ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 08, pp.35739-35743, August, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

CHENNELLU – A RESISTANT VARIETY OF TRADITIONAL RICE CULTIVAR IN KERALA AGAINST BACTERIAL BLIGHT

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ARTICLE INFO

Article History:
Received 21st May, 2016
Received in revised form
30th June, 2016
Accepted 25th July, 2016
Published online 20th August, 2016

Key words:

Rice,
Chennellu,
Bacterial blight,
Xanthomonas oryzae pv. *oryzae.*

ABSTRACT

Chennellu is one of the medicinal rice of Kerala and are indigenous to Northern Kerala. Chennellu in Kannur district is used for treat in diarrhea and vomiting, another type of Chennellu called 'Valiya Chennellu' from Wayanad district is used for recovering from jaundice. Chennellu, the red rice, is also a good source of Vitamin B1. In Asia, a large number of the population consumes rice in every meal and rice accounts for more than 70% of human caloric intake in many countries. Even though the average cost of rice production is highest in Kerala when compared to other states in India; it is a hot spot for pests and diseases. The high humidity and temperature of the rice growing environments during the cropping periods increases the incidence of diseases. Bacterial blight is one of major disease of rice in Kerala caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and is a reason for severe damage to rice crop in Kerala. Adukkan, Ayiramkana, Chennellu, Chomala, Gandhakasala, Kayma, Valichoori, Veliyan and Thondi were the local rice varieties in North Kerala used for finding out the susceptibility of *Xoo*. The field grew 45 days old rice plants were inoculated with 2.5µl, 5µl, 10µl and 25µl of the supernatant of 48-hour old *Xoo* broth culture at a concentration of approximately 10^8 CFU/mL ($0.5OD_{600}$nm). Inoculations by creating wounds were carried out manually on the leaves with sterile forceps. Plants were visually examined after 21 days for bacterial blight severity, and the lesion length of each leaf was measured. Disease reactions were categorized according to the lesion length. Variation in lesion length across different concentration was tested individually for each rice variety with one-way ANOVA. During bacterial infection, the mean lesion length was least at the concentration of 2.5µl in Chennellu (0.5cm). In this study, it was concluded that Chennellu is the resistant rice cultivar against bacterial blight among other traditional rice cultivars commonly used.

Citation: Showmy, K. S. and Yusuf, A. 2016. "Chennellu – A resistant variety of traditional rice cultivar in Kerala against bacterial blight", *International Journal of Current Research*, 8, (08), 35739-35743.

INTRODUCTION

Bacterial blight is one of the important diseases of rice and present throughout of the rice-growing regions. Reports from the Philippines, Indonesia, and India estimated that bacterial blight affects the 60–75% transplanted seedlings. It also causes considerable losses in all cultivars of rice in India. In some cases, Rice yield losses by bacterial blight can reach up to 50% when plants are infected at the maximum tillering stage. Bacterial blight of rice badly affects thee conomy of India. It is facing the loss of US $60 million even if two per cent of its total paddy yield was affected by the bacterial blight of rice. The loss would be a staggering US $2.4 billion if the same quantity of paddy was affected globally (Swapan, 2013).

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Bacterial blight was first noticed by the farmers in Fukuoka prefecture Kyushu Island, Japan, as early as in 1884-85 and caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is a devastating pathogen of rice. It has also been extensively studied as a model disease of rice to understand the host-pathogen interactions, bacterial pathogenesis and defense responses in monocotyledonous plants (Rumdeep et a.l, 2012). *Xoo* is a rod-shaped, round-ended, Gram-negative bacteria. They move short distances in infected crops. Xoo enters the rice leaf through hydathodes at the leaf tip and leaf margin and also through wounds or openings caused by emerging roots at the base of the leaf sheath then gain access to the xylem (Ou, 1985). Bacteria multiply in the intercellular spaces of the underlying epithelium and spread into the plant through the xylem (Noda and Kaku, 1999). After few days bacterial cells fill the xylem vessels and ooze out through hydathodes, forming beads or strands of exudate on the leaf surface, a characteristic sign of the disease.

Effectors of Gram-negative plant pathogens are delivered into plant cells via a type III secretion system (T3SS). The T3SS consists of a Hrp pilus, resembling the flagellar biosynthetic complex, but producing a needle-like appendage hence the T3SS is encoded by hrp genes. Apart from hrp gene-encoded T3SS, T2SS (Type II secretion system) are also be found to the virulence of phytopathogenic bacteria (Alfano & Collmer, 1996). T2SS consists of several degradative enzymes such as pectatelyases, cellulases, xylanases and proteases and play important roles in the interaction of *Xoo* with its host (Chatterjee *et al*., 2003; Ray *et al*., 2000; Sun *et al*., 2005). The management of bacterial blight of rice can be improved with the use of biological control agents. The resistant varieties induce defense-related genes after the interaction with pathogens. These defense-related genes assist for the potential use of biological control of plant diseases and development of innovative resistant varieties etc. this study reports the incidence of disease resistance in *Oryza sativa* Indica group against the bacterial blight.

MATERIALS AND METHODS

Plant material

Seeds of nine native varieties of rice cultivars were collected from different agro-ecological zones. Adukkan, Ayiramkana, Chennellu, Chomala, Gandhakasala, Kayma, Valichoori, Veliyan and Thondi were used for the study. Seeds of these rice varieties were disinfested with 0.1% $HgCl_2$ (mercuric chloride) for 1 min, followed by washing with sterilized distilled water and kept for germination in disposable petriplates.

Inoculum preparation

Xoo procured from Kerala Agricultural University, Vellanikara was used for the present study. Cultures were incubated on Nutrient agar (NA) medium at 27˚C for 48 h. Analytical grade chemicals from Himedia were used. Each bacterial colony on the slants were suspended in sterilized distilled water and adjusted to concentrations of approximately 10^9cfu/ml prior to inoculation.

Treatment of rice seeds with *Xoo*

Seeds of 9 local varieties of rice were kept for germination in humid petriplates for seed infection. Rice seeds were treated with cultures of *Xoo* having the dilution at 10^8 to 10^9CFU/ml when the coleoptiles of rice plants formed. 30 seeds of each variety, in triplicate, both treated and control were kept for germination in sterile Petri dishes.

Pathogen inoculation on leaves

Two days old cultures of *Xoo* having aconcentration of approximately 10^8 CFU/mL ($0.5OD_{600}$nm) were inoculated intonutrient broth medium and were incubated on a shaker (130 to 140 rpm) for 48–72 h at 28°C. The supernatant of *Xoo* broth culture after centrifugation at 5000rpm for 10min was inoculated in different volumes such as 2.5µl, 5µl, 10µl, and 20µl. Inoculation was carried out on manually created wounds on the plants with sterile forceps.

Determination of Lesion length

Within 4-7 days after incubation, plantlets were planted in the field having pond soil and cow dung in the Botanical Garden of Calicut University. The experiment was laid out in randomized block design in plots of 6 x 4 m^2 size. Separate plots were used for different isolates. 45-day old rice plants were used for the bacterial challenge. The study on morphological variability has been carried out by analyzing the leaf lesion length of nine cultivars against the pathogen. The lesion length from the cut leaf tip was measured in centimeters (cm) after 21 days of inoculation.

Total Protein estimation

Total protein from the three-week old leaves of X*oo* infected rice varieties were extracted and the total protein content was measured by Lowry's assay using bovine serum albumin (BSA) as the standard (Lowry *et al*., 1951).

Statistics

Analysis of variance was carried out to find out the significance of variations induced by the treatments in the case of the different cultivars. Hypersensitivity reactions were categorized according to lesion length. The lesion length was 0 to 6cm classified as resistance (R) and more than 6 cm as susceptible (S).

RESULTS

Morphological variability

Effect of *Xoo* on germinated seeds of rice

No lesion was observed in seedlings by bacterial infection with germinated seeds. Infectivity of rice varieties at seedling stage with respect to their changes in morphological parameters by *Xoo* was observed (Table 1).Graphical representation of morphological characters of the cultivars studied on bacterial treated seeds is illustrated in Figure 1.

Discussion and Conclusion

Bacterial leaf blight (BLB) caused by *Xoo* is a major seed-borne pathogen of rice and is a threat to rice production in both temperate and tropical rice-growing regions, due to its high epidemic potential. BLB of rice causes considerable losses in all cultivars of rice in India. Resistance to bacterial blight is one of the most important studies in agro-biodiversity and this will help to develop and grow many resistant cultivars. During1970s, the survey of rice breeders in 10 Asian countries listed bacterial blight as the second most important disease. There was no yield at all in some places in Kerala with the effect of bacterial blight (Swapan, 2013). Hence it has also become a major problem in many rice growing areas of Kerala. This epidemic disease also occurred in Palghat district of Kerala during 1998 and destroyed the crop (Ganamanickam *et al*., 1999). The present world population of 6.5 billion is likely to reach 8 billion by 2020.

Table 1. Morphological characters of the cultivars studied on bacterial treated seeds

Characters	Cultivars								
	Adukkan	Ayiramkana	Chennellu	Chomlala	Ghandakasala	Kayma	Valichoori	Veliyan	Thondi
									1.Plant height (cm.)
Control	56.5	39	37.5	35	55	49.5	27.5	31	26
Treatment	56.33	30	49.67	28.33	35	50.67	21	28	29.33
Range	0.17	9	12.17	6.67	20	1.17	6.5	3	3.33
Rank	IV	VIII	I	VII	IX	III	VI	V	II
									2.Number of leaves
Control	5	3.5	5	4	5	4	4	4	4
Treatment	4.67	3.33	4.33	4	4	4	4	4	3.67
Range	0.33	0.17	0.67	0	1	0	0	0	0.33

Table 2. Cultivar differences of bacterial blight severityas determined by lesion length

Treatment	Rice cultivar																	
	Adukkan		Ayiram kana		Chennelu		Chomala		Ghandakasala		Kayma		Valichoori		Veliyan		Thondi	
	Mean ± SE	CD@5 %	Mean ± SE	CD@5 %	Mean ± SE	CD@5 %	Mean ± SE	CD@ 5%	Mean ± SE	CD@5 %	Mean ± SE	CD@5 %	Mean ± SE	CD@5 %	Mean ± SE	CD@5 %	Mean ± SE	CD@ 5%
Control	0.133 ± 0.05	0.67	0.433±0.03	1.98	0.367±0.03	0.33	0.55±.06	1.44	0.35±.03	0.46	0.167±.02	0.85	0.183±.02	0.68	0.317±.02	0.72	0.283±.02	0.06
2.5	0.733 ± 0.064		1.8±0.298		0.5±0		1.833±.15		4±0*		1.33±0.08		0.833±.15		1.5±.13*		0.5±0*	
5	1.033 ± 0.02*		3.5±0.56*		0.667±.075		4.167±.37*		5.3±.15*		2.17±.2*		1.07±.02*		1.5±.13*		1±0*	
10	4.3 ± 0.15*		6.5±0.129*		1±0*		6.17±0.2*		7±0*		2.5±.13*		2.17±.15*		3.83±.075*		1.5±0*	
20	3.67 ± 0.15*		6.5±0.129*		2.33±.075*		7.67±.15*		8±0*		2.5±.13*		2.667±.07*		6±.13*		2±0	

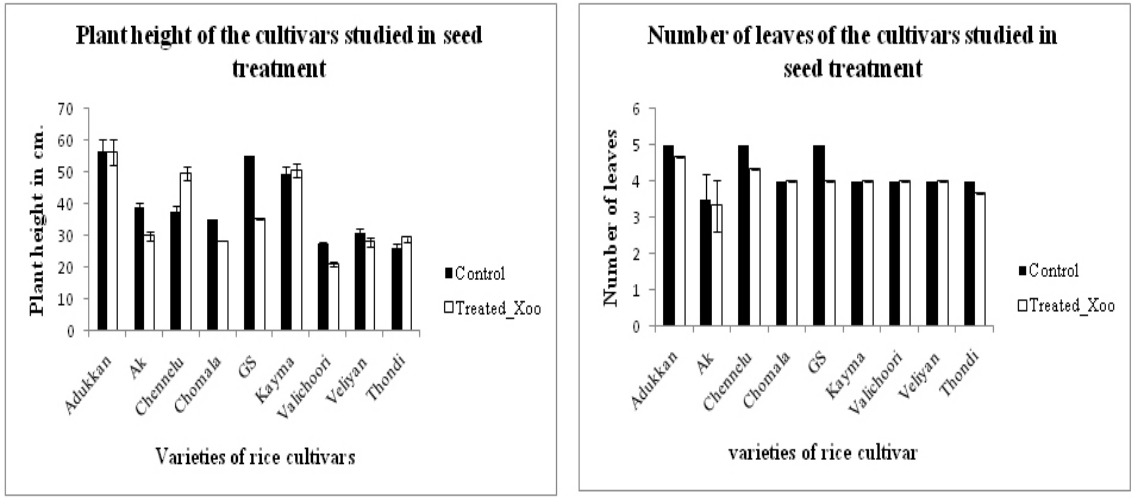

Figure 1. Graphical representation of morphological characters of the cultivars studied on bacterial treated seeds

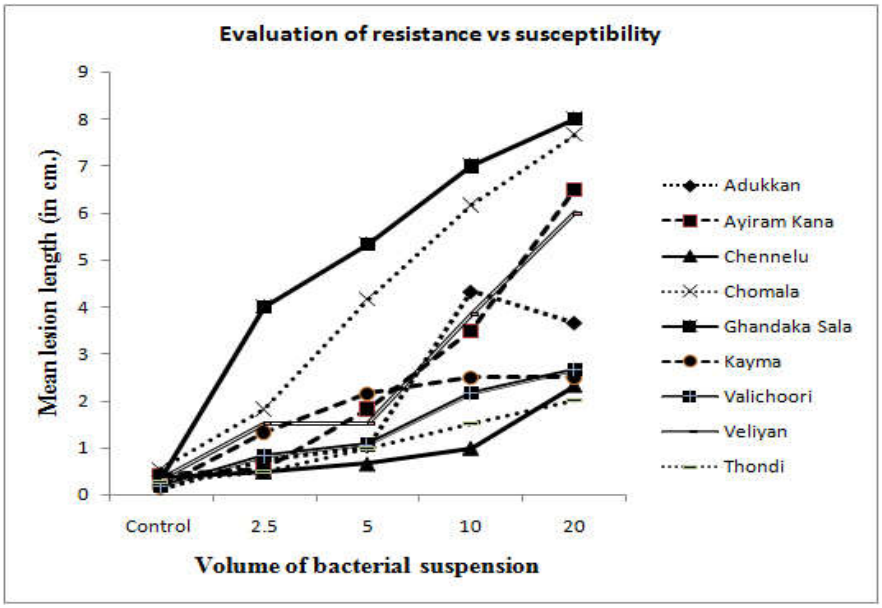

Figure 2. The mean lesion length in different rice varieties among different treatments

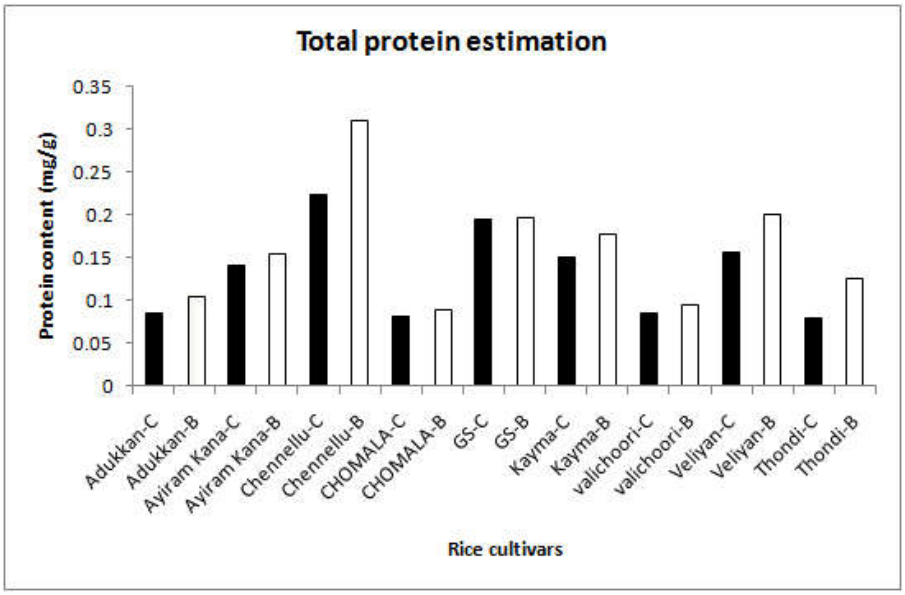

Figure 3. Effect of Xooe on protein content (mg/µl) of rice cultivar

Thus rice production must be improved by making durable resistance to high yielding rice varieties. In Kerala normally, hybrid varieties are used for high yield but they lack resistance to biotic and abiotic stresses. Nine different commonly used native rice cultivars of North Kerala were selected because of their resistance to stress. Bharathkumar *et al*. (2008) improved bacterial blight resistance in two hybrid varieties Jyothi and IR50 via backcross breeding. Kerala is famous for different varieties of rice which include aromatic, medicinal and those fit for human consumption. Aromatic rice varieties are famous for their fragrance when cooked and have high market value. Gandhakasala, Jeerakasala, and kayama are the main scented varieties of Wayanad. Several rice varieties are used either as medicine or as amost important ingredient in medicinal preparations in Kerala. Most of the medicinal rice cultivars in Kerala are lost, some varieties viz., Chennellu, Njavara, Karuthachembavu, Kunjinellu, etc. are still grown by farmers. Chennellu, red rice, is native to North Kerala. Chennellu with bright redgrains, grown as an upland variety inparts of Kannur district is used in the treatment of diarrhoea and vomiting, another type of Chennellu known as 'Valiya Chennellu' with straw coloured grains is grown in wet lands in Wayanad district used to recovering from jaundice (Leena Kumary, 2007).

In this study, all the plant materials are native rice cultivars in North Kerala. No lesion was observed when the rice seeds were treated with the pathogen. This itself shows the resistance of native cultivars. But their morphological parameters discuss that chennellu shows high resistance than the others. The difference in plant height was maximum in Chennellu with an average of 49.67cm in treatment and 37.5 cm in control and the difference between them was 12.17. The difference between treated and control was minimum in Ghandakasala

with 35cm as average in treatment and 55cm as average in control. Then the 45-day old rice leaves were inoculated with supernatant of *Xoo* showed variation in the lesion pattern and this is due to the inherent capacity of developing resistance by the development of various antibacterial proteins by the plants. Chennellu shows least lesion length in all concentrations of pathogen inoculation. The estimation of total protein content in the leaves of *Xoo* treated rice plants showed an increase in the protein content which was prominent in Chennellu. Similar results were obtained in Fenugreek plants (Singh & Singh, 2010). According to them,non-inoculated plants exhibited a lesser amount of protein than inoculated plants. This preliminary study illustrates that Chennellu is a resistant cultivar from others against bacterial blight. Based on the disease severity we screened the native rice cultivars in Kerala for the study of Pathogenesis-related proteins. Use of native varieties shows high resistance to bacterial blight and hopefully this study may leads to the detailed study of defense-related genes and also for the crop improvement.

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