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Constructing and expressing acyl-homoserine lactone lactonase (*aiiA*) gene for enhancing *Solanum tuberosum* resistance against soft rot disease

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Abstract. *Suryanti E, Rusamana I, Wahyudi AT, Akhdiya A, Sukamdjaja D. 2022. Constructing and expressing acyl-homoserine lactone lactonase (*aiiA*) gene for enhancing* Solanum tuberosum *resistance against soft rot disease. Biodiversitas 23: 1780-1787.* Expression of the genes associated with pectinase production in soft rot disease of *Dickeya* spp. are regulated by quorum sensing mechanism using acyl homoserine lactone (AHL). Degradation of AHL using AHL lactonase can be developed to reduce pectinase production in *Dickeya* spp. The *aiiA* gene encodes AHL lactonase from *Bacillus* spp. This study aims to construct binary vector of inserted *aiiA* gene (pCAB10) from *Bacillus cereus* B10 and express the *aiiA* gene in potato Medians cultivar genome. The *aiiA* gene was cloned into binary vector and transformed into *Agrobacterium tumefaciens* LB4404. The *Agrobacterium*-mediated genetic transformation was conducted with co-cultivation technique. The transgenic plant was confirmed through PCR and selected through kanamycin and soft rot disease resistance to *Dickeya dadantii* A3. The result showed that *aiiA* gene was successfully integrated to pCAB10 and introduced to *A. tumefaciens* LB4404. The percentage of pCAB10 transformation was 57.6%, while the regeneration efficiency was 11.9% with 50 mg/L acetosyringone addition. Four transgenic potato Medians cultivar exhibited significantly higher resistance against soft rot disease than non-transgenic plants. Mahl1 clone had the highest resistance to *D. dadantii* with soft rot disease incidence as 7.1% and percentage of soft rot disease suppression as 92.85%.

Keywords: *aiiA* gene, *A. tumefaciens* LB4404, pCAB10, *Solanum tuberosum*

INTRODUCTION

Potato (*Solanum tuberosum*) is multipurpose crop species, used for food, feed and industrial processing. Potato blackleg and soft rot disease are among the most significant bacterial diseases affecting global potato production. Bacterial soft rot disease was caused by *Dickeya* and *Pectobacterium* (Charkowski 2018). The soft rot infection increasingly causes severe economic losses at potato crops in Western and Northern Europe, Finland, Georgia**,** Israel, and Indonesia (Toth et al. 2011; Tsror et al. 2011; Degefu et al. 2013; Ismiyatuningsih et al. 2016). Soft rot bacteria disease can occur at the seedling or storage stage, then infect the other healthy potato around quickly. Soft rot bacteria are found on plant surface and in soil where they may enter the plants via wound sites or through natural openings on the plant surface. The bacteria enter plant tissue, spread and multiply in the intercellular spaces (Reverchon and Nasser 2013). The soft rot bacteria can degrade plant cell wall and tissue by releasing pectolytic enzymes, such as pectinase, protease and cellulase. These enzymes can cause maceration and rotting of parenchymatous tissues on all plant organs, resulting in plant death (Reverchon and Nasser 2013). Infection of pectolytic bacteria and soft rot disease in potato could decrease potato production for long period.

The virulence factor of some phytopathogenic bacteria, such as soft rot bacteria *Dickeya* are regulated through quorum sensing (QS) mechanism (Zhang et al. 2019). QS is bacterial communication mechanism to regulate gene expression. QS mechanism coordinate gene expression of social behavior, such as biofilm formation, virulence factor, antibiotic synthesis, biofilm formation, adhesion, swarming competence, sporulation, and enzyme production (Schuster et al. 2013). QS mechanism depends on the density of bacterial cells that produce and accumulate signal molecules or autoinducers (AIs) (Zhang et al. 2019). AIs compounds bind the regulatory proteins when the concentration is within a certain threshold (quorum) and then stimulate gene transcription. N-acyl homoserin lactone (AHL) is universal AIs compounds that play a major role in the QS process of Gram-negative pathogenic bacteria such as *Dickeya* spp., *Vibrio fisheri, Chromobacterium violaceum, Aeromonas hydrophyla, Serratia marcescens*

(Keivit and Iglewski 2000). The disruption of QS pathogenic bacteria could inhibit expession of virulence gene mediated QS mechanism (Zhang et al. 2019. The AHL is degraded by using enzymes to degrade AHL. These mechanisms that are known as quorum quenching (QQ). AHL degradation enzyme was reported that could interfere signal of QS mechanism in *C. violaceum* and *Dickeya chrysanthemi* (Hosseinzadeh et al. 2016).

AHL lactonase is one of AHLs degradation enzymes, which was first identified from *aiiA* gene of *Bacillus* strain 240B1 (Dong et al. 2000). Previous study reported that the *aiiA* gene encoding the AHL lactonase enzyme is widespread among *Bacillus* sp. strains including *Bacillus cereus*, *B. thuringiensis*, *B. anthracis* and *B. mycoides* (Huma et al. 2011). Krzyzanowska et al. (2012) stated that the Rhizosphere bacteria that have capability to hydrolyze N-AHLs and to reduce soft rot maceration on potato tuber of various *Pectobacterium* spp. and *Dickeya* spp. infections. Application of AHL lactonase producing bacteria as biocontrol agent significantly attenuate soft rot/black leg disease in potato, chicory, and orchid (Sari et al. 2016; Garge and Nerurkar, 2017; Khoiri et al. 2017; Fan et al. 2020). The expression of AHL lactonase in *Dickeya chrysanthemi* decreased virulence properties including swarming motility and production of pectinolytic enzymes. This condition suppressed soft rot maceration symptoms on African violet plants (*Saintpaulia ionantha)* and potato tuber after *D. chrysanthemi* inoculation (Hosseinzadeh et al. 2016). Han et al. (2015) reported that expression of *aiiA* gene in transgenic plants could decrease tissue maceration and increase resistance to *E. carotovora* (*Pectobacterium carotovorum*) infection. Based on previous study, AHL lactonase as have high potency as qourum quenching agent. Qourum quenching (QQ) is mechanism to suppress QS mechanism through AHL degradation. These discruptions could cause virulence gene in bacteria to reduce pathogenic bacteria infection. The qourum quenching (QQ) was successfully applied as preventive approach to control soft rot bacteria infection (Chen et al. 2013; Fan et al. 2020).

Genetic engineering through genetic transformation is one of the biotechnology to produce new variety of plants. *Agrobacterium tumefaciens*-mediated transformation can be used to introduce genes from other species into the target plant genome (Păcurar et al. 2011). This method shows higher gene expression stability in plants (Kado 2014). This particular method can also be used to introduce *aiiA* gene from *Bacillus* spp. into potato genome. This study aims to construct a binary plasmid pCAB10 carrying *aiiA* gene from *B. cereus* B10 and to introduce *aiiA* gene into Medians cultivar potato for producing AHL lactonase, therefore inhibiting quorum-sensing signaling on *D. dadantii*. Ultimately, the AHL lactonase production of transgenic plants may be used to treat and protect Medians cultivar potato from *D. dadantii* infection.

MATERIALS AND METHODS

Materials and culture condition

Bacillus cereus B10 (source of *aiiA* gene) was obtained from Dr. Iman Rusmana, M. Si. collection, while *A. tumefaciens* strain LB4404, *Escherichia coli* DH5α and *E. coli* DH1 were obtained from Plant Microbiology Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, IPB University, Indonesia collection. pGEMT-Easy (Promega) and pCAMBIA2300 plasmid were used as binary vector construction. *E. coli* DH5α and *E. coli* DH1 were cultured in Luria Bertani (LB) 77 at 37°C. *B. cereus* B10, *Dickeya* spp., and *A. tumefaciens* LB4404 were cultured in LB liquid medium at 28°C. In vitro medians cultivar potato plants were collected from Research Center of Agricultural Biotechnology and Genetic Resources. In vitro potato plantlets were cultured in Murashige and Skoog (MS) medium. This research was conducted from August 2017 until May 2019 at Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, IPB University and tissue culture Laboratory, Center for Agricultural Biotechnology and Genetic Resources Research (BB BIOGEN), Bogor.

DNA isolation and amplification of *aiiA* gene

B. cereus B10 is the source of *aiiA* gene isolated from soil Cangkuang forest, Sukabumi, Indonesia. Genomic DNA of this bacteria extraction was performed by Presto Mini gDNA procedure Bacteria Kit (*Geneaid*). The concentration and purification of DNA were calculated using Nanodrop 2000 (*Thermo Scientific Wilminton DE, USA*). Amplification of *aiiA* gene *B. cereus* B10 was performed using *aiiA*F (5'-GGA**AGA TCT**ATGACAGT AAAGAAGCTTTATTTCG-3') and *aiiA*R (5'-**CATGCC** ATTGTCAACAAGATACTCCTAATGATGT-3') primer (bold underlined words are restriction enzyme sites of *Bgl*II dan *Nco*I). The amplification was done with an initial denaturation step at 94ºC for 2 minutes followed by 30 cycles of amplification at 95ºC for 30 seconds, annealing at 55ºC for 45 seconds, elongation at 72ºC for 30 seconds, then followed by final elongation at 72ºC for 10 minutes (Dong et al. 2000). Purification of *aiiA* fragments from PCR product was performed by Gel/PCR DNA Fragments Extraction Kit (*Geneaid*).

Subcloning *aiiA* gene into *Escherichia coli* DH5α

The pGEMT-Easy was used as vector for cloning *aiiA* gene into *Escherichia coli* DH5α. Ligation reaction of *aiiA* gene with pGEMT-Easy was done following pGEM-T-Easy Vector Systems Kit (Promega). The *aiiA* gene cloning into *E.coli* DH5α was performed by Transform Aid Bacterial Transformation Kit (*Thermo Scientific*). The recombinant cells were observed on selected medium (LB agar medium containing 100 mg/L ampicillin, and 40 mg/L X-Gal) after 18 hours of incubation (Sambrook and Russel 2001). Recombinant plasmid was isolated using ATP™ Plasmid Mini Kit (AIP). Confirmation of recombinant cells was done with amplification recombinant plasmid using primer T7 (5'-TAA TAC GAC TCA CTA TAG GG-3') and SP6 (5'-ATT TAG GTG ACA CTA TAG-3') with digested

recombinant plasmid using *Bgl*II dan *Nco*I restriction enzyme. PCR was performed using T7-SP6 primer for 30 cycles with the condition as followed: pre-denaturation at 94°C for 2 minutes, denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, extension at 72°C for 1 minute, and post extension at 72°C for 10 minutes. DNA fragments were run on 1% agarose gel electrophoresis for 45 minutes and 90 Volt.

Binary vector construction

Construction of binary plant transformation vector pCAMBIA2300 carrying *aiiA* gene (pCAB10) into *E. coli* DH5α was done based on Sambrook and Russel (2001). pCAB10 was transformed into *E. coli* DH5α through electroporation technique using MicroPulserTM BIO-RAD electroporator (Nickoloff 1995). As 100 µL recombinant cells were grown on selected medium (LB agar medium containing 100 µg/mL kanamycin) and were incubated for 24 hours at 37ºC. Confirmation of recombinant cells was done by amplifying *aiiA* gene of recombinant plasmid using primer *aiiA*F and *aiiA*R. Recombinant plasmid was digested with *Bgl*II dan *Nco*I restriction enzyme. The DNA fragments were run on 1% agarose gel electrophoresis for 45 min in 90 Volt.

Transformation of pCAB10 into A. *tumefaciens* strain LB4404

The introduction of pCAB10 into *A. tumefaciens* strain LB4404 was done with the Tri Pariental Mating (TPM) assay (Handayani et al. 2014). Three bacteria: *E. coli* DH5α as donor containing pCAB10 plasmid, *E. coli* DH1 containing helper pRK2013 plasmids and *A. tumefaciens* LBA4404 as the recipient strain were used in TPM assay. The three bacteria were grown in solid LB and incubated for 24 hours to prevent conjugation. *A. tumefaciens* LBA4404 recombinant were grown on selected medium (LB agar medium containing 100 mg/L kanamycin and 50 mg/L streptomycin. *A. tumefaciens* LBA4404 recombinant were then identified using colony PCR with *aii*AF and *aii*AR primers. Recombinant plasmid *A. tumefaciens* LBA4404 was digested with *Bgl*II dan *Nco*I enzyme to confirm of transformation pCAB10 into A. *tumefaciens* strain LB4404.

Plant genetic transformation

Transformation *aiiA* gene was done with co-cultivation methods and indirect regeneration (Gea et al. 2017). *A. tumefaciens* LBA4404 harboring a binary vector pCAB10 was used in the present study. This bacterial strain was cultured on LB medium. *A. tumefaciens* LBA4404 were grown for 15 hours at 24ºC on LB. *A. tumefaciens* LBA4404 with OD_{600}= 0.5 were centrifuged at 6000 rpm for 15 minutes and pellet taken was dissolved in 50 mL co-cultivation medium (MS medium, 2 mg/L 2.4 D, 3 mg/L BA, 50mg/Lacetosyringone, pH 5.8). This suspension was used as the bacterial inoculum for infection explants. Internode with 0.5-1 cm size was combined with pellet suspension of *A. tumefaciens* LBA4404, then shaken for 15 minutes at 150 rpm. Ex-plants were dried on sterile tissue paper for 15 minutes at room temperature and grown on solid co-cultivation medium (MS medium, 2 mg/L 2.4 D, 3 mg/L BA, 40mg/L acetosyringone, 2.5 mg/L phytagel, pH 5.8) in the darkroom for 3 days.

Selection and recovery of the transgenic plants

After 3 days of co-cultivation, explants were rinsed in sterile water containing 500 mg/L cefotaxime for 10 minutes and dried on sterile tissue paper for 15 minutes at room temperature. Explants were transferred into the callus initiation medium CI (MS medium, 3 mg/L BAP, 1mg/L IAA, 1mg/L GA3, 2.5 mg/L phytagel, 500 mg/L cefotaxime). Ex-plants were grown on CI medium at 24-25ºC in light condition of 2000-3000 lux for 8 hours. After 15 days, callus was selected on MS medium containing 50 mg/L kanamycin. After shoots has appeared from the callus, shoots were sub-cultured on MS medium.

Detection *aiiA* gene from cDNA putative transgenic plants

Total genomic RNA was isolated from each putative transgenic and non-transformed plants using total RNA Mini Kit (*Geneaid*). Total RNA was synthesized into cDNA using RevertAid Strand cDNA Synthesis Kit (*Thermo Scientific*). The *aiiA* gene was amplified from cDNA putative of transformed and non-transformed plants using *aii*AF and *aii*AR primers.

Evaluation of resistance putative transgenic plants

The resistance of four putative transgenic plants were tested with the inoculation of *D. dadantii*. Putative transformed was cut by scissors from infected *D. dadantii* (1.2×10^9 mL^{-1} cells). Each putative transformed plants were replicated four times with each replication using 7 plantlets. Un-transfomed plants were infected by *D. dadantii* used as control. Plants were incubated at 25-27ºC for three weeks. Disease incidence was calculated by following the formula (Talibi et al. 2012).

$$DI = n / N \times 100\%$$

Where:
DI : Disease incidence (%)
N : Number of plants
n : Number of plants observed

RESULTS AND DISCUSSION

Binary vector construction

The cloning of *aiiA* gene in pGEMT-Easy into *E. coli* DH5α has been successfully conducted. The recombinant cells were shown by white colonies growing on selected medium on pGEMT-Easy plasmid containing genes encoding protein resistance to ampicillin. The amplification of recombinant plasmid using T7 and SP6 primers with 900 bp fragment DNA had confirmed whether *aiiA* gene had been inserted in pGEMT-easy (Figure 1A). 900 bp amplicons are total sequences of DNA inserts and restriction sites in multiple cloning sites (MCS). The digestion result of recombinant plasmid using *Bgl*II and

*Nco*I had separated DNA inserts and pGEMT-easy vector. The size of *aiiA* gene was 750 bp and pGEMT-Easy was 3015 (Figure 1B).

The result of binary vector construction showed *aiiA* gene had been successfully constructed in (pCAB10) and transformed into *A. tumefaciens* LB4404. Transformation pCAB10 of into *A. tumefaciens* LB4404 was performed by tri-parental mating (Figure 2A). pCAB10 was transferred from *E. coli* DH5α as donor into *A. tumefaciens* LBA4404 as recipient through conjunction process by pRK2013 plasmid of *E. coli* DH1 helper. *E. coli* DH5α containing pCAB10 was resistant to kanamycin because pCAB10 carries kanamycin-resistant genes while *E. coli* DH1 was resistant to streptomycin and *A. tumefaciens* LBA4404 was resistant to streptomycin. The result indicated only *A. tumefaciens* LB4404 transformant grown on selective medium (Figure 2B). The *A. tumefaciens* LB4404 transformant grow in selective medium because containing pCAB10 carries kanamycin-resistant. The pCAB10 *A. tumefaciens* LB4404 transformant was identified by PCR of recombinant plasmid. The results showed *aiiA* gene had amplified from recombinant plasmid as 750 bp (Figure 3A). Recombinant plasmid (pCAB10) was divided into two DNA fragments, i.e. pCAMBIA2300 DNA fragment on 8701 bp and *aiiA* DNA fragment on 750 bp after digested using *Bgl*II and *Nco*I restriction enzyme (Figure 3B).

The introduction of *aiiA* gene can be done through *Agrobacterium*-mediated transformation procedure. *A. tumefaciens*-mediated transformation system has some advantages, such as simple operation, high transformation efficiency, and easy DNA integration (Kado 2014). Binary vector is known as the shuttle vector to be replicated in *E. coli* DH5α and *A. tumefaciens.* A binary vector is a standard tool with high efficiency for the transformation of higher plants mediated by *A. tumefaciens* (Murai 2013).

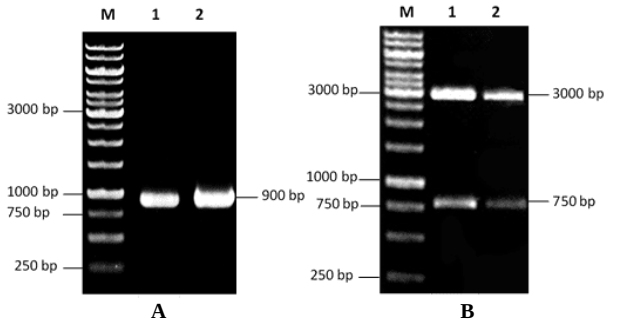

Figure 1. A PCR product of recombinant plasmid in *E. coli* DH5α by T7 and SP6 primer. M: 1 kb marker, lane 1-2 amplicon DNA targets. B. Restriction result of recombinant plasmid with *Bgl*II and *Nco*I. M: 1 kb marker; lane 1-2 restriction fragment

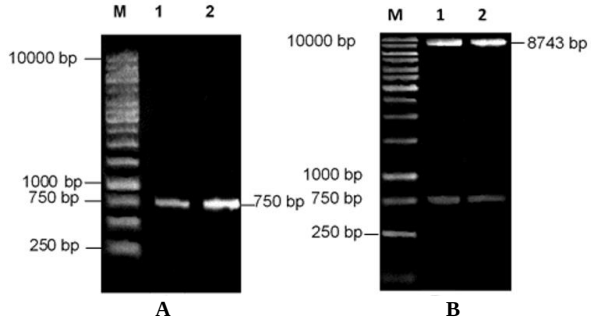

Figure 3. A. PCR product of pCAB10 from *A. tumefaciens* LB4404 by *aii*AF and *aii*AR primer. M: 1 kb marker; lane 1-2 amplicon DNA target, B. Restriction result of pCAB10 recombinant plasmid with *Bgl*II and *Nco*I. M: 1 kb marker; lane 1-2 amplicon DNA target

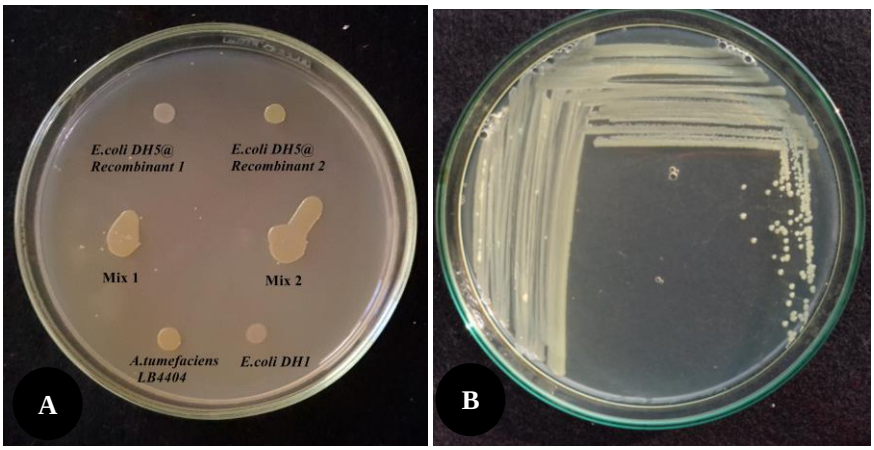

Figure 2. A. Results of tri-parental mating grown on LA plate-medium without antibiotics, Mix 1-2*: E. coli* DH5α+ *E. coli* DH1α+ *A. tumefaciens* LB4404 wild type; B. *A. tumefaciens* LB4404 transformants cells on LA medium containing 100 mg/L kanamycin and 50 mg/L streptomycin

Transformation, selection, and recovery of the putative transgenic *aiiA*

The explants used in this transformation process on the Medians potato cultivar were internodes/stem segments from 2-month-old in vitro plants. The number of transformant callus growing on the selection media containing 100 mg/L kanamycin indicated the success of the transformation process. The transformation results showed the shoots and leaves grew from any callus on selective medium containing 100 mg/L kanamycin 60 days incubation (Figure 4A). The transformant shoot was only found in explants with addition of acetosyringone for 15 days in cocultivation stage. The non-transformed callus was brown colored and dead on selective medium (Figure 4B). Kanamycin can cause toxicity to plant explants because of protein synthesis inhibition in chlorophyll and mitochondria (Shuja et al. 2021). The disruption of protein synthesis results in inhibition of chlorophyll synthesis. Kanamycin will indirectly inhibit photosynthesis, resulting in inhibition of cell division and growth and eventually death (Duan et al. 2009). Syahril et al, (2018) reported 100 mg/L kanamycin addition in selective medium that causes the non transformant *Chrysanthemum morifolium* to turn yellowish green and calluses are not formed, these concentrations have explant signs that they will inhibit the formation of new cells or callus until finally explants die. The non-transformed sugarcane shoots died after four weeks of 100 mg/L kanamycin inoculation (Rastogi et al. 2018). In this study, 100 mg/L kanamycin has been used successfully as a selection agency to select the transformed callus containing the aiiA gene.

Transgenic shoots or callus from explants without acetosyringone addition were not found to grow on the selective medium (Table 1). The timing of co-cultivation and addition of asetosyringone are key efficiency of *Agrobacterium*-mediated gene transformation. This study constructed the introduction of *aiiA* gene into potato Medians potato cultivar through co-cultivation methods in medium containing 50 mg/L asetosyringone. The addition of asetosyringone in co-cultivation medium can increase the infection of *Agrobacterium* and gene of interest integration into the host plant. Acetosyringone is compound secreted at wounded site of dicotyl plants which enhances *Agrobacterium*-mediated gene (Păcurar et al. 2011). The success of the transformation process in explants was influenced by the concentration of acetosyringone in cocultivation stage. The addition of acetosiringone with a concentration of 50 mg/L can increase the efficiency gene transformation in *S. tuberosum* cv. granola (Mbau et al. 2018). Based on previous study we conducted 50 mg/L acetosiringone in cocultivation stage. The acetosyringone concentration added in co-cultivation medium will determine the transformation success in some plants, such as rice, wheat, sugar cane, potatoes, and other plants (Rashid et al. 2011; Mayavan et al. 2013; Sawant et al. 2018).

The co-cultivation of *Agrobacterium* transformant and internode of potato as explants in this study can be done for 15 minutes in co-cultivation liquid medium and co-cultivation in solid medium can be done for three days in dark condition. Co-culture time for T-DNA integration is important to *A. tumefaciens* mediated transformation system that required time for *A. tumefaciens* to integrate T-DNA with DNA genomic plants host. T-DNA transfer occurs only when *A. tumefaciens* is present at the wounded site for more than 16 hours (Kumar et al. 2006). Dark conditions at the time of co-cultivation can increase the efficiency of *Agrobacterium* infection into host cells. This is due to the presence of light that inhibits the work of the flaABC operon to form flagellin, thus decreasing the amount of flagella forms and eventually causing decreased *Agrobacterium* movement ability (Rosen and Ron 2011). The existence of light also causes *A. tumefaciens* disruption process of T-DNA region transfer to the chromosomes of plant cells, therefore decreasing the transformation efficiency.

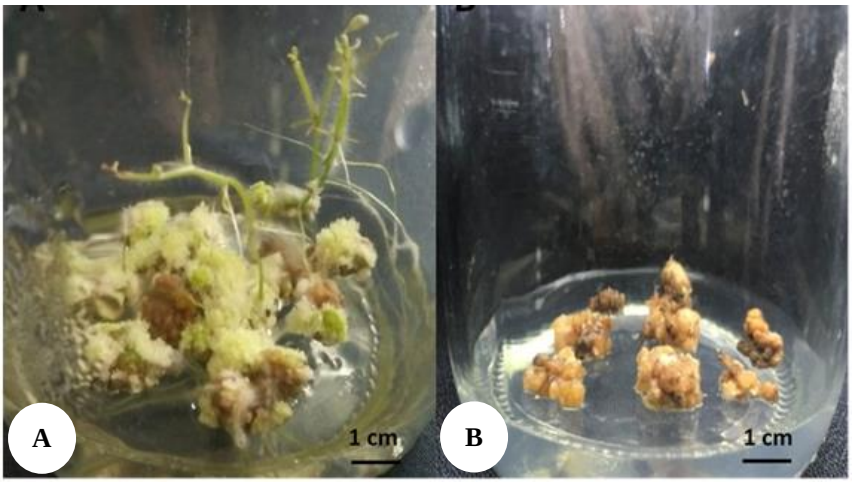

Figure 4. A. The adventitious shoot and leaves putative transgenic from callus with asetosyringone co-cultivation treatment on selective medium (100 mg/L kanamycin) after 60 days cultivation. B. Callus without asetosyringone cocultivation treatment on selective medium (100 mg/L kanamycin) after 60 cocultivation

Table 1. Transformation and regeneration efficiency *aiiA* gene of *B. cereus* B10 in Median potato cultivar

Treatment	Explants total	Callus total	Number of kanamycin resistant callus	Number of regenerated callus	Transformation eficiency	Regeneration efficiency
50 mg L^{-1} AS	210	189	109	13	57.9%	11.9%
0mg L^{-1} AS	210	163	0	0	0%	0%

Analysis of *aiiA* gene expression and transgenic putative resistance of *D. dadantii*

The 13 putative transgenics of adventitious shoot were transferred to selective regeneration medium (MS medium containing 50 mg/Lkanamycin). As many as 4 of 13 putative Genetic Modified Organism (GMO) transgenic shoots were taken randomly for the detection of *aiiA* gene and *D. dadantii.* resistant assay. The results of *aiiA* gene detection indicated that *aiiA* gene was successfully inserted into the 4 putative transgenic plants marked by the presence of ± 750 pb of *aiiA* gene, whereas *aiiA* gene fragment was not amplified from non-transgenic plant/control (Figure 5). The integration of *aiiA* gene into putative transgenic plants was confirmed by PCR analysis from cDNA putative transgenic plants using primer *aii*AF dan *aii*AR. cDNA samples were taken randomly from putative transgenic plants (Mahl1, Mahl2, Mahl3, and Mahl4) and non-transgenic plant.

As many as 28 plants from 4 transgenic putative plants were infected by *D. dadantii.* and the resistance level was observed. The in vitro analysis on *D. dadantii.* resistance showed that all non-transgenic putative plants experienced symptoms of soft rot after 12 days of infection. The disease symptoms can be seen in Figure.6A-F. The four putative transgenic plants have the ability to withstand *D. dadantii.* infection (Table 2). Four transgenic putative plant clones carrying *aiiA* gene had higher resistance to *D. dadantii* than non-transgenic plants. Disease incidence of soft rot disease occurred on four transgenic putative clones between 7.1-21.4% and percentage of soft rot disease suppression was between 78.57-92.85%. Mahl1 clone had the highest resistance to *D. dadantii* with soft rot disease frequency of 7.1% and percentage of soft rot disease suppression of 92.85%. These results show that *aiiA* gene has been expressed by putative transgenic plants at the transcription level based on the resistant analysis of putative transgenic plant infected *D. dadantii.* This can also be stated that *aiiA* gene was successfully expressed on transgenic Medians cultivar potato plants.

The putative transgenic potato can produce AHL lactonase, therefore reducing the AI compounds. Resistant analysis showed whether 4 putative transgenic Medians cultivar potato (Mahl1, Mahl2, Mahl3, and Mahl4) had higher resistance from *D. dadantii.* infection than nontransgenic potato (Control). The *Cauliflower mosaic virus* (CaMV) 35S promoter was used in introduction of *aiiA* gene to Medians cultivar potato plant. The CaMV 35S promotor is known as high-level expression of chimeric genes in most tissues and has broad range of species (Porto et al. 2014). The resistance of transgenic plants was influenced by *aiiA* gene expression regulated by CaMV 35S. The *aiiA* gene expression in plants leads to AHL lactonase production constitutively, thus decreasing the symptoms of soft rot or black leg disease.

Inactivation of AHL compounds may reduce the production of pectinolytic enzymes produced by *Dickeya* spp., thus declining the pathogenicity level of *Dickeya* spp. This study describes the resistance of anti-soft rot plants can be provided through the introduction and expression of *aiiA* gene into DNA genomic Medians cultivar and it is also suitable for gene transformation in other plants. Another study described that transgenic tobacco, *Amorphophallus konjac*, *Eucalyptus* spp. and Chinese cabbage with *aiiA* gene expression were able to inhibit soft rot disease caused by *Erwinia carotovora* SCG1 (Dong et al. 2001; Ouyang and Li, 2016; Ban et al. 2016; Vanjildorj et al. 2009). Insertion and expression of AHL lactonase enzyme in plants can be developed for inhibiting QS mechanism in various bacteria.

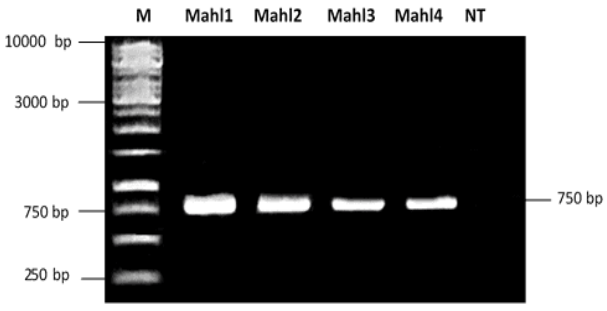

Figure 5. The *aiiA* gene fragment from CDNA Median cultivar potato plant, M: 1 kb marker; 1-4 putative transgenic plants; 4 NT: non putative transgenic plants/control

Table 2. Level of resistance transgenic potato plants expressing *aiiA* gene of *B. cereus* B10 againt *D. dadantii A3*

Cultivar	Number of shoot	Number of infection	Disease incidence (%)	Disease suppression (%)	Level of resistance
Control	28	28	100	-	Susceptible
Mahl 1	28	2	7.1	92.85	Resistant
Mahl 2	28	3	10.7	89.24	Resistant
Mahl 3	28	4	14.2	85.71	Resistant
Mahl 4	28	6	21.4	78.5	Resistant

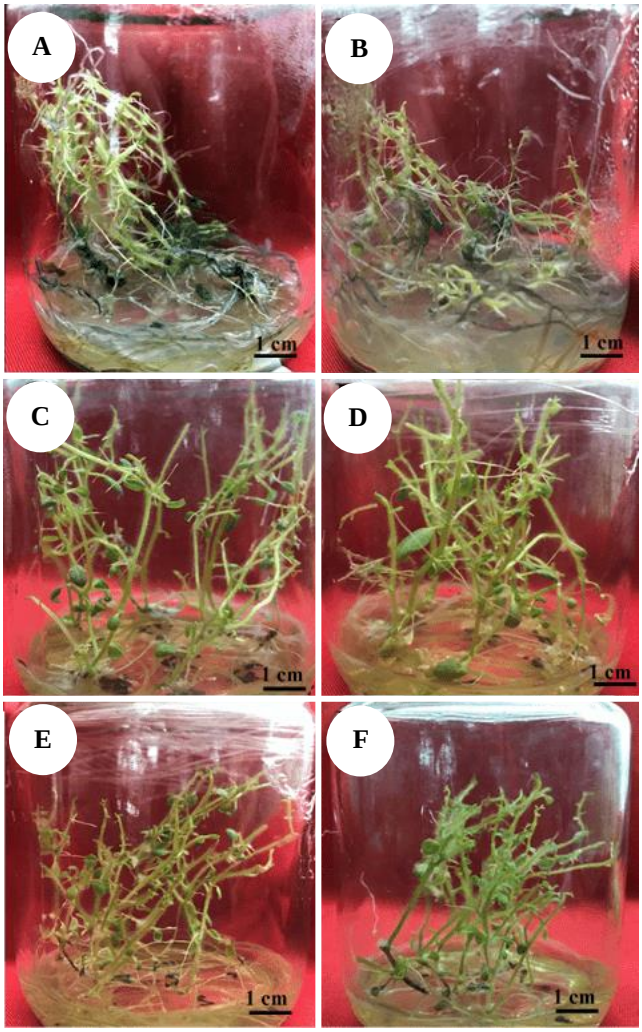

Figure 6. Soft rot symptoms in potato plantlets after 12 days of infection using *D. dadantii* A3. A-B. Non transgenic plants. C. Putative transgenic plants Mah1 clones. D. Putative transgenic plants Mahl2. E. Putative transgenic plants Mah3 clones. F. Putative transgenic plant Mah4 clones

In conclusion, the *aiiA* gene from *Bacillus cereus* B10 had successfully constructed into pCAB10 as binary vector to integrate *aiiA* gene in potato through *A. tumefaciens* mediated transformation system. The *aiiA* gene had also been successfully integrated and expressed into Medians cultivar potato. This expression of aiiA gene increased resistant ability on transgenic potato against soft rot infection of *D. dadantii* A3.

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