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Original Article

Molecular profiling of bacterial blight resistance in Malaysian rice cultivars

Perfil molecular da resistência à ferrugem bacteriana em cultivares de arroz da Malásia

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Abstract

Bacteria blight is one of the most serious bacterial diseases of rice worldwide. The identification of genetic potential against bacterial blight in the existing rice resources is a prerequisite to develop multigenic resistance to combat the threat of climate change. This investigation was conducted to evaluate alleles variation in 38 Malaysian cultivars using thirteen Simple Sequences Repeats markers and one Sequence Tagged Sites (STS) marker which were reported to be linked with the resistance to bacterial blight. Based on molecular data, a dendrogram was constructed which classified the rice cultivars into seven major clusters at 0.0, 0.28 and 0.3 of similarity coefficient. Cluster 5 was the largest group comprised of ten rice cultivars where multiple genes were identified. However, *xa*13 could not be detected in the current rice germplasm, whereas *xa*2 was detected in 25 cultivars. Molecular analysis revealed that Malaysian rice cultivars possess multigenic resistance.

Keywords: rice, genetic potential, SSR, STS, multigene resistance.

Resumo

A ferrugem bacteriana é uma das doenças bacterianas mais graves do arroz em todo o mundo. A identificação do potencial genético contra a ferrugem bacteriana nos recursos de arroz existentes é um pré-requisito para desenvolver resistência multigênica no combate à ameaça da mudança climática. Esta investigação foi conduzida para avaliar a variação de alelos em 38 cultivares da Malásia usando 13 marcadores Simple Sequences Repeats (SSR) e 1 marcador Sequence Tagged Sites (STS), que foram relatados como associados à resistência à ferrugem bacteriana. Com base em dados moleculares, foi construído um dendrograma que classificou as cultivares de arroz em sete grandes agrupamentos a 0,0, 0,28 e 0,3 de coeficiente de similaridade. O Cluster 5 foi o maior grupo composto por 10 cultivares de arroz, no qual múltiplos genes foram identificados. No entanto, xa13 não pôde ser detectado no germoplasma atual de arroz, enquanto xa2 foi detectado em 25 cultivares. A análise molecular revelou que as cultivares de arroz da Malásia possuem resistência multigênica.

Palavras-chave: arroz, potencial genético, SSR, STS, resistência multigênica

1. Introduction

Rice (*Oryza sativa* L) is an important staple food for over 50% of the global population. Bacteria blight caused by *Xanthomonas oryzae* is the most destructing bacterial disease of rice (Nino-Liu et al., 2006;Mew 1987). The symptoms of bacterial blight in rice are characterized through drying and yellowing of leaves which usually started from the upper most tips of leaves kept proceeds downward to the petioles of leaves. Usually, the temperatures from 25–34 °C found to be favourable for the development of this disease, with median type of relative humidity above 70% (ChuKwu et al., 2019) The rice crop infected by bacterial blight can lose 10–20% and even up to

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80% of its yield, while major grains yield loss in the range of 2.5 to 16% in the tropics and subtropics (Dossa et al., 2020). The disease resistance breeding programme has been found to be more effective to combat with the losses of bacterial blight in rice, while the use of Single nucleotide polymorphisms (SNPs), Next-generation sequencing (NGS) and marker assisted backcross breeding are recent advanced techniques (Chen et al., 2020; Jamaloddin et al., 2020; Kumar et al., 2019). The modern omics techniques and approaches including the genomics, proteomics, transcriptomics, interactomics, metabolomics, etc. may be most helpful techniques for the identification of desired genes along with their products, usually involved the pathogen perception through host as well as response which is manifested from the host against all types of pathogenic attacks in crop plants (Kumar et al., 2020). A CRISPR–Cas9 genome-edited Kitaake rice kit has been developed for the identification and evaluation for the efficacy of effector-binding elements (EBEs), in SWEET gene promoters while a software to predict the optimal resistance genes for bacterial blight of rice (Eom et al., 2019).

The most effective and sustainable method of bacterial blight disease management is the cultivation of resistant cultivars (Habarurema et al., 2012; Yang et al., 2003). The subspecies of rice including japonica, javanica, and indica are consisted of a huge reservoir of rice germplasm which have been developed through intermingling of rice cultivars and landraces (Noreen et al., 2020). Till today, 40 genes have been identified for bacterial blight resistance (Hajira et al., 2016). Several genes (*xa4, xa5, xa7*, *xa13* and *xa21*) have been cloned and identified to produce novel cultivars that are resistant to bacterial blight (Perumalsamy et al., 2010). In addition, several genes such as *xa2*, *xa4*, *xa7*, *xa30*, *xa33* and *xa38* were found through physical mapping (Bhasin et al., 2012; Cheema et al., 2008; Nino-Liu et al., 2006; Sun et al., 2003; Yang et al., 2003; Song et al., 1995). In general, a single resistance gene against some race-specific pathogen is usually incorporated into the breeding programs. However, this method is not durable for long term breeding programs (Suh et al., 2009). Rice cultivars containing multiple resistance genes have been shown to deliver durable resistance against bacterial blight (Muhammad et al., 2016; Pradhan et al., 2015, Rajpurohit et al., 2011).

Molecular marker technologies are useful tools for the identification of desirable resistance genes as well as analysis of genetic diversity in plants (Erayman et al., 2014; Prabakaran et al., 2010; Davierwala et al., 2001). The molecular genetic diversity would contribute to preserve the desired alleles variation of genes for bacterial blight resistance. Microsatellite markers would be efficient in the profiling of alleles variation of resistance genes for bacterial blight in Malaysian rice cultivars (Song et al., 2014). This is essential to enrich gene pool in rice germplasm which could be utilized for crop improvement. Hence, present study was focused to identify the presence of genes, linked to bacterial blight resistance, in Malaysian rice cultivars and genetic diversity analysis.

2. Materials and methods

2.1. Plant materials

A total of 38 Malaysian rice cultivars were used in this study. The seeds of these rice cultivars were collected from Malaysian Agriculture Research and Development Institute (MARDI) as given in Table 1.

2.2. Seed germination and DNA extraction

The seeds samples were subjected to surface sterilization as described by Pradhan et al. (2014). Germinated were transferred into seedling trays. The leaves of two-week-old seedlings were used to extract the genomic DNA as described by Ikeda et al. (2001), where liquid nitrogen is not required for DNA extraction. 0.02 g of fresh leaf tissue was used for DNA extraction. Before slicing the plant tissue, the scissors were sterilized with absolute ethanol. Sliced pieces of leaves were transferred to 1.5 ml microtube. 200 µl of TE buffer (10 mM Tris-HCI, 1 mM EDTA, pH 8.0) were added and the leaves were grounded by using a grinder. Tubes were placed in boiling water (100 ºC) for 20 minutes. 800 µl of TE buffer (10 mM Tris-HCI, 0.1 mM EDTA, pH 8.0) were added and then mixed by vortex for 25 second and centrifuged at 14000 rpm at room temperature for 3 minutes to elute the DNA products. Lastly, the supernatant was transferred to a new 1.5 ml microtube and stored at -20 ºC for further analysis.

2.3. Polymerase chain reaction

PCR reactions were carried out in a 25 µL volume containing 6 µl of Template DNA, 0.125 µl of 10 mM dNTP mix, 0.25 unit of Taq polymerase, 2.5 µl 10x Taq buffer, 2 µl of 2 µm forward and reverse primer, 2.8 µl of 25 mM $MgCl_2$ and 11.325 µl of sterile nucleus free water. PCR amplification was performed in a gradient thermocycler programs with the initial denaturation of 94°C for 2 minutes. It was followed by 39 cycles of denaturation at 94 °C for 30 seconds, annealing temperature around 2 ºC less than TM value of the respective primers for 45 seconds, 72 °C for 90 seconds and then the final extension at 72 °C for 10 minutes. Lastly, the PCR products were held at 4°C. PCR products were kept at -20°C for further analysis.

2.4. Microsatellite selection and cluster analysis

The molecular markers were selected from the literature which were reported to be linked with the genes controlling resistance against bacterial blight in rice. The banding patterns, the band size and the numbers of bands as well as the visibility of these bands were used as criteria in the selection of thirteen Simple Sequences Repeats (SSR) markers and one Sequence Tagged Sites (STS). The description of these markers is given in Table 2.

The banding pattern of each amplified PCR products were scored as "+" indicating the presence of resistance and "- "indicating the absence of resistance gene. The data was exported to PAST software for cluster analysis and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram (Hammer et al., 2001) for genetic diversity analysis. Dice coefficient subprogram

Table 1. List of Malaysian rice cultivars, with year of release.

No.	Rice variety	Accession No.	Year of release	No.	Rice variety	Accession No.	Year of release
1	Seberang	04559	1985	20	Pulut Hitam 9	08363	1991
2	Ria	01478	1973	21	Mahsuri	00826	1966
3	MR220	11634	2003	22	Bahagia	00167	1964
4	MR81	04630	1986	23	MR84	04633	1988
5	Manik	04556	1984	24	Sri Malaysia II	02672	1974
6	MR232	12047	2006	25	MR263	-	2010
7	Murni	01041	1972	26	Sekembang	04553	1979
8	MR123	07488	1990	27	Maswangi (MRQ74)	11787	2005
9	MR159	08638	1995	28	MR219	11633	2001
10	MR106	07487	1990	29	Jaya (Malaysia)	00581	1965
11	MR167	08646	1997	30	Pulut Siding	04555	1981
12	Sri Malaysia I	02125	1974	31	MR127	07489	1991
13	MRM 16 (Padi Merah)	-	2010	32	Kadaria	04554	1981
14	Muda	04557	1984	33	Malinja	00839	1968
15	Sekencang	04552	1979	34	MR253	12095	2010
16	Setanjung	04551	1979	35	Masria	00860	1972
17	MR185	08455	1995	36	Puteri (MRQ50)	09345	1999
18	Makmur	04558	1984	37	MR103	07486	1990
19	Pulut Malaysia I	02123	1974	38	MR211	1629	1999

in UPGMA software implemented in PAST software was used to categorize the Malaysian rice cultivars for the resistance potential of each cultivar. The different levels of resistance in the rice cultivars were reported in the form of phylogenetic tree. PIC analyses were performed using PIC calculator software.

3. Results and discussion

Cluster analysis was used to identify the genetic relationship among the rice cultivars. Various patterns of resistance against bacterial blight were observed in the rice cultivars (Figure 1). This dendrogram was constructed based on Nei's genetic distance. UPGMA dendrogram separates 38 germplasm into two major clusters. The genetic distance between the rice cultivars pairs was calculated based on the dice coefficient from combined data for 14 primers, ranged from 0 to 1 (Nei, 1972). The genetic similarity among the pair-wise is ranged from 0.00 to 0.91 with an average of 0.39 among the rice varieties. Result of genetic similarity showed the presence of broad range of genetic variability in Malaysian rice cultivars at the molecular level. Rice cultivars with similar disease resistant patterns were clustered into the same group and it is useful for exploitation of desired alleles variation in Malaysian rice (Song et al. 2014).

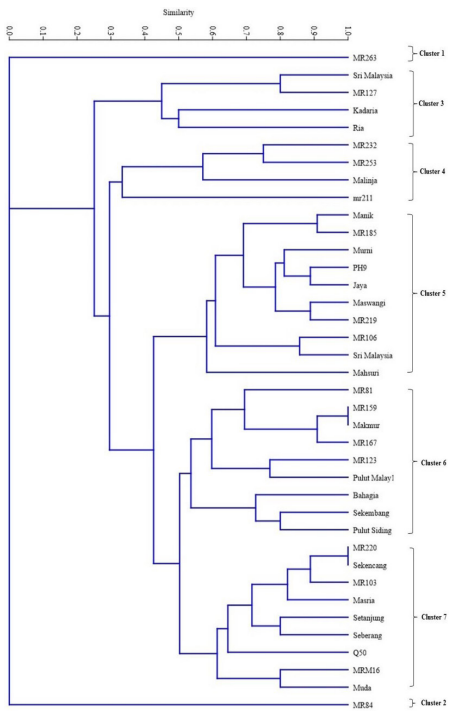

Figure 1. UPGMA dendrogram showed the patterns of resistance to bacterial blight in Malaysia rice varieties, based on scoring of 13 SSR and 1 STS.

Table 2. Description of selected molecular markers.

No.	Marker (Gene)	Location *	Primer sequence	References
1	RM122 *(xa5)*	5	F: GAGTCGATGTAATGTCATCAGTGC	Shanti et al. (2010)
			R: GAAGGAGGTATCGCTTTGTTGGAC	
2	RM21 *(xa5)*	11	F:ACAGTATTCCGTAGGCACGG	Pha and Lan (2004)
			R:GCTCCATGAGGGTGGTAGAG	
3	RM206 *(xa10)*	11	F:CCCATGCGTTTAACTATTCT	Chen et al. (1997)
			R:CGTTCCATCGATCCGTATGG	
4	RM159 *(xa5)*	5	F:GGGGCACTGGCAAGGGTGAAGG	Pinta et al. (2013)
			R:GCTTGTGCTTCTCTCTCTCTCTCTCTC	
5	*xa13 promoter (xa13)*	8	F:TCCCAGAAAGCTACTACAGC	Hajira et al. (2016)
			R:GCAGACTCCAGTTTGACTTC	
6	RM224 *(xa4)*	11	F: ATCGATCGATCTTCACGAGG	Chen et al. (1997)
			R: TGCTATAAAAGGCATTCGGG	
7	*pTA 248 (xa21)*	11	F:AGACGCGGGAAGGGTGGTTCCCGGA	Davierwala et al. (2001)
			R:AGACGCGGGTAATCGAAAGATGAAA	
8	RM251 *(xa7/8)*	3	F: GAATGGCAATGGCGCTAG	Chen et al. (1997)
			R: ATGCGGTTCAAGATTCGATC	
9	RM317 *(xa2)*	4	F:CATACTTACCAGTTCACCGCC	He et al. (2006)
			R:CTGGAGAGTGTCAGCTAGTTGA	
10	RM13 *(xa5)*	5	F:TCCAACATGGCAAGAGAGAG	McCouch et al. (1988)
			R:GGTGGCATTCGATTCCAG	
11	RM167 (xa3/4)	11	F:GATCCAGCGTGAGGAACACGT	Wu and Tanksley (1993)
			R:AGTCCGACCACAAGGTGCGTTGTC	
12	RM230 *(xa13)*	8	F:GCCAGACCGTGGATGTTC	Chen et al. (1997)
			R:CACCGCAGTCACTTTTCAAG	
13	RM347 *(xa11)*	3	F: CACCTCAAACTTTTAACCGCAC	Goto et al. (2009)
			R: TCCGGCAAGGGATACGGCGG	
14	RM263 *(xa8)*	2	F: CCCAGGCTAGCTCATGAACC	Chen et al. (1997)
			R:GCTACGTTTGAGCTACCACG	

*: Chromosome number.

Based on the dendrogram result, the cophenetic correlation coefficient is 0.778 for binary fission for presence and absence of resistance in 38 rice cultivars. High cophenetic correlation coefficient indicates that closed relationship between the matrix binary data and the dendrogram constructed. Cluster and dendrogram constructed were robust as the cophenetic correlation coefficient is very high (Mayer et al., 2010). Rice cultivars were grouped using unweighted pair group method with arithmetic means. High level of genetic similarity indicated that having the closed relationship between the rice cultivars pairs. It provides information to identify the genetic diversity among the rice cultivars (Khan et al., 2015). The results also provide the baseline in the understanding the relationship between the resistance genes and Malaysian cultivars. The dendrogram indicated that rice genotypes had similar banding pattern were grouped in the same cluster. Genotypes having the large value of similarity indicated narrow level of genetic distance among the genotypes. A low value of genetic similarity indicated high diversity among the cultivar pairs.

Genetic similarity among the Malaysian rice cultivars pair-wise varies from 0.00 to 0.91. Maswangi (MRQ74) possesses multiple resistance genes while MR84 and MR263 do not possess any resistance gene (Table 3). Genetic similarity between Maswangi (MRQ74) and MR84 is 0.000 which is the least level of genetic similarity (Figure 1). The presence of multiple resistance genes in Maswangi (MRQ74) would be useful for the development of durable bacterial blight resistant cultivars in breeding programs (Evamoni et al., 2014). Rice cultivars had the high level of genetic similarity showed small genetic distance among the pair-wise. SSR and STS markers distinguish the thirty-eight rice cultivars based on genetic distance and

Table 3. Scoring of bands of molecular markers in Malaysian rice cultivars.

Rice cultivars		Scoring of bands in respective molecular markers (-: Absent +: Present) (1-14 markers as given in Table 2)														
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**# genes**
1	Seberang	-	-	-	-	-	+	+	-	+	-	-	-	+	-	4
2	Ria	-	-	-	-	-	-	-	-	+	-	-	-	+	-	2
3	MR220	-	-	+	-	-	+	+	-	+	-	-	-	-	-	4
4	MR81	-	-	-	-	-	+	-	-	-	-	-	-	-	-	1
5	Manik	+	-	+	+	-	-	+	-	-	+	-	-	-	+	6
6	MR232	-	+	-	-	-	-	+	+	-	-	+	-	-	+	5
7	Murni	+	-	-	+	-	+	-	-	+	+	-	-	-	+	6
8	MR123	-	-	+	+	-	+	-	-	+	-	+	-	+	+	7
9	MR159	-	-	-	+	-	+	+	-	+	-	-	-	+	-	5
10	MR106	+	+	+	-	-	-	+	+	+	+	+	-	-	-	8
11	MR167	-	-	-	+	-	+	+	+	+	-	-	-	-	-	5
12	Sri Malaysia I	+	+	+	-	-	-	+	+	+	+	+	-	-	-	8
13	MRM16 (Padi Merah)	-	-	-	-	-	+	-	+	+	-	-	-	+	+	5
14	Muda	-	-	-	-	-	+	+	-	+	-	-	-	+	+	5
15	Sekencang	-	-	+	-	-	+	-	-	+	-	-	-	-	-	3
16	Setanjung	-	+	+	+	-	+	+	-	+	-	-	-	+	-	7
17	MR185	+	-	+	-	-	-	+	-	+	+	-	-	-	+	7
18	Makmur	-	-	-	+	-	+	+	+	+	-	-	-	+	-	5
19	Pulut Malaysia I	-	-	+	+	-	-	-	+	+	-	-	-	-	+	5
20	Pulut Hitam 9	+	-	+	-	-	+	+	+	+	+	-	-	-	+	8
21	Mahsuri	+	+	-	+	-	-	-	-	-	+	-	-	+	+	6
22	Bahagia	-	+	-	-	-	+	-	+	-	-	+	-	+	+	6
23	MR84	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
24	Sri Malaysia II	-	-	+	-	-	-	+	-	-	-	-	-	-	-	2
25	MR263	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
26	Sekembang	-	+	-	-	-	+	-	+	+	-	+	-	-	-	5
27	Maswangi (MRQ74)	+	+	+	+	-	+	+	-	+	+	-	-	+	+	10
28	MR219	+	+	-	+	-	+	+	-	+	+	-	-	-	+	8
29	Jaya (Malaysia)	+	-	+	+	-	+	-	+	+	+	-	-	+	+	9
30	Pulut Siding	-	-	-	-	-	+	-	+	+	-	+	-	-	+	5
31	MR127	-	-	-	-	-	-	+	-	-	-	-	-	+	-	2
32	Kadaria	-	-	-	-	-	-	-	-	-	-	+	-	+	-	2
33	Malinja	-	-	-	-	-	-	+	-	-	-	+	-	-	-	2
34	MR253	-	-	+	-	-	-	+	-	-	-	+	-	-	+	4
35	Masria	-	-	+	-	-	+	-	-	+	-	-	-	-	-	3
36	Puteri (MRQ50)	-	-	-	-	-	+	-	-	+	-	-	-	-	-	2
37	MR103	-	-	+	-	-	-	-	-	+	-	-	-	-	+	3
38	MR211	-	-	+	-	-	-	+	-	-	-	-	-	-	+	3
Numbers of cultivars		**10**	**9**	**17**	**12**	**0**	**21**	**20**	**12**	**25**	**10**	**10**	**0**	**14**	**18**	

Number of genes.

genetic similarity. Rice genotype with low level of genetic distance was found to be closed in the dendrogram using dice coefficient method. The resistant genes for bacterial blight reported by Chen et al. (2011) and Hajira et al. (2016) could not be detected in here, using RM 230 and *xa* 13 promoter, respectively. The maximum and minimum frequencies of *xa* 2 and *xa* 5 were observed in the current studies, respectively (Table 3). MR84 and MR263 did not show the presence of any gene for bacterial blight resistance, whereas Maswangi showed the presence of 10 genes (Table 3). Similar studies have been reported by Sombunjitt et al. (2017), where resistance for bacterial blight was investigated in Thai rice.

Based on the UPGMA dendrogram, a total of 38 rice cultivars had been separated into seven major groups. MR84 and MR263 were grouped into Cluster 1 and Cluster 2 alone. Both cultivars do not possess any resistance genes against bacterial blight and thus no similarity was found as compared with other rice cultivars. These two cultivars exhibited the least genetic similarity index (0.00) so maximum genetic distance was observed in UPGMA dendrogram. RIA, Kadaria, MR127 and Sri Malaysia 2 were found in cluster 3 at the similarity coefficient 0.45. In this cluster, all rice cultivars showed the presence of two bacterial blight resistance genes. Cluster 4 comprised of four Malaysian rice cultivars (MR232, MR253, Malinja and MR211) at the similarity coefficient 0.35. These rice varieties also harboured 2 bacterial blight resistance genes and different types of resistance genes were found in this group as compared to the cluster 3. Cluster 5 was the largest group comprised of 10 rice cultivars (Manik, MR185, Murni, Pulut Hitam 9, Jaya, Masawangi (MRQ74), MR219, MR106, Sri Malaysia 1 and Mahsuri) at similarity coefficient 0.6. This group comprised of rice cultivars that carrying multiple resistance genes against bacterial blight. Rice cultivars in cluster 5 had maximum bacterial blight resistance gene as compared to another cluster. Cluster 6 was the second largest group comprised of nine rice cultivars. MR81, MR159, Makmur, MR167, MR123, Pulut Malaysia, Bahagia, Sekembang and Pulut Siding were grouped into cluster 6. In cluster 6, rice cultivars possessed bacterial blight resistance genes, ranged from 5 to 8. MR220. Sekencang, MR103, Masria, Setanjung, Seberang, Q50, MRM16 and Muda were grouped into cluster 7. Rice cultivars in cluster 7 had maximum four bacterial blight resistance genes. Malaysian rice germplasm were clustered based on the bacterial blight resistance genes using molecular markers. SSR markers provide the guidance to identify the suitable gene donor and recipients of the resistance genes for future rice breeding programs (Evamoni et al., 2014). Based on UPGMA dendrogram, highest level of resistance response in Malaysian rice varieties were observed in cluster 5 and cluster 6. This information would be significant in improving the stability and resistant potential of rice cultivars. Wang et al. (2017) inducted *xa*10 gene in *japonica* rice cv Nipponbare and confirmed the resistance against bacterial blight. Current study revealed the presence of this gene in 17 Malaysian cultivars, it reflects that current Malaysian rice germplasm possesses resistance against this biotic stress. This information would be useful in pyramiding the genes of resistance in certain cultivars which are lacking resistance genes against bacterial blight e.g. MR 263 (Evamoni et al., 2014). Basmati rice is reported to be very sensitive to bacterial blight, however Baliyan et al. (2018) integrated the resistance in Basmati rice by marker assisted selection.

4. Conclusion

Evaluation of alleles variation, linked to resistance genes among rice cultivars, is the essential strategy to explore the resistance potential of rice germplasm against bacterial blight. Various alleles for different bacterial blight resistance genes were detected in Malaysian rice cultivars. A dendrogram separated the 38 Malaysian rice varieties into seven major clusters at 0.0, 0.25 and 0.3 of similarity coefficient. Cluster 5 is the largest group comprised of 10 rice cultivars carrying the multiple resistance genes. Maswangi (MRQ74) would be a potential donor parent for bacterial blight resistance in Malaysian rice breeding program. Presence of resistance genes in Malaysian rice varieties would facilitate further the rice breeders to develop the resistant cultivars carrying durable resistance genes against bacterial blight. Multigene resistance is a robust strategy in the era of climate change for a sustainable food security.

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