Resistance Test of 6 Eggplant (*Solanum melongena* Linn.) Genotype Against Bacterial Wilt Disease (*Ralstonia solanacearum*) in the Greenhouse and on the Field

Awang Maharijaya[1(✉)], Devi Oktavia[1], Giyanto Giyanto[2], Heri Harti[3], and Kusuma Darma[3]

[1] Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Bogor, Indonesia
awang.maharijaya@gmail.com
[2] Department of Plant Protection, Faculty of Agriculture, IPB University, Bogor, Indonesia
[3] Center for Tropical Horticulture Studies, Institute of Research and Community Services, IPB University, Bogor, Indonesia

Abstract. Proper testing parameters of eggplant resistance to bacterial wilt in the greenhouse are needed for efficient and effective development of resistant eggplant varieties in order to increase eggplant fruit productivity and quality. This research aims to obtain the most accurate observed resistance testing parameters in greenhouse which also represent field test response and to obtain genotypes that resistant and have similar productivity with commercial variety from the test. This research was conducted at Cikabayan greenhouse with artificial inoculation (puncture method) and at Leuwikopo research station (180 m asl). A randomized complete block design single factor was randomized to 3 replications. The treatment factors were genotypes consisting of 4 genotypes belonging to PKHT and 2 commercial varieties. The result showed resistance response of the eggplant against bacterial wilt disease through inoculation method in the greenhouse were significantly correlated with field test response. The best and most accurate observed parameters in greenhouses is bacterial colonization level. Resistance test showed that PKHT-80 and MUSTANG is resistant to bacterial wilt disease while PKHT-90 and PRINCE F1 are moderate resistance. Growth and development of all six genotypes generally are not significantly different. PKHT-80 is resistant and has high productivity on land that could compete commercial varieties.

Keywords: Bacterial wilt disease · Correlation · Eggplant · Genotype · Parameter · Resistance

1 Introduction

Eggplant (*Solanum melongena* L.) is one of important vegetable in Indonesia due to rate of consumption, ability to produce, and economic value. Production of eggplant in Indonesia is about 509,724 t in 2016. Demand for this commodity is increase by

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years with consumption rate about 5.98%. This commodity is takes fifth place in the export of vegetables followed cabbage, potato, shallot, and chili [1]. The farmers prefer to cultivated the eggplant because this plant is more tolerant to drought and heavy rain than other vegetables.

The production of eggplant in Indonesia faces the problem of wilt disease cause by Ralstonia solanacearum. The disease might reduce the production of eggplant between 15–95% [2]. The disease can attack many plants from Family Solanaceae including eggplant, chili, potato, tomato, etc. There are several strategies to overcome the disease, i.e., resistant variety, culture technique, bio-control agent, and fungicide.

The development of resistant varieties to control plant diseases has been carried out widely in the world. Using resistant variety to controlling of plant disease has several advantages. It is specific to the target disease, environmental safety, easy to adopt by farmer without additional cost, and compatible with other methods of plant disease control such as natural enemies, cultural practices, and also improves the efficiency of pesticides in pest management. On the other hand, development of resistant varieties of plant requires a great deal of expertise and resources, time consuming, and absence of adequate levels of resistance in the available germplasm [3].

The development of resistant variety needs screening method to determine and selection the resistant plant obtained from breeding program. Currently, conventional testing method in open field is less efficient especially when used for large populations because requires a high cost and a long time to get the result. Limited-scale in green house testing is preferred because It's can be conducted in shorter time, more cost efficiency, smaller area, more thorough assessment on controlled environmental conditions, and as well as a lower risk to contaminating new diseases in the field [4]. Beside that, green house testing requires less labor, easier to be carried out, and give more precision in result than open field testing [5, 6].

This research has two objectives. First, to obtain accurate and reliable parameters for screening of plant resistance in the green house that represent the field conditions. Second, to obtain genotype of eggplant that resistant to bacterial wilt and have at least the same productivity as the commercial varieties.

2 Methodology

This study was conducted in green house of Cikabayan and on field of Leuwikopo, IPB University Dramaga located at 180 m asl. Six genotypes of eggplant were used for this study, consist of four PKHT genotype i.e., PKHT-021, PKHT-046, PKHT-080, and PKHT-090, and two commercial genotypes i.e., Prince 07 F1 and Mustang F1. Isolate of R. solanacearum race 3 biovar 2 obtained from Department of Plant Protection, IPB University.

Seeds of eggplant were sowing in pot tray filled with media mixture of cocopeat: soil: manure with ratio 1:1:1 (v/v/v). Three weeks-old eggplant seedlings are transplanted to polybag or to field. For green house testing, six genotypes of eggplants were grown in polybag. One month after transplanting, the plants were injected with 0.12 ml of R. solanacearum with concentration 109 colony forming unit (cfu) at the base of the stem. For open field testing, the eggplant seedlings are cultivated on the mulched-bed

plots. Plants are planted in double rows with a plant spacing of 50 cm between rows and 50 cm in rows. Inoculation of R. solanacearum in the field occurs naturally, no artificial inoculation is carried out like testing in a greenhouse. Plants in green house and on field are fertilized with 250 ml AB mix solution with concentration 5 ml L^{-1} until 4 weeks after transplanting. After that, the plants are fertilized with 250 ml NPK (15-15-15) with concentration 5 g L^{-1}.

Resistance level of each genotype is assessed based on four parameters, namely: disease severity (DS), bacterial colonization (BC), plant height (PH), dichotomous height (DH), flower number (FN), and productivity (P). The severity of bacterial wilt disease on eggplants was observed by calculating the percentage of plants with wilt disease symptoms. A plant is declared to be infected with bacterial wilt disease if at least one leaf shows symptoms of wilting. Observation of disease severity carried out at 28 days after inoculation (DAI) for the green house testing and every day at 3–8 weeks after planting (WAP) for field testing. The severity of bacterial wilt disease will be calculated using the disease incidence formula proposed by Wang [7]. Classification of the severity of bacterial wilt disease using disease resistance criteria according to Maharijaya [8].

The bacteria colonization shows aggressiveness of bacteria to infect the plant base on intensity of vascular browning and ooze. Bacterial colonization assessment was carried out according to the method developed [9] at 30 DAI in green house and 31 DAP on field. Level of bacteria colonization classified according to Ishikawa [9]. Plant growth was measured by PH, DH, and FN. Measurement taken weekly at 1–5 WAP in green house and at 2–9 WAP on field. The productivity of plant is calculated based on the weight of the fruit harvested in three harvests times (three weeks). The productivity observation was carried out for on the field testing only but not for in greenhouse testing because there were several genotypes in the greenhouse that did not produce fruit even though the plants were in good health.

This experiment was conducted using a completely randomized block design with a single factor (6 genotypes) and 3 replications as a group. Each experimental unit had one additional plant that was not inoculated as a diversification factor. Data collected during this study were analyzed by Pearson correlation method to find out the relationship and the closeness of the response from the two tests. Effect of the treatment determined by F test at a significant level of $\alpha = 1$ and 5%. The further test used was Duncan Multiple Range Test (DMRT) at a significant level of $\alpha = 5\%$. The whole process of data analysis using Microsoft Office Excel and SAS software.

3 Results and Discussion

3.1 Wilt Disease Incidence

Early symptoms of bacterial wilt disease appear at 3 WAI in green house or 3 WAP on field. The wilted plant is the initial symptom of *R. solanacearum* infection, and then the plant become withered. Plant's leaves turning pale yellow and even browning which eventually died in one to two weeks (Fig. 1). Withering plant often accompanied by excretion of bacterial ooze when the severe infection occurred.

Fig. 1. Symptoms of bacterial wilt disease on eggplant: early wilt (left) and browning or dried plant (right).

During the infection process, bacteria produce several types of enzymes such as pectinase, cellulase, protease, and produces exopolysaccharide (EPS). EPS is an extracellular compound with a high molecular weight, which is deposited in the vascular tissue and obstructs the flow of air from the roots to the top of the plant. The blockage causes lay symptoms [10]. At mild levels of infection, wilt disease usually appears on only a part of the branch. At the level of severe infection, the entire stem will wilt permanently, the plant dries, browns and eventually dies [11]. If the plant in this phase is cut at the base of the stem and immersed in water, a milky white bacterial ooze will come out which indicates the plant has been infected by *R. solanacearum* [12].

3.2 Correlation Between Parameters in Greenhouse and on Field

The value of the correlation coefficient ranges between -1 to $+1$. Two parameters are said to be very closely related if they have an r value close to $+1$ or -1 [13]. Two parameters are said to be closely correlated if they have a value of $0.5 < r \leq 0.8$ and very closely related if they have a value of $0.8 < r \leq 1$ [14].

Parameter of disease severity in the greenhouse test shows strongly positively correlated to the parameter of disease severity ($r = 0.62$), significantly positive correlation with bacterial colonization ($r = 0.58$), and strongly negatively correlated to productivity ($r = -0.60$) on the field test. Bacterial colonization parameter in the greenhouse also has a very significant positive correlation with disease severity ($r = 0.69$) and bacterial colonization ($r = 0.64$) parameters on field test. There was a very significant negative correlation ($r = -0.64$) between bacterial colonization in greenhouse and productivity on the field.

Correlation coefficient of bacterial colonization in greenhouses and disease severity on field is slightly higher than correlation coefficient of disease severity in greenhouse and disease severity on field (Table 1). This indicates that the bacterial colonization is a more accurate parameter for resistance testing than the disease severity in the greenhouse. It is not easy to observe the symptoms of wilt disease in green house due to the less significant difference between healthy and mild-infected plants. Bacterial colonization parameter is indeed more accurate for resistance testing, but the procedure to obtain data for bacterial colonization is more complicated than disease severity. Collecting bacterial

Table 1. Linear correlation between parameters in greenhouse and on field

Parameter		Field					
		DS	BC	PH	DH	FN	P
Greenhouse	DS	0.62**	0.58*	−0.27	0	−0.38	−0.60**
	BC	0.69**	0.64**	−0.23	−0.13	−0.23	−0.64**
	PH	−0.05	−0.19	0.48*	0.01	0.27	0.07
	DH	−0.43	−0.01	0.09	−0.36	−0.08	0.34
	FN	0.12	0.27	0.22	−0.07	0.11	−0.02

** = strongly corelated; * = significantly corelated; DS = disease severity; BC = bacterial colonization; PH = plant height; DH = dichotomous height; FN = flower number; P = productivity

Table 2. Linear correlation between parameters on field

Parameter		Field				
		DS	BC	PH	DH	FN
Field	BC	0.80**				
	PH	−0.17	−0.17			
	DH	0.36	0.26	0.22		
	FN	−0.49*	−0.3	0.70**	−0.12	
	P	−0.93**	−0.83**	0.36	−0.24	0.57*

** = strongly corelated; * = significantly corelated; DS = disease severity; BC = bacterial colonization; PH = plant height; DH = dichotomous height; FN = flower number; P = productivity

colonization data needs more labor and time for cutting and measuring plant stems, whereas disease severity data takes by visual observations immediately. This makes the bacterial colonization parameter less appropriate to be applied to large populations or to woody perennials plants. The bacterial colonization parameter is more suitable and more accurate for greenhouse testing because It's generally has a small population size with herbaceous plants such as eggplant.

Disease severity both in greenhouse and field had a very significant positive correlation with bacterial colonization with r values of 0.65 and 0.80, respectively (Table 2 and Table 3). Same result has been shown by Mandal et al. (2014) that disease severity has positive correlation with bacterial colonization. Plant resistance related parameters, that is disease severity and bacterial colonization in the field testing showed a very significant negative correlation with the production parameters. This indicated, more resistant plants to bacterial wilt disease, then that plants that can grow better and produce higher yield.

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Table 3. Linear correlation between parameters on greenhouse

Parameter		Greenhouse				
		DS	BC	PH	DH	FN
Greenhouse	BC	0.65**				
	PH	0.09	−0.11			
	DH	−0.26	−0.44	0.49*		
	FN	0.41	0.15	0.18	0.06	0.60**

** = strongly corelated; * = significantly corelated; DS = disease severity; BC = bacterial colonization; PH = plant height; DH = dichotomous height; FN = flower number

Table 4. Quantitiave resistance parameters of eggplant in the greenhouse and on field

Genotype	Greenhouse*			Field*			Response of Resistance
	Disease Severity (%)	Bacterial Colonization	Bacterial Colonization (negative control)	Disease Severity (%)	Bacterial Colonization	Productivity (ton ha^{-1})	
PKHT-21	66.67^{ab}	1.83^{ab}	0	81.67^{a}	4.00^{a}	0.00^{b}	S
PKHT-46	73.33^{ab}	2.60^{a}	0	90.00^{a}	4.00^{a}	0.00^{b}	S
PKHT-80	33.33^{c}	0.50^{c}	0	5.00^{c}	0.00^{b}	4.89^{a}	R
PKHT-90	46.67^{bc}	1.33^{b}	0	15.00^{c}	1.67^{ab}	4.16^{a}	MR
PRINCE F1	53.33^{abc}	1.50^{b}	0	41.67^{b}	1.33^{b}	2.51^{a}	MR
MUSTANG F1	41.00^{c}	1.17^{bc}	0	11.67^{c}	1.00^{b}	3.74^{a}	R

Numbers followed by the same letter in the same column show no significant difference at the DMRT test at level of 5%

3.3 Resistance of Eggplant Against Bacterial Wilt Disease

Evaluation of six genotype eggplant against bacterial wilt disease obtained each two genotypes of susceptible (PKHT-21 and PKHT-46), moderate resistant (PKHT-90 and PRINCE F1), and resistant (PKHT-80 and MUSTANG F1). This study showed that the disease severity among genotypes was significantly different for both the greenhouse test and the field test (Table 4). PKHT-80 showed the lowest percentage response of disease severity but it was not significantly different from the response of PKHT-90 and MUSTANG F1 both in greenhouse test and on field test. Meanwhile, PRINCE F1 has a higher disease severity value than the PKHT-80, PKHT-90 and MUSTANG F1. These results indicated that genotypes belonging to PKHT (PKHT-80 and PKHT-90) can compete with the commercial varieties in term of resistance against bacterial wilt disease. Resistant plants have defense mechanisms against pathogen, and then suppress the intensity and severity of the disease.

Disease severity in the greenhouse is lower than on the field. This can be due to the number of pathogens in the greenhouse is more limited than pathogen on the field. The environment in the greenhouse is more sterile and more controllable than on the field so that pathogens in the greenhouse grow and multiply slower than pathogen on field. Beside that, the pathogen inoculated into plants in a greenhouse may be has lower virulence because the bacteria were propagated from laboratory cultures that had been stored in the laboratory. The virulence of pathogenic microorganisms often decreases when the pathogens are kept in culture for relatively long periods of time. If the culturing of the pathogen is prolonged sufficiently, the pathogen may lose virulence completely [15].

Susceptible genotypes generally have a high infection rate begin from the early vegetative phase, while resistant genotypes have a low infection rate and symptoms of wilt disease appear at the end of the vegetative phase (Fig. 2). Resistant plants have effective passive and active mechanisms to inhibit pathogen infection compared to susceptible plants. The passive defense mechanism of plant is pathogen independent, it is always available and it is not affected by the presence of pathogen. Otherwise, active defense mechanism is pathogen dependent, that is triggered when the pathogen infects the plant, for example, tylosis and phytoalexins.

Tylosis is responsible to treats pathogens that attack the xylem tissue. Tylosis can block the xylem tissue to limit the movement of pathogens. Phytoalexins are substances produced by plants to inhibit the growth and development of pathogens [15]. A study on *Arabidopsis thaliana* and tomato showed that the regulation of phytoalexin biosynthesis is important for developing plant varieties that are resistant to *R. solanacearum* [16]. Another study on tobacco showed that riboflavin compounds can induce the synthesis of scopoletin (tobacco plant phytoalexin) as a plant defense mechanism against *R. solanacearum* and *Phytophthora parasitica* [17].

The defense mechanism of the plant which is an internal factor of the host plant affects the interaction with the disease. Resistant plants that have better defense will suppress the interaction of the disease triangle longer than susceptible plants. The yield of plant attack rate will be slower than that of susceptible plants [15].

3.4 Bacterial Colonization

The correlation coefficient between bacterial colonization in the greenhouse and the disease severity in field is increased from week to week. Correlation between bacterial colonization in the greenhouse at 30 DAI with the severity of the disease in the field can be found significantly in 6 WAP (Table 5). This indicated that the observation of bacterial colonization level in the greenhouse at 30 DAI was quite representative and could be used as indicator for disease development on the field at 6 WAP.

Bacterial colonization in eggplant was significantly different between genotypes, both in the greenhouse and on the field testing. Susceptible genotypes (PKHT-46 and PKHT-21) have high bacterial colonization value. In contrast, resistant genotypes (PKHT-80 and MUSTANG F1) have low bacterial colonization value. Bacterial colonization in PKHT-90 is not significantly different with commercial genotype PRINCE F1 and MUSTANG F1 (Table 4). PKHT-90 with medium bacterial colonization value express only a mild symptom of bacterial wilt disease. This result is indicated that

Table 5. Correlation between bacterial colonization in greenhouse and disease severity on field at 3–8 WAP

Disease severity of plants on field at	Correlation coefficient value with bacterial colonization in greenhouses at 30 DAI
3 WAP	0.290nd
4 WAP	0.306nd
5 WAP	0.340nd
6 WAP	0.507*
7 WAP	0.527*
8 WAP	0.647**

nd - not significant difference; * - significant difference at level of 5%; ** - significant defference at level of 1%;

PKHT-90 is supposed be a tolerant plant, but this assumption still needs to be further confirmation.

Bacterial colonization was assessed by observing vascular browning (discoloration) in the pith of the plant stem. Discoloration is usually accompanied with a soft and wet texture of tissue. This condition indicates the presence of extracellular compounds excreted by bacteria [18]. Extracellular compounds with high molecular weight, such as polygalacturonase, endoglucanase, and toxin are play role as the pathogenicity or virulence factors of *R. solanacearum* [19, 20].

3.5 Plant Productivity

There is a very significant difference in productivity between susceptible genotypes and resistant genotypes. Susceptible genotypes (PKHT-21 and PKHT-46) did not produce fruit at all, while moderate resistant genotypes (PKHT-90 and PRINCE F1) and resistant genotypes (PKHT-80 and MUSTANG F1) had the same level productivity (Fig. 3). This study shows that resistant genotypes belonging to PKHT can compete with commercial genotypes in terms of productivity, even though these commercial genotypes are hybrid varieties.

Hybrid varieties are generated from a cross of two or more parents (pure lines) that have superior properties [21]. Hybrid superiority is associated with the phenomenon of heterosis [21, 22]. Heterosis also named hybrid vigor is defined as the phenomenon whereby a progeny exhibits phenotypic superiority over its parents with regard to traits such as growth rate and yield [23]. The heterosis phenomenon causes F1 plants have more vigor growth, grow faster, and produce higher yields than non-hybrid varieties.

However, the superiority of hybrid varieties was not obtained in the second generation (F2) and subsequent populations [24]. In addition, the price of hybrid varieties is more expensive than non-hybrid varieties. Therefore, PKHT-80 and PKHT-90 have the potential to compete with commercial hybrid varieties because they are not hybrid varieties so that can be planted more than once generation without reducing their productivity significantly.

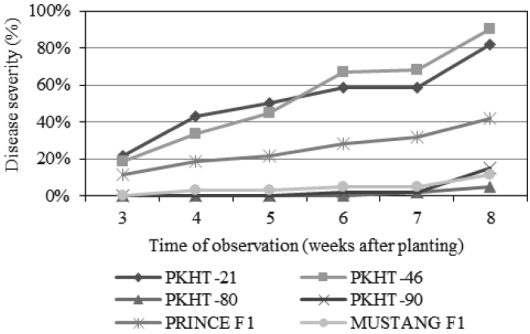

Fig. 2. Development of disease severity of bacterial wilt disease on six genotype of eggplant on field at 3–8 weeks after planting.

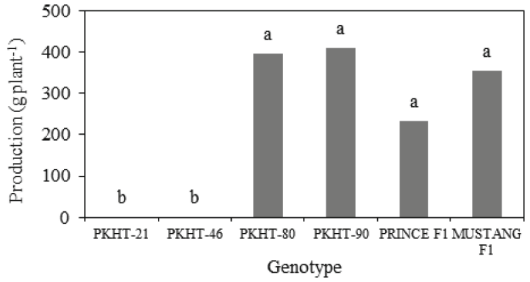

Fig. 3. Production of six genotype eggplant on field testing.

4 Conclusion

Artificial inoculation in greenhouse could be an alternative method to determine resistance of eggplant against bacterial wilt disease. Bacterial colonization is the most accurate and reliable parameter to determine plant resistance against bacterial wilt in greenhouse testing. PKHT-080 and MUSTANG F1 are resistant, PKHT-90 and PRINCE F1 are moderately resistant, whereas PKHT-21 and PKHT-46 are susceptible to bacterial wilt disease. PKHT-80 and PKHT-90 are resistant to bacterial wilt disease and has a productivity that can compete with commercial hybrid varieties.

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