The Bioscan 6(1) : 5-10, 2011
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

CHARACTERIZATION OF *XANTHOMONAS ORYZAE* PV. *ORYZAE* FROM MAJOR RICE GROWING REGIONS OF KARNATAKA

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KEY WORDS
Rice
Xanthomonas oryzae pv. *oryzae*
Field survey
Characterization

Received on :
03.11.2010
Accepted on :
19.02.2011

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ABSTRACT
Among many bacterial diseases bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* is a major biotic constraint and is wide spread in Asia, including India. In the present study field survey was undertaken in the major rice growing regions of Karnataka, a southern state of India which revealed the presence of bacterial leaf blight incidence ranging from 12 to 75%. The pathogen was isolated from the seeds and infected plant materials collected during field survey. *Xanthomonas oryzae* pv. *oryzae* was detected from rice seeds and also from plant material and its identity was confirmed by morphological, physiological and biochemical tests, hypersensitive and pathogenicity tests. The scenario and severity of bacterial leaf blight in Karnataka is discussed in the present study.

INTRODUCTION

Rice (*Oryzae sativa* L.) is life for millions of people and possibly the oldest domesticated food grain (Anonymous, 2009a) and it is the important staple food in Asia. Over 90% of the world rice is grown and consumed here, where 60% of the world population lives. Rice accounts for up to 60% of the energy intake of 3 billion Asians (Guyer *et al.,* 1998). India has the largest area under rice cultivation (43 million hacter) and with production of 87.80 million ton, next only to China. Indian rice production target for the year 2025 is 140 million ton which can be achieved only by increasing rice production two million ton per year in the coming decade (Anonymous, 2006). The major rice growing states of India are West Bengal, Uttarpradesh, Madhya Pradesh, Andrapradesh Karnataka, Kerala, Haryana, Tamil Nadu etc. In Karnataka it is grown in an area of 1.3 million hacter with production of 2.82 mt/h (Anonymous, 2009b). Rice crop is prone to number of bacterial diseases among which bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* is a serious problem and threat to rice production in both tropical and temperate rice growing regions due to its high epidemic potential (Mew, 1987). The disease occurs in the host plant at the seedlings, vegetative and reproductive stages but bacterial leaf blight infection at the tillering stage causes severe blighting of leaves resulting in yield loss upto 75% depending on weather, location and particular rice cultivar used (Ou, 1985).

The pathogen is seed-borne (Reddy *et al*., 1989; Singh *et al*., 1984) and has been considered as an important quarantine organism in many countries. Sowing infected seeds can lead to reduced germination, vigor and yield. Thus seed-borne bacteria act as a primary source of inoculum may lead to extremely high field incidence, a seed infection usually occurs during the three distinct phases of seed production, seed development and seed maturation, the pathogen can infect the seed and developing plant leading to systemic infection (McGee, 1995). More complete knowledge of the mechanism of seed transmission may lead to better method of controlling disease.

The aim of the present study is to find out bacterial leaf blight incidence across rice growing regions of Karnataka, and to characterize *Xanthomonas oryzae* pv. oryzae bio chemically from the isolates collected from different agro climatic regions of the state.

MATERIALS AND METHODS

Field survey and collection of seed samples

Field survey was undertaken in major rice growing regions of Karnataka India. During the month of June to Nov 2009 and Feb to June 2010. During the field survey the plants were inspected at the nursery stage, after transplanting, and at the flowering stage. Bacterial leaf blight incidence was recorded, among the randomly selected subplots of $1m^2$ each (10 subplots/hectare). Plants were diagnosed as infected on the basis of typical symptoms of bacterial leaf blight, *viz.,* yellow

water soaked lesions at the margin of the leaf blade, the lesions run parallel along the leaf, bacterial discharge appears on young lesion early in the morning that looks like a milky dew drop, as the disease progress the leaf dries up with white lesions and the leaf blade as wavy margin. The suspected seeds and plant parts were collected separately from the field, labeled and brought to the laboratory for the further studies.

Screening of seed samples

Seeds of forty one different rice cultivars were collected from farmers fields of various agro-climatic region, public and private seed agencies in Karnataka. The collected seed samples were subjected to different seed health testing methods *viz.*, direct plating, liquid assay and seedling symptom test to determine the seed-borne *X. oryzae* pv. *oryzae* infection.

Direct plating

The seed samples were surface sterilized with 1% sodium hypochlorite solution for three min, followed by repeated washing with distilled water three times and blot-dried, then plated directly (25 seeds/plate) on to Yeast Dextrose Calcium carbonate agar medium (YDC; Yeast extract-10.0g, Calcium carbonate-20.0g, Agar-20.0g, Distilled water-950 mL, Dextrose-20.0g, Distilled water-50.mL, the two solutions were autoclaved separately and mixed well when temperature of the medium was 50ºC). Plated seeds were incubated at 28 ± 2ºC for 24 to 72hr and observed for the presence of bacterial colonies based on the morphological characters such as shiny, raised, mucoid, pale yellow at first, straw yellow later. The suspected colonies were subjected to different biochemical, hypersensitive reaction and pathogenicity tests for confirmation of *X. oryzae* pv. *oryzae*. The experiment was carried out in four replicates of 100 seeds each and repeated twice.

Kernel plating

Three layers of filter papers were placed in each Petridish, filter paper was soaked with 0.15% of carbendazim solution and plates were sterilized at 121ºC for 20 min. The kernels from 100 seeds were removed and 25 were placed in filter paper, the plates were incubated at 28 ± 2ºC for 24hr, plates were transferred into deep freezer for 12 to 18hr, then plates were removed and incubated for 72hr and observed for development of yellow mucoid bacterial colonies on kernel.

Liquid assay method

Four hundred seeds of each sample were ground to a coarse powder and suspended in 200 mL of sterile saline (0.85% sodium chloride) and keep for 2 hr on a rotatary shaker at 150 rpm. The samples were serially diluted in 4x 1:10 concentration and streaked 50 μL of undiluted and diluted suspension on growth factor medium plates. The plates were incubated at 26 $\pm$ 2ºC for 2 to 4 days and observed for small shiny yellow colonies. Number of colonies were counted and recorded, the experiments were repeated twice (Mortensen, 1994).

symptom test

200 seeds were soaked in water for few min, four replicates of 50 seeds were put on the paper towel in equal distance and incubated them at 30 ± 2ºC for 9 days. After 9 days seedlings were examined for typical symptoms of bacterial leaf blight disease (ISTA, 1999).

Isolation of the pathogen

The bacteria were isolated directly from the seed sample and from infected plant material. Test for bacterial ooze from the suspected portion of the plants is carried out by immersing the freshly cut portion of the leaf on to glass slide containing a drop of water and observed under the compound microscope. Leaf parts from where ooze was observed were selected for further isolation of the bacteria. Leaf sections were surface sterilized with 1% (w/v) sodium hypochlorite for three min followed by repeated washing with distilled water, blot dried and plated on to YDC and NA media. The plates were incubated at 28 ± 2ºC for 24-72hr, after 48 hr plates were observed for the presence of bacterial colonies, were pure cultured on to YDC slants. All bacterial isolates were maintained at 4ºC for short term storage and for long term the bacteria was stored in 40% glycerol at -80ºC.

Biochemical characterization of bacterial isolates

Identification and characterization of the bacterial blight pathogen was carried out by subjecting the bacterial isolates to various biochemical tests, such as Gram staining, potassium hydroxide (KOH) solubility test, Kovac's oxidase test (Hilderbrand and Schroth, 1972) starch hydrolysis, Lipase activity and Arginin dehydrogenase test (Lelliot and Stead, 1987), gelatin hydrolysis, oxidative/fermentative metabolism of glucose and catalase tests. The strains were also subjected to the hypersensitive reaction in tobacco (*Nicotiana tobaccum*) plants (Carlton *et al*., 1998) and pathogenicity test (Kauffman and Rao, 1972). Each test was conducted with four replicates and repeated twice.

RESULTS

Field survey and collection of seed sample

During the present study ten different district *viz.*, Mandya, Mysore, Ramanagar, Davangere, Koppala, Hassan, Madikeri,Haveri, ChamarajaNagar and Tumkur, which are known for their rice cultivation were serveyed. The popular rice cultivar *Thanu, Jaya, MTU 1001, BR, Ankur Sona, Emergency Sona* and *Sonamahsuri* etc., were common in all district. The details of the rice cultivars and the district were tabulated in the table (Table 1; Fig. 1). Among the field visited in both the consecutive seasons none of the fields were completely free from bacterial leaf blight disease incidence. In each district a minimum 15 plots were visited, which were separated by atleast 5 km. The bacterial leaf blight incidence ranged from 12 to 37% in both the consecutive season. Highest disease incidence of 37% was recorded in the cultivar Jyothi in Davanagere District. The rice cultivar Doddi record the least disease incidence of 1.6% in Madikeri District (Table 1, Fig. 1). The disease incidence was more prevalent in the district where maximum area under the rice cultivation. The disease incidence was less prevalent where few fields were under rice cultivation.

Screening of seed sample

Forty one rice cultivars were collected from ten districts surveyed. The minimum seed sample size collected from different sources is 25g, all the collected seed sample were subjected to seed health testing methods, *viz*,. direct plating,

Table 1: Field Survey for Bacterial Leaf Blight Incidence in Karnataka State

District	Plot No.	Place	Rice Cultivar	Disease Incidence (%)
Ramanagar	1	Nagavara	Rajabhoga	30
	2	Nagavara	Jaya	25
	3	Bevur	Mysore mallige	24
	4	Byrapatna	Aishwarya	22
	5	Doddamallur	Rajabhoga	28
	6	Kylancha	Arbisona	24
	7	Anjuvadi	JGL	28
	8	Byramangala	Jaya	24
	9	Kyathapura	Jaya	21
	10	Shanabhoganahalli	Aishwarya	23
	11	Shanabhoganahalli	Jaya	21
	12	Jalamangala	Jaya	25
	13	Doddamallur	Rajabhoga	24
	14	Anjuvadi	JGL	26
	15	Anjuvadi	JGL	26
Mandya	1	Modechakanahalli	Gowrisanna	24
	2	S R Patna	Thanu	20
	3	Pandavapura	Mandyavijaya	27
	4	Sundahalli	MTU1001	25
	5	Ragimudanhalli	Thanu	4
	6	Sabbanakuppe	Ankursona	24
	7	Sabbanakuppe	Thanu	22
	8	Heremarahalli	Navara	13
	9	Haralahalli	Jaya	30
	10	Haralahalli	IR 20	32
	11	Haralahalli	BR	32
	12	Shivahalli	Naga	6
	13	Ejjalaghatta	IR 64	16
	14	Gejjalagere	Kannathumba	12
	15	Mallavlli	BR	30
Koppala	1	Gangavathi	64 Sona	28
	2	Hosabandi haralla	64 Sona	27
	3	Hallebandi haralla	B T Mallige	18
	4	Shanapura	Emergencysona	25
	5	Karatage	MTU 1010	16
	6	Shivapura	Mysore mallige	26
	7	Basapura	Sonamasuri	22
	8	Hagakere	Ankursona	24
	9	Hittanhallu	Tamil Nadu sona	24
	10	Muneerabad	Ankursona	26
	11	Nalegundi	BT Mallige	20
	12	Shanapura	MTU 1001	14
	13	Gangavathi	64 Sona	30
	14	Muneerabad	Sonamahsuri	22
	15	Gangavathi	Ankursona	18
Davanagere	1	Thavenige	Jyothi	37
	2	Basapura	Sonamasuri	30
	3	Bada	IR 64	34
	4	Bada	JGL	30
	5	Nellakhudere	Swarna	26
	6	Kabbla	Ankursona	26
	7	Pogalu	Sonamahsuri	32
	8	Heerepogalu	IR 64	32
	9	Camp	Jyothi	33
	10	Thavenige	Jyothi	34
	11	Thavenige	JGL	28
	12	Sangahalli	Swarna	29
	13	Nellakhudere	Sonamahsuri	32
	14	Heerepogalu	IR 64	35
	15	Kabbla	Jyothi	36
Mysore	1	Periyapatna	Rajabhoga	30
	2	Nanjangudu	Rathanchoodi	32

Cont.....Table 1: Field Survey for Bacterial Leaf Blight Incidence in Karnataka State

District	Plot No.	Place	Rice Cultivar	Disease Incidence (%)
	3	Periyapatna	DPT Sona	25
	4	Bettadapura	Rajamudi	27
	5	Hejjige	Thanu	28
	6	Bokkahalli	Rajabhoga	32
	7	Thoremavu	MTU 1001	26
	8	Kempisiddanhundi	BR	24
	9	Hullimavu	Thanu	26
	10	Chenamgere	Jyothi	30
	11	Chapardahalli	Thanu	27
	12	Hadinaru	1001	28
	13	T Narasipura	Rajabhoga	29
	14	Bannur	Sonamahsuri	34
	15	Gargeswari	IR 64	26
Hassan	1	A Kallenahally	Rasi	24
	2	Srinivasapura	Rasi	22
	3	Alfonas nagara	IR 20	14
	4	Kachenahalli	Jyothi	30
	5	Thumbenahalli	Ankursona	24
	6	Agrahara	IR 64	30
	7	Doddakuncha	MTU1001	24
	8	Hoovenahalli	Rasi	20
	9	Bechenahalli	Jyothi	32
	10	Gorur	Ankursona	26
	11	Hosahalli	Ankursona	23
	12	Hosahalli	IR 64	32
	13	Hosur	Rasi	24
	14	Harihara	Ankursona	24
	15	Padavalippe	MTU1001	28
Madikeri	1	Kudlur	IR 20	22
	2	Kanive	Rajamudi	26
	3	Hebale	IR 64	32
		Manajoor	Thanu	30
	3	Shirangala	1001	27
	4	Maddapura	Sonamahsuri	23
	5	Seegehosuru	Mysore mallige	18
	6	Kajoor	Doddi	1.6
	7	Chikkattur	BKB	25
	8	Banavara	Thanu	25
	9	Banavara	Rajmudi	23
	10	Hebale	Intana	10
	11	Chikkattur	Kerala	6
	12	Gummankolli	Hemavathi	12
	13	Kajoor	MTU 1001	14
	14	Manajoor	Doddi	1.3
	15	Maddapura	Rathanachoodi	21
Tumkur	1	Honnudike	IR 20	24
	2	Thovinkere	Hamsa	22
	3	Bellavavi	IR 8	21
	4	Hokkodi	Jaya	26
	5	Hadalvadi	Doddi	18
	6	Honnenahalli	IR 65	21
	7	Honnenahalli	Mangala	18
	8	Bettadahalli	Puddidoddi	12
	9	Chellur	Rasi	22
	10	Thuruvekere	Sona	25
	11	Vokkodi	Jaya	26
	12	Mudagere	Rasi	28
	13	Kunigal	Hamsa	24
	14	Mudagere	Rasi	22
	15	Honnenahalli	Mangala	20
Haveri	1	Ukkadagathri	Hybrid 64	25
	2	Koolihallu	MTU 1010	22

Cont....Table 1: Field Survey for Bacterial Leaf Blight Incidence in Karnataka State

District	Plot No.	Place	Rice Cultivar	Disease Incidence (%)
	3	Nittuhalli	JGL	28
	4	Nittuhalli	MTU 1010	24
	5	Holebannevri	Sonamahsuri	21
	6	Mudenur	Ankursona	28
	7	Makanur	BR	32
	8	Nagenahalli	MR 1001	26
	9	Kavalethu	JGL	25
	10	Kuruvathi	Hybrid64	23
	11	Kudherehalla	Bhagylakshmi	16
	12	Hallebaneri	Sonamahsuri	18
	13	Mudenur	BR	31
	14	Nagenahalli	MTU1010	29
	15	Kuruvathi	MTU1010	26
C R Nagar	1	Kollegala	Jaya	24
	2	Mudigunda	Jaya	22
	3	Sathegala	Ankursona	21
	4	Maddur	Sonamahsuri	26
	5	Agara	DPT Sona	23
	6	Mahamballi	Jyothi	28
	7	Kuntur	Jaya	23
	8	Honnur	IR 64	18
	9	Kestur	Thanu	20
	10	Yeriur	Thanu	26
	11	Surapura	IR 64	24
	12	Gowdahalli	Jaya	22
	13	Tagarpura	Jyothi	24
	14	Chinikalli	MTU1001	21
	15	Maddur	Ankursona	27

Filed survey was undertaken during Jul-Nov 2009 and Feb-Jun 2010 rice plants were observed for the typical bacterial leaf blight disease symptoms at different growth stages, disease incidence was calculated based on the number of plants with symptoms and total number of plants counted in each subplot.

liquid assay, seedling symptom test and Kernal plating method. Among the forty one rice sample screened none of the cultivar found free from *X. oryzae* pv. *oryzae* infection. The rice cultivar *Rathnachoodi* was recorded minmum of 4% *X. oryzae* pv. *oryzae* infection and the cultivar *Jyothi* recorded the highest infection of 75% when the seed samples were subjected to direct plating method (Table 2).

The cultivar *Pusa basumati* recorded the lowest amount of $63x10^4$cfu/g and the cultivar *Jyothi* recorded the highest amount of $310x10^4$cfu/g, when the seed samples were subjected to the liquid assay method. However in seedling symptom test the highest incidence of 68% was recorded in the cultivar *Jyothi* and lowest incidence of 8% was recorded in the *Sona mahsuri* cultivar and also *Uma* cultivar, where as in Kernel plating method the lowest incidence of 4% was recorded in *Uma* cultivar and highest incidence of 48% was recorded in *Jyothi* (Table 2).

The typical *X. oryzae* pv. *oryzae* colony showing yellow mucoid shining growth around the seed and kernal were observed in direct plating, kernal plating and liquid assay method. In seedling symptoms test seedlings were observed for typical symptoms of bacterial leaf blight disease. The isolates of *X. oryzae* pv. *oryzae* were purified and used for further studies.

Characterization of *Xanthomonas oryzae* pv. *oryzae isolates*

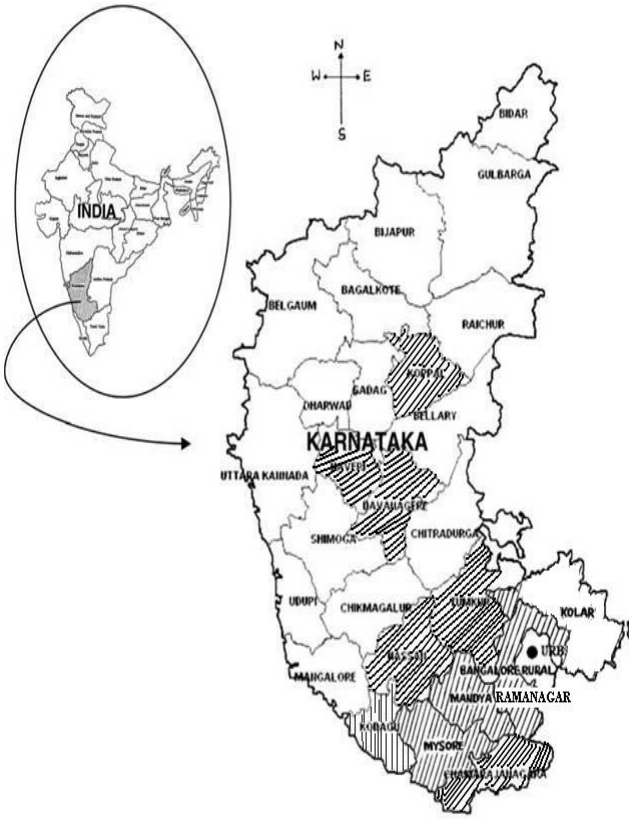

Figure 1: Geographical area surveyed for bacterial leaf blight incidence
Karnataka state map showing different district and the location of the current studies marked (?). Field survey for the bacterial leaf blight incidence carried out for two consecutive seasons.

The isolated bacteria stained pink-red and showed thin viscid mucoid strand indicating positive for KOH test, and gram negative nature of the bacteria (Table 3). A clear zone of hydrolysis was formed around the bacterial colonies, when the plates were flooded with Lugol's iodine. Hence the bacterium indicated positive for starch hydrolysis (Table 3). The inoculated Tween 80 agar plates showed the presence of white precipitate around the colonies of the bacteria, hence the bacterium indicated positive for lipase activity (Table 3). After 2 to 3 days of incubation, test isolates showed the liquefaction of the gelatin media when compared to the control, hence the bacterium indicated positive for gelatin hydrolysis (Table 3). After the incubation period, O/F medium showed color change from green to yellow indicating acid production from glucose. There was no change in color of O/F medium when it was covered with white petroleum jelly (Table 3). Necrosis was observed in tobacco plants within 24 hr of infiltrated with bacterial cells, where as sterile distilled water infiltrated leaf regions did not show any change in the leaf color, which served as control (Table 3). Rice plants inoculated with the suspected *X. oryzae* pv. *oryzae* isolates showed bacterial blight symptoms. Control plants inoculated with distilled water did not show any symptoms (Table 3).

DISCUSSION

In the present study, field survey was undertaken in the major

Table 2: Screening of Different Rice Seed Samples for the incidence of *X . oryzae* pv. *oryzae* Collected From Different Paddy Growing regions of Karnataka

Districts	Cultivar	Incidence of *X . oryzae* pv. *oryzae* DPM(%)	LAM 10^4cfu/gm	SST(%)	KPM(%)
Mandya	Jaya	27 ± 3.4^{cd}	225 ± 1.8^{h}	34 ± 3.6^{fg}	26 ± 0.7^{de}
	Thanu	64 ± 1.2^{ab}	245 ± 9.5^{gh}	50 ± 2.8^{j}	47 ± 3.3^{jk}
	Gowri sanna	20 ± 4.3^{ab}	106 ± 2.1	14 ± 1.7^{ab}	32 ± 2.8^{fg}
	Navara	14 ± 1.0^{ab}	71 ± 3.3	3 ± 4.8^{ab}	20 ± 1.2^{bc}
	Naga	18 ± 0.8^{ef}	186 ± 4.7	16 ± 0.9^{ab}	20 ± 2.2^{bc}
	Kananthumba	13 ± 1.9^{ef}	170 ± 2.8^{de}	26 ± 0.8^{bc}	13 ± 0.2^{ab}
	MTU-1001	18 ± 0.5^{ab}	122 ± 22.3^{ab}	18 ± 2.8^{ab}	15 ± 2.3^{ab}
	Dehradunbasumati	30 ± 0.7^{a}	176 ± 7.1^{ab}	28 ± 1.4^{bc}	28 ± 1.8^{de}
	Mandya Vijaya	41 ± 7.5^{ab}	216 ± 16.4^{cd}	45 ± 4.2^{hi}	32 ± 5.5^{fg}
Mysore	Rathna choodi	4 ± 0.4^{a}	96 ± 4.0^{ab}	14 ± 1.0^{ab}	4 ± 4.8^{ab}
	Rajboga	51 ± 0.7^{j}	198 ± 4.5^{ab}	36 ± 4.0^{fg}	38 ± 4.0^{bc}
	Rajamudi	31 ± 2.6^{ab}	115 ± 3.1^{ab}	31 ± 3.6^{ef}	32 ± 2.8^{gh}
Tumkur	Uma	10 ± 0.8^{ab}	124 ± 9.6^{ef}	8 ± 0.9^{a}	4 ± 0.8^{a}
	Panni	29 ± 1.0^{ef}	104 ± 0.8^{cd}	22 ± 2.2^{ab}	36 ± 3.3^{gh}
	DPT Sona	29 ± 0.6^{ef}	125 ± 3.1^{ef}	20 ± 0.7^{ab}	27 ± 2.3^{de}
	Pusa Basumti	13 ± 1.9^{ef}	63 ± 2.3^{a}	10 ± 1.4^{ab}	36 ± 4.2^{gh}
Ramanagar	Athria	23 ± 0.5^{ab}	76 ± 1.8^{ab}	12 ± 1.8^{ab}	23 ± 0.8^{cd}
	Arbisona	27 ± 7.5^{cd}	195 ± 7.4^{bc}	15 ± 1.2^{ab}	18 ± 1.0^{ab}
	Aishwarya	20 ± 0.1^{ab}	125 ± 7.1^{ef}	10 ± 1.4^{ab}	16 ± 2.3^{ab}
Hassan	Rasi	30 ± 2.6^{ab}	175 ± 2.8	28 ± 1.4^{cd}	33 ± 2.8^{ef}
	JGL	22 ± 8.8^{bc}	112 ± 1.8	16 ± 0.9^{ab}	22 ± 2.1^{ab}
	Ankur sona	30 ± 0.7^{ef}	115 ± 2.2	47 ± 3.9^{ij}	33 ± 1.4^{fg}
C R Nagar	Jaya	55 ± 3.4^{ab}	268 ± 3.2^{g}	50 ± 3.2^{j}	45 ± 5.7^{ab}
	Mahsuri	22 ± 1.1^{bc}	175 ± 4.2^{ab}	29 ± 1.0^{de}	24 ± 2.2^{de}
Davangere	Jyothi	46 ± 4.4^{ij}	310 ± 4.3^{i}	68 ± 10.1^{ij}	48 ± 2.2^{jk}
	IR 64	29 ± 15.4^{de}	$220 \pm 6.5f^{g}$	48 ± 5.8^{ij}	34 ± 3.0^{gh}
	Sona Mahsuri	10 ± 0.4^{ab}	110 ± 4.4^{cd}	8 ± 1.4^{a}	16 ± 1.5^{ab}
	Swarna	10 ± 0.6^{ab}	96 ± 2.4	14.6 ± 2.0^{ab}	15 ± 1.7^{ab}
Haveri	IR 64	47 ± 1.7^{ij}	160 ± 1.2^{ab}	22 ± 2.4^{ab}	38 ± 3.7^{gh}
	IET 7191	31 ± 1.7^{ef}	160 ± 11.2^{ab}	28 ± 2.8^{bc}	18 ± 1.7^{ab}
	IR 36	20 ± 5.4^{ab}	97 ± 2.3^{bc}	25 ± 0.9^{bc}	38 ± 4.6^{ab}
	Malgudi sanna	30 ± 0.6^{de}	196 ± 4.2	33 ± 1.8^{fg}	36 ± 4.3^{gh}
Koppala	Emergencymahsuri	18 ± 2.5^{ab}	148 ± 3.8^{ef}	13 ± 2.6^{ab}	22 ± 1.4^{de}
	Shirala Mahsuri	28 ± 0.2^{cd}	105 ± 1.0^{cd}	21 ± 1.2^{ab}	24 ± 0.8^{ef}
	Mysore Mallige	20 ± 5.4^{bc}	71 ± 3.3^{ab}	18 ± 3.1^{ab}	18 ± 1.7^{ab}
	Tamilnadu sona	27 ± 0.8^{cd}	86 ± 2.5^{ab}	25 ± 1.4^{bc}	26 ± 1.4^{de}
	A. R. Mallige	40 ± 0.4^{hi}	175 ± 7.1^{ab}	35 ± 4.1^{gh}	35 ± 4.2^{gh}
Madikeri	Rasi	25 ± 1.0^{bc}	150 ± 3.4^{ef}	21 ± 2.08^{ab}	16 ± 2.5^{ab}
	BPT 5204	50 ± 1.9^{j}	250 ± 3.2^{h}	45 ± 5.4^{hi}	36 ± 3.3^{gh}
	IR 501	27 ± 0.6^{cd}	175 ± 7.3^{ab}	15 ± 1.2^{ab}	28 ± 3.6^{de}
	PHB	33 ± 1.0^{ad}	110 ± 2.5^{ab}	13 ± 1.8^{ef}	35 ± 4.1^{ab}

*values are the mean ± SE of four replicates of 100 seeds each and repeated twice.DPM: Direct plating method, LAM: Liquid assay method, SST: Seedling symptom test, KPM: Kernel plating method.

Table 3: Biochemical Characterization of *Xanthomonas oryzae* pv. *oryzae*

Sl. No.	Biochemical test	Results
1	Gram's reaction	-
2	KOH test	+
3	Starch hydrolysis	+
4	Kovacs hydrolysis	-
5	Lipase activity	+
6	Gelatin hydrolysis	+
7	Arginine test	+
8	O/F test	+/-
9	Catalase	+
10	Hypersensitivity test	+
11	Pathogenicity test	+

All tests were conducted in four replicates and were repeated twice '+' indicates positive reaction, '-' indicates negative reaction.

rice growing region of Karnataka and the study revealed that the bacterial leaf blight disease incidence ranged from 12 to 75%. Bacterial leaf blight of rice is highly destructive, wide spread disease and is a threat to rice production in both temperate and tropical rice growing region due to its high epidemic potential (Mew, 1987) it is particularly destructive in Asian countries during heavy rains of monsoon. The disease occurs in the host plants at the seedling, vegetative, and reproductive stages, but bacterial leaf blight infection at the tillering stage causes severe yield loss of up to 75% depending on weather, location and particular rice cultivar (Ou, 1985). *Xanthomonas oryzae* pv. *oryzae* is a seed-borne, occurring in glumes and occasionally with in the endosperm, seed collected from heavily diseased fields seedlings grown from such seeds usually shows disease symptoms and die at an early stage (Srivastava and Rao, 1969). In our studies none of the field surveyed were free from disease incidence. Most of the popular cultivars of rice *viz*, IR20, Jaya, Jyothi, IR64 were recorded

more than 30% of the disease incidence.

In an attempt to screen different rice samples for the incidence of *X. oryzae* pv. *oryzae* we have used the assays like direct plating method, liquid assay method, seedling symptom test, kernel plating method, the results of *X. oryzae pv. oryzae* is highly variable the results of these four seed health testing methods can be still highly encouraging. Any of these test can be used for the detection of *X. oryzae pv. oryzae* from rice seed samples based on morphological, biochemical, physiological, pathogenicity test and hypersensitive reaction, we identified casual agent of bacterial leaf blight of rice as *X. oryzae pv. oryzae,* all isolates of *X. oryzae pv. oryzae* produce blight symptoms on rice, these results suggest that the isolates obtained from different field don't differ in their degree of virulence. These results well correlate with the study of Ghasemie *et al*., (2008). Characterize large number of *X. oryzae pv. oryzae,* isolates by pathogenicity test and phenotypic analysis.

The present study clearly demonstrates that bacterial leaf blight disease is prevailing in all the surveyed regions of the state with varied degree of disease incidence. This different degree of disease incidence may be due to the prevailing agro climatic conditions and nature of host cultivar. Hence the present work suggest that the periodic field survey will be necessary to understand the progression of blight disease in newer rice cultivar. Finally more evaluation of their resistance to this serious bacterial disease should also be conducted. Future studies are needed to understand the mode of infection of this pathogen to the newer rice cultivar more knowledge on the ecological behavior of *X. oryzae pv. oryzae,* and its host cultivar is required to develop sound control strategies.

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