EFFECTS OF LATE BLIGHT RESISTANT POTATO CONTAINING RB GENE ON THE SOIL MICROBES, PESTS AND PLANT DISEASES

Pengaruh Kentang Tahan Hawar Daun yang Mengandung Gen RB pada Mikroba Tanah, Hama, dan Penyakit Tanaman

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ABSTRACT

Late blight caused by *Phytophthora infestans* is an important disease on potato. Several potato hybrids have been generated by crossing local varieties (Atlantic and Granola) with Katahdin SP951 which contains late blight resistance gene RB. Prior to release, these hybrids need to be evaluated for their environmental effects on non-target organisms and natural pests and diseases. The objectives of the study were to investigate the effect of LBR potato hybrids on beneficial soil microbes, pests and diseases. The trial was conducted in the confined field trial (CFT) in Lembang, West Java. The parental non-transgenic (NT) clones (Granola, Atlantic and Katahdin) and LBR hybrids (four clones of Atlantic x Katahdin SP951 hybrids; 10 clones of Granola x Katahdin SP951) were planted at a plant spacing of 30 cm x 70 cm. Fungicide applications were used as treatments (no spray, five and twenty times sprays). The experiment was arranged in a randomized completely block design with three replications. The parameters determined were populations of N_2 fixing and P solubilizing bacteria, soil C/N ratio as well as natural pests and diseases. The results showed that the transgenic LBR potato hybrids did not have negative effect on N fixing bacteria. The bacterial populations were around 10^{10-11} cells g^{-1} soil before planting, 10^{12} cells at 1.5 months after planting (MAP) and 10^8 cells after harvest. For P- solubilizing bacteria, their populations were 10^{10} cells before planting, 10^{12} cells at 1.5 MAP and 10^{11} cells g^{-1} soil after harvest. The soil C/N ratio of the transgenic plot was not statistically different compared to non-transgenic plot, i.e. 12-15 before planting, 10-11 at 1.5 MAP, and 10 after harvest in non-spray plot. Pests and diseases such as *Alternaria solani, Liriomyza*, potato tubber moth, aphid and mites on the transgenic and non-transgenic plots were statistically not different. The resistance score for *A. solani* was 7.2 (parental tansgenic) and 7.6 (parental non-transgenic); for *Liriomyza* it was 2.07 (parental transgenic) and 2.32 insect per plant (parental non-transgenic), the PTM was 0.63 (parental transgenic) and 0.73 insect per plant (parental non-transgenic), aphid and mites were 0.75 (parental transgenic) and 1.68 insects per plant (parental non-transgenic). The study indicated that LBR potato hybrids did not have any negative impacts on non-target organisms.

[***Keywords*:** Potato, RB gene, transgenic plants, *Phytophtora infestans*, soil microbes, pests, diseases]

ABSTRAK

Penyakit busuk daun yang disebabkan oleh Phytophthora infestans *merupakan penyakit penting pada kentang. Beberapa hibrida kentang telah dihasilkan dari persilangan varietas lokal (Atlantic dan Granola) dengan Katahdin SP951 yang mengandung gen resisten hawar daun RB. Sebelum dilepas, hibrida ini perlu dievaluasi efek lingkungannya terhadap organisme bukan sasaran serta hama dan penyakit alami. Penelitian ini bertujuan mengetahui pengaruh kentang LBR transgenik pada mikroba tanah berguna serta hama dan penyakit alami. Penelitian dilaksanakan di Lapangan Uji Terbatas (LUT) di Lembang, Jawa Barat. Tanaman kentang transgenik LBR (empat klon Atlantic x Katahdin hibrida SP951, 10 klon Granola x Katahdin SP951) serta tanaman kontrol nontransgenik (NT) (Granola, Atlantic, dan Katahdin) dan kontrol transgenik Katahdin SP951 ditanam dengan jarak tanam 30 cm x 70 cm. Penyemprotan fungisida (tidak disemprot, disemprot 5 dan 20 kali) digunakan sebagai perlakuan. Penelitian disusun dalam rancangan acak lengkap, tiga ulangan. Parameter yang diamati yaitu populasi bakteri penambat N_2 dan bakteri pelarut P, nilai C/N tanah, serta hama dan penyakit alami. Hasil penelitian menunjukkan bahwa transgenik LBR tidak menimbulkan dampak negatif terhadap bakteri pengambat N dengan populasi 10^{10}-10^{11} sel sebelum tanam, 1.0^{12} sel pada umur 1,5 bulan setelah tanam (BST), dan 10^8 sel g^{-1} tanah setelah panen. Populasi bakteri pelarut P sekitar 10^{10} sel sebelum tanam, 1.0^{12} sel pada umur 1,5 BST, dan 10^{11} sel g^{-1} tanah setelah panen. Nilai C/N rasio tanah dari plot transgenik tidak berbeda dengan non-transgenik, sekitar 12-15 sebelum tanam, 10-11 pada 1,5 BST dan 10 setelah panen pada petak yang tidak disemprot pestisida. Hama dan penyakit seperti* Alternaria solani, Liriomyza, potato tubber moth, *apid, dan tungau pada petak transgenik dan non-transgenik tidak berbeda. Pada akhir pengamatan, skor resistensi untuk* A. solani *adalah 7,2 (tetua tansgenik) dan 7,6 (tetua non-transgenik);* Liriomyza *rata-rata 2,07 (tetua transgenik) dan 2,32 serangga per tanaman (tetua non-transgenik), PTM rata-rata 0,63 (tetua transgenik) dan 0,73 serangga per tanaman (tetua non-transgenik) serta apid dan tungau rata-rata 0,75 (tetua transgenik) dan 1,68 serangga per tanaman (tetua non-transgenik). Studi ini menunjukkan bahwa tanaman kentang LBR transgenik tidak menimbulkan dampak negatif terhadap organisme bukan sasaran serta hama dan penyakit.*

[***Kata kunci:** Kentang, gen RB, tanaman transgenik,* Phytophtora infestans*, mikroba tanah, penyakit tanaman*]

INTRODUCTION

Late blight caused by *Phytophthora infestans* is one of the most important diseases on potatoes in the world (Edwards 1956; Song *et al.* 2003; Fry 2008). An estimated loss due to the disease was USD3.25 billion per annum worldwide (Fry and Goodwin 1997). Aggressiveness of *P. infestans* has increased in the last two decades (Brurberg *et al.* 2011; Cooke *et al.* 2011) might be due to the intensive use of multiple fungicide application in humid condition, and development of resistance to fungicides based on the potential for sexual recombination within pathogen populations which could create new strains (Peters *et al.* 2014; Naerstad *et al.* 2013).

Transgenic potato containing late blight resistance (LBR) gene (Katahdin) which is resistant to *P. infestans* has been developed using *Agrobacterium tumefaciens* and tested for many years (Halterman *et al*. 2008; Halterman and Middleton 2012). Hybrids of Katahdin LBR potato with local varieties Atlantic and Granola have been developed in Indonesia and evaluated at a confined field trial to the late blight (Ambarwati *et al*. 2011). Prior to release, these hybrids should be evaluated their safety to environment as regulated in the Government Released Regulation No. 21/2005 concerning the Biosafety of Genetically Engineered Products (Herman 2009). Some environmental aspects that need to be evaluated are their effects on beneficial soil microorganisms including changes in the chemical compositions of root exudates which may interfere beneficial soil microorganisms (Liu *et al.* 2005).

Most studies suggested that transgenic plants caused transient and minor changes in soil microbial community structures (Oger *et al*. 1997). Some studies also showed that soil microbial population in transgenic potato lines was not significantly different than that of non-transgenic ones (Milling *et al*. 2004; Demaneche *et al.* 2008; Ikeda *et al*. 2006). By using 16S- and 18S-rDNA denatured gradient gel electrophoresis (DGGE) fingerprints techniques, Milling *et al*. (2004) showed no significant differences between two potato cultivars and the transgenic line. Similar results were obtained for the rhizosphere samples using the eubacterial, α and β-proteobacterial and fungal specific primers.

Engineering of transgenic potato resistant to *P. infestans* aims to reduce late blight disease while does not give adverse, however no effect to the environment especially beneficial soil microorganisms. Previous result by Ikeda *et al*. (2006) showed that there was no difference in ribosomal intergenic spacer analysis (RISA) profile and terminal restriction fragment length polymorphism (T-RFLP) of soil bacteria on transgenic tomatoes containing 3-hydroxyl 3 methylglutaryl coenzyme reductase compared to soil from the non-transgenic rhizosphere.

The objectives of the study were to investigate the effect of LBR potato hybrids on beneficial soil microbes such as N_2-fixing and P-solubilizing bacteria and the response of tested clones for natural pests and diseases.

MATERIALS AND METHODS

Experimental Site and Planting Condition

This experiment was conducted in an endemic late blight confined field trial (CFT) of the Indonesian Vegetables Research Institute (IVEGRI) in Lembang, West Java, from January 2012 to March 2012. Location of the CFT was far from human residential, and no related species were planted throughout the experiment. The northern part of the plot was cultivated with cauliflower, while the southern and western parts were fallow and the eastern part was road/garden way (± 4 m wide).

Planting condition of genetically modified potato clones followed Ambarwati *et al.* (2009). Experimental plants were exposed to natural condition and no artificial inoculation was applied. Susceptible clones (Granola, Atlantic and non-transgenic Katahdin) were planted earlier as a border around the plot. Sweet corn was planted in five rows around the experimental field as borders, and two susceptible potato varieties (Atlantic and Granola) were planted as natural source for *P. infestans* inoculum.

Each clone was represented by ten plants. Plant spacing was 30 cm x 70 cm. Fungicide application was used as treatment in three levels, i.e. no spray, five-time spray and twenty-time spray. Local farmers commonly sprayed potato plants with fungicides of more than 40 times during a planting cycle.

Basal fertilizers, i.e. composted manure was applied at 30 t ha^{-1}, whereas NPK (15-15-15) at the rate of 800 kg ha^{-1} was applied three-fourth rate at planting time and the rest at 30 days after planting. Pest management was conducted as needed using the standard potato cultural practices, but no fungicide was used throughout the experiment.

Materials

Plant materials used were transgenic (T) clone, Katahdin SP951 (clone number 79) containing RB gene which confers late blight disease, and selected transgenic hybrid, i.e. four hybrid transgenic (HT) of Atlantic x Katahdin SP951 (clone number HT 26, 27, 28, 29) and 10 hybrids of Granola x Katahdin SP951 (clone number HT 38, 42, 43, 47, 62, 63, 65, 66, 69 and 70). As non-transgenic (NT) controls were No. 74 = NT Atlantic, No. 75 = NT Granola, and No. 76 = NT Katahdin. The plant materials were propagated using stem cutting method. Stem cuttings were obtained from mother stock in MS (Murashige and Skoog) medium and then transferred onto medium for root development. Plantlets were then transferred into plastic trays containing a mixture of rice husk charcoals and decomposed dung and maintained in the greenhouse.

Experimental Design

The environmental conditions were evaluated at the same time with agronomical measurement. Therefore, the experiment was designed for measuring agronomic characters and arranged in a randomized complete block design using three replications. The first treatment was potato clones, consisted of fourteen LRB transgenic clones (four LBR hybrids of Atlantic x Katahdin SP951 and 10 hybrids of Granola x Katahdin SP951). As the controls were three non-transgenic (NT) clones (NT Granola, NT Atlantic and NT Katahdin) and a transgenic (T) clone Katahdin SP951. The second treatment was three levels of fungicide application, namely no-spraying, five-time spraying and twenty-time spraying.

Data Collection

Soil Sample

Soil samples were taken three times during the experiment, i.e. before planting, at 1.5 months after planting (map) and after harvest. Soil samples were taken from the rhizosphere of control plots, i.e. non-transgenic (NT) clones, i.e. NT Atlantic (74), NT Granola (75) and NT Katahdin (76), and transgenic (T) clone Katahdin SP951 and transgenic hybrids) for each treatment and replication. Soil samples from the control plots were mixed for the same replication, and also 18 soil samples from the transgenic plots (CI0, CII0, CIII0, CI5, CII5, CIII5, CI20, CII20, CIII20, TI0, TII0, TIII0, TI5, TII5, TIII5, TI20, TII20 and TIII20, in which C representing control non-transgenic, T representing transgenic, and I, II, and III representing replication.

Beneficial Soil Microorganism Population

The beneficial soil microorganism population was determined using plating method on selected media, conducted in the Molecular Biology Laboratory of the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Bogor, West Java. Each sample was taken about 1 g and then added with 10 ml of saline buffer and diluted into 10^{-1} to 10^{-9}. The solution (100 µl) was then spread-plated into appropriate medium in duplicate plates. Plates were then incubated at 29°C for 2 weeks, and the colony forming units (CFU) were counted every day for each dilution, and the population were then determined.

Nitrogen Fixing and Phosphate Solubilizing Bacteria

Nitrogen fixing bacteria were cultured on Burke N-free selective medium containing 5 g DL-malic acid, 4 g KOH, 0.5 g K_2HPO_4, 0.1 g $MgSO_4.7H_2O$, 0.01 g $MnSO_4.H_2O$, 0.05 g $FeSO_4.7H_2O$, 0.2 g NaCl, 0.01 g $CaCl_2$, 0.002 g $Na_2MoO_4.2H_2O$, 2 ml alcoholic bromothymol blue (BTB), and 1.75 g agar, at pH 6.8 (Newton *et al*. 1953). While P solubilizing bacteria were grown on Pikovskaya's medium containing (per liter) 10 g glukosa, 5 g $Ca_5(PO_4)_3OH$, 0.2 g NaCl, 0.2 g KCl, 0.1 g $MgSO_4.7H_2O$, 2.5 mg $MnSO_4.H_2O$, 2.5 mg $FeSO_4.7H_2O$, 0.5 g yeast extract, 0.5 g $(NH_4)_2SO_4$ at pH 6.8, and 15 g agar (Pikovskaya 1948). Plate was incubated at 29°C for 6 days. Phosphate solubilizing bacteria could be differentiated from non-solubilizing P based on clear-zones formed on the media as P was dissolved from the tricalcium phosphate compound.

C/N Ratio

C/N value of the soil was analyzed in soil laboratory of the Center for Soil Research, Bogor. Soil samples were taken three times, i.e. before planting, during plant growing (1.5 month after planting), and after harvest. Carbon (C) content was determined using Wakley and Black (1934), and N content was determined using a method of Kjedalh (Bremner 1996).

Natural Pests and Disease

Investigations on other pests and diseases were conducted to determine whether the RB gene in the genetically modified potato influencing the change in resistance or susceptibility of the plant to other pests and diseases compared to the parental clones. Therefore in this experiment, all the existing pests and diseases in the field were observed and compared to the controls, i.e. NT Atlantic (74), NT Granola (75), and NT Katahdin (76) and the transgenic hybrids. All of the pests and diseases in the CFT were observed and scored every 2 weeks.

Plant resistance to diseases was determined by visual inspection of the symptoms of the diseases using the scale given by Colton *et al*. (2006), Henfling (1979) and Halterman *et al*. (2008) as follows: 0 = 100% infected tissue; 1 = >90%; 2 = 81-90%; 3 = 71-80%; 4 = 61-70%; 5 = 41-60%; 6 = 26-40%; 7 = 11-25%; 8 = <10%, and 9 (0%). Pests were observed on every number of plants.

Data Analysis

Data were analyzed using Tukey's Honestly Significant Difference (HSD) and Least Significant Difference (LSD).

RESULTS AND DISCUSSION

Populations of N-Fixing and P Solubilizing Bacteria

Microbial populations in the soil samples fluctuated before planting, in the middle of plant growing and after harvest. Before planting, N-fixing bacteria populations ranged from was 10^{10} to 10^{11} cells g^{-1} soil, and at 1.5 map it increased at 10^{12} cells g^{-1} soil, then decreased after harvest at 10^{8} cells g^{-1} soil. Statistical analysis showed that there was no significant difference in bacterial population between control non-transgenic plots and transgenic plots (Table 1). It means that cultivation of transgenic potato do not cause negative impact on the N-fixing bacterial population. Bacterial population with different fungicide applications, without treatment compared to 20 times application, from the soil samples taken before planting was significantly different. This difference could be canged by different soil conditions or crops planted before the CFT.

Population of P-solubilizing bacteria had a similar pattern as that of N-fixing bacteria. The statistical analysis of the data was shown in Table 2. There were no differences in bacterial populations between non-transgenic plots and transgenic plots and those in the soil taken in the middle of plant growing (1.5 map) and after harvest. It means that transgenic plants do not have negative impact on N-fixing bacterial population. However, bacterial populations were significantly different between treatments from the soil sample taken before planting. This condition is predicted due

Table 1. Population of N-fixing bacteria in the late blight resistant potato rhizosfere from the confined field trial (CFT) at Lembang, West Java, 2012.

Traetment[1)]	Bacterial population (viable cells g^{-1} soil)		
	Before planting (**)	1.5 month after planting (ns)	After harvest (ns)
C0	9.5 x 10^{10ab}	5.8 x 10^{12}	6.75 x 10^{8}
T0	1.1 x 10^{11a}	5.35 x 10^{12}	7.15 x 10^{8}
C5	1.16 x 10^{11a}	5.0 x 10^{12}	6.71 x 10^{8}
T5	1.16 x 10^{11a}	6.1 x 10^{12}	5.96 x 10^{8}
C20	4.9 x 10^{10bc}	3.45 x 10^{12}	9.0 x 10^{8}
T20	3.3 x 10^{10c}	6.5 x 10^{12}	7.63 x 10^{8}

[1)]C = soil samples from the rhizosphere of control non-transgenic plants; T = soil samples from the rhizosphere of transgenic plants; 0, 5, 20 = number of fungicide application. *) significant at P < 0.05, **) very significant at P < 0.01, ns) not significant. Numbers in the same column followed by the same letter are not significantly different.

Table 2. Population of P-solubilizer bacteria in the LBR potato rhizosphere from the confined field trial (CFT) Lembang, West Java, 2012.

Treatment	Mean value of bacterial population (viable cells g^{-1} soil)		
	Before planting (**)	1.5 month after planting (ns)	After harvesting (ns)
C0	2.56 x 10^{10}a	4.8 x 10^{12}	4.06 x 10^{11}a
T0	2.35 x 10^{10}a	4.8 x 10^{12}	4.46 x 10^{11}a
C5	2.0 x 10^{10}a	4.5 x 10^{12}	4.38 x 10^{11}ab
T5	2.7 x 10^{10}a	4.5 x 10^{12}	4.5 x 10^{11}a
C20	1.2 x 10^{10}b	4.2 x 10^{12}	4.23 x 10^{11}ab
T20	1.56 x 10^{10}b	4.2 x 10^{12}	4.45 x 10^{11}ab

[1)]C = soil samples from the rhizosphere of control non-transgenic plants; T = soil samples from the rhizosphere of transgenic plants; 0, 5, 20 = number of fungicide application.

**)very significant at P < 0.01, ns) not significant. Numbers in the same column followed by the same letter are not significantly different.

to the difference in soil fertility or planting conditions before the trial.

C/N Ratio

C/N values were not significantly different between soil samplse taken from the transgenic and non-transgenic plots. However, the values were significantly different at 1.5 map and after harvest, due to the different fungicide treatments (Table 3). It means that planting RB potato does not affect soil fertility. Therefore; concerns on the direct and indirect effects of RB potato on soil microorganism populations can be neglected because population of beneficial micro-organisms and C/N ratio in the soil from the transgenic rhizosphere was not significantly different compared to those in non-transgenic one.

Natural Occurrence of Other Diseases and Pests in the CFT

Pests and diseases observed in the CFT Lembang in 2012 were potato tuber moth (*Phthorimaea operculella*), *Liriomyza*, aphid and mites. While diseases observed in the CFT were *Alternaria solani* and bacterial blight/fusarium.

Alternaria solani

A. solani was found in non-sprayed plots and those sprayed five and 20 times. Disease score was presented in Table 4. Statistical analysis using Tukey test showed that resistance scores among the control plants and test plants for observation 1, 2 and 3 were not significantly different. It means that the hybrid clones (HT 26, 27, 28, 29, 38 and 42) have the same resistance score with the non-transgenic parents (HT 74, 75, 76) and transgenic parent (clone number 79). However, the scores were significantly different for the 4th and 5th observation. Hybrid clones number HT 27, 38 and 62 had the higher resistance score compared to other hybrid clones. These clones might be chosen as candidate for superior clones beside their resistance to *P. infestans*.

The resistance score among the fungicide application treatments in all observation 1, 2, 3, 4 and 5 were significantly different, whereas 20 time spraying lowered *A. solani* infection (Table 5). It means that fungicide application might help plant to grow healthier and less infected by *A. solani*.

Table 3. The C/N ratio of the soil in the LBR potato rhizosphere from the confined field trial (CFT) at Lembang, West Java, 2012.

Traetment	Mean value of bacterial population (viable cells g^{-1} soil)		
	Before planting (**)	1.5 month after planting (ns)	After harvesting (ns)
C0	12.67	10.00ab	10.33ab
T0	15.33	11.00a	10.67a
C5	13.67	8.67ab	9.67ab
T5	13.67	8.33b	9.33ab
C20	15.33	8.67ab	8.33b
T20	11.67	9.67ab	9.00ab

[1)]C = soil samples from the rhizosphere of control non-transgenic plants; T = soil samples from the rhizosphere of transgenic plants; 0, 5, 20 = number of fungicide application.

**)very significant at P < 0.01, ns) not significant. Numbers in the same column followed by the same letter are not significantly different.

Table 4. Tukey test for resistance score for *Alternaria solani* on potato at first to fifth observations the confined field trial (CFT) at Lembang, West Java, 2012.

Clones	Resistance score of *A. solani*				
	1(ns)	2	3(ns)	4	5
NT Atlantic (74)	8.98a	8.90a	7.87a	8.05a	7.52ab
NT Granola (75)	8.98a	8.93a	7.74a	8.76a	7.95a
NT Katahdin (76)	8.97a	8.91a	7.70a	8.22a	7.60ab
T Kahtadin SP951 (79)	8.85a	8.88a	7.53a	8.04a	7.20ab
26	8.95a	8.81a	8.59a	7.54a	7.44ab
27	8.93a	8.79a	8.73a	8.26a	7.66a
28	8.81a	8.6ab	8.30a	6.58a	4.29bc
29	8.96a	8.86a	7.68a	8.04a	6.84ab
38	8.97a	8.87a	8.47a	8.27a	7.93a
42	8.94a	8.84a	7.70a	8.11a	7.55ab
43	8.80a	8.40b	6.73a	3.70b	2.21c
47	8.98a	8.84a	8.74a	8.33a	7.65a
62	8.97a	8.86a	8.70a	8.35a	7.44ab
63	8.97a	8.82a	8.76a	7.92a	6.84ab
65	8.97a	8.83a	8.64a	7.80a	7.35ab
66	8.95a	8.84a	7.60a	8.01a	6.99ab
69	8.97a	8.84a	6.47a	6.76a	7.03ab
70	8.83a	8.84a	7.54a	7.78a	7.14ab

1-5 = resistance score 1-5. NT = non- transgenic, T = transgenic, 26-29 = hybrids between SP951 and Atlantic, 38-70 = hybrids between SP951 and Granola.

Table 5. Tukey test for insectice treatments on potato plant at first to fifth observation in the confined field trial at Lembang, West Java, 2012.

Treatments	Resistance score				
	1	2	3	4	5
No-spray	8.9b	8.7b	7.1b	7.5b	6.9ab
Spray 5 times	8.9b	8.7b	8.3a	7.4b	6.6b
Spray 20 times	9.0a	8.9a	8.4a	8.1a	7.2a

Liriomyza

Potato leaf miner fly (*Liriomyza huidobrensis*) was observed in five times examination at 2 week interval. This pest occurred only at unsprayed plot. The number of insects increased in the late phase of plant (Table 6). All clones were infected by *Liriomyza*, but it was not significantly different. It means that the parental and hybrids have the same response to the *Liriomyza* in the CFT.

Potato Tubber Moths

PTM or *Phthorimaea operculella* was observed in five times at 2-week interval on unsprayed plot in clones, control and tested plants. PTM population was not significantly different among the clones (Table 7). It means that the transgenic and non-transgenic clones have similar response toward the PTM in all observations times.

Table 6. Mean values of *Liriomyza* population on potato plant at first to fifth observation in the confined field trial at Lembang, West Java, 2012.

Clones	Liriomyza population (insects per plant)				
	1 (ns)	2 (ns)	3 (ns)	4 (ns)	5 (ns)
NT Atlantic (74)	0.45	0.00	0.42	0.83	1.58
NT Granola (75)	0.58	0.00	0.50	1.23	2.97
NT Katahdin (76)	0.32	0.00	0.35	0.67	2.32
T Katahdin SP951 (79)	0.43	0.00	0.65	1.55	2.07
HT 26	0.43	0.55	0.78	1.44	1.45
HT 27	0.66	0.07	0.43	1.37	2.67
HT 28	0.33	0.00	0.40	1.27	2.00
HT 29	0.42	0.33	0.62	1.17	3.70
HT 38	0.80	0.00	0.43	0.67	2.51
HT 42	0.72	0.00	0.42	1.55	4.51
HT 43	0.48	0.33	0.81	1.40	0.82
HT 47	0.83	0.33	0.96	2.27	3.51
HT 62	1.00	0.08	0.62	1.51	1.85
HT 63	0.38	0.07	0.58	0.90	1.23
HT 65	0.45	0.33	0.92	0.40	1.20
HT 66	0.72	0.00	0.40	0.60	1.00
HT 69	0.53	0.27	0.33	0.77	1.67
HT 70	0.23	0.00	0.30	1.17	3.68

NT = non-transgenic, T = transgenic, HT = hybrid transgenic, 26-29 = hybrid between SP951 and Atlantic, 38-70 = hybrid between SP951 and Granola.
ns = not significant.

Table 7. Mean values of potato tuber moth population on potato clones at first to fifth observation in the confined field trial at Lembang, West Java, 2012.

Clones	PTM population (insects per plant)				
	1 (ns)	2 (ns)	3 (ns)	4 (ns)	5 (ns)
NT Atlantic (74)	0.35	15.57	0.28	0.52	1.08
NT Granola (75)	0.37	16.25	0.30	0.38	1.47
NT Katahdin (76)	0.27	15.53	0.20	0.52	0.75
T Katahdin SP951 (79)	0.38	16.90	0.37	0.62	0.63
HT 26	0.43	15.72	0.72	0.68	1.00
HT 27	0.85	16.27	0.68	1.13	1.09
HT 28	0.25	15.77	0.79	0.88	3.12
HT 29	0.35	15.38	0.20	0.50	1.73
HT 38	0.82	16.40	0.66	0.72	1.93
HT 42	0.33	15.50	0.30	0.87	1.32
HT 43	4.63	11.50	0.22	0.70	0.22
HT 47	0.62	15.95	0.85	0.94	0.89
HT 62	0.82	16.92	0.65	1.18	1.37
HT 63	0.37	15.73	0.58	0.23	0.68
HT 65	0.37	16.37	0.65	0.47	0.84
HT 66	0.73	16.40	1.07	0.56	1.42
HT 69	0.42	16.72	0.37	0.45	1.80
HT 70	0.33	14.67	0.53	0.37	0.47

NT = non-transgenic, T = transgenic, HT = hybrid transgenic, 26-29 = hybrid between SP951 and Atlantic, 38-70 = hybrid between SP951 and Granola.
ns = not significant.

Aphid

Aphid especially green aphid was observed scatter in potato leaves especially at the bottom of leaves. Observation on non-transgenic control (Atlantic and Granola) and transgenic control (T Katahdin SP951), and on tested clones HT 28, 38, and 43 on unsprayed plot at 10 weeks after planting showed that both of transgenic and non-transgenic clone responses towards aphid were not significantly different (Table 8).

Mites

Mites were observed only in unsprayed plot showen by brown spot on underside of leaves. Some numbers of plants were attacked by mites including non-transgenic control (NT Granola) and transgenic control (T Katahdin SP951) and hibrid transgenic 70, 28, 66, 62, 47, 38, 27, 42, 29, 69. However, the data were not significantly different (Table 9).

Table 8. Mean values of aphid population on potato plant in the confined field trial at Lembang, West Java, 2012.

Clones	Aphid population (insect per plant)
NT Atlantic (74)	0.00
NT Granola (75)	1.85
NT Katahdin (76)	1.68
T Katahdin SP951 (79)	0.75
HT 26	0.00
HT 27	1.43
HT 28	0.98
HT 29	0.00
HT 38	0.93
HT 42	2.05
HT 43	0.77
HT 47	0.00
HT 62	0.00
HT 63	0.00
HT 65	0.00
HT 66	0.00
HT 69	0.00
HT 70	1.42

NT = non-transgenic, T = transgenic, HT = hybrid transgenic, 26-29 = hybrid between SP951 and Atlantic, 38-70 = hybrid between SP951 and Granola.

Table 9. Mean values of mite population on potato plant in the confined field trial at Lembang, West Java, 2012.

Clones	Mite population (insect per plant)
NT Atlantic (74)	0.00
NT Granola (75)	0.00
NT Katahdin (76)	0.00
T Katahdin SP951 (79)	0.00
HT 26	0.00
HT 27	0.00
HT 28	2.03
HT 29	0.00
HT 38	1.38
HT 42	0.00
HT 43	0.42
HT 47	0.00
HT 62	0.00
HT 63	0.00
HT 65	0.00
HT 66	1.57
HT 69	0.00
HT 70	1.92

NT = non-transgenic, T = transgenic, HT = hybrid transgenic, 26-29 = hybrid between SP951 and Atlantic, 38-70 = hybrid between SP951 and Granola.

CONCLUSION

Planting the transgenic LBR potato did not affect the beneficial bacterial populations in the rhizosphere, including N-fixing bacteria and P-solubilizing bacteria, and C/N value of the soil. LBR potato did not change the response of the naturally occurrence of pests and diseases. However, the resistance scores of the diseases were different during the stage of plant growth and fungicide treatment. *Alternaria solani* was observed in unsprayed and sprayed plots, while PTM, aphid and mites were only observed in unsprayed plots. Clones number 43 was more susceptible compared with other clones including control resistance and parent at observation 4, while at observation 5, clones number 27, 38, and 47 were more resistant. No significant different in all clones due to plant pest PTM, aphid and mites was found. From these results, it is concluded that planting transgenic potato containing RB gene did not have impact to the environment based on the parameters tested.

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