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Inheritance of bacterial leaf blight resistance in rice cultivar, Ajay

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Abstract

Nature and mode of inheritance of bacterial leaf blight (BLB) resistance of rice cultivar, Ajay (IET4141-CR98-7216) was studied against a highly virulent culture of *Xanthomonas oryzae pv. Oryzae* from Northern India corresponding to pathotype II reported earlier from India. The segregation pattern for BLB reaction in F_2 and F_3 generations from the cross of cultivar Ajay with the susceptible cultivar, Taichung Native I suggested that BLB resistance in cultivar Ajay is controlled by independently inherited duplicate genes. No relationship was observed between any polymorphic isozyme of either peroxidase or esterase and BLB resistance gene(s) in cultivar Ajay.

Key words : Bacterial leaf blight resistance, isozyme, inheritance, *Oryza sativa*

Introduction

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae pv. oryzae* Ishiama sp. Nov. nom. Rev. is a major constraint limiting rice yield throughout south east Asia, including India [1]. Although the environmental factors contribute significantly towards the spread of this disease, the inherent susceptibility of rice cultivars is primarily responsible for outbreak of the epidemics [2]. For an effective and long lasting control of any plant disease, information on its mode of inheritance and the allelic relationships of the genes from resistance donors is useful [3]. So far, 21 genes for resistance to *X. oryzae pv. oryzae* have been identified and designated as *Xa1* through *Xa21* [4]. However, none of the known genes for BLB resistance were found to be effective against all the isolates to BLB pathotypes prevalent in India [5]. In such a situation combining diverse genes from different sources may prove useful for genetic control of this disease. Since the selection of more than one gene in segregating populations using avirulent pathogen cultures is difficult, particularly at adult plant stage, biochemical and molecular markers can effectively be used. Co-segregation of various isozymes loci in relation to resistance genes has been exploited for quick selection of such genes [6]. The present study was therefore, planned to examine the inheritance of BLB resistance of a high yielding cultivar Ajay in relation to the isozymes of peroxidase and eastrase.

Materials and methods

The susceptible cultivar Taichung Native 1 (TN1) was crossed with the resistant cultivar Ajay (IET4141-CR98-7216) during 1996 in the experimental farm of Department of Genetics and Biotechnology, Punjab Agricultural University, Ludhiana. The F_1 generation was grown during Rabi 1996-97 to get F_2 seed. The F_2 generation was single plant harvested during kharif 1997 and the seed from each F_2 plant was separately sown during 1998 in two metre long rows alongwith earlier saved F_1 and F_2 seed during kharif 1997 and 1998, respectively. All the plants of F_1, F_2 and F_3 generations were inoculated using a single colony culture of *X. oryzae pv. oryzae* corresponding to pathotype II [7] virulent on the single gene lines carrying *Xa1, Xa3, xa5, Xa7, xa8, Xa10, xa11, Xa14, Xa18* and having avirulence on *Xa21*. The inoculation was done following leaf clipping method of Kauffman *et al.*, [8] at heading stage using 48 hours old culture of the pathogen maintained in Waki-moto medium. The observations on BLB reaction were recorded on a 0-9 scale according to Standard Evaluation System (SES) of the International Rice Research Institute, Philippines. The plants with score 0-5 were classified resistant and those with scores more than 5 were classified as susceptible. The number and nature of resistance genes present in cultivar Ajay was ascertained by fitting appropriate genetic ratio in F_2 and F_3 generations.

The starch gel electrophoresis technique [9] was used to examine polymorphism for the isozymes of peroxidase and esterase from the crude flag leaf extract of each plant (prepared by grinding 1g leaf sample/1.5 ml (W/V) 0.1M Tris Hcl buffer). The two isozymes were visualized by immersing the gel slices into different staining mixtures. For peroxidase, the gel was benzidine stained using the method of Veech [9]. For esterase,

method of Harris and Hopkinson [10] was used for staining the gels.

Chi square (x^2) test was applied to fit appropriate genetic ratios in F_2 and F_3 generations.

Results and discussion

The disease reaction of cultivar Ajay, TN_1 and all the plants in F_1, F_2 and F_3 generations from the cross was recorded and the segregation ratio in F_2 and F_3 generation is given in Table 1. The BLB score

Table 1. Segregation for bacterial leaf blight reaction in F_2 and F_3 generations from the cross of cultivar Ajay with TN1.

Generation and segregation pattern								
F_2				F_3				
No. of plants		Postulated ratio	X^2	No. of families			Postulated ratio	X2
R	S			HR	Segr.	HS		
126	8	15;1	0.02	39	55	10	7:8:1	2.99

R = Resistant; S = Susceptible; HR = Homozygous resistant; Segr. = Segregating; HS = Homozygous susceptible.

for cultivars Ajay and TN_1, were 1.0 and 9.0 respectively. The F_2 generation contained 126 resistant and 8 susceptible plants segregating in 15 resistant and 1 susceptible ratio (x^2 = 0.02). In F_3 generation, 39 homozygous resistant, 55 segregating and 10 homozygous susceptible families were obtained which fit in a 7 homozygous resistant : 8 segregating 1 homozygous susceptible ratio (x^2 = 2.99). These F_2 and F_3 ratios, indicate that the BLB resistance in cultivar Ajay is conditioned by duplicate genes.

The electrophoretic assay for peroxidase activity revealed six anodal bands and three cathodal bands for cultivar Ajay (Figs. 1 and 2). The susceptible parent TN_1 showed six anodal and two cathodal bands.

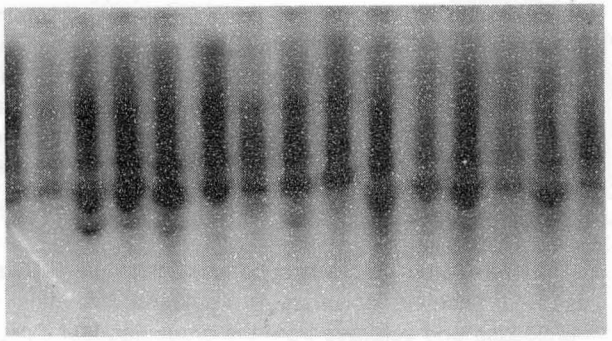

Fig. 1. Presence of isoperoxidase band C_2 in resistnat parent, F_1 and resistant/susceptible F_2 plants from the cross Ajay/TN_1 (R = BLB resistant plants, S = BLB susceptible plants)

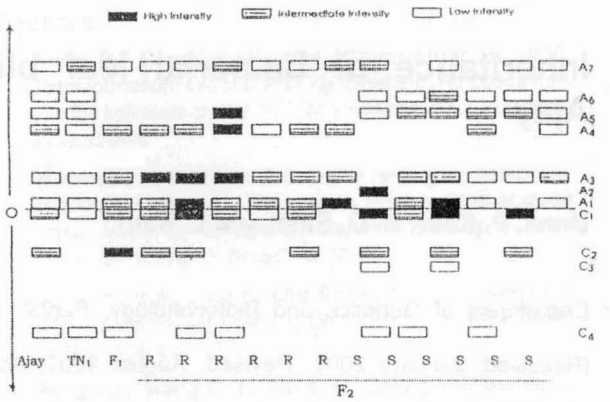

Fig. 2. Zymogram indicating presence/absence of isoperoxidase band C_2 in parents, F_1 and resistant/ susceptible F_2 plants from the cross Ajay/TN_1 (R = BLB resistant plants, S = BLB susceptible plants)

Cathodal band C_2 present in cultivar Ajay was absent in TN_1. Therefore, the inheritance of the band C_2 has been studied. The band C_2 showed its presence in F_1 and it segregated in F_2 plants. Out of the 134 F_2 plants studied, 67 plants the band C_2 showed its presence. The expression of this band in F_1 and high number of F_2 plants expressing this band indicates that the gene controlling this band may be dominant. The electrophoretic assay of eastrase activity revealed four anodal and one cathodal band for cultivar Ajay (Figs. 3 and 4). The susceptible cultivar TN_1 showed three anodal and one cathodal band. Anodal band A_5 of cultivar Ajay was absent in TN_1. Therefore, the inheritance of band A_5 was studied. This band was also present in all the F_1 plants and segregated in F_2 generation. Out of the 134 plants studied, 72 plants showed presence of this band while in the remaining plants, this band was absent. The gene determining this band appeared to be dominant but the F_2 generation

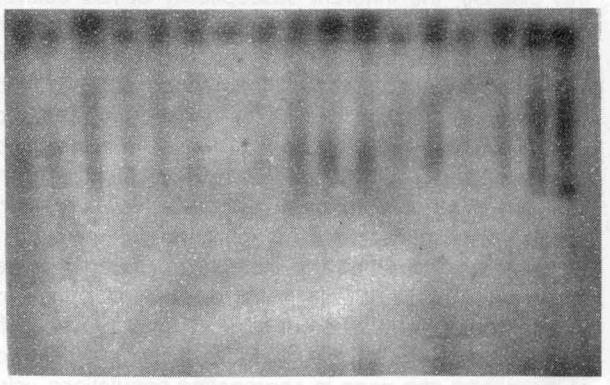

Fig. 3. Presence of isoperoxidase band C_2 in resistnat parent, F_1 and resistant/susceptible F_2 plants from the cross Ajay/TN_1 (R = BLB resistant plants, S = BLB susceptible plants)

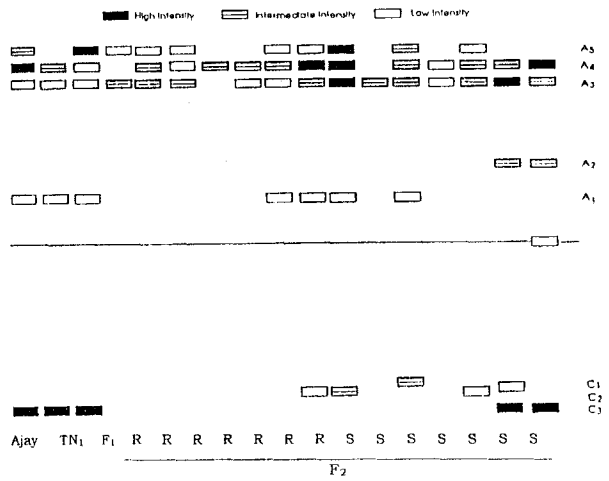

Fig. 4. Zymogram indicating presence/absence of estarase band A_5 in parents, F_1 and resistant/ susceptible F_2 plants from the cross Ajay/TN_1 (R = BLB resistant plants, S = BLB susceptible plants)

did not fit into a single gene ratio. Inability to fit in any segregation ratio for isoperoxidase C_2 and esterase A_5 band may be due to instability of these bands. Therefore, no conclusion on the inheritance of the gene(s) determining any of the isozyme band has been drawn from the present observations. Both resistant and susceptible plants in F_2 generation and homozygous resistant and homozygous susceptible families in F_3 generation showed the presence and absence of isozyme bands C_2 and A_5 for peroxidase and esterase, respectively.

Therefore, no relationship between isozymes of either pedoxidase or esterase expressing as polymorphic bands and BLB resistance gene(s) in cultivar Ajay could be established. Isozymes have been used as marker loci in earlier studies on resistance to *Xanthomonas oryzae pv. oryzae* in rice [11, 12]. Although, in the present study no association between any of the two BLB resistance genes in cultivar Ajay and the isozyme loci was observed further studies involving more enzymes as markers may be useful. Cultivar Ajay is the best source of resistance to BLB against large number of pathogen cultures [5] from northern India. Moreover, the BLB resistance of cultivar Ajay against the presently used highly virulent culture has shown oligogenic inheritance, the use of this cultivar in developing new BLB resistant cultivars may be comparatively easy.

References

1. **Mew T. W., Alvarez A. M., Leach J. E. and Swing J.** 1993. Focus on bacterial blight of rice. Pl. Dis., **77**: 5-12.
2. **Khush G. S. and Virmani S. S.** 1985. Breeding rice for disease resistance. In Russell GE (ed.). Progress in Plant Breeding., pp. 239-79, Butterworths, London.
3. **Yoshimura A., Mew T. W., Khush G. S. and Omura T.** 1984. Genetics of bacterial leaf blight resistance in breeding lines of rice. Phytopathology, **74**: 773-77.
4. **Ogava T.** 1993. Methods and strategy for monitoring race distribution and identification of resistance genes to bacterial leaf blight (*Xanthomonas compestris pv. oryzae*) in rice. Jpn. Agri. Res. Quat., **27**: 71-80.
5. **Goel R. K., Kaur L. and Saini R. G.** 1998. Effectiveness of diffrent *Xa* genes against *Xanthomonas oryzae pv. oryzae* population causing bacterial leaf blight of rice in Punjab (India). Rice Genet. Newslett., **15**: 131-33.
6. **Tanksley S. D. and Rick C. M.** 1980. Isozymic linkage map of the tomato: applications in genetics and breeding. Theor. Appl. Genet., **57**: 161-170.
7. **Reddy M. T. S. and Reddy A. P. K.** 1992. Occurrence of pathotypes of *Xanthomonas compestris pv. oryzae* in India. Indian J. Mycol. Pl. Pathol., **22**: 205-206.
8. **Kauffman H. E., Reddy A. P. K., Hsieh S. P. Y. and Merca S. D.** 1973. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. Pl. Dis. Reptr., **57**: 537- 41.
9. **Veech J. A.** 1969. Localization of peroxidase in infected tobaccos susceptible and resistant to black shank. Phytopathology, **59**: 556-71.
10. **Haris H. and Hopkinson D. A.** 1976. Section 3.1.3.2. *In*: Handbook of enzyme electrophoresis in Human Genetics, Elsevier Science Publishers, North Holland, Amsterdam.
11. **Ye Z. Y. and Liu J.F.** 1984. The relationship between resistance to *Xanthomonas compestriv pv. oryzae* and peroxidase isozyme activity in rice. J. Nanjing Agri. College, **2**: 39-46.
12. **Wang R. H., Chen W. D., Liu G. F. and Zhang Y. K.** 1997. Genetic correlation between peroxidase activity and resistance to bacterial leaf blight in rice. J. South China Agric. Univ., **18**: 75-80.