

RESISTANCE EVALUATION ON POPULATIONS OF CROSSES BETWEEN TRANSGENIC POTATO KATAHDIN *RB* AND NON-TRANSGENIC ATLANTIC AND GRANOLA TO LATE BLIGHT (*Phytophthora infestans*) IN CONFINED FIELD TRIAL

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ABSTRACT

Late blight resistance gene (*RB* gene) isolated from *Solanum bulbocastanum*, is a broad resistance gene against all races of *Phytophthora infestans*. The gene was transformed into Katahdin event SP904 and SP951 using *Agrobacterium tumefaciens* and these transgenic plants have been crossed with susceptible potato cultivars Atlantic and Granola. Populations of the crosses have been molecularly characterized for the integration of the *RB* transgene. The study aimed to evaluate the resistance of the populations of crosses between transgenic Katahdin *RB* and susceptible non-transgenic parents (Atlantic and Granola) to late blight in a confined field trial at Pasir Sarongge, Cianjur, West Java. A total of 84 clones originated from four populations were evaluated for resistance to late blight. These included 22 clones of Atlantic x transgenic Katahdin SP904, 16 clones of Atlantic x transgenic Katahdin SP951, 19 clones of Granola x transgenic Katahdin SP904, and 27 clones of Granola x transgenic Katahdin SP951. Observations of the late blight infection were conducted when late blight symptoms were detected, i.e. at 56, 60, 63, 70, and 77 days after planting (DAP). The result showed there were high variations in the resistance level of all the 84 clones tested. Clones of crosses between susceptible parents (Atlantic or Granola) and resistant parents (transgenic Katahdin SP904 or Katahdin SP951) showed a similar pattern based on the area under disease progress curve (AUDPC) value, i.e. 377.2 greater than the AUDPC of the resistant parents (180.1), but smaller than that of the susceptible parents (670.7). Observation at 77 DAP resulted four resistant potato clones having resistance score of 7.0-7.6, higher than the transgenic parents Katahdin SP904 (4.6) and Katahdin SP951 (6.8), i.e. clone B8 (Atlantic x transgenic Katahdin SP951) with resistance score of 7.6 and clones B26 (Atlantic x transgenic Katahdin SP951), C183 (Granola x transgenic Katahdin SP904), and D89 (Granola x transgenic Katahdin SP951) with resistance score of 7. These four transgenic potato resistant clones need to be further developed as promising potato clones to late blight.

[**Keywords:** Transgenic potato, *RB* gene, *Phytophthora infestans*, confined field trial]

INTRODUCTION

Potato breeding and extensive selection for late blight resistance caused by *Phytophthora infestans* have been carried out since 1900s. At least 11 race-specific late blight resistance (R) genes originated from *Solanum demissum* have been incorporated into various potato cultivars. However, all of these genes have already been overcome by new virulent races of the pathogen (Wastie 1991; Umaerus and Umaerus 1994).

In the 1970s, potato breeding with emphasis on vertical resistance was replaced by breeding for horizontal resistance (Wastie 1991). Various efforts were made to seek new sources of resistance, as the result a number of wild, tuber-bearing Mexican potato species have been identified to carry valuable resistance to late blight, thus providing a potential source of resistance for breeding programs (Black 1970). *Solanum bulbocastanum* ($2n = 2x = 24$), a wild diploid potato species from Mexico is considered to be a promising source of resistance and confers broad spectrum to all known races of the late blight, but sexually incompatible with potato (Hermsen and de Boer 1971; Song *et al.* 2003).

Gene cloning and transformation technique have provided a means for bypassing sexual incompatibility between *Solanum* species (Staples 2004). *RB* gene isolated from *S. bulbocastanum* for resistance to late blight, was transformed into Katahdin event SP904 and SP951 using *Agrobacterium tumefaciens* (Song *et al.* 2003). Katahdin plants transformed with the *RB* gene showed broad spectrum resistance against all known races of *P. infestans*, both in the greenhouse and in field experiments (Song *et al.* 2003;

Lozoya-Saldana *et al.* 2005; Kuhl *et al.* 2007; Halterman *et al.* 2008). Therefore, transgenic Katahdin SP904 and SP951 were used as resistant donor parents in crosses with susceptible potato cultivars Atlantic and Granola. The crosses have been molecularly characterized for the integration of the *RB* transgene (Ambarwati *et al.* 2009). To confirm that the integrated *RB* gene is correlated with resistance phenotype, further evaluation on the expressions to late blight is needed. The evaluation should be conducted in a confined field trial as regulated by the Government Regulation No. 21 of 2005 concerning the Biosafety of Genetically Engineered Products (Herman 2009). Resistance evaluation on populations of crosses between transgenic potato Katahdin *RB* and non-transgenic Atlantic and Granola to late blight in confined field trial has not yet been reported. The study aimed to evaluate the resistance of the populations of crosses between transgenic Katahdin *RB* containing the *RB* gene and susceptible non-transgenic parents Atlantic and Granola against late blight in a confined field trial.

MATERIALS AND METHODS

The experiment was conducted in an endemic late blight confined field trial station of the Bogor Agricultural University at Pasir Sarongge (1,120 m asl), Cianjur, West Java, from March to December 2008. Transgenic Katahdin SP904 and SP951 were obtained from Wisconsin University through the collaboration of Indonesia - USAID - ABSP (Agricultural Biotechnology Support Project) II. Four populations were evaluated for resistance to late blight. These include 22 clones of Atlantic x transgenic Katahdin SP904, 16 clones of Atlantic x transgenic Katahdin SP951, 19 clones of Granola x transgenic Katahdin SP904, and 27 clones of Granola x transgenic Katahdin SP951. These transgenic clones were chosen because preliminary study showed that they positively contained *RB* gene based on PCR analysis (Ambarwati *et al.* 2009). Female parents (Granola and Atlantic), male parents (transgenic Katahdin SP904 and SP951), non-transgenic Katahdin, and wild species *S. bulbocastanum* PT29 were used as control.

Planting in Confined Field Trial

Tubers were grown in pot, 30 cm in diameter, containing a mixture of manure, compost, and rice husk in a volume of 3:2:1. 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. The border plants were used as the source of disease inoculum. Border plants were planted around the plot, and every three clones in the plot were interspersed with a border plant. Each clone was represented by three plants. Plant spacing was 30 cm x 70 cm. Experiments were arranged in a randomized complete block design using three replications.

Location of confined field trial was far from human residential, and no related species were planted throughout the experiments. The northern part of the plot was fallow land, while the southern part was cultivated with carrot and onions. Pepper and tomato planting area was located at the western part of the plot, which was restricted by side road/garden's way (± 3 m wide). In the east, there was fallow land which adjacent with land which carrot and cabbage were grown. Basal fertilizers of compost 30 t ha⁻¹ and NPK (15-15-15) 800 kg ha⁻¹ were applied three-fourth rate at planting and the rest was applied at 30 days after planting (DAP). Pest managements were conducted as needed using the standard potato cultural practices, but no fungicide was used throughout the experiment.

Evaluation of Late Blight Resistance

Observations of the late blight infection were conducted when the late blight symptoms were detected, i.e. at 56, 60, 63, 70, and 77 DAP. Parameters observed include the late blight resistance score, disease intensity, and area under disease progress curve (AUDPC).

The late blight resistance score was determined by visual observation of the plants. The score was determined based on the percentage of infected leaf tissue (Table 1). An average score for the resistance

Table 1. Scoring of late blight infection based on the percentage of infected leaf tissue.

Score	Percent of infected leaf tissue
0	100
1	>90
2	81-90
3	71-80
4	61-70
5	41-60
6	26-40
7	11-25
8	<10
9	0

Source: Henfling (1979); Halterman *et al.* (2008).

of each clone was determined based on the three replicates. Plants were scored as resistant if the resistance score was > 7.0 ($< 25\%$ infection) (Song *et al.* 2003; Colton *et al.* 2006; Halterman *et al.* 2008) and were scored as susceptible if the resistance score was < 6.9 ($> 25\%$ infection) (Song *et al.* 2003).

Disease intensity was calculated using the formula as follows:

$$P = \frac{\sum n \times v}{N \times Z} \times 100\%$$

P = disease intensity

n = number of plants from each category of attack

v = scale of each category of attack

N = number of plants assessed

Z = highest score of scale

Area under diseases progress curve (AUDPC) is the accumulation of daily percent infection values. The AUDPC was analyzed based on Landeo (1999, unpubl.) as follows:

$$\text{AUDPC} = \sum_{i=1}^n [(X_{i+1} + X_i)/2] [t_{i+1} - t_i]$$

X_i = infected leaf area (%) at the i^{th} observation

t_i = time (days) at the i^{th} observation

n = total number of observations

RESULTS AND DISCUSSION

The first observation of late blight resistance score was started at 56 DAP when the border plants, i.e. Atlantic, Granola, and non-transgenic Katahdin exhibited 75-80% late blight infection. Leaves of all these susceptible plants displayed typical spreading late blight lesions, i.e. water-soaked areas and extensive rotting developed at later time points.

The average of late blight infection of susceptible controls (Atlantic, Granola, and non-transgenic Katahdin) observed at different periods is presented in Table 2. All plants showed few disease lesions with disease score of 7.1-8.5 and disease intensity of 5.6-20.8% at 56 DAP, however, at 63 DAP disease intensity was greater ($> 25\%$), therefore all these plants were considered as susceptible to the pathogen. These infected plants become a new source of inoculum for other healthy plants. Decrease in the level of resistance along with increasing disease intensity was significantly observed at the end of experiment (77 DAP). This illustrates that natural infection of the late blight in the experiment site was high, therefore, any resistance symptoms on the tested clones are justified.

On the contrary, disease resistance level on the four population check clones was stable as presented in Table 3. These clones, i.e. Atlantic x transgenic Katahdin SP904 (A), Atlantic x transgenic Katahdin SP951 (B), Granola x transgenic Katahdin SP904 (C), and Granola x transgenic Katahdin SP951 (D) demonstrated a high resistance score at 56 DAP. Most of the clones (98%) tested remained resistant, except clones A94 and B59 which have the same score (6.8) as that shown by the susceptible clone Atlantic. Differences in resistance level amongst clones of each cross occurred at 60 and 63 DAP. All clones of Granola x transgenic Katahdin SP904 (C) and Granola x transgenic Katahdin SP951 (D) were still resistant, while 4.8-20% of Atlantic x transgenic Katahdin SP904 (A) and Atlantic x transgenic Katahdin SP951 (B) were susceptible.

Number of resistant plants decreased with increase in the period of observation. Individually observation of clones from the four populations at 70 DAP showed that there were 31 (37.8%) resistant clones, i.e. 3 clones from A crosses, 5, 8 and 15 clones from B, C, and D crosses, respectively, with resistance scores

Table 2. Average of late blight (*P. infestans*) natural infection on three susceptible potato clones at a confined field trial in Pasir Sarongge, Cianjur, West Java, March-December 2008.

Observation period (DAP) ¹	Atlantic		Granola		Non-transgenic Katahdin	
	Resistance score (0-9)	Disease intensity (0-100%)	Resistance score (0-9)	Disease intensity (0-100%)	Resistance score (0-9)	Disease intensity (0-100%)
56	7.1	20.8	7.3	19.4	8.5	5.6
60	6.8	24.7	6.9	23.5	8.0	11.1
63	5.8	35.9	6.1	31.9	6.4	28.6
70	0.7	92.1	0.8	91.1	0.7	92.1
77	0.0	100.0	0.0	100.0	0.0	100.0

¹DAP = days after planting.

Table 3. Resistance evaluation on potato populations in an endemic late blight (*P. infestans*) confined field trial at various observation periods, Pasir Sarongge, Cianjur, West Java, March-December 2008.

Observation period (DAP) ¹⁾	Clones							
	Atlantic x transgenic Katahdin SP904 (A) ²⁾		Atlantic x transgenic Katahdin SP951 (B) ³⁾		Granola x transgenic Katahdin SP904 (C)		Granola x transgenic Katahdin SP951 (D)	
	No. of clones tested	No. of resistant clones	No. of clones tested	No. of resistant clones	No. of clones tested	No. of resistant clones	No. of clones tested	No. of resistant clones
56	22	22 (100.0)	16	16 (100.0)	19	19 (100.0)	27	27 (100.0)
60	21	20 (95.2)	16	15 (93.8)	19	19 (100.0)	27	27 (100.0)
63	21	18 (85.7)	15	12 (80.0)	19	19 (100.0)	27	27 (100.0)
70	21	3 (14.3)	15	5 (33.3)	19	8 (42.1)	27	15 (55.6)
77	21	0 (0.0)	15	2 (13.3)	19	1 (5.3)	27	1 (3.7)

¹⁾DAP = days after planting, ²⁾One clone die at 60 DAP, ³⁾One clone die at 63 DAP. Numbers in parentheses are percentages of resistant clones.

of 7.0-8.7. Atlantic, Granola, and non-transgenic Katahdin were categorized susceptible (Table 2), while transgenic Katahdin SP904 and Katahdin SP951 had scores of 7.0, and 8.3, respectively. Populations of crosses between Granola x transgenic Katahdin SP951 showed the highest percentage of resistance (55.6%), followed by Granola x transgenic Katahdin SP904 (42.1%), Atlantic x transgenic Katahdin SP951 (33.3%), and Atlantic x transgenic Katahdin SP904 (14.3%). This result was supported by observation that Granola was slightly more resistant than Atlantic, at least until 63 DAP. Similarly, transgenic Katahdin SP951 was more resistant than transgenic Katahdin SP904 at each observation period.

Four clones, i.e. B8 (disease score 7.6), and B26, C183, D89 (disease score 7.0) were categorized resistant at 77 DAP. The above resistance scores were higher than those shown by transgenic Katahdin SP904 and Katahdin SP951, with scores of 4.6 and 6.8, respectively. No symptom of late blight infection was observed on *S. bulbocastanum* from which the *RB* gene was cloned during these experiments.

Clones of crosses between susceptible parents (Atlantic or Granola) and resistant parents (transgenic Katahdin SP904 or Katahdin SP951) showed a similar pattern in AUDPC. AUDPC values of these clones (377.2) were in the range between the susceptible and resistant parents. The range was greater than that shown by resistant parents (180.1), but lower than that of susceptible parents (670.7) (Fig. 1). The highest total AUDPC was demonstrated by Atlantic (1,302.4) followed by Granola (1,268.3) and non-transgenic Katahdin (1,187.7).

Similar field experiments of *RB* transgenic potato clones for resistance to *P. infestans* have been

conducted using artificial inoculation with single or multiple isolates in Michigan Agricultural Experimental Station (Kuhl *et al.* 2007) and Minnesota (Bradeen *et al.* 2009). They reported that late blight symptoms were first detected at 9 days after inoculation (DAI), and susceptible cultivars, such as Atlantic and Russet Burbank showed 100% infection at 28 DAI. In our study, the experiments were conducted using natural inoculation, thus the appearance of the disease symptom depends on the availability of inoculum in the field and field condition.

All clones positively contained the *RB* transgene (verified by PCR) were further tested to the field disease evaluation. However, until the end of observation (77 DAP), independent clones showed variation in resistance phenotypes in the field. Four clones, i.e. B8, B26, C183, and D89 showed resistance scores of 7.0-7.6, indicating that these clones were still be infected by *P. infestans* at the infection level of < 25%. This means that potato clones contained *RB* gene could delay disease development, but these plants did not become immune to the pathogen. Further studies are being conducted to evaluated the resistance of these clones to the pathogen in other field experiments. The result, however, is in agreement with the report of Bradeen *et al.* (2009) that among 57 *RB* transgenic potato clones tested in the field, 13 (23%) were highly resistant and 26 (46%) were moderately resistant at 30 DAI. Furthermore, Bradeen *et al.* (2009) stated that *RB*-mediated foliar blight resistance is lacking in a clear macroscopic hypersensitive reaction although it induces delay and spread of pathogen development in infected plant.

Rasmussen *et al.* (1998) reported that the presence of resistance non-coding genes in one parent could

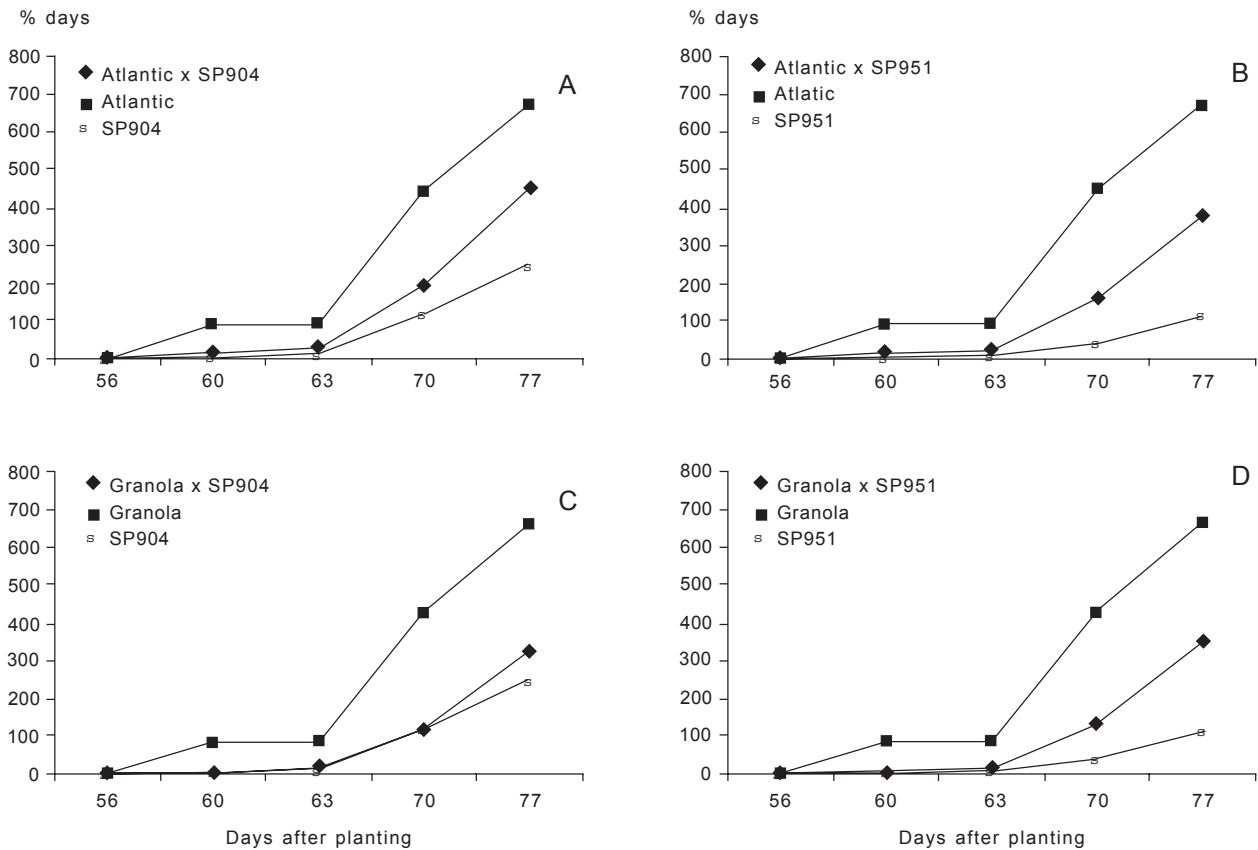


Fig. 1. Area under disease progress curve of late blight (*P. infestans*) on clones Atlantic x transgenic Katahdin SP904 (A), Atlantic x transgenic Katahdin SP951 (B), Granola x transgenic Katahdin SP904 (C), Granola x transgenic Katahdin SP951 (D) at various observation periods.

reduce the expression of resistance gene in other parents. Kuhl *et al.* (2007) reported that potato clones contained *RB* gene, but showed susceptible phenotype. Transcription analysis of the clones showed that *RB* transcript of the transgenic clones was not detected. This result was due to partial deletion/rearrangement of the *RB* transgene in the tested clones. Recently, it has been reported that independent potato clones carrying the *RB* transgene were varied in resistance response to late blight, that might be correlated with the amount of *RB* transcript (Bradeen *et al.* 2009; Kramer *et al.* 2009; and Millett *et al.* 2009). Bradeen *et al.* (2009) reported that the transcription level among *RB* transgenic clones of the genotypes Katahdin, Dark Red Norland, Russet Burbank, and Superior varied from 0.01 to 0.73 with the average 0.1, while 14 clones showed no *RB* transcript. There was no *RB* transcript in non-transgenic Katahdin, whereas transgenic Katahdin SP951 showed transcript level of 0.04. Based on quantitative RT-PCR, Bradeen *et al.* (2009) reported that the transgenic clones accumulate only low to modest levels of the *RB* transcript, that might correlate with

the endogenous promoters of *RB* gene. The *RB* gene, like many other disease resistance genes of the nucleotide binding site-leucine rich repeat (NBS-LRR) gene class, is constitutively transcribed throughout plant development, even in the absence of the pathogen (Bradeen *et al.* 2009; Kramer *et al.* 2009).

Comparison of phenotypic appearances of the confined field trial evaluation on clones derived from four crossing populations is shown in Figure 2. Clones tested to late blight in a confined field trial showed variations in phenotypic resistance due to several reasons such as variations in level of *RB* gene transcription. At 77 DAP, Atlantic, Granola, and non-transgenic Katahdin showed 100% infection (Fig. 2 b, c, d) similar to susceptible clones A10 (Fig. 2 h) and B59 (Fig. 2 i). Clones B8, C183, and D77 (Fig. 2 j, k, l) were resistant and showed disease intensity of less than 25%, while *S. bulbocastanum* PT29 as a source of resistance gene exhibited no symptom of *P. infestans* (Fig. 2 g).

Resistant clones obtained from this preliminary study are valuable genetic resources for late blight resistance breeding programs. These clones need to



Fig. 2. Resistance evaluation of potato late blight (*P. infestans*) on populations of crosses between transgenic potato and non-transgenic one in confined field trial at Pasir Sarongge, Cianjur, West Java at 77 days after planting; (a) border Katahdin, (b) Granola, (c) Atlantic, (d) non-transgenic Katahdin, (e) transgenic Katahdin SP904, (f) transgenic Katahdin SP951, (g) *Solanum bulbocastanum* PT29, (h) clone A10 (Atlantic x transgenic Katahdin SP904), (i) clone B59 (Atlantic x transgenic Katahdin SP951), (j) clone B8 (Atlantic x transgenic Katahdin SP951), (k) clone C183 (Granola x transgenic Katahdin SP904), (l) clone D77 (Granola x transgenic Katahdin SP951).

be further developed before they are used as practical resistance potato varieties. We hope these resistant potato clones will reduce huge amount of fungicide application as traditionally used by local farmers as many as 20-30 times during a single season of potato crop (Adiyoga 2009).

CONCLUSION

Eighty-four *RB* clones from four crossing combinations showed variation in phenotypic resistance to late blight caused by *P. infestans* in confined field trial. Clones of crosses between susceptible parents

(Atlantic or Granola) and resistant parents (transgenic Katahdin SP904 or Katahdin SP951) showed a similar pattern in AUDPC. AUDPC value of these clones (377.2) was in the range between the susceptible and resistant parents, that was greater than the resistant parents (180.1) but smaller than susceptible parents (670.7).

Observation at 77 DAP resulted four resistant clones, i.e. B8 (Atlantic x transgenic Katahdin SP951) with a resistance score of 7.6, and clones B26 (Atlantic x transgenic Katahdin SP951), C183 (Granola x transgenic Katahdin SP904), and D89 (Granola x transgenic Katahdin SP951) with a resistance score of 7.0. This resistance score was higher than that of transgenic Katahdin SP904 and Katahdin SP951, i.e. 4.6 and 6.8, respectively.

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