INTERNATIONAL JOURNAL OF PLANT PROTECTION
VOLUME 13 | ISSUE 1 | APRIL, 2020 | 30-35

● ISSN-0974-2670 | Visit us : *www.researchjournal.co.in*

IJPP

RESEARCH PAPER

DOI : 10.15740/HAS/IJPP/13.1/30-35

Standardization of culture age, crop growth stages and different methods of inoculation of *Xanthomonas oryzae* pv. *oryzae*, A cause bacterial leaf blight in rice (*Oryza sativa* L.)

■ Sonika Deep[1], Durga Prasad*[2] **and** Subhashish Sarkhel[3]

[1]Department of Agriculture, Jharkhand Rai University, **Ranchi (Jharkhand) India**
[2]Department of Plant Pathology, Banda University of Agriculture and Technology, **Banda (U.P.) India**
[3]Department of Plant Pathology, Dr. Kalam Agricultural College, **Kishanganj (Bihar) India**
(Email : thesonikadeep900@gmail.com, subhashishiari@gmail.com)

ARITCLE INFO

Received : 31.01.2020
Revised : 15.02.2020
Accepted : 28.02.2020

KEY WORDS :
Bacterial leaf blight, Culture age, Rice, TN-1. *Xanthomonas oryzae* pv. *oryzae*

***Corresponding author:**
Email : dp.shubh@gmail.com

ABSTRACT

Bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most dreaded diseases of rice across the world, particularly affecting the majority of rice growing regions of Asia. In India, rice crop is severely affected by BLB and accounts for 6- 74 per cent of estimated yield loss. Since selection of efficient screening method is important for identification of resistance against BLB, so, three investigations were conducted to find out the most efficient *Xoo* culture age, crop growth stage and inoculation method to evolve an efficient and reliable methodology of screening of rice genotypes for identification of resistance against BLB. In the investigations, the rice TN-1 was used as test variety and it maintained as one hill per pot. To find out the appropriate age of *Xoo* culture for its successful inoculation in the host, the plants were inoculated with different ages of *Xoo* cultures at maximum tillering stage. The most effective inoculum age observed for BLB inoculation was 36 hours and 48 hours old culture of *Xoo,* both in terms of disease severity and incubation period. In case of appropriate crop growth stage for successful inoculation of *Xoo*, the most susceptible crop growth stage was found to be booting stage followed by panicle initiation stage. Among the different methods of inoculation; the most efficient method was clip + dip followed by clip method.

How to view point the article : Deep, Sonika, Prasad, Durga and Sarkhel, Subhashish (2020). Standardization of culture age, crop growth stages and different methods of inoculation of *Xanthomonas oryzae* pv. *oryzae,* A cause bacterial leaf blight in rice (*Oryza sativa* L.). *Internat. J. Plant Protec.*, **13**(1) : 30-35, **DOI : 10.15740/HAS/IJPP/13.1/30-35,**

INTRODUCTION

Rice is the world's leading food crop, cultivated worldwide over an area of about 158.91 million hectare (m ha) with an annual production of about 471.87 m tons (milled basis) and average productivity of 4.43 tons per ha (USDA, 2017). India has the largest area about 44.11 m ha under rice with total production (105.48 m tons) and average productivity of 2.40 tons per ha in 2014-15 (Anonymous, 2016). The total grain demand will increase from 201 m tonnes in 2000 to about 291 and 377 million tonnes by 2025 and 2050, respectively (Khatkar *et al*., 2016). To meet the growing food needs of increasing population in the country, there is a need to raise rice productivity in the region. Efforts for enhancing the productivity are limited by a number of biotic and abiotic stresses. The crop suffers from a number of devastating diseases caused by fungi, bacteria, viruses, nematodes, phytoplasmas and a number of environmental factors (Mew, 1992). In India, annual crop losses are estimated about Rs. 6000-7000 crore, losses contributed to diseases are 26 per cent, weeds 23 per cent, insects 20 per cent and rest by birds and nematodes (Raju, 2000). Bacterial leaf blight (BLB) is a typical vascular disease, systemic in nature; the disease causes infection in nursery at seedling stage, after transplanting and later at booting or heading stage (Chahal, 2005). It is one of the most destructive diseases of rice in Asia (Mew *et al.,* 1993). Since the introduction and cultivation of new, high-yielding but susceptible rice varieties over a large acreage in recent years, the disease has become one of the most serious problems of rice cultivation in India (Srivastava, 1967). In India, the losses had been estimated to vary from 6- 74 per cent (Adhikari *et al.,* 1995 and Gnanamanickam *et al*., 1999). At present in fact, no efficient and reliable control measure is available for BLB of rice sofar (Mary *et al.,* 2001b and Singh, 2009). Host resistance is a good approach for management of BLB of rice, however, sometimes; it is also not stable due to variability and emergence of new races in pathogen. In view of above facts, investigations were carried out to standardize the age of bacterium culture, crop growth stages and inoculation techniques for an effective screening method for identification of host resistance against BLB.

MATERIAL AND METHODS

Collection of BLB samples:

The experiments were conducted in laboratory of Department of Plant Pathology, Bihar Agricultural College (BAC), Sabour (Bihar) during *Kharif* 2017. The diseased leaves of rice cv. TN-1 showing typical bacterial blight (BB) symptoms were collected in brown paper bags from Agriculture Experimental Farm, BAC, Sabour and brought to the Laboratory for further processing.

Isolation and pathogenicity test:

Isolation of the bacterium *Xanthomonas oryzae* pv *oryzae* was carried out using infected leaves of rice plant collected from Agriculture Experimental Farm of BAC, Sabour. The sample showing typical leaf blight and bacterial oozing from the cut section during microscopy were used for isolation of bacterium. The diseased portion with healthy tissues was cut into 0.5 to 1 cm pieces. These diseased pieces were disinfected in 1 per cent sodium hypochlorite solution for 30 seconds, followed by three subsequent washing with sterilized distilled water in aseptic condition to remove the traces of NaOCl. The diseased bits were then suspended in a test tube containing 3 ml of sterilized distilled water and squeezed gently with sterilized scalpel. When the water became slightly turbid due to oozing of bacterial cells, the suspension was serially diluted up to 10^3 dilutions in 9 ml sterile water blanks. This suspension was streaked on nutrient agar (NA) medium with the help of sterilized wire loop. The inoculated plates were incubated at room temperature (27 ± 2^{o}C) for 48 hrs. After the incubation period, observations were made for the development of well separated, typical, light yellow coloured bacterial colonies resembling *Xoo*. The typical colony of *Xoo* was sub-cultured on NA plates to get pure culture. Cultures on NA slants were preserved for longer duration at 4°C. The isolated *Xoo* proved pathogenic to rice (TN-1) using Koch's postulate, which confirmed that the culture isolated was of *Xanthomonas oryzae* pv. o*ryzae*.

Identification of efficient *Xoo* culture age, crop growth stage and inoculation method:

Three experiments were conducted to find out the most efficient *Xoo* culture age, crop growth stage and inoculation method to evolve a reliable methodology of screening for host resistance. First experiment was conducted to identify the appropriate age of *Xoo* culture for its successful inoculation. In the experiment, the highly susceptible variety TN-1 was inoculated with 12, 18, 24, 36, 48 (standard), 72, 96 and 120 hrs old culture of *Xoo*. In another experiment, to find out the appropriate age of

rice plant for successful inoculation of Xoo, the highly susceptible variety TN-1 was inoculated at seedling, maximum tillering, panicle initiation, boot leaf (standard) and at panicle emergence stages. In third experiment, different methods of inoculation *viz*., pin prick and spraying of bacterial suspension, pin prick, spraying of bacterial suspension, clip inoculation and clip and dip inoculation method (standard) were standardized to find out the appropriate method for successful inoculation of *Xoo*. The experiments mentioned above were conducted in Complete Randomized Design (CRD) with five replications using TN-1 as a test variety. The TN-1 plants maintained as one hill per pot were inoculated at maximum tillering stage. In first and second experiments, the inoculation was done by clipping of the tip of 4-5 leaves of single hill with scissor dipped in the bacterium suspension (10^8 cells /ml) followed by dipping the cut ends of leaf in the same suspension. Post-inoculation incubation period and severity were recorded using 0-9 SES scale (IRRI, 1996) and data were analyzed statistically (Sukhatme and Amble, 1985).

RESULTS AND DISCUSSION

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

Identification of the most efficient *Xoo* culture age for inoculation:

While studying the effect of inoculum age on BLB, two parameters *viz.*, disease severity and incubation period were taken in to account. Based on disease severity, the most effective inoculum age for inoculation of BLB was found to be of 36 hours old inoculum and 48 hours old inoculum. Both 36 hours and 48 hours old cultures in this experiment produced 100 per cent disease severity in the TN-1 susceptible variety. It was followed by 72 hours old inoculum with 92.84 per cent disease severity. The lowest disease severity was produced by that of 12 hours old inoculum with 79.24 per cent disease severity. When incubation period is taken into account, the lowest incubation period *i.e.* 12.6 days was exhibited when the inoculation was done by 24, 36 and 48 hours old cultures, while, the highest incubation period (15.2 days) resulted in 12 hours old culture (Fig. 1). These results were found to be in agreement with the experimental findings of Gupta *et al.* (1986) and Suryadi (1990) where, they found that 48 hours old culture was most effective for induction of BLB in rice. The plant pathogenic bacterium takes some time to acclimatize to its surroundings, when it is sub cultured and placed in a fresh medium, which represents the lag phase of the bacterial growth curve, preceding the log phase in which bacterium starts to divide exponentially. Therefore, the

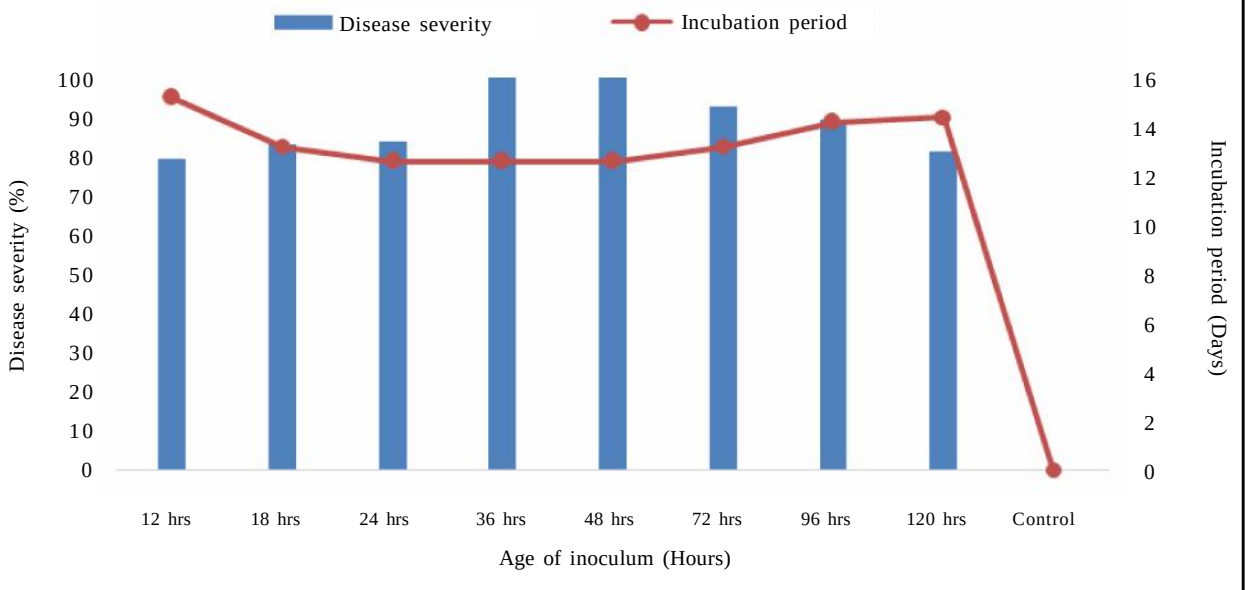

Fig. 1: Effect of inoculum age on bacterial leaf blight

12 hour old culture didn't have the sufficient bacterial population density and caused least disease severity. While in case of 36 hours and 48 hours old cultures had shown significant population density of actively dividing bacterium and thus, caused highest disease severity. Subsequently older inoculums also caused less disease severity because of reduced bacterial activity caused by exhaustion of nutrients, accumulation of waste products and inhibitory metabolites.

Identification of the most susceptible crop growth stage for *Xoo* inoculation:

It is evident from the perusal of the data presented in Fig. 2 that as all the crop growth stages of rice exerted a significant influence on the development of bacterial blight disease. The maximum BLB severity was recorded in the booting stage of rice (100%) followed by panicle initiation stage (91.52) while the minimum BLB severity was recorded in maximum tillering stage (88.38%). Based on the study of incubation period data, the minimum days required for first appearance of symptom was that in case of booting stage (12.4 days) while highest incubation period was for maximum tillering and panicle emergence stage (13.2 days). Similar results were also found by Patel (2008), where they observed highest BLB severity at booting stage of rice crop. The maximum number of rice plants found infected by *Xoo*, which resulted in development of BLB were in the 40 to 60 days of age group. The results are also in agreement with that of Mahmood and Singh (1970) and Koch and Mew (1991). They reported that fastest increase of this disease occurred between 30 and 50 days after sowing. Chauhan (1973) reported maximum numbers of plants were infected in the 40 to 60 days age group. The severity of disease decreased with further increase in age of plant, resulting in to only few mm yellowing and drying of leaves. The booting stage of rice coincides with greater number of hydathodes development, the water pores which are the means of entry of *Xanthomonas oryzae* pv. *oryzae* into the plants, which will lead to increase the number of bacterial population in the vascular system of the rice plants. Since BLB is a vascular disease, it will present an ideal condition for the bacterium to cause disease at booting stage and therefore, the booting stage had emerged as the most susceptible stage of rice.

Standardization of different methods of *Xoo* inoculation:

The maximum disease severity was recorded in case of clip + dip method of inoculation (100%) which is followed by clip method while the minimum BLB severity was found in Pin prick method of inoculation (91.08%). The minimum incubation period was also found to be in case of in clip + dip method (11.8 days) while maximum

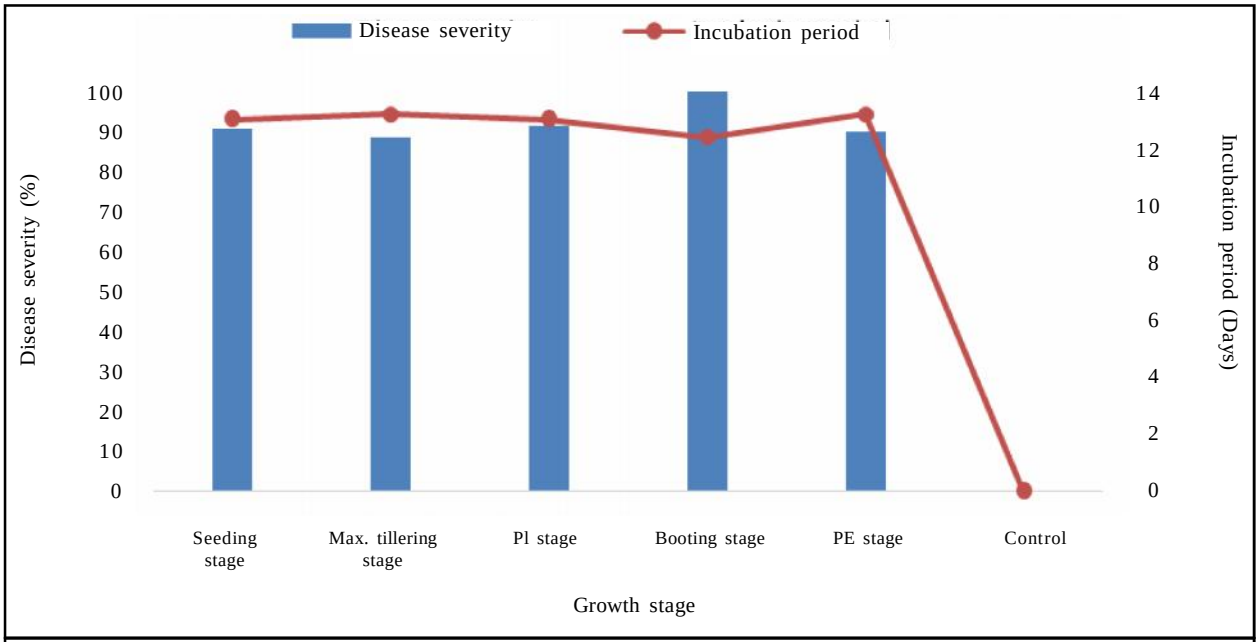

Fig. 2 : Effect of crop growth stages on bacterial leaf blight

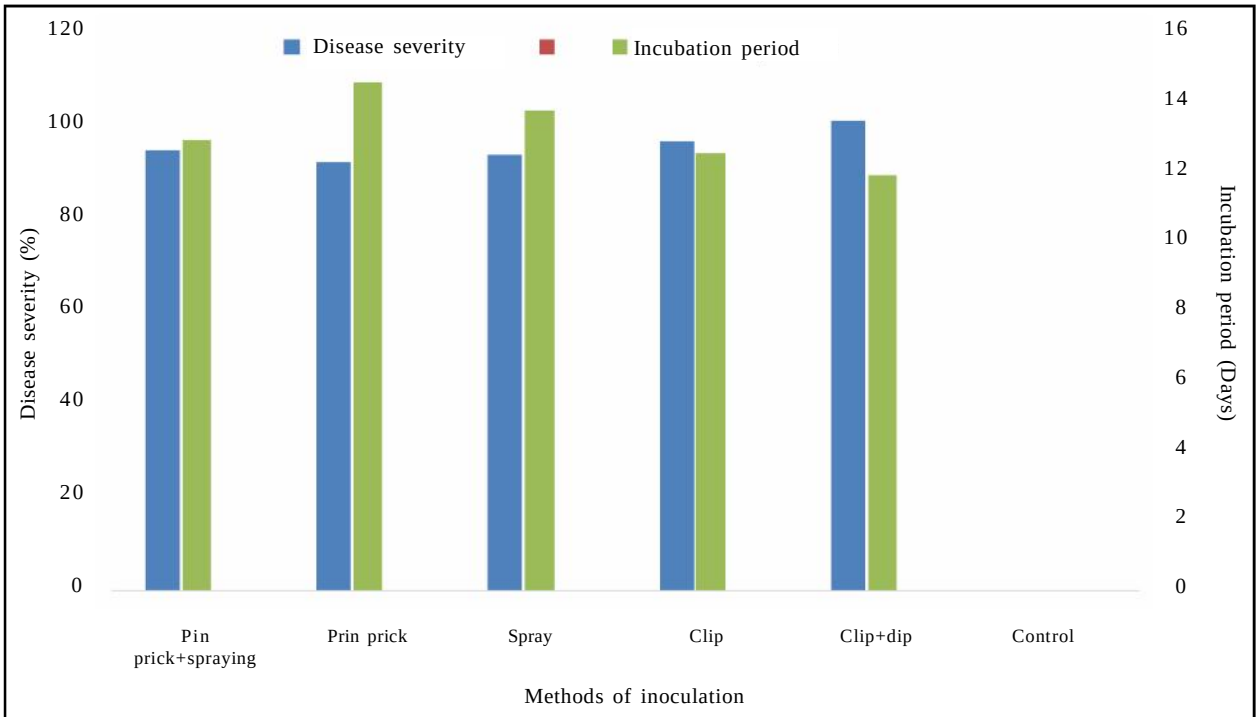

Fig. 3 : Standardization of different methods of BLB inoculation

(14.4 days) was obtained in pin prick method of inoculation (Fig. 3). Therefore, the results proved that the best method of inoculation was clip + dip method of inoculation. These results were found to be in agreement with Kauffman *et al.* (1973) and Prasad *et al.* (2018). The clip inoculation method has recommended by IRRI and DRR, Hyderabad for screening BLB nursery by artificial inoculation. Other methods of inoculation like pin prick, spraying and pin prick + spraying expressed lesser development of BLB symptom as compared to clipping method. Das and Rabindran (2012) observed the best methods in production of the disease by injuring the leaf with entomological pins (Pin-prick method) and smearing the bacterium. BLB is a vascular disease and hence if the organism in bacterial suspension is filtrated into the vascular system of the plant, it gets an easy approach to spread more rapidly and pronounce the disease symptoms of blighting as compared to other methods of inoculations. Clipping of rice with scissor dipped in bacterial suspension and dipping of cut rice leaves in the bacterial suspension facilitates the entry of bacterium into the vascular system of rice to the best and therefore, produces maximum disease symptoms with highest disease severity taking minimum period of incubation.

REFERENCES

Adhikari, T.B., Vera Cruz, C.M., Zhang, Q., Nelson, R.J., Skinner, D.Z., Mew, T.W. and Leach, J.E. (1995). Genetic diversity of *Xanthomonas oryzae pv.oryzae* in Asia. *Appl. & Environ. Microbiol.*, **61** (3):966-971.

Anonymous (2016). Annual Report. Department of Agriculture, Cooperation & Farmers Welfare, Ministry of Agriculture & Farmers Welfare, Government of India, Krishi Bhawan, New Delhi-110 001.

Chahal, S.S. (2005). Disease scenario in rice during post-green revolution era in Punjab. *Pl. Dis. Res.*, **20** : 1-11.

Chauhan, H.L. (1973). Studies on bacterial leaf blight of paddy (*Oryza sativa* L.) caused by *Xanthomonas oryzae* (Uyeda and Ishiyama) Dowson. M.Sc. (Ag.) Thesis, Gujarat Agricultural University, S.K. Nagar.

Das, L. and Rabindran, R. (2012). Molecular variability in *Xanthomonas oryzae* pv.*oryzae* isolates of Tamil-Nadu. *Indian J. Plant Protec.*, **40**(2):123-128.

Gnanamanickam, S.S., Priyadarsini, V.B., Narayanan, N.N., Vasudevan, P. and Kavitha, S. (1999). An overview of bacterial blight disease of rice and strategies for its management. *Curr.*

Sci., **77**(11):1435-1443.

Gupta, A.K., Sharma, S.C. and Saini, R.G. (1986). Variation in pathogenicity of some Indian *isolates of X. oryzae pv. oryzae*. *Phytopathology*, **76** (9):881-883.

IRRI (1996). Standard Evaluation System Manual. International Rice Research Institute, Manila, Philippines. p. 35.

Kauffman, H.E., Reddy, A.P.K., Hsieh, S.P.Y. and Nerca, S.D. (1973). An improved technique for evaluating resistance of rice varieties to *Xantomonas oryzae*. *Plant Disease Reporter*, **57** : 537-541.

Khatkar, B.S., Chaudhary, N. and Dangi, P. (2016). Production and Consumption of Grains: India. In: Wrigley, C., Corke, H., and Seetharaman, K., Faubion, J., (eds.) Encyclopedia of Food Grains, 2nd Ed., pp. 367-373. Oxford: Academic Press.

Koch, M.F. and Mew, T.W. (1991). Effect of plant age and leaf maturity on the quantitative resistance of rice cultivars to *X. c.* pv. *oryzae*. *Plant Disease*, **75**(9):901-904.

Mahmood, M. and Singh, M.N. (1970). Investigations on bacterial blight of paddy caused by *Xanthomonas oryzae* (Uyeda and Ishiyama) Dowson. *Science & Culture*, **36**(3):170-171.

Mary, C.A., Nair, S.K. and Saraswathy, P. (2001 b). Efficacy of prophylactic and curative sprayings on the control of bacterial blight of rice. *Tropical Agric.*, **39**(1):73-75.

Mew, T.W., Alwarez, A.M., Leach, J.E. and Swings, J. (1993). Focus on bacterial blight of rice. *Plant Disease*, **77**(1) : 5-12.

Mew, T.W. (1992). Bacterial blight. In: Compendium of rice diseases. Webster, R.K. and Pamela, S.G. eds. St. Paul: Amer. *Phytopathol. Soc.*, pp. 10-11.

Patel, S.J. (2008). Management of bacterial blight of rice. Ph. D. (Ag.) Thesis. Dept. of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari – 396 450, Gujarat, 302 pp.

Prasad, D. Singh, R. and Deep, S. (2018). *In-vitro* and *In-vivo* Efficacy of Antibacterial Compounds against *Xanthomonas oryzae* pv. *oryzae*, A Cause of Bacterial Leaf Blight of Rice. *Internat. J. Curr. Microbiol. App. Sci.*, **7**(05): 2960-2969.

Raju, R.A. (2000). *Glimpses of Rice Technology*. Agro Bios., New Delhi.

Singh, R.S. (2009). *Plant Diseases*. Oxford & IBH. Pub. Co. Pvt. Ltd. pp.64-65.

Srivastava, D.N. (1967). Epidemiology and control of BLB of rice in India. In: proceedings of asymposium in rice diseases and their control by growing resistant varieties and other measures. Japan Agriculture, Forestry and Fisheries Research Council, 11:18.

Sukhatme, P.V. and Amble, V.N. (1985). *Statistical Methods for Agricultural Workers*, Publication and Information Division, New Delhi, pp. 553.

Suryadi, Y. (1990). Development of kresek symptoms on some rice varieties. *Internat. Rice Res. Notes*, **15**(3):13.

USDA (2017). World Agricultural Production, United States Department of Agriculture, Foreign Agricultural Service/Office of Global Analysis, International Production Assessment Division (IPAD). Circular Series WAP 08-17.