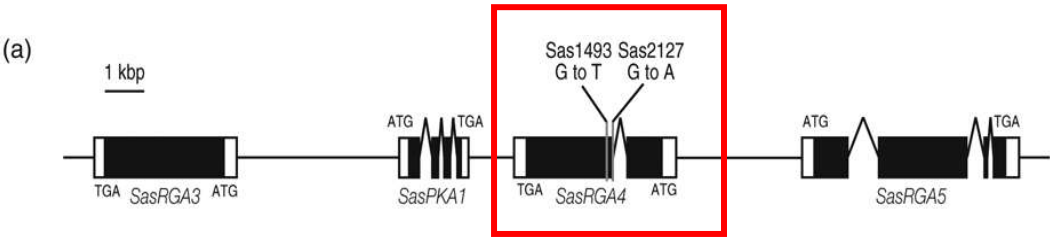
栽培試料（13系統）

No.	Plant species		Origin, Status	Cultivar/Accession name	Gene	備考
1	<i>Oryza sativa</i> L.	paddy rice	日本・育成	ひとめぼれ	<i>Pii, Piks, Pia-</i>	
2	<i>O. sativa</i> L.	paddy rice	インド・不明	Kasalath	<i>Pi13, Pia-, Pik-</i>	
3	<i>O. sativa</i> L.	paddy rice	バングラデシュ・在来	Tupa 729	?	
4	<i>O. sativa</i> L.	upland rice	日本・在来	七面鳥糯	?	
5	<i>O. sativa</i> L.	paddy rice	日本・在来/育成 中国・在来	白稻	?	DNA抽出できず
6	<i>O. sativa</i> L.	paddy rice	日本（山形）・育成	森田早生/森多早生	?	
7	<i>O. sativa</i> L.	paddy rice	日本・在来	かばしこ	?	
8	<i>O. sativa</i> L.	paddy rice	日本・育成	新山田穂2号	?	
9	<i>O. sativa</i> L.	paddy rice	日本（青森）・育成	ムツニシキ 水稻農林218号 ふ系88号	<i>Pikh, Pia</i>	
10	<i>O. sativa</i> L.	paddy rice	日本（愛知）・育成	愛知旭	<i>Pia, Pi19(t)</i>	
11	<i>O. sativa</i> L.		台湾	104 (Peh-kuh-tsao-tu)	<i>Pia</i>	
12	<i>O. sativa</i> L.	paddy rice	日本（青森）・育成	ふ系86	<i>Pia</i>	
13	<i>O. sativa</i> L.	paddy rice	日本（埼玉）・育成	関東51	<i>Pik, Pi19(t), Pia-</i>	
14	<i>O. barthii</i> A. Chev.	wild rice		W0720	?	

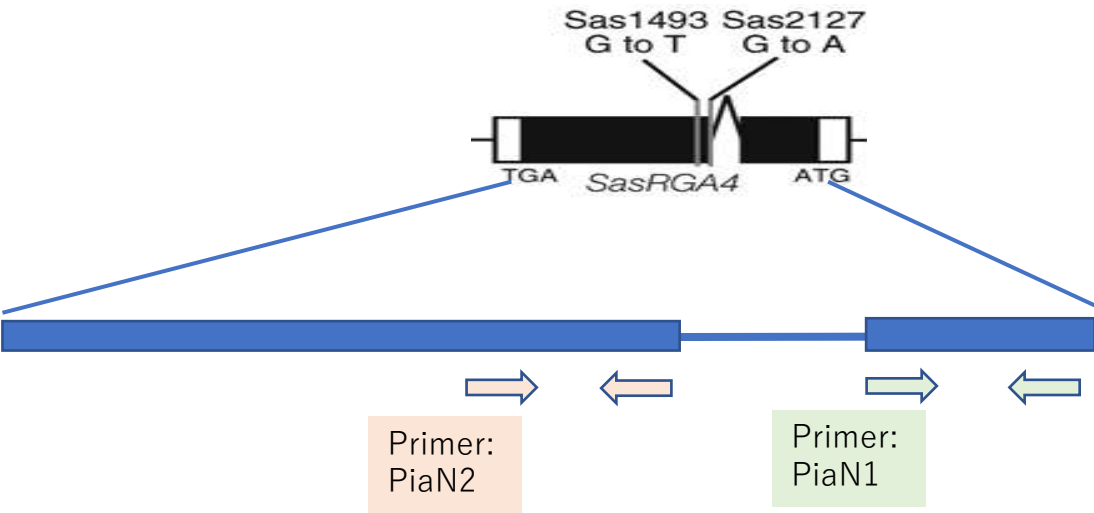
データベース由来の配列：ササニシキ・愛知旭（*Pia*）：AB604621，日本晴（*Pia-*）：Os11g0225100 + α

分担

__班：RGA4 exon1 ...Primer: PiaN1
__班：RGA4 exon2 ...Primer: PiaN2



Okuyama et al. 2011
A multifaceted genomics approach allows the isolation of the rice Pia-blast resistance gene consisting of two adjacent NBS-LRR protein genes
<https://doi.org/10.1111/j.1365-313X.2011.04502.x>



<i>Pia-1</i> (<i>RGA4</i>)	Os11g0225100 chr11:6541924..6546026 (-strand)
5'UTR	288 bp
exon1	842 bp
intron	393 bp
exon2	2203 bp
3'UTR	377 bp

Primer name	Primer sequence	Note	
PiaN1F	5'- ACAGACGCTATGCATGG AGG-3'	プライマー長: 20 bp Tm値: 59.894 °C GC含量: 55.000 %	F: 486-505 R: 976-993 産物長: 509bp CC motifを含む
PiaN1R	5'- CGTGCCTCTCGAATCTCC C-3'	プライマー長: 19 bp Tm値: 59.933 °C GC含量: 63.158 %	
PiaN2F	5'- GAAAGCTTGCCCAGAGA GGT-3'	プライマー長: 20 bp Tm値: 59.963 °C GC含量: 55.000 %	F: 1728-1747 R: 2271-2290 産物長: 563bp Sas2127を含む exon2前半 NBS motifを含む
PiaN2R	5'- GCCGACGAACATATTTG GGC-3'	プライマー長: 20 bp Tm値: 59.971 °C GC含量: 55.000 %	

プライマー設計に用いた配列（１）

日本晴 (*Pia-*)

Os11g0225100

Class=Sequence_position=chr11:6541924..6546026 (-strand)

Description: Nucleotide binding site-leucine rich repeats (NBS-LRRs) protein, Resistance to the blast fungus, (Nipponbare: susceptible to the blast fungus carrying the AVR-Pia)

Locus: Os11g0225100

Transcript variants: Os11t0225100-01

<https://rapdb.dna.affrc.go.jp/locus/?name=Os11g0225100>

…プライマー配列

…exon配列

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>Os11g0225100 class=Sequence_position=chr11:6541924..6546026 (-strand)
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[illegible]

プライマー設計に用いた配列（2）

ササニシキ・愛知旭（Pia）

Oryza sativa Japonica Group Os11gRGA4 gene for NBS-LRR type protein, partial cds, cultivar: Sasanishiki, Aichi-asahi

Locus: AB604621 6300bp DNA linear PLN 25-JUL-2016

Accession: AB604621

Version: AB604621.1

<https://www.ncbi.nlm.nih.gov/nuccore/336088135>

…プライマー配列
…exon開始位置

>AB604621.1 Oryza sativa Japonica Group Os11gRGA4 gene for NBS-LRR type protein, partial cds, cultivar: Sasanishiki, Aichi-asahi

TTTGTCTTTCCCTCTCTTTTCGTTTATCTGGGGGAGGTGATGATCCATGCCATGATTGATTACTTGTAGTCCACATATTGTAAGGTCCACGAGTAATCGAGTGACTCGAATCGTGATAGGTTGTCTATATCTTAAGAGAAAATTTCTTCTATATCCCTGAAAATT
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プライマー間の配列 [PiaN1F/R]

>Os11g0225100_primerN1FR

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>AB604621.1_2505cut_primerN1FR

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GGTCTACTCATCGGCTGCGGCGCAGGTGGATGAGCCATGGCCGTCGTGCTCATCTGCCTCAG
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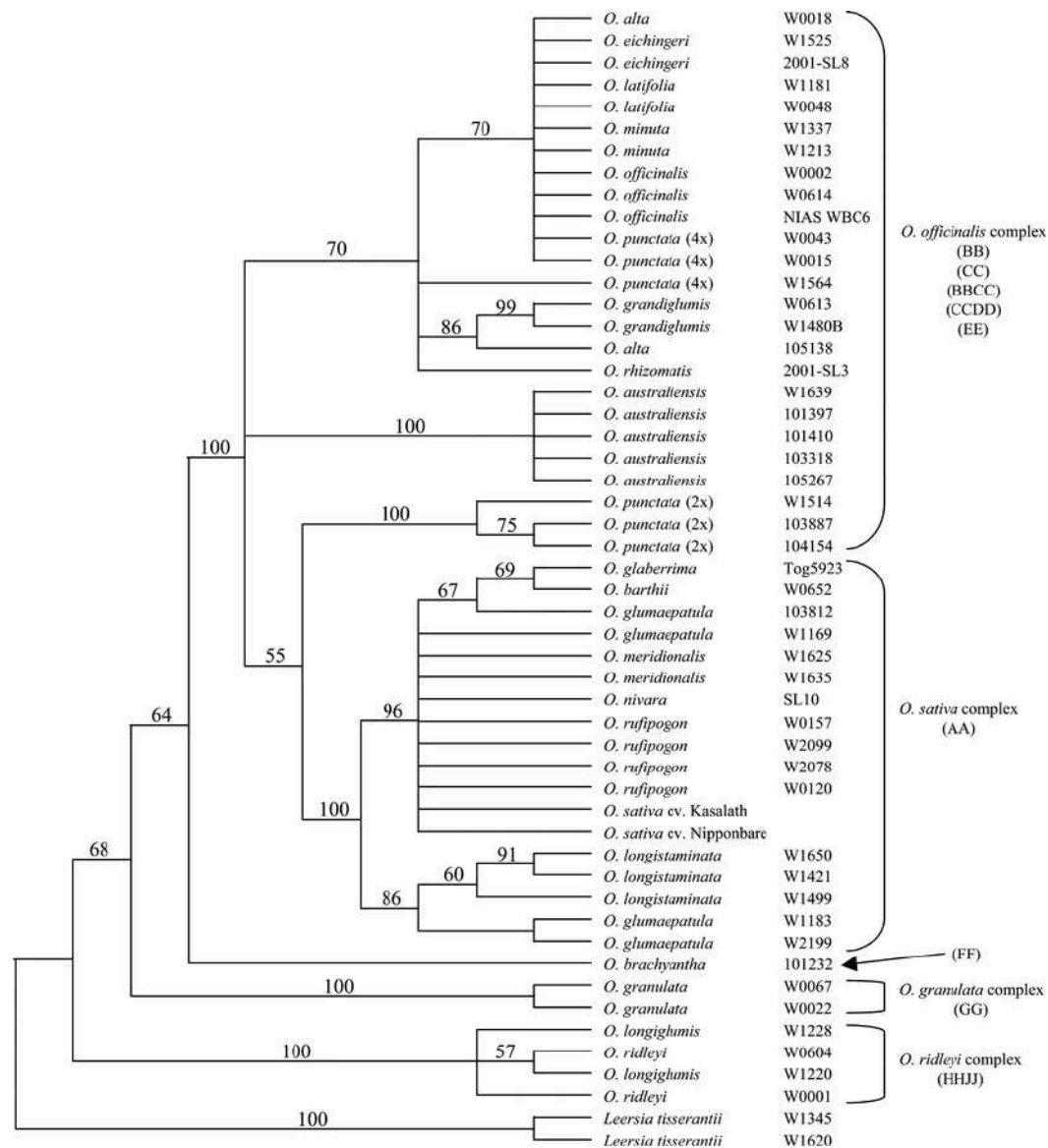
プライマー間の配列 [PiaN2F/R]

>Os11g0225100_primerN2FR

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>AB604621.1_2505cut_primerN2FR

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TGAGGCGATGGTTGGCCGAGGGATTTGTAGAACCACTGTCTTCATCGAGTAACATAGATTCCA
CGGCTGCTTTCAATGTGCTAATGGACCGGAACATCATTGAGCCCATCAATGTAAGCAATAATG
ATAAAGTCAAGACATGCCAAACGTACGGTATGATGCGTGAATTCATATCGCATATGTCAATCT
CTCAGAACTTTGTCACCTTTTTCTGTGATGACAAGTTCGTGCCCAAATATGTTTCGTCGGC



O. sativa complex (AA)

O. sativa
O. rufipogon
O. glumaepatula
O. meridionalis
O. nivara
O. glaberrima
O. barthii
O. longistaminata

Fig. 1 Phylogenetic tree of the genus *Oryza* based on 12 organelle single sequence repeats and their flanking sequences, using the maximum parsimony method. The tree is a strict consensus of the 4,394 most parsimonious trees. Tree length = 356, consistency index = 0.6657, retention index = 0.9043, rescaled consistency index = 0.6020. The numbers above the nodes represent bootstrap values expressed as percentage of 1,000 bootstrap replications

Nishikawa et al. 2005 Phylogenetic analysis of *Oryza* species, based on simple sequence repeats and their flanking nucleotide sequences from the mitochondrial and chloroplast genomes
 Theor Appl Genet. 2005 Feb;110(4):696-705.
<https://doi.org/10.1007/s00122-004-1895-2>

イネ関係の公開ゲノム配列

O. sativa complex

[AA]

Oryza sativa Japonica Group (Japanese rice) Cultivar: Kitaake

Genome assembly Osativa_Kitaake_v2.0

https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_009797565.1/

Oryza sativa (Asian cultivated rice) Cultivar: 93-11

Genome assembly ASM386521v1

https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_003865215.1/

Oryza sativa (Asian cultivated rice) Cultivar: Tetep

Genome assembly Tetep-1.0_genome

https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_004348155.2/

Oryza sativa Indica Group (long-grained rice) Cultivar: Zhenshan 97

Genome assembly ZS97RS3

https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_001623345.3/

Oryza glaberrima (African rice) Biological material: IRGC:96717

Genome assembly OglRS2

https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000147395.1/

Oryza rufipogon Isolate: MA-2024a

Genome assembly Oruf_Collapsed_v1.0

https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_046718375.1/

Oryza barthii Biological material: IRGC:105608

Genome assembly ObarRS3

https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_000182155.4/

Oryza glumipatula

Genome assembly OgluRS3

https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_000576495.2/

Oryza longistaminata

Genome assembly ASM980554v1

https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_009805545.1/

Oryza meridionalis

Genome assembly OmerRS3

https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_000338895.3/

O. officinalis complex

Oryza punctata [BB • BBCC]

Genome assembly OpunRS2 Biological material: IRGC:105690

https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_000573905.2/

Oryza officinalis [CC]

Genome assembly Oryza_officinalis_v1.0 Strain: W0002

https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_008326285.1/

O. granulata complex

Oryza meyeriana var. *granulata* [GG]

Genome assembly ASM522336v2

https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_005223365.2/

Pia関連資料

Okuyama et al. 2011

A multifaceted genomics approach allows the isolation of the rice Pia-blast resistance gene consisting of two adjacent NBS-LRR protein genes

<https://doi.org/10.1111/j.1365-313X.2011.04502.x>

Yoshida et al. 2009

Association Genetics Reveals Three Novel Avirulence Genes from the Rice Blast Fungal Pathogen *Magnaporthe oryzae*

The Plant Cell, Volume 21, Issue 5, 1573–1591

<https://doi.org/10.1105/tpc.109.066324>

Kanzaki et al. 2012

Arms race co-evolution of *Magnaporthe oryzae* AVR-Pik and rice Pik genes driven by their physical interactions

The Plant Journal, 72, 894–907

<https://doi.org/10.1111/j.1365-313X.2012.05110.x>

齋藤 ら, 2016

総説：イネいもち病抵抗性NLR免疫受容体によるエフェクターの認識と抵抗性誘導機構

日植病報 82:296–300

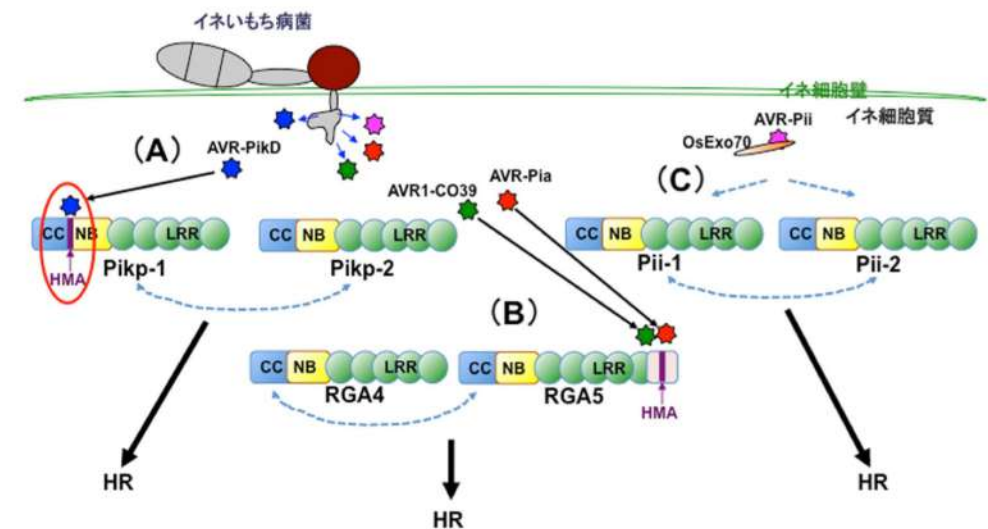
<https://doi.org/10.3186/jiphytopath.82.296>

Shimizu et al. 2022

A genetically linked pair of NLR immune receptors shows contrasting patterns of evolution

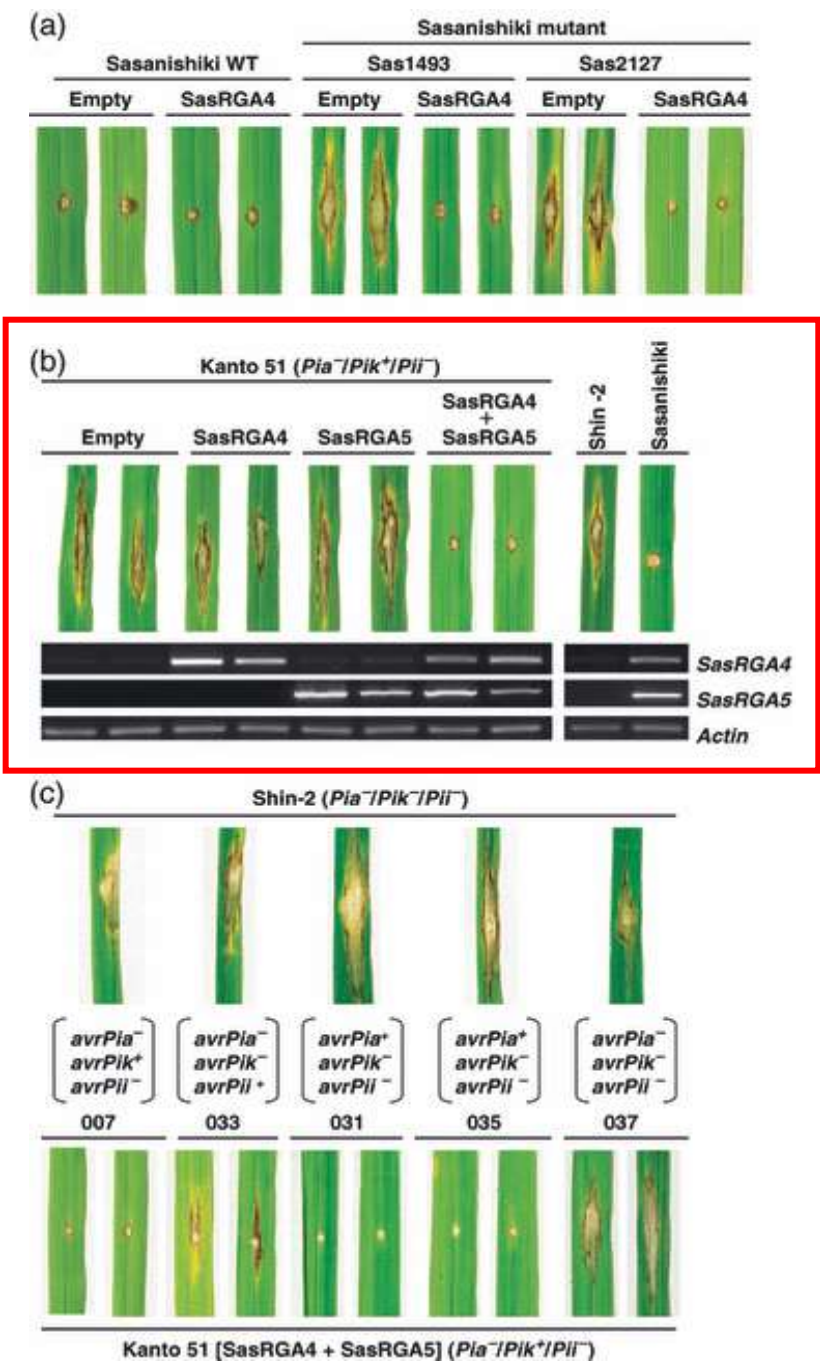
PNAS, Vol. 119 No. 27 e2116896119

<https://doi.org/10.1073/pnas.2116896119>



第1図 いもち病菌 AVR とイネ CC-NLR タンパク質との相互作用によって誘導されるイネいもち病抵抗性の分子機構

(齋藤ら, 2016)



*Pia*遺伝子座は*RGA4*と*RGA5*の2つの遺伝子で構成される

Okuyama et al. 2011
A multifaceted genomics approach allows the isolation of the rice *Pia*-blast resistance gene consisting of two adjacent NBS-LRR protein genes
<https://doi.org/10.1111/j.1365-313X.2011.04502.x>

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Locus Transcript

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RAP ID		Description	Position	Gene Symbol			Gene Name		
Locus ID	Transcript ID			RAP-DB	CGSNL	Oryzabase	RAP-DB	CGSNL	Oryzabase
<input type="checkbox"/> Os11g0225100	Os11t0225100-01	Nucleotide binding site-leucine rich repeats (NBS-LRRs) protein, Resistance to the blast fungus, (Nippponbare: susceptible to the blast fungus carrying the AVR-Pia) (Os11t0225100-01)	chr11:6541924..6546026 (- strand)	Os11gRGA4, RGA4, Pia	RGA4	Os11gRGA4, OsRGA4, Pias-1	R-gene analog 4	R-GENE ANALOG 4	R-gene analog 4
<input type="checkbox"/> Os11g0225300	Os11t0225300-01 Os11t0225300-02	Nucleotide binding site-leucine rich repeats (NBS-LRRs) protein, Resistance protein, Resistance to the blast fungus, (Nippponbare: susceptible to the blast fungus carrying the AVR-Pia) (Os11t0225300-01) Hypothetical conserved gene. (Os11t0225300-02)	chr11:6554522..6561687 (+ strand)	Os11gRGA5, RGA5, Pia	RGA5	Os11gRGA5, OsRGA5, RGA5-A, RGA5-B, Pi5, Pias-2	R-gene analog 5	R-GENE ANALOG 5	R-gene analog 5

1 to 2 of 2 hits 10 per page

IRGSP-1.0

6541500 b

chr11

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Predicted locus

Predicted genes

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5,000,000

10,000,000

15,000,000

20,000,000

25,000,000

chr11

chr11:6540861..6566420 (25.56 Kb)

Go

6,545,000

6,550,000

6,555,000

6,560,000

6,565,000

IRGSP-1.0

Zoom in to see sequence

Representative transcripts

Os11t0225000-01

serine/threonine protein kinase-related domain containing protein

Os11t0225200-00

Hypothetical protein

Os11t0225100-01

Nucleotide binding site-leucine rich repeats (NBS-LRRs) protein, Resistance to the blast ...

Os11t0225300-01

Nucleotide binding site-leucine rich repeats (NBS-LRRs) protein, Resistance protein, Resistance to the blast...

Os11t0225566-00

Hypothetical protein

Os11t0225300-02

Hypothetical conserved gene

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rap-db: The Rice Annotation Project Database
https://rapdb.dna.affrc.go.jp/

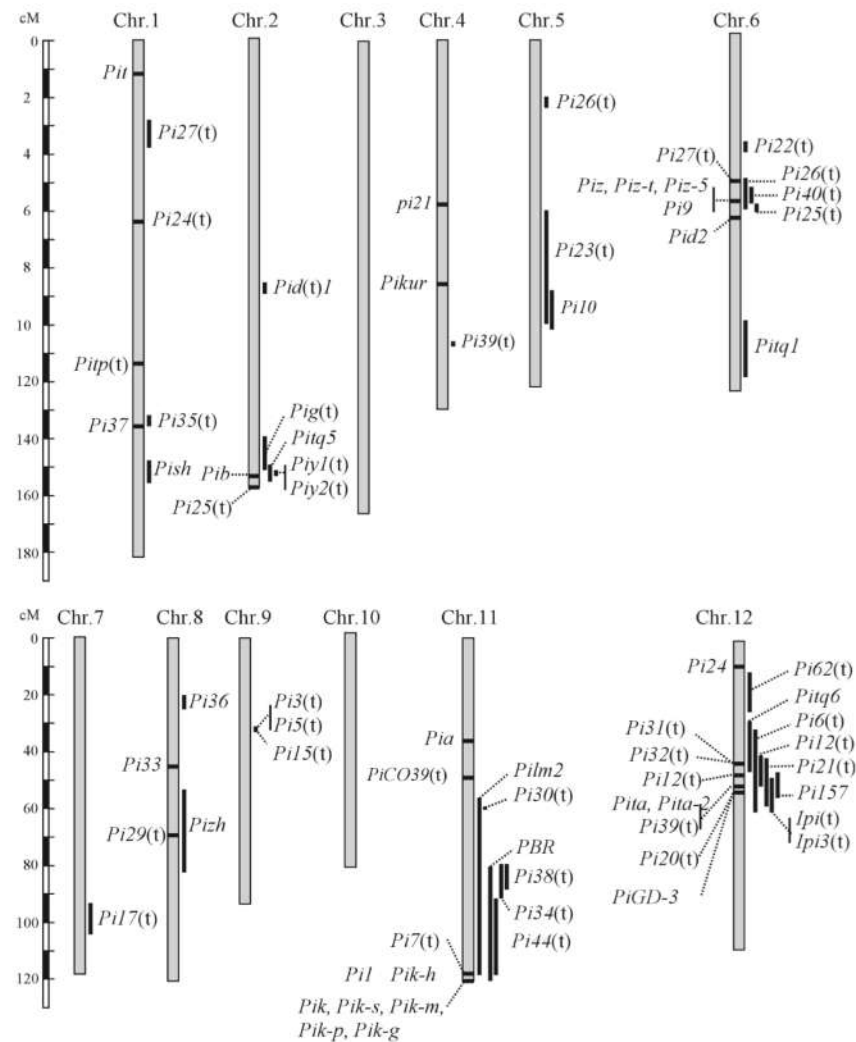


Fig. 1. Putative location of the blast resistance genes reported by 2008
The genetic location of each gene is based on the public databases (Oryzabase and Gramene) and the references for each gene (see Table 1).

Koide et al. 2009
Resistance Genes and Selection DNA Markers for Blast Disease in Rice
(*Oryza sativa* L.) JARQ, Volume 43, Issue 4, Pages 255-280
<https://doi.org/10.6090/jarq.43.255>

