➔DOI : 10.15740/HAS/AJBS/9.2/261-266
e ISSN-0976-8343 | ■ Visit us : *www.researchjournal.co.in*

Asian Journal of Bio Science, Volume **9** | Issue 2 | October, 2014 | 261-266
Received : 27.07.2014; **Revised :** 06.09.2014; **Accepted :** 18.09.2014

RESEARCH **P**APER

Traditional landraces of rice for blast (*Magnaporthe oryzae*) resistance and analysis of biochemical components involved in disease reaction

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Rice blast disease is the major biotic threat to rice production and the pathogen *Magnoporthe oryzae* is highly genetically diverse. Rice germplasm are the important reservoirs of valuable traits possessing specialty uses and tolerance to various biotic and abiotic stresses. The present study was aimed to evaluate the collected landraces of rice against blast and to understand the biochemical changes in landraces in response to blast infection. Field evaluation of 186 rice landraces collected from Karnataka state, along with improved lines resulted in identification of land races with consistent resistance to blast. The key biochemical factors involved in disease reaction, Phenol, orthodihydroxy phenol, protein and enzyme PAL at different crop growth stages was studied. Total phenol, OD phenol and PAL accumulation and increase were rapid and more in resistant landraces as well as in improved lines due to blast infection. Landrace Beesginsali, Siddasala and Casebatta showed highly resistant reaction with disease grade either 1 or 0 and lesion type A or B. Enhancement of defense responsive biochemical components was quick and more in these landraces. These resistant landraces may serve as source of novel allele/genes to blast for future study.

Key words : Rice, Landraces, Blast, Resistance, Biochemical factors

How to cite this paper : Sowmya, H.M., Prashanthi, S.K. and Hegde, Yashoda R. (2014). Traditional landraces of rice for blast (*Magnaporthe oryzae)* resistance and analysis of biochemical components involved in disease reaction. *Asian J. Bio. Sci.,* **9** (2) : 261-266.

INTRODUCTION

Rice (*Oryza sativa* L.) is the second most important cereal crop and the staple food crop of about 65 per cent of the world's population. Rice production and productivity is limited by various biotic stresses. Diseases, especially the blast caused by *Magnoporthe oryzae* is one of the most devastating diseases in rice, and its occurrence during all stages of plant growth decreases the yield and grain quality to a greater extent. In India rice blast appears in almost all rice growing areas, and it accounts up to 50 per cent loss in upland ecosystem of Eastern India (Widawsky and O'Toole, 1990). Of the various strategies employed to manage this disease, enhancement of host resistance is considered to be the most important. However, breeding for resistance has always been challenging due to considerable genetic variability (Bonman *et al.,* 1986) in the pathogen, which generally breaks down the stability of resistance (Lee and Cho, 1990). Hence, the best option is to evaluate and select the parental and breeding lines in blast 'hotspots', where the pathogen population is highly diverse and the inoculum persists throughout the season (Correa-Victoria and Zeigler, 1993).

Rice germplasm are the reservoirs of valuable traits. India with diverse rice growing ecosystems, is a rich source of rice diversity. Landraces are niche-specific and they also have several specialty uses apart from being tolerant to biotic and abiotic stresses. Since the landraces have evolved and adapted to diverse ecosystems, they could be the potential sources of valuable genes/alleles for blast resistance. Therefore, identification of those landraces with broad spectrum blast resistance can be targeted further for gene/ allele mining, and serve to transfer such alleles to important varieties (Ramkumar *et al.,* 2010).

An effort was made to evaluate rice landraces collected

from Karnataka state for blast resistance and to dissect the biochemical factors responsible for disease reaction.

RESEARCH METHODOLOGY

The present study was carried out at Agriculture Research Station, Mugad, Dharwad and evaluated for blast resistance during the wet seasons of 2010 to 2012 :

Field evaluation of rice landraces and improved lines for blast resistance :

The landraces collected earlier were multiplied at Agriculture Research Station, Mugad, Dharwad, and total 186 landraces were evaluated for blast resistance during the wet seasons of 2010 to 2012 which represents a rainfed upland situation of South India. Landraces were screened under field conditions in Uniform Blast Nursery (UBN) where each landrace was planted in two rows of 1.0 mt length with a row spacing of 10×20 cm and the trial was replicated. After every ten rows, a line of susceptible check, HR-12 was sown and each nursery bed was surrounded by HR-12 to facilitate uniform spread of blast disease. Observation on leaf blast and neck blast severity and lesion type was recorded by following SES 0-9 scale (IRRI, 2002). Line with 0-3 disease grade was considered resistant, 4-5 as moderately resistant, and 6-9 as susceptible. Lesion type was scored (Anonymous, 2005), wherein A (no symptoms or reddish flecks only), B (minute reddish flecks or distinct circular spots without central ashy zone) and C (circular spots with central ashy zone and with brown margin) indicated resistant reaction, whereas D (spindle shaped lesions, 3-5 mm diameter), E (large spindle lesions, >5 mm and up to several cm in length), E (D) or E (C) (burning of leaves due to coalition of either C type or D type lesions) represented susceptibility. The data from all the seasons was pooled and grouped either as Immune, Resistant, Moderately resistant and Susceptible.

Further, five representative lines showing resistant, moderately resistant and susceptible reaction were selected to understand the biochemical events involved in such disease reaction. Key biochemical factors responsible for host resistance *viz.*, Phenol, orthodihydroxy phenol, proteins, and enzyme PAL were estimated at different crop growth stages during disease progression *viz.*, Before disease onset (30 days crop), Forty five days old crop (Leaf blast initiation), Seventy five days old (Leaf blast at peak), At panicle maturity (Neck blast stage). The disease severity level on susceptible check HR-12 was considered as an indication for disease progression in field. The samples were drawn from UBN at A.R.S.Mugad and estimations were done in the Department of Plant Pathology and Department of Biochemistry, University of Agricultural Sciences, Dharwad (Karnataka).

Estimation of Biochemical Components involved in disease reaction :

Estimation of total phenol :

The total phenols present in plant samples was estimated by following Folin-Ciocalteau reagent method (Bray and Thorpe, 1954). One ml of alcoholic extract of sample was taken in a test tube to which one ml of Folin Ciocalteau reagent was added followed by two ml of sodium carbonate solution (2%). The tubes were shaken well and heated in a hot water bath for exactly one minute and then cooled under running tap water. The colour developed was diluted to 25 ml with distilled water and its absorbance was read at 650 nm in spectrophotometer. The amount of phenols present in sample was calculated from a standard curve prepared from catechol.

Estimation of Ortho dihydroxy Phenol :

The ortho dihydroxy phenols were estimated by the method of Arnow (1937). 1 ml of the alcohol extract was pipetted into a test tube, to which one ml of 0.05 N HCL, one ml of Arnow's reagent, 10 ml of distilled water and 2 ml of 1 N NaOH were added. Soon after the addition of NaOH the contents of the test tube turned pink colour. The intensity of which was read at 515 nm in spectrophotometer.The OD-phenol was then determined from standard curve of catechol.

Total protein :

Protein estimation was done by following the procedure of Lowry *et al*. (1951). Bovine serum albumin was used as the standard. The samples were diluted to 100 µg protein concentration per ml and known aliquats of the sample were made up to 1 ml with distilled water. To this 5ml alkaline copper solution was added and mixed well. After 10 min 0.5 ml of 1 N Folin Ciocalteau reagent (FCR) was added and mixed well. The colour developed after 30 min was measured spectrophotometrically at 660nm against a reagent blank.

Phenylalanine ammonia lyase (PAL) :

Assay and determination of phenylalanine ammonia lyase was carried out by adopting the procedure given by Sadasivam and Manikam (1996). Five hundred mg of the plant material was homogenized in 5 ml of cold 25 mM borate-HCl buffer, pH 8.8 containing 5 mM mercapta ethanol (0.4 ml/l). The homogenate was centrifuged at 12,000 g for 20 min. Use the supernatant as enzyme source. To 0.2 ml of enzyme extract, 0.5 ml of borate buffer, 0.2 M, pH 8.7, 1.3 ml water was added and mixed. To this 1 ml of L-phenyl alanine, pH 8.7 was added. This mixture was incubated at 32°C for exactly one hour. After incubation for one hour, the reaction was stopped by adding 0.5 ml of 1 M trichloroacetic acid. Similarly, a zero hour control was maintained in which L-phenyl alanine was added after trichloroacetic acid. Then the absorbance was measured at 290 nm using UV

spectrophotometer. Simultaneously the standard graph for trans-cinnamic acid was also prepared. The PAL activity was expressed as ìmole trans-cinnamic acid formed per mg protein per min. The quantity of cinnamic acid formed was calculated by using a standard curve of cinnamic acid. Simultaneously, Protein content of the enzyme extract was estimated by the procedure of Lowry *et al*. (1951). The specific activity of the enzyme was expressed as ìg of cinnamic acid formed per mg of protein per hour.

RESEARCH FINDINGS AND ANALYSIS

The findings of the present study as well as relevant discussion have been presented under the following heads :

Evaluation of landraces for blast resistance:

In order to evaluate the potential of landraces against blast, Total 186 landraces were evaluated in field conditions in UBN in ARS, Mugad, U.A.S.Dharwad. Among the 186 landraces evaluated across the locations, fifteen showed disease severity of = 3 grade in all the seasons tested (Table 1). Beesginsali, Siddasala and Casebatta showed highly resistant reaction disease grade either 1 or 0 and with lesion type A or B. These landraces may be further used as donors in breeding programmes. Earlier studies on the genetic variability in *M. oryzae* populations of South India (Srinivasachary *et al*, 2002; Prashanthi *et al*., 2010) and revealed that the pathogen is highly diverse and are site specific though stem from diverse lineages. Hence these fifteen landraces, which were resistant across the seasons in this study, must have encountered a wide range of *M. oryzae* population indicating the probable presence of novel genes/ alleles for broad spectrum blast resistance in them. Hence these landraces constitute an ideal material for gene/allele

Table 1 : Grouping of rice landraces and varieties based on blast reaction

Groups	Leaf blast	Neck blast
Immune	0	0
Resistant	Karikantiga, Ondukaddi Mingola, Vanasurya, Kavalikannu, Kulanjipille,	Karikantiga, Ondukaddi, Mingola, Vanasurya, Kavalikannu, Kulanjipille,
Lesion type: A or B	Beesginsali, Mysoremallige, Budda, Bidar local-2, Kempusali, Gowrisanna, Orrugalu, Siddasala, Casebhatta IR-64, Vajram, MGD101, Jasmine-85	Beesginsali, Mysoremallige, Budda, Bidar local-2, Kempusali, Gowrisanna, Orrugalu, Siddasala, Casebhatta IR-64, Vajram, Jasmine-85
Moderately resistant	Kiruvanna, Mote bangarkaddi,Vasane sanna, Karigajivile, Maisali, Guddenellu, Turumari, Bili kalive, Dodiga, Mornowmi guddbatta, Waner-l, Khaima, Mugad-181, Mysore sanna, Konnur batta,	Gopal dodiga, Turumari, Beli kalavi, Dodiga, Mobane, Bangar kovi, Honne kattu, Farm valya, Sanna Mullare, Honasu, Mutalaga, Kareisade, Chippiga, Jiggu Varatiga, Kari kantiga, Bilidadi Moratiga, Sanna valya, Raj Khaima, Kannanur local,
Lesion type: C or D	Kannanur local, Navalisali, Antarsali, Hakkalsali, A-90, Ollefarm batta, Dodda gowri, Sannavalya, Sundarsali, Alursanna, Bilinellu, Gulwadi Sannakki, Jamsal, Kalsal, Bilidodi moratiga, Sanna batta, Atikaya Kabbaga, Urichippiga, Ratnachuda, Vasanesanna batta, Sanna mullare, Moradda, *Sasyari, Early sona, Pramod, Rajamani, Shankar poonam, Abhilash, Annada, Rasi, Amruth, Prasanna, MTU-1001	Kannanur batta, Mysore sanna, Nadantar Sali, Betiga, Navali Sali, Hakkal Sali, Orrugalu, Sagar selection-3, Udarsali, Bolasali, Jeersali, MS, Kempunellu, Adnenkelti, Doddibatta, Kiru vanna, Biliya, Karisadi, Nyare minda, Bili hegge, Dodda valya, Madras batta, Hakkal budda, Antar Sali, A-90, Maisali, Hy -449-1, Korma, Karibatta, Jedi kuni Valle farm batta, Madras sanna, Saliva hana, Maibra-2, Dodda gouri, Sagar selection-1 Nere guli, Ratna chuda, Valya, , Karikalavi, PNR-162, Avinash, MTU-1001, Jaya, Vandana, ADT-38, Surksha, IR-20, Sasyari, Moradda, Pramod, Manila, Rajamani, ADT-43, Divya, Co-45, Abhilash, Mandya vijay, Indrani, Lunishree, Rasi, Hemavati, ADT-39,
Susceptible Lesion type: E(C) or E(D)	Champakali, Murukata batta, Zadagi, Karkaladodiga, Doddamullare, Chitiga mugad, Shetiga, Bolasali, Jeersali, Somasali, Nizamshait, Karibatta, Kunkumsali, Valya, Kothimirisal, Kyasakki, Anekombina batta, Holesalu Chippiga, Gheersali, Chinnaponni, Kumud , Kagisali , Kasturi basmati, Yallakki Sali, Halhagana batta, Edikuni, Suggikaime, Navara, Halaga, Y-4,Mascat, Yedi kuni *Ponni, Sampige, Intan, Mandyavijay, Sona mahasuri, Jagganath, HR-12, BPT-5204, Mascat, Bidar local-1	Ratan Sagar,Bidar local-1, Champakali, Hegge, Guj Budda, Marnami gudabatta, Holesalu chippiga, Sorata, Kagisali, Y4, Medum Sali, Dodda Mullare, Karkala dodiga, Bidar local-1, Khaima, Chitiga, Chitiga Mugad, Mascat, Wari M.S., shetagi, Karidadi jaya, Bidar local-2, Bilidadi goratiga, Nizamshait, Bilinelu, Batukoli, Hy-449-1, Early sona, Shankar poonam, Pranava, MTU-9992, Annada, Tellahamsa, Prasanna, Swathi, Mote bangar kaddi, Intan, BPT-5204, HR-12

mining for blast resistance.

Estimation of biochemical components involved in disease reaction :

Five representative lines showing resistant, moderately resistant and susceptible reaction were used for estimation of Phenol, orthodihydroxy phenol, proteins and enzyme PAL which are the key factors of defense reaction.

Estimation of total phenol :

There was significant increase (0.59 mg/g to 1.35 mg/g) in total phenol content from before disease initiation stage (thirty days) to disease peak stage in field (seventy five days), but there was slight decrease in mean total phenol content in neck blast stage (1.18 mg/g). This trend was common for all the lines evaluated. However, resistant lines had higher phenol content with a mean of 1.19 mg/g compared to moderately resistant and susceptible lines which had a mean value of 1.09 mg/g and 0.67 mg/g (Table 2). At forty five days, when leaf blast disease was initiated, the maximum phenol content was recorded in resistant line Vajram (1.5 mg/g) followed by IR-64 and moderately resistant landrace Antarsali (1.0 mg/g). Phenol content was least in susceptible landrace Y-4 (0.42 mg/g). At seventy five days, when leaf blast was maximum in field, phenol content enhanced to maximum level in resistant lines (mean 1.66 mg/g) followed by moderately resistant lines (mean 1.58 mg/g).

Ortho dihydroxy-Phenol :

There was significant increase in the OD-Phenol content from before disease initiation (thirty days) to peak stage (seventy five days) (Fig.1). The mean ortho-di-hydroxy-phenol content increased from before disease initiation stage to neck blast stage (0.63 to 1.21 mg/g). In general, ortho-di-hydroxy-phenol content was more in moderately resistant (mean of 1.05 mg/g) and resistant lines (0.99 mg/g) than susceptible lines (0.76 mg/g). OD phenol accumulation was more in moderately resistant and resistant lines than in susceptibles with increase in crop age and disease pressure.

Table 2 : Total phenol content in different rice genotypes at different stages of infection

Genotypes	Total phenols (mg/g)				Mean
	Leaf blast			Neck blast	
	Before	Initiation	Peak		
Resistant					
Casebatta	0.48	0.97	1.20	1.0	0.91
Jasmine 85	0.84	0.98	1.40	1.10	1.08
IR-64	0.52	1.0	1.60	1.40	1.13
Vajram	0.75	1.50	2.10	2.0	1.58
Siddasala	0.45	0.90	2.0	1.81	1.29
Mean	0.60	1.07	1.66	1.46	1.19
Moderately resistant					
A-90	0.83	1.0	1.66	1.51	1.25
Dodiga	0.93	0.95	1.87	1.10	1.21
Mysore sanna	0.52	0.60	1.20	1.04	0.84
Rasi	0.70	0.85	1.71	1.30	1.14
MTU-1001	0.75	0.90	1.50	1.0	1.03
Mean	0.74	0.86	1.58	1.19	1.09
Susceptible					
HR-12	0.37	0.51	0.95	0.74	0.64
Bidar local-1	0.48	0.66	0.92	0.82	0.72
Y-4	0.41	0.42	0.96	0.72	0.62
Intan	0.42	0.47	0.85	0.84	0.64
Karkala dodiga	0.53	0.70	0.93	0.91	0.76
Mean	0.44	0.55	0.92	0.80	0.67
Grand mean	0.598	0.827	1.39	1.15	0.99
	S.E. ±	C.D. (P=0.01)			
Genotype (G)	0.009	0.03			
Stage (S)	0.004	0.01			
Genotype x stage	0.018	0.06			

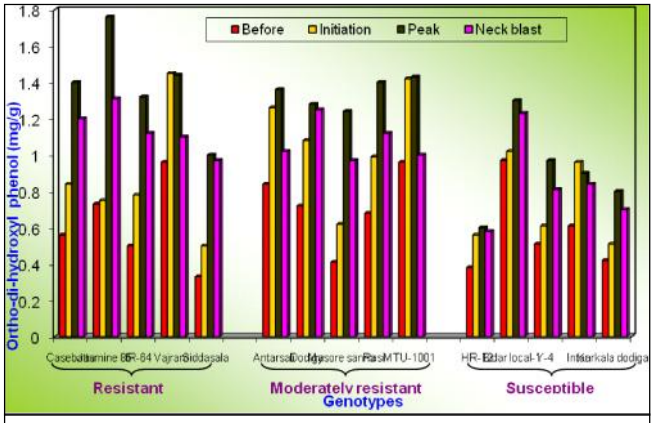

Fig. 1 : Ortho-hydroxyl Phenol content in different genotypes at different

Total phenol accumulation and increase was rapid and more in resistant landraces as well as in improved varieties immediately after blast initiation. This may be one of the probable reasons for the landraces to be resistant consistently to blast infection. Many plant phenolic compounds are antimicrobial in nature, function as precursors of various structural polymers, such as lignin, or serve as signal molecules. Positive correlation between the amount of phenolic content and degree of resistance to plant disease has been evidenced by several workers. Earlier reports support the finding of present investigation that, resistant genotypes recorded high phenols than susceptible genotypes at different phases of disease progress (Ralph and Nicolson, 1992). Similarly accumulation of ortho di hydroxy phenol content between before disease initiation to peak stage of infection was more in resistant lines than moderately resistant and susceptible lines.

Total proteins :

The protein content decreased gradually soon after the blast initiation (Fig.2). Susceptible lines had significantly higher protein content with a mean of 1.80 mg/g compared to moderately resistant (1.34 mg/g) and resistant lines (1.04 mg/g). At disease initiation stage highest protein content was recorded in susceptible HR-12 (2.30 mg/g) followed by Intan and Karkala dodiga (1.90 mg/g) and protein accumulated quickly in these lines as disease increased. Protein accumulation was least in resistant land race Siddasala at seventy five days when disease pressure in field was peak. The protein biosynthesis of the host is widely assumed to be significant feature of pathogenesis, particularly during incompatible reaction. In the present findings, susceptible genotypes recorded more of total protein content than the moderately resistant and resistant varieties. Similar association of protein content with susceptibility has been reported in ragi – blast association (Byre Gowda, 1997). This protein increase is principally due to protein synthesis of the pathogen rather than the host (Staples and Ledbetter, 1958). In addition, high protein content of the host provides more precursors for the growth and development of the pathogen. The results of the present investigation showed that in general the susceptible genotypes have more protein content making them more attractive for *M.oryzae*.

Phenylalanine ammonia lyase:

The phenylalanine ammonia lyase activity showed variations among *viz.*, resistant, moderately resistant and susceptible lines (Fig. 3). In general, resistant lines recorded significantly higher PAL activity than moderately resistant and susceptibles. Highest PAL activity was observed in resistant landrace *viz.*, Casebatta (1.21 ìmole trans-cinnamic acid mg^{-1} protein hr^{-1}) followed by resistant line Vajram (1.11 ìmole cinnamic mg^{-1} protein hr^{-1}). Least PAL activity was observed in susceptible check HR-12 (0.47 ìmole trans-cinnamic acid). Mean PAL activity was highest in resistant lines (1.06 ìmole trans-cinnamic acid) compared to moderately resistant (0.802 ìmole trans-cinnamic acid) and susceptible lines (0.53 ìmole trans-cinnamic acid).

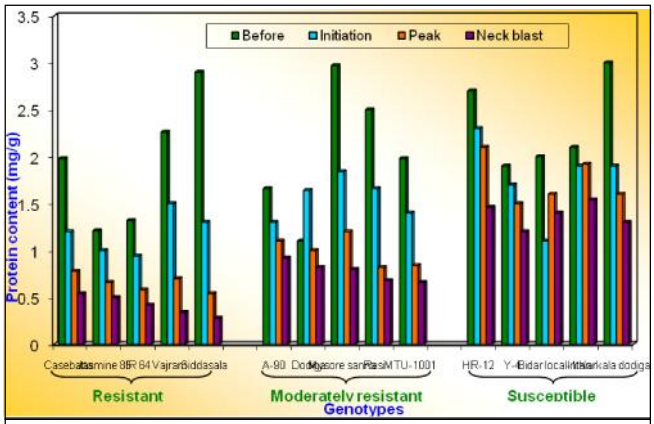

Fig. 2 : Protein content in different rice genotypes at different stages

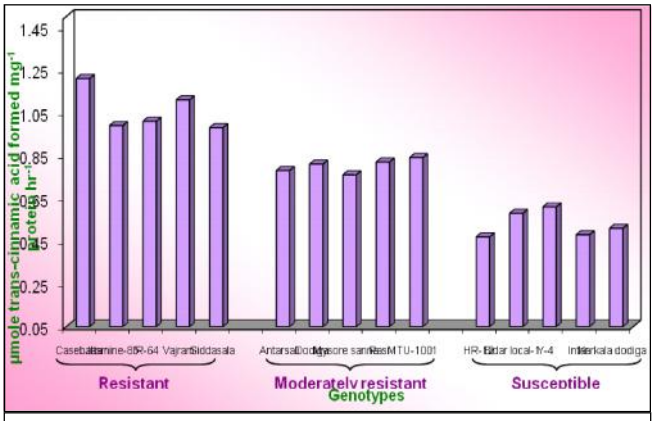

Fig. 3 : Activity of Phenylalanine ammonialyase in different rice genotypes PAL

Phenylalanine Ammonia Lyase (PAL) is the key enzyme in the phenylpropanoid metabolism which brings about deamination of phenylalanine and forms transcinnamic acid which gives rise to various compounds mainly like phenolics which are reported to have role in disease resistance. These results confirm the earlier findings that resistant rice cultivars had higher PAL activity than that of susceptible and PAL activity was positively correlated with the degree of resistance (Zhang *et al.*, 1987).

The landrace collection evaluated in this study revealed the significant variation among them for blast resistance as evidenced by the defense enzyme/biochemical components. This confirms the worthiness of the germplasm collection maintained at our institute as a source of novel genes/alleles for broad spectrum blast resistance.

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