Jurnal Perlindungan Tanaman Indonesia, Vol. 23, No. 1, 2019: 16–22
DOI:10.22146/jpti.34912
Available online at http://jurnal.ugm.ac.id/jpti
ISSN 1410-1637 (print), ISSN 2548-4788 (online)

Research Article

The Effectiveness of Several Plant Extracts to Induce Rice Plant Resistance against Bacterial Leaf Blight - (*Xanthomonas oryzae* pv. *oryzae*)

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Received April 25, 2018; revised September 4, 2018; accepted October 2, 2018

ABSTRACT

Resistant plants are one of the disease control techniques that considered to be effective. Resistant plants can be produced in various ways including the application of plant extracts. The aim of this study was to examine the ability of several plant extracts to increase the resistance of rice plants to bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). A total of 13 plants were extracted and applied in two methods, which were seed treatment and seedling treatment which sprayed on two-week old rice seedlings. *Xoo* bacteria were inoculated on rice plants two weeks after planting. The observations on the intensity of BLB disease infection showed that water hyacinth extract (*Eichhornia crassippes*), spiny amaranth (*Amaranthus spinosus*) and jasmine leaves (*Jasminum grandiflorum*) can suppress the development of BLB disease in both application methods. The application of plant extracts as inducing agents needs to be repeated to maintain the activated plant defense mechanism.

Keywords: plant extract, resistance induction, seed treatment, seedling treatment

INTRODUCTION

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae pv. oryzae* (*Xoo*) is one restraining factor for rice production. This disease causes rice yield loss up to 90% (Rafi *et al*., 2013). BLB disease can be found in various ages of rice growth. Young rice plants infected by *Xoo* show symptoms of wilt followed by symptoms of blight, starting from the edge of the leaf. *Xoo* enters the plant through stomata or wounds and then attacks the plant systemically by invading xylem vessels to cause symptoms of blight (Mew *et al*., 1993).

Plant disease control that relatively easy and efficient is by using resistant varieties. However, BLB disease is relatively difficult to control. The use of BLB-resistant rice plants is thought to cause a more severe explosion of BLB disease (Mew *et al*., 1993). Development of resistant varieties through plant breeding programs is time consuming. Along with the development of pathogens, the resistance of resistant varieties is relatively easy to break. Therefore another novel alternative is needed to increase plant resistance to disease.

Plants respond to pathogen attacks by activating a series of defense mechanisms. However, activated plant defenses are often formed later which in the mean time pathogens are able to invade plant tissues and causing disease on plants (Agrios, 2005). Various resistance trigger factors have been reported to be able to activate plant defenses against pathogens so that plants can react faster when the pathogen infects. Sumardiyono *et al*. (2015) stated that increasing the resilience of plants by activating the plant defense systems is one of the environmentally friendly control methods. Various plant extracts are reported to increase plant resistance to disease. It has been reported that basil extract can increase the resistance of rice plants to sheath blight disease (*Rhizoctonia solani*) (Pal *et al*., 2011), spiny amaranth and neem plant extracts against the disease of brown spot (*Bipolaris oryzae*) in rice (Harish *et al*., 2007) and pagoda plant extract (*Clerodendrum japonicum*) against CMV disease in chili plants (Hersanti, 2005).

However, although in general the resistance-inducing agents in plants have a broad spectrum and can last a long time, the response of host plants to

the application of plant extracts as resistance-inducing agents varies depending on the application method, plant type and environment (Walters *et al*., 2013). To be effective in activating plant resistance to disease, application of resistance induction needs to be carried out from the start. Generally, induction agents are applied as seed treatments or treatments in the nursery phase (Goellner & Conrath, 2008), therefore it is necessary to explore the ability of various plant extracts to induce resistance to BLB disease using different application methods.

MATERIALS AND METHODS

Preparation of Plant Extracts

Various plants include pagoda (*Clerodendron paniculatum*), four o'clock flower (*Mirabilis jalapa*), water hyacinth (*Eichornia crassipes*), spiny amaranth (*Amaranthus spinosus*), neem (*Azadirachta indica*), jasmine (*Jasminum grandifolium*), Indian camphorweed (*Pluchea indica*), chik weed (*Ageratum conyzoides*), basil (*Oicimum basilicum*), Siam weed (*Chromolaena odorata*), Madeira-vine (*Anredera cordifolia*) and galangal rhizome (*Alpinia galangal*) washed and rinsed with distilled water for drying. Extracts were made by adding distilled water to obtain an extract concentration of 10% (b/v). Extracts were filtered through series of filtration, which were centrifugation, filter paper and Whatman paper No. 1 (Suganda *et al*., 2002).

Treatment of Rice Seeds and Seedlings with Plant Extracts

Ciherang rice varieties were soaked for 24 hours to break dormancy. Seeds were then dried with filter paper. Seeds were induced by immersing seeds in plant extract solution (10% b/v) for one hour with the entire surface of the seeds were submerged. After soaking the seeds were grown in the seeding tray. While for seedling treatment, 2 weeks old rice seedlings were sprayed with plant extracts (10% b/v) until the extract dripping. The next day seeds were transferred from the seeding tray to the bucket filled with paddy soil (Suganda *et al*., 2002).

Bacterial Propagation and Xoo *Inoculation of* Xoo

Xoo were obtained from the Indonesian Center for Rice Research in Sukamandi, West Java. Bacteria were grown on glucose yeast extract media (GYE, 20 g glucose, 10 g yeast extract, and 2% agar for each liter of distilled water). As a source of inoculum, a single *Xoo* colony from GYE media in petridish was then inoculated into 250 ml GYE liquid media which was then shaken out (100 rpm) for 24 hours at room temperature. The bacterial suspension was measured and the density adjusted using a spectrophotometer with OD_{600} to reach bacterial cell density of 10^7 cfu/ml. Tween 20 was added to reach a concentration of 0.5%. Inoculation was carried out by dipping rice leaves (age 2 weeks after transplanting) which had been injured in bacterial suspension for 2 minutes (Niño-Liu *et al.*, 2005).

Observation

Observation of the BLB disease severity started from 4 WAP (weeks after planting). Disease severity (I) was calculated using the following formula (Rafi *et al*., 2013):

$$I = \left[\frac{n(1) + n(3) + n(5) + n(7) + n(9)}{tn}\right] \times 100\%$$

I = disease severity; n = number of leaves showing the percentage score of leaves attacked; tn = total number of leaves observed. Disease scores were based on the categories shown in Table 1.

Detached Leaf Assay

Plants that have been applied with various plants extracts, both in seed treatment and seedling treatment, were used as plant material in detached leaf assay methods. From the previous experiment, healthy leaves that did not show symptoms of bacterial leaf blight were selected. The leaves were then cut 15 cm long and cleaned by dipping the leaves for 30 seconds into sterile distilled water. The leaves were air then dried. After drying, the leaves were stored in a plastic box coated with moist tissues. The cleaned leaves were then punctured with sterile needles that have been soaked in alcohol.

Table 1. Scoring of rice leaf blight disease (Rafi *et al*., 2013)

Disease score	Percentage of leaves infected (%)
0	0
1	>1−10 %
3	>11−30 %
5	>31−50 %
7	>51−75 %
9	>76−100 %

Leaves were punctured at four spots for each leaf, then dripped with 5 µl of bacterial *Xoo* suspension (10^7 cfu/ml) at each point of the wound. The tip of the leaf was then covered with a tissue that has been moistened. Observations made after symptoms appeared by measuring the symptom length of BLB in each leaf using a micrometer (Akhtar *et al.*, 2008).

RESULTS AND DISCUSSION

The BLB Disease Severity after Application of Plant Extracts by Seed Treatment and Seedling Treatment Methods

In general, the average of BLB disease severity on rice plants, which the seeds were treated with various plant extracts, fluctuated at each observation time. These fluctuations of the BLB disease severity were thought to be influenced by plant extracts treatments. The BLB disease severity tended to increase in almost all of the plant extract treatments except in the treatments of water hyacinth extract, jasmine, neem, Indian camphorweed and basil (Table 2). The BLB disease severity at 8 WAP of those treatments were decreased compared to the initial disease finding (4 WAP). Nevertheless, statistical analysis did not show any significant difference in each treatment compared to the control.

At 4 WAP, it can be seen that the BLB disease intensity was relatively similar in each treatment. There was no noticeable effect of the seed treatment with plant extracts on BLB disease severity. It was suspected that the application of plant extracts to rice seeds was not been able to activate the plant's defense mechanism against BLB disease. However, in subsequent observations, there was difference in the level of *Xoo* infection in each treatment. Even in some treatments, rice plants showed higher disease intensity compared to controls. At 5 WAP, the treatment of Indian camphorweed extract showed higher BLB disease severity than controls or other treatments. However, in subsequent observations, BLB disease intensity was seen to decrease. This was suspected that the Indian camphorweed extract need longer period of time to be able to activate the rice plant's defense system.

Meanwhile, in observations of 6 WAP, 7 WAP and 8 WAP, rice plants treated with Madeira-vine extract showed higher BLB disease severity compared to controls or other extract treatments. At 8 WAP, the intensity of BLB disease at Madeira-vine extract treatment reached 41.83%. It was suspected that, Madeira-vine extract when applied as seed treatment was not able to stimulate the rice defense mechanisms against BLB disease. At the end of observation (8 WAP), it was seen that only seed treatment with extracts of water hyacinth plants, spiny amaranth, neem jasmine, Indian camphorweed and galangal having the disease intensity which was relatively different from the control treatment. Treatment with other plant extracts did not show any difference compared to controls.

Table 2. Bacterial leaf blight severity in rice plants following seeds treatment with various plant water extracts

Treatment	The bacterial leaf blight disease severity (%)				
	4 WAP	5 WAP	6 WAP	7 WAP	8 WAP
A. Control	26.1a	23abcd	22.62b	33.1ab	30.47ab
B. Pagoda	26a	20abcd	21.03b	21.6bcd	27.23ab
C. Four o'clock	14.73a	22.96abcd	23.33b	17.0cd	27.56ab
D. Water hyacinth	23.23a	12.56cd	20.07b	16.4cd	22.6b
E. Spiny amaranth	17.5a	14.4bcd	16.16b	25.4bcd	17.13b
F. Neem	21.9a	26.6abc	15.20b	14.3d	15.3b
G. Jasmine	22.76a	20.06abcd	17.33b	18.1cd	20.76b
H. Indian camphorweed	19.26a	29.63a	21.77b	21.9bcd	16.73b
I. Galangal	20.06a	16.93bcd	16.30b	26.04bcd	20.86b
J. Chik weed	25.53a	16.93abcd	19.13b	27.3bcd	27.33ab
K. Basil	26.8a	23.8abcd	18b	29.3bc	21.83b
L. Siam weed	19.3a	27.3ab	18.83b	24.1bcd	26.83ab
M. Madeira-vine	20.56a	21.16abcd	32.25a	43.7a	41.83a

Remarks: Means followed by the same letter in one column were not significantly different based on the Duncan test at the level of 5%; WAP =Week After Planting.

Walters *et al*. (2005) stated that activation of the defense mechanism in plants following the inducing agent application occurs directly or will be actived when the plant is infected with pathogens. It is suspected that this happened in the treatment of rice seeds with plant extracts, the defense system of rice plants was only activated when the plants infected by pathogens.

Different situations were found in the seedling treatment with plant extracts (Table 3). If in the seed treatment, at 2 weeks after *Xoo* inoculation (4 WAP) the effect of extracts in inducing plant resistance was not seen, in the seedling treatment, at 4 WAP different level of BLB disease severity among treatments were detected. BLB severity was observed to be high in most of treatments. The treatment with extracts of Indian camphorweed, Madeire-vine and four o'clock flower were seen able to reduce the disease severity compared to controls. It was suspected that the resistance mechanism of the plant was activated directly. However, the plant resistant against BLB did not occur permanently. This was found in the subsequent observations, BLB disease severity was detected increasing rapidly.

At the observations of 6 WAP, 7 WAP, and 8 WAP, the BLB disease severity fluctuated. Some treatments showed higher BLB intensity compared to controls. At 8 WAP, only seedling treatments with extract of pagoda, water hyacinth, spiny amaranth, jasmine and Madeira-vine showed lower BLB severity which significantly different than controls. While the other treatments showed higher intensity of BLB compared to controls.

BLB Disease Patches Symptoms Length in Detached Leaf Assay

The effect of plant extracts on the BLB length lesion is presented on Table 4. Not all treatments of plant extracts on rice seeds can inhibit the growth of the lesion. It can be seen that in some extract treatments, the length of BLB lesion was found longer than the control. Even in some treatments, there was a significant increase in lesion length. The growth size of the lesion was faster than the lesion growth on the control. This was found on rice plant which its seed was treated by water extracts of neem, galangal, Madeira-vine, and Indian camphorweed. It was suspected that the effectiveness of neem, galangal and Indian camphorweed extracts in inducing rice plant resistant against *Xoo* was decreased. Because in the *in planta* method, those plant extracts were able to reduce the *Xoo*'s infection at 8 WAP (see Table 2). Whereas, the Indian camphorweed extract did not show any ability to reduce the BLB disease severity from the beginning.

However, there were several seed treatments by plant extracts which inhibit the development of lesions and significantly different from controls. These treatments were extracts of pagoda plants leaves, water hyacinth, four o'clock flower, and jasmine.

Table 3. The bacterial leaf blight disease severity in rice plants following seedling treatment with various plant extracts

Treatment	Bacterial leaf blight disease severity (%)				
	4 WAP	5 WAP	6 WAP	7 WAP	8 WAP
A. Control	12.73def	31.1b	22.62a	33.10a	27.07bc
B Pagoda	21.13cd	29.5b	22.12a	27.73abc	24.17c
C. Four o'clock	9.23def	14.63b	20.79ab	20.43abcd	26.98bc
D. Water hyacinth	38.67ab	31b	22.44ab	14.38d	19.07c
E. Spiny amaranth	45.9a	33.07ab	20.07ab	16.37cd	21.87c
F. Neem	29.97bc	20.20b	18.26ab	20.92abcd	26.73bc
G. Jasmine	43.13a	48.98a	39ab	23.75abcd	24.8c
H. Indian camphorweed	5.57f	15.40a	16.37ab	13.60bcd	37.67ab
I. Galangal	22cd	22.92b	17.33ab	18.10bcd	32.77a
J. Chik weed	22.4cd	21.84b	20.21ab	16.93cd	31.5bc
K. Basil	19.23cde	23b	17.73ab	29.94ab	30.73bc
L. Siam weed	19cde	23b	17.83ab	24.73abcd	32.89abc
M. Madeira-vine	7ef	19.53b	19.13ab	27.33abc	24.89c

Remarks: Means followed by the same letter in one column were not significantly different based on the Duncan test at the level of 5%; WAP=Week After Planting.

Table 4. The length of bacterial leaf blight lesion on the leaves of rice plants treated with various plant extracts as seed treatment

Treatment	The length of BLB lesion (mm)	
	3 DAI	7 DAI
A. Control	0.2035b	0.2779e
B. Pagoda	0.24c	0.2892e
C. Four o'clock	0.1996c	0.2577d
D. Water hyacinth	0.0352a	0.0365a
E. Spiny amaranth	0.1835b	0.2392c
F. Neem	0.3575d	0.4902i
G. Jasmine	0.2438c	0.2956e
H. Indian camphorweed	0.1910b	0.3348f
I. Galangal	0.2671c	0.4283h
J. Chik weed	0.1942b	0.2594d
K. Basil	0.1817b	0.2244c
L. Siam weed	0.1171a	0.1460b
M. Madeira-vine	0.2636c	0.3588g

Remarks: Means followed by the same letter in one column were not significantly different based on the Duncan test at the level of 5%; DAI = Days After Inoculation.

The best seed treatment of plant extracts to inhibit the development of bacterial leaf blight was water extract of water hyacinth plants. There was no significant increase in the size of the lesion on the 3 DAI (Day After Inoculation) and 7 DAI.

The leaf extract of pagoda plant has an active compound in the form of a 34 kDa protein which can cause tobacco to be immune to the virus infection, this allows the leaf extract of pagoda plants to also affect rice plants (Verma *et al*., 1998; Kurnianingsih *et al*., 2012). Kumalasari *et al*. (2015) in their study reported that water hyacinth extract can inhibit the development of Cucumber mosaic virus (CMV) for cucumber plants. Up to now, there have been no studies reporting the use of water hyacinth extract to increase plant resistance to bacterial leaf blight. The induction of resistance to bacterial blight (*Xanthomonas campestris* pv. *malvacearum*) in cotton plants was previously reported by Satya *et al*. (2007) who used zimmu (*Allium sativum* L. x *Allium cepa* L.) plant extract as an inducing agent. The resistance of rice plants to *Xoo* is reported to be formed when rice plants are applied with malabar nut (*Adathoda vasica*) by increasing the production of PR proteins (pathogenesis-related) such as peroxidase, PAL (phenylalanine ammonia-lyase), β-1,3-glucanase and compound formation of phenol (Govindappa *et al*., 2011).

It was suspected that water hyacinth extract contains inhibitors therefore it can suppress the development of diseases in plants (Kumalasari *et al*., 2015). The ability of the four o'clock flower extract in inducing disease resistance was thought to be because the four o'clock flower extract contained active compound which was able to increase salicylic acid in which able to express pathogenesis-related protein genes (Hersanti, 2005). Goellner and Conrath (2008) stated that salicylic acid plays role as inducing signal that triggers the formation of defense genes in the form of PR proteins. These proteins can be found in healthy plants, but the concentration will be greatly increased when the plant is infected with pathogens. Meanwhile, the other treatments showed no significant effect on rice plants.

Following the observation on the size of lesion of the seedling treatment-rice plants, not all of the treatments were able to inhibit the lesion development (Table 5). At the 3 DAI, it can be seen that the treatment with water hyacinth extract showed good inhibitory effect in suppressing the development of BLB disease. However, at 7 DAI, the treatment of water hyacinth extract and other treatments did not show any significant effect on BLB. Suganda (2002) reported that inducing plant resistance using the seedling treatment method did not make the plant immune but only increased the degree of resistance and inhibited disease progression. In this study, the results of experiments in greenhouse and in leaf detached assay showed treatments that consistently

Table 5. Length bacterial leaf blight lesion on the leaves of rice plants treated with various plant extracts as seedling treatment

Treatment	The length of BLB lesion (mm)	
	3 DAI	7 DAI
A. Control	0.1938a	0.2038 a
B. Pagoda	0.2754b	0.3056 a
C. Four o'clock	0.1921a	0.2038 a
D. Water hyacinth	0.1377a	0.1438 a
E. Spiny amaranth	0.2032a	0.215 a
F. Neem	0.2767b	0.2871 a
G. Jasmine	0.2311b	0.2340 a
H. Indian camphorweed	0.2108a	0.2142 a
I. Galangal	0.1717a	0.1890 a
J. Chik weed	0.2438 a	0.2513 a
K. Basil	0.1275	0.1502 a
L. Siam weed	0.1717 a	0.2392 a
M. Madeira-vine	0.1914 a	0.1982 a

Remarks: Means followed by the same letter in one column were not significantly different based on the Duncan test at the level of 5%; DAI = Days After Inoculation.

inhibited the development of BLB disease when applied both as seed treatment and seedling treatment methods.

The effectiveness of an inducing agent in activating the defense mechanism is influenced by various factors ie the host plant itself and environmental factors. Plant varieties influenced the effectiveness of inducing agents in increasing plant resistance. Romero and Ritchie (2004) stated that in susceptible varieties, the defense mechanism will be more quickly formed compared to resistant varieties. Activation of defense systems requires certain amount energy and nutrients in which could be used for plant growth and development (Goellner and Conrath, 2008; Walters *et al*., 2005). Therefore, under stressed environment condition, plants will divert their energy for growth rather than being used to activate the defense mechanism.

Furthermore, other factor that influence the effectiveness of inducing agents in activating plant defense mechanism is method of delivery. The application of an inducing agent needs to be done as early as possible when the plants are young. Thus, the plant defense mechanism can be activated and can provide protection since the plants are young (Walters *et al*., 2013). In addition, the application of an inducing agent also needs to be repeated. This is because not all activated plant defense mechanisms can last long (Luna *et al*., 2015). Similar situation happened to rice plants treated with plant extracts. Some treatments of plant extracts, such as Indian camphorweed and Madeira-vine extracts, need to be sprayed on rice leaves repeatedly. Single spraying application on rice leaves was thought unable to maintain the resistance of the activated plants. While other plant extract treatments may not able to activate the rice defense mechanism against *Xoo*. Only extracts of water hyacinth, spiny amaranth, and jasmine in both application methods (seed and seedling treatment) were thought to increase the resistance of rice plants against BLB disease.

CONCLUSION

The treatment of rice seeds and seedlings with extracts of water hyacinth leaves, spiny amaranth and jasmine increased the resistance of rice plants to BLB disease. The activated defense mechanism in rice plants by those treatments is unknown.

ACKNOWLEDGMENTS

We would like to thank to the Universitas Padjadjaran through financial support from the Padjadjaran Internal Grants (HIU) for the 2016 Academic Leadership Grant (ALG) scheme to Tarkus Suganda, in which this research could be performed smoothly.

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