Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(4): 1329-1331
Received: 22-05-2020
Accepted: 23-06-2020

Yamini SN
Department of Plant Pathology, College of Agriculture, PJTSAU, Hyderabad. Indian Institute of Rice Research, Rajendranagar, Hyderabad, Telangana, India

Bharati NB
Department of Plant Pathology, College of Agriculture, PJTSAU, Hyderabad. Indian Institute of Rice Research, Rajendranagar, Hyderabad, Telangana, India

Laha GS
Department of Plant Pathology, College of Agriculture, PJTSAU, Hyderabad. Indian Institute of Rice Research, Rajendranagar, Hyderabad, Telangana, India

Corresponding Author:
Yamini SN
Department of Plant Pathology, College of Agriculture, PJTSAU, Hyderabad. Indian Institute of Rice Research, Rajendranagar, Hyderabad, Telangana, India

Population dynamics of *Xanthomonas oryzae* pv. *oryzae* in cultivars with different levels of resistance

Yamini SN, Bharati NB and Laha GS

DOI: https://doi.org/10.22271/phyto.2020.v9.i4r.11930

Abstract
Bacterial blight (BB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most destructive diseases of rice in majority of the rice growing countries especially in Asia. As the chemical control of this disease is not very successful, greater emphasis has been given on host plant resistance. In the present investigation, rate of multiplication of *Xoo* was observed in three different rice varieties having different levels of resistance against BB *viz*., TN1 (susceptible), Ajaya (moderately resistant) and Improved Samba Mahsuri (ISM) (resistant). The results revealed that rate of multiplication was faster in susceptible TN1 after 5th day, population shot up at POI in TN1 and gradually decreased after 7th day probably due to the rapid death of leaf tissue and unavailability of nutrients, and however the movement of the bacteria was also high in TN1 when compared to other two varieties Ajaya, Improved samba mahsuri. However, there were significant differences in *Xoo* population at 1 cm below POI. At 1 cm below of POI, *Xoo* multiplied rapidly in TN1 compared to resistant variety, ISM.

Keywords: Modified wakimoto's agar (MWA), rifampicin

Introduction
Rice (*Oryza sativa* L.) is the most important staple food for the ever increasing Indian population and is the world's leading food crop. It is cultivated over an area of about 158 M ha with a production of about 725 Mt in the world (FAO, 2016-17). Bacterial blight (BB) is a major production constraint in India especially in irrigated and rain fed lowland ecosystem (Laha *et al.*, 2009) [8]. The disease appeared in epidemic form in north western India during 1979 and 1980, in Pallakad region of Kerala during 1998 (Priyadarishini and Gnanamanickam, 1998) [9] and several parts of Andhra Pradesh during 2010 and 2013 (Yugander *et al.*, 2014) [12].

Materials and methods
Development of rifampicin resistant mutant for population studies
Preparation of stock solution
To ensure that there is no contamination with other strains of BB pathogen a rifampicin mutant by using rifampicin (antibiotic) was developed. Rifampicin stock solution is prepared by dissolving 100 mg in 10 ml of distilled water to get a 10,000ppm stock solution.

Purification of *rif* resistant culture
After incubation when pin head sized colonies were observed, they were picked using a sterilised inoculation loop and developed on rifampicin 100 ppm plates into a pure culture.

Raising of plants in glasshouse
Seeds of three different varieties *viz.*, TN-1, Ajaya, Improved Samba Mahsuri were sown in earthen pots in glass house and were raised up to 40-45 days by following all the agronomic practices.

Inoculation of plants with rif mutant
Inoculation of plants with rif mutant was carried out by double pin prick method, marked with the help of marker at a point beside the point of inoculation for further identification.

Recording of observations by taking leaf discs
Observations of colony forming units are to be taken at regular intervals and even at zero hour *i.e.*, immediately after inoculation to know the inoculum load, at 3, 5, 7, 9 days after

inoculation 3 points *i.e.*, at point of inoculation, 1 cm above and below the point of inoculation5 mm leaf disc using a sterile punch were taken and these leaf discs were ground using 1 ml sterile water in mortar and pestle, the extract is serially diluted up to 10^{-3} and 10^{-4} concentrations using sterile water blanks (0.9 ml). Hundred microlitre of this diluted extract is placed on to rifampicin mixed MWA plates, spread evenly using a glass spreader and were incubated at 25±2 ^{0}C for 48 hrs, pin head sized colonies were to be observed and to be counted at each concentration, distance and variety.

Results and discussion

In the present investigation, rate of multiplication of *Xoo* was observed in three different rice cultivars having different levels of resistance against BB. Rifampicin resistant mutant IX-020rif100 was found equally virulent as the wild type strain of IX-020. Analysis of *Xoo* population at the point of inoculation (0 hour; 0 day) revealed that about 6 x 10^3 bacterial cells were released into the middle of each leaf through double pin-prick. *Xoo* population was measured from 3rd day at point of inoculation (POI), 1 cm above the POI and 1 cm below POI at 2 days interval on 3 rice varieties. The data pertaining to the *Xoo* population at different days are presented in Tables 1, 2 and 3.

On 3rd day after inoculation there was no significant difference between the varieties with respect to *Xoo* population. But highest population (log value 5.60) was observed in TN1 and least (log value 5.10) in Improved Samba Mahsuri (ISM).After 5th day, population shot up at POI in TN1. and gradually decreased after 7th day probably due to the rapid death of leaf tissue and unavailability of nutrients (Table 1).

In Ajaya and ISM, the peak population of *Xoo* was recorded on 9th day of inoculation mainly because of slower tissue death. However, there were significant differences in *Xoo* populations at 1 cm below POI. At 1 cm below of POI, *Xoo* multiplied rapidly in TN1 compared to resistant variety, ISM (Table 3). This may be due to production and accumulation of antibacterial substances in response to infection in ISM. Similar observations were recorded at 1 cm above point of inoculation (Table 2). In present investigation, movement of *Xoo* was tested on the 11th day after inoculation in all the three cultivars and data are presented. It was observed that there was movement of *Xoo* in TN1 up to 9 cm above and below POI but in Ajaya it was only up to 6 cm and in ISM it was only up to 3 cm (Table 4). This variation in movement can be attributed to differences in resistance levels among the cultivars. As *Xoo* is a vascular pathogen, the ability of a rice cultivar depends largely on its ability to restrict the bacterial multiplication and movement. It can be inferred from the results mentioned above that initial establishment of *Xoo* is equal in all three cultivars but the multiplication and movement of *Xoo* is impeded in resistant cultivar (ISM).

Table 1: Population dynamics (log10CFU/5 mm diameter leaf disc) of *Xanthomonas oryzae* pv. *oryzae* (IX-020^{rif100}) at point of inoculation

Cultivars	log10CFU/5 of IX-020^{rif100}/Days after inoculation				
	3rd day	5th day	7th day	9th day	11th day
TN-1	5.60	7.15	7.07	6.7	6.58
Ajaya	5.57	6.46	7.18	7.43	6.67
ISM	5.10	6.16	6.59	7.02	6.84
CV (%)	3.22	4.95	4.57	3.13	2.62
LSD (*P=0.05*)	1.18	0.81	0.82	0.40	0.26

Table 2: Population dynamics (log10CFU/5 mm diameter leaf disc) of *Xanthomonas oryzae* pv. *oryzae* (IX-020^{rif100}) at 1 cm above POI

Cultivars	log10CFU/5 of IX-020^{rif100}/ Days after inoculation				
	3rd day	5th day	7th day	9th day	11th day
TN-1	6.02	6.56	7.04	6.81	7.3
Ajaya	5.43	6.72	6.32	7.04	6.82
ISM	5.43	6.04	6.14	6.83	6.08
CV	4.26	4.63	5.63	5.3	5.04
LSD (*P=0.05*)	0.52	0.57	0.75	1.05	0.89

Table 3: Population dynamics (log10CFU/5 mm diameter leaf disc) of *Xanthomonas oryzae* pv. *oryzae* (IX-020^{rif100}) at 1 cm below POI.

Cultivars	Days after inoculation				
	3rd day	5th day	7th day	9th day	11th day
TN-1	6.02	6.24	7.86	7.38	7.49
Ajaya	5.77	6.27	6.75	7.06	6.87
ISM	5.42	5.82	5.94	7.12	6.51
CV	5.08	5.15	5.69	2.76	2.82
LSD (*P=0.05*)	1.13	0.97	0.27	0.28	0.28

Table 4: Movement of *Xanthomonas oryzae* pv. *oryzae* in three cultivars having different level of resistance.

Distance	Population (log10CFU/5 mm diam leaf disc) of *Xoo* (IX-020$^{rif\ 100}$) after 11 days of inoculation		
	TN-1	AJAYA	ISM
POI	*7.02±0.09	6.89±0.08	6.78±0.09
3cm above	6.50±0.18	5.89±0.09	3.56±0.05
6cm above	5.56±0.02	5.02±0.15	ND
9cm above	ND	ND	ND
3cm below	6.64±0.05	5.68±0.02	2.05±0.16
6cm below	5.54±0.08	5.23±0.06	ND
9cm below	4.08±0.06	ND	ND

*- log transformed values of CFU/ml at 5mm leaf disc.
Values after ± are standard error values ND= Not divided.

References

1. Adhikari TB, Basnyat RC, Mew TW. Virulence of *Xanthomonas oryzae* pv. *oryzae* on rice lines containing single resistance genes and gene combinations. Plant Disease. 1999; 83:46-50.
2. Banito Essohouna. Ago kadaï, Amos onasanya and Yacouba. Screening of local rice germplasm against bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* IRRI (ed) Bacterial blight of rice. IRRI, Manila, Phillippines, 2012.
3. Bhapkar DG, Kulkarni NB, Chavan VM. Bacterial blight of paddy. Poona Agriclture College Magazine. 1960; 51:36-46.
4. Borkar SG, Verma JP. Population dynamics of *Xanthomonas campestris* pv. *malvacearum* in compatible and incompatible reaction of cotton cultivars. Acta Phytopathol Acad Sci Hungaricae. 1985; 20:31-34.
5. Buddenhagen IW, Silva J, Ou SH. First year report of the cooperative project on comparison of the virulence of *Xanthomonas oryzae* strains from different Asian countries. University of Hawaii, Honolulu, 1969.
6. Chauhan HL. Studies on bacterial leaf blight of paddy (*Oryza sativa* L.) caused by *Xanthomonas oryzae* (Uyeda & Ishiyama) M.Sc. (Agri.) Thesis submitted to G.A.U., S.K. Nagar, 1973.
7. FAO. 2016-17. FAOSTAT Agriculture data (online). In: http://www.fao.org/and http://faostat.fao.org./. Rome, Italy: Food and Agriculture Organization (FAO).

8. Laha GS, Virakthamath BC, Priyanka. Bacterial blight of rice and its management. Technical Bulletin No.4. Directorate of Rice Research (ICAR), Rajendranagar, Hyderabad-500 030, Andhra Pradesh, India, 2009, 37.
9. Priyadarisini BV, Gnanaminckam SS. Focus on bacterial blight of rice Plant Disease. 1998; 83:781.
10. Srivastava DN, Rao YP. Paddy farms should beware of bacterial blight. Indian Farming. 1964; 13:32-33.
11. Swings J, Vanden Mooter M, Vauterin L, Hoste B, Gillis M, Mew TW *et al*. Reclassification of the causal agents of bacterial blight (*X. c. pv. oryzae*) and bacterial leaf streak (*X. c. pv. oryzae*) of rice as pathovars of *Xanthomonas oryzae* (ex Ishiyama, 1922) sp. nov.,nom. rev., 276 International Journal of Systematic Bacteriology. 1990; 40:309-311.
12. Yugander A, Sundaram RM, Srinivas Prasad M, Laha GS. Outbreak of Bacterial Blight of Rice in Guntur District of Andhra Pradesh. DRR Newsletter. 2013; 11:5.