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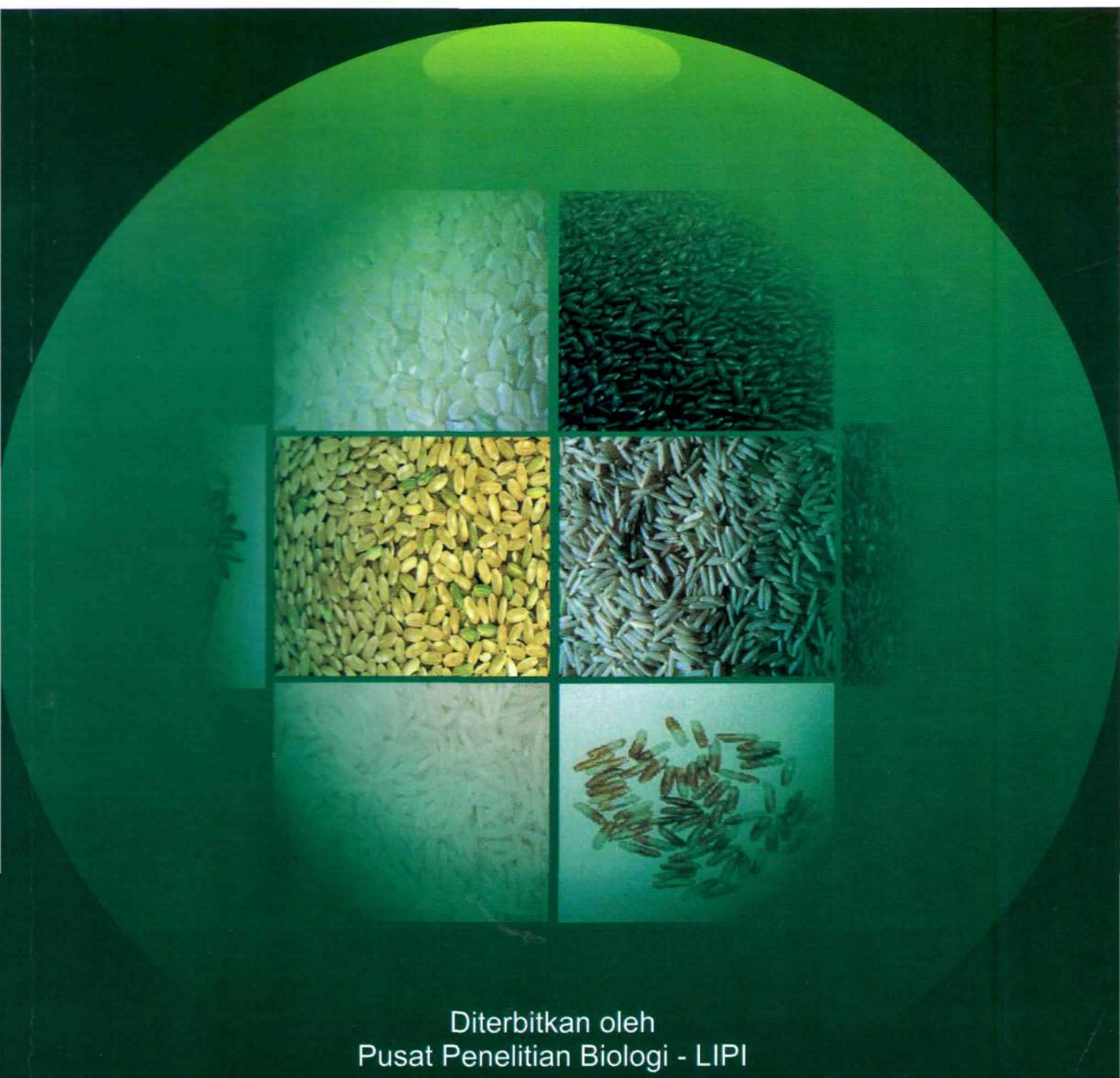
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Keterangan foto cover depart: Keragaman genetik plasma nutfah padi beras putih dan beras warna, sesuai makalah di halaman 143 Foto: Dwinita W Utami - Koleksi BB Biogen-Badan Pengembangan dan Penelitian Pertanian-Departemen Pertanian.

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VIRULENCE OF *Xanthomonas oryzae* pv. *oryzae* AND REACTION OF RICE GENOTYPES TO THE RACES OF THE PATHOGEN¹

[Virulensi *Xanthomonas oryzae* pv. *oryzae* dan Reaksi Genotipe Padi Terhadap Ras Patogen]

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ABSTRAK

Tujuan dari penelitian adalah untuk mempelajari hubungan inang-patogen pada kondisi pengujian rumah kaca dan lapangan. Virulensi isolat-isolat *Xanthomonas oryzae* pv. *oryzae* (XOO) yang termasuk kelompok ras III, dan VIII telah dikarakterisasi pada genotipe padi yang mempunyai perbedaan ketahanan terhadap penyakit hawar daun bakteri (HDB). Hasil penelitian menunjukkan perbanyakan populasi bakteri pada genotipe padi tahan (IR 36) dan rentan (TN-1) hampir sama saat awal infeksi; namun perkembangan gejala HDB yang ditunjukkan oleh panjang gejala lesio dan kurva perkembangan penyakit (AUDPC) pada genotipe TN-1 lebih cepat dibandingkan IR 36. Berdasarkan hasil pengujian di lapangan Kebun Percobaan Pusakanagara, Jawa Barat hampir semua genotipe padi yang diuji menunjukkan reaksi rentan (R) sampai sangat rentan (SR) terhadap ras VIII XOO saat pengamatan fase generatif.

Kata kunci: virulensi, *Xanthomonas oryzae* pv. *oryzae*, genotipe padi

ABSTRACT

The objective of the trials is to study host-pathogen relationships under green house test and field experiment. The virulence of *Xanthomonas oryzae* pv. *oryzae* (XOO) isolates of races III, and VIII is characterized on rice genotypes with different resistance to bacterial blight (BB) disease. The result shows that the multiplication of the bacterial population on the resistant (IR 36) and susceptible (TN-1) rice genotype is almost similar at initial stage of infection; however, BB symptom expression as indicated by lesion length and area under disease progress curve (AUDPC) are much faster on TN-1 than that on IR 36 genotype. Based on rice field trial in Pusakanagara Expt. St; W. Java; almost all rice genotypes tested shows susceptible (S) to highly susceptible (HS) against XOO race VIII at generative stage observation.

Keywords: virulence, *Xanthomonas oryzae* pv. *oryzae*, rice genotypes

INTRODUCTION

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (XOO) is one of the most serious diseases of rice in Indonesia and other Asian rice growing countries, as result of the introduction of improved but susceptible rice genotypes. The disease has also been found in Latin America, USA and Northern Australia (Mew, 1989).

The apparent BB symptom was easily seen on leaves of susceptible rice plant. Lesion was initiated at the margin of rice leaves with typical pale green to yellowish and eventually leaves become whitish to yellow in color (Ou, 1985). The disease has become serious because many improved high-yielding cultivars shows less resistance degree to the pathogen when grown under high level Nitrogen (N) and close spacing. In the last few years, damage caused by BB disease in Indonesia was seriously increased and

threaten decreased of rice productivity. In the 2006 planting season, BB has been increased infected area of 74,243 ha, whereby 61 ha was almost completely damaged (Ditlantan, 2007). Introduction of XOO in infested seed into an area that was previously blight-free present will serve as a potential threat to commercial rice production (Mew, 1989). Mean annual BB infected areas in Indonesia in the 2004/2005 wet season was 19,935 ha and yield losses in severely infected fields was ranging from 20% to 30% (Kadir *et al.*, 2004).

Until now the successful BB disease control was rely upon planting resistant varieties that shown more effective and economical, and breeding resistance against the disease has long been developed (Koch, 1989; Zhang and Mew, 1989). Cruz *et al.* (2000) and Wang *et al.* (2000) reported that the use of broad-spectrum durable resistance is a desirable trait for protecting the rice crop against multiple strains/races

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of a pathogen or multiple pathogens. Virulence of each races differed among rice cultivars. Races of XOO were reported in Indonesia as well as in Japan and the Philippines on the basis of infection of rice cultivars with specific resistance at reproductive stage of plant growth (Eamchit and Mew, 1982; Hifhi and Kardin, 1998).

The XOO bacteria were reported to survive and multiply on leaves surface of rice and plant debris, so that they can be served as primary source of infection (Koch, 1989). The elucidation to the interactions mechanism between bacterial pathogen and rice was considered as one of the important aspect in disease control. It was pointed out several workers that knowledge of the pathogenic variability of this bacterium is necessary to exploit genetic pools for developing rice with stable resistance to the pathogen (Reddy and Ou, 1976; Nayak *et al.*, 2008).

Broaden understanding of the relationship between a host plant and virulence pathogen requires information on development of pathogen within the host based on symptom development and bacterial multiplication in leaves. Hence, the objective of the trials was to study host-pathogen relationships between rice genotypes against races III and VIII of the XOO pathogen in the screen house; as well as a study of rice resistance to race VIII under field condition. This paper reported population dynamics of XOO pathogen on rice genotypes differs in their resistance and genotypes response against different races of XOO.

MATERIALS AND METHODS

The study was conducted in the Bacteriology Laboratory (ICABIOGRAD-Bogor); screen house (ICRR-Sukamandi) and Pusakanagara Exp. St (ICRR-Sukamandi) in the 2008 planting season.

Virulence of bacterial XOO isolates races in and Vm on rice genotypes TN-1 and ER 36

Three seedlings of rice genotypes TN-1 (cross between with *Xal4* R gene) and IR 36 (cross between IR1561-228//IR 24/O.nivara//CR94-13 with *Xa-4-R* gene) were planted each in a 30 cm plastic pot containing 'Ultisol' rice soil. The experiment was laid out in factorial using randomized complete design with

six replications. When rice plant at generative stage (45 days after transplanting), it was inoculated with XOO pathogen. The bacterial isolates XOO races III and VIII were used to inoculate fully develop rice leaves, respectively. The bacteria were previously grown either on Wakimoto agar (WA) or Peptone Sucrose Agar (PSA) media (Parry and Collow, 1984). The 24 hour-old bacterial suspension grown on PSA was measured for its concentration using spectrophotometer ($OD_{620nm}=0.4$) with equal to bacterial capacity of 10^8 cfu/ml (Lelliot and Schaad, 1984). It was further diluted with 0.01 M PBS pH 7.3 to gave final bacterial concentration of 10^7 cfu/ml. The rice plant was inoculated using needle prick method and disease assessment was done by measuring bacterial population at 24 h interval after inoculation until BB disease symptoms appears in all treatments. Bacterial cells population per cm^2 of leaves was counted using procedure of Di *et al.* (1991). BB disease severity was measured based on lesion length (cm) which observed at 11,14,17 and 21 days after inoculation (DAI). Disease progress curve was estimated using formula proposed by Shaner and Finney (1977) as follows:

$$AUDPC = 2 \cdot \frac{(X_{i+1} + X_i)}{2} [(t_{i+1} - t_i)]$$

where x_i = cumulative BB lesion length expressed as a proportion at the i^{th} observation, t = time at the i^{th} observation, and n = total number of observations of BB lesion length.

Reaction of rice genotypes against XOO races VIII

The experiment was conducted at Pusakanagara Expt. Station (ICRR). Out of 12 rice genotypes/lines were tested for their reaction to BB disease (XOO race VIII). Rice materials tested with their characteristics were presented in Table 1.

The test entries were pre-germinated in petri dishes for 48 hours. At least ten to fifteen seedlings in each entry are planted in a 10 cm long row using 30 x 40 cm plastic trays; then the rice was transplanted in the field. Rice genotypes (21 days after sowing) were planted in plot size of 4 X 5 m^2 using 25 X 25 cm^2 plant spacing. The experiment was arranged in complete randomized design with three replications. Plants were fertilized using recommended NPK fertilizer i.e. urea,

Table 1. Rice genotypes materials that were tested in the rice field (Pusakanagara, Expt. St. - ICRR Sukamandi in the 2008 planting season)

Lines/genotypes	AEZ	Source of origin
B10-1 -AC-BLB/BLAS-05	Wet land	<i>BB Biogen</i>
B10531E-KN-14-3-0-LR-B376-1	Wet land	<i>BB Padi</i>
OBS 1735/PSJ	Wet land	<i>Batan</i>
BP 11252-2-PN-12-2-2-2-1-7-MR-6	Wet land	<i>BB Padi</i>
BIO-8-AC-BLB-05	Wet land	<i>BB Biogen</i>
OBS 1740/PSJ	Wet land	<i>Batan</i>
BP 3300-2C-2-3	Wet land	<i>BB Padi</i>
OBS 1739/PSJ	Wet land	<i>Batan</i>
B10531E-KN-14-10-LR-B375-12	Wet land	<i>BB Padi</i>
Ciherang (control)	Wet land	<i>BB Padi</i>
Inpari 1 (control)	Wet land	<i>BB Padi</i>
Cimelati (control)	Wet land	<i>BB Padi</i>

Notes: AEZ= agroecology zone

Table 2. BB score based on SES-IRRI scale in the field (IRRI, 1996)

Score	Lesion area (%)	Reaction
1	1 - 5	R
3	6 - 12	MR
5	13 - 25	MS
7	26 - 50	S
9	51 - 100	HS

Noted: R= resistant, MR= moderately resistant, MS=moderately susceptible, S=susceptible, HS= highly susceptible

ISP and KCl at rate of 250 kg, 100 kg, 100 kg/ha, respectively. Pest and weeds infestation were controlled as needed based on IPM concept.

The bacterial isolates XO0 race VIII was selected and used to inoculate each ten fully develops leaves of rice genotypes, respectively. Bacterial inoculums concentration was adjusted to a capacity of 10^8 cells/ml. Inoculation was done at 45-60 days after transplanting by clipping method. Rice leaves were clipped 5 cm from the tip with a pair of scissors (sterilized in boiling water for 10 min) that had been dipped into the inoculums of bacterial XO0 race VIII. The rating of disease reaction on inoculated leaves was assessed based on a modified 1 to 9 scale of the Standard Evaluation System for Rice (SES) (IRRI, 1996) at 21 DAI or until susceptible (S)-check is showing maximum of BB disease severity (Table 2).



Figure 1. Typical colony of XO0 on slant WA medium (a) and single bacterial cells with lypopolysaccharides that observed under SEM 10.000 x (b)

RESULTS

Virulence of bacterial XO0 races **III** and **VIII** on rice genotypes **TN-1** and **IR 36**

Degree of host susceptibility in the build up of BB was studied in this experiment. Colonies developed in the WA medium that was counted based on serial dilution plate count technique showed typical uniform appearance (Figure 1).

The result shows that bacterial population on TN-1 was much higher than that of IR 36 genotype (Figure. 2). For both cultivars, the population of XO0 races increased from an initial level of 10^6 cells per leaf to around 10^{10} cells per leaf during observations. The bacterial population derived from TN-1 leaves sample was ranging from 6.9 to 7.5 log cfu/cm² at 48 h after inoculation, whereas the bacterial population has increase ranging from 9 to 11 log cfu/cm² at 10 days

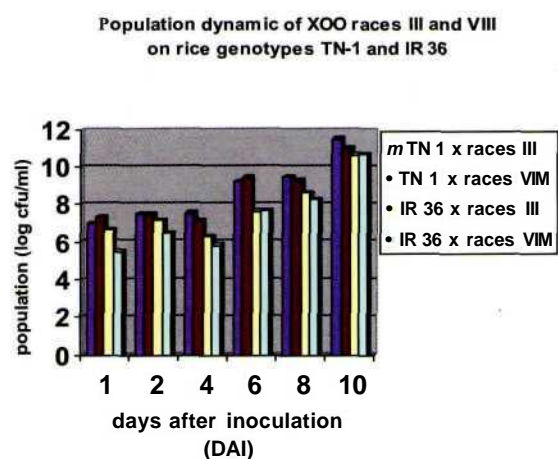


Figure 2. Population dynamic of XOO races III and VIII on rice genotypes TN-1 and IR 36

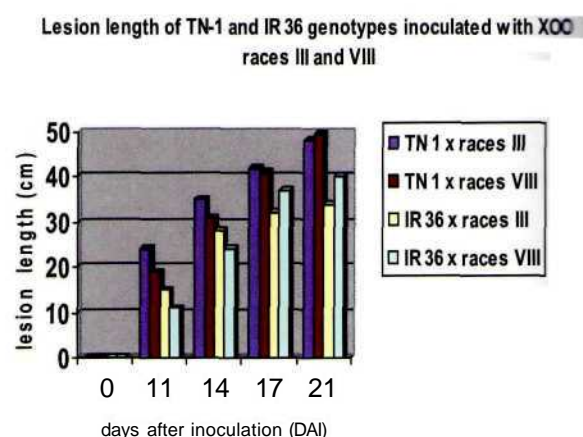


Figure 3. BB disease lesion length on genotypes TN-1 and IR 36 inoculated with XOO races III and VIII

Table 3. Virulence of XOO races III and VIII on rice genotypes TN-1 and IR 36 based on BB disease progress curve (AUDPC)

Genotypes	AUDPC inoculated with XOO race III	AUDPC inoculated with XOO race VIII	AUDPC Mean genotypes
TN-1	498.5	423	460.75 a
IR 36	384	360.5	372.15 b
AUDPC Mean of XOO races	441.25 a	391.65 b	

Notes: Means followed by the same letter are not significantly different by Duncan Multiple Range Test (DMRT; $P=0.05$)

after inoculation. In general, the multiplication of the causal bacterium in the resistant and susceptible genotypes was almost the same in the initial stage of infection (Figure. 2). It was pointed out that successful infection of a host plant by a bacterium involves the movement of the bacterium towards the host, and proliferation of the bacterium inside the host immediately following entrance (Gnanamanickam *et al.*, 1999).

The difference in disease infection was indicated by disease lesion length (Figure 3). On each cultivar, lesions developed uniformly downward from the point of inoculation. The length of lesion from the leaf tip varied suggesting virulence index occurred on each XOO isolates/races on both rice genotypes TN-1 and IR 36. This difference in virulence factor may affect varied reaction on each genotypes. In case of disease response of rice genotypes to the races III and VIII of

the bacterium, there was a gradual increased in the BB disease severity at different interval of observations.

Mean of BB infection rates indicated by AUDPC of each genotypes against XOO races III and VIII is ranging from 360.5 to 498.5 (Table 3). Significantly greater differences AUDPC for most observations of BB lesion length was obtained for rice cv. TN-1 as compared with IR 36 genotypes grown in green house test.

Reaction of rice genotypes against XOO race VIII

The results reveals that Susceptible (S) and Highly Susceptible (HS) responses were observed on nine rice genotypes to XOO race VIII at generative stage in the field inoculation test (Pusakanagara Expt St). The reaction of rice genotypes originated from different institution with different inherited resistance genes is presented in Figure 4. BB disease severity was ranging from 32% to 83%. Reaction of BIO-1 -AC-

BLB/Blas-05, BIO-8-AC-BLB-05 and BP11252-2-PN-12-2-2-1-7-MR6 against race **VIII**, showed **BB** severity less than 35%; whilst control check treatment (Ciherang, Inpari 1 and Cimelati) showed HS reactions (>50%).

DISCUSSION

Bacterial isolates can be divided into virulence groups (or races) based on their interaction with certain rice host. To date it was reported at least 12 races occurred in rice ecosystem (Hifhi and Kardin, 1998; Kadir *et al*, 2004). Agrios (1997) pointed out that genetic variability was present within species pathogen where specific races of the pathogen infect plant on specific variety and location; whilst other races could not infect plants.

The result support previous study where XOO multiplied slightly greater extent on differential rice (Mohiuddin and Kauffman; 1975). Reddy and Kauffinan (1973) reported that multiplication of XOO strains reaching a *plateau* value shortly after symptoms first appear. The initial BB symptom expression on rice leaves TN-1 appeared at 98 h after inoculation, whilst on IR 36 symptom apparently appeared at 168 h after inoculation. The symptom expression on host tissue was probably due to physical force on cell membrane in related to accumulation of bacterial cells mass (Preece, 1982; Huang and De Cleene, 1989). After 21 days after inoculation, cultivars showed the same pattern of host-pathogen interaction coincide with previous study as described by other workers (Mew *et al*, 1982; Cruz *et al*, 2004). TN-1 (*Xa-14* gene,) was susceptible, whilst IR 36 (*Xa-4* gene) was resistant to both races. The virulence of the XOO races is narrow in East and South East Asia but broad in South Asia (Mew, 1989).

The result suggested that multiple disease readings expressed as an AUDPC, provide more complete assessment of the foliar symptoms on rice due to XOO infection. As seen in figure 1, the bacterial virulence of XOO might be correlated with total EPS (extra cellular polysaccharides) content. It was reported that on bacterial pathogens such as *Clavibacter michiganense* subsp. *insidiosum* the presence of an abundant EPS would increase bacterial virulence on

potato plant (van Alfen *et al*, 1987).

Nine rice genotypes showed susceptible reaction to XOO race VIII in the field test. The ability of rice genotypes to withstand the spread of BB severity was suggested depends on the restricting capacity of the tissue system against multiplication and movement of the pathogen (Reddy and Kauffinan, 1973; Suryadi and Machmud, 1990). Host plant resistance can be classified into Vertical resistance (VR) and Horizontal resistance (HR) with considering both genetic and epidemiological aspects. VR is monogenic and is effective against specific strains/races (Zhang and Mew, 1989).

In this experiment none of genotypes tested showed resistance against XOO race VIII. A similar test at Sukamandi Expt. Farm, Kadir *et al*, (2009) reported that almost 60% rice genotypes were susceptible to race VIII; however two genotypes i.e., Bio 38-AC-BLB 05 and Bio 63-AC-bals/BLB 03 showed resistant against XOO races IE, IV and VIII at seedling test. Agrios (1997) pointed out that morphological and physiological characteristics races of the pathogen could not easily differentiated; however the ability to infect groups of differential cultivars did occur. This phenomenon may explain why cultivars become resistant in some geographical area, whilst others showed susceptible in other areas which related to different races and virulence genetic factors (Bashim, 1998). According to gene for gene concept (Flor, 1971), for each resistance in one genotype there was a close correspondence or relationships with the pathogen gene of virulence. Mew (1989) pointed out that BB resistance had been detected in many rice genotypes. In some cases, it may controlled by a single gene or in other cases influenced by many genes (Cruz *et al*, 2004). IR BB7 (*Xa-7*), IR BB58 and IR BB59 showed moderately resistant (MR) to BB (Kadir *et al*, 2007). Cruz *et al*, (2004) stated that in Indonesia was suggested to use *Xa-7* and *xa-5* gene for resistance individually in creating resistant genotype such as crossing with IR64 (*Xa-4*) background that have been released as cultivars Code and Angke. Based on three years experiment (four seasons) in the rice field trials at different location in C. Java and W. Java; Kadir *et al*, (2004) pointed out that IR BB7 containing *Xa-7* resistance gene may be

used as resistance donor parent to overcome dominance races of BB pathogen in the field.

CONCLUSION

In screen house test, BB symptom expression as indicated by lesion length and area under disease progress curve (AUDPC) were much faster on TN-1(*Xa-14*) than that of IR 36 (*Xa-4*) genotype.

Nine rice genotypes showed susceptible reaction to XOO race VIII in the field test.

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