Jurnal Ilmu Pertanian Indonesia (JIPI), Januari 2025
(Indonesian Journal of Agricultural Sciences)
ISSN 0853-4217 EISSN 2443-3462

Vol. 30 (1): 40–47
http://journal.ipb.ac.id/index.php/JIPI
DOI: 10.18343/jipi.30.1.40

Molecular and Growth Responses of *Musa acuminata* var. Barangan Post Application of Beneficial Endophytic Bacteria

Hanifah Yusadi, Arief Pambudi, Yunus Effendi*

(Received May 2024/Accepted December 2024)

ABSTRACT

Endophytic bacteria reside in plants' roots and can benefit plant growth. The study aimed to evaluate the application of an endophytic bacterial consortium in enhancing the growth of banana plants and identify genes that maximally assist in nutrient utilization during banana plant growth. One-month-old banana plants were inoculated by soaking them for one hour in a 500 mL suspension of endophytic bacteria. *In vivo* observations were conducted in the greenhouse at Al-Azhar University Indonesia over 40 days, during which morphological and physiological growth were calculated. The results revealed that control plants exhibited lower growth than those treated with endophytic bacteria. The application of endophytic bacteria led to increased growth in banana barangan plants, as evidenced by improvements in leaf length, leaf width, plant height, and chlorophyll levels. Furthermore, a semi-quantitative analysis of banana plants treated with *Cytobacillus depressus*, *Bacillus stratophericus*, and *B. mycoides* revealed genes significantly contributing to growth. These genes, including *WRKY33, Ma03_92660, Ma01_901890, Ma04_936790, and Pho-1,2*, exhibited their highest expression levels starting from the 28th day of the experiment.

Keywords: plant growth, endophytic consortium bacteria, semi-quantitative real-time PCR, identify genes

INTRODUCTION

Pisang barangan (*Musa acuminata* Linn.) is an herbaceous plant from Southeast Asia, particularly Indonesia. Bananas are among the plants with high growth potential. However, significant problems remain in banana cultivation due to awareness levels and agroecosystem conditions. Furthermore, Indonesia faces considerable obstacles in banana production, including uneven planting patterns, inadequate management of banana culture, soil fertility, and fertilizer supplies (Blandina et al. 2019). These factors have an overall impact on plant growth, resulting in a decrease in banana production.

Several studies have documented the use of microbes to improve the availability of key nutrients in soil and manage plant diseases. Plant organs such as roots, leaves, stems, shoots, and flowers are home to a diverse range of beneficial bacterial populations called endophytes. These endophytic bacteria can promote plant growth and protect against diseases (Parniske 2018; Eid *et al*. 2019; Lin *et al*. 2022). Endophytic bacteria play an important role in plant development by converting nutrients into easily accessible forms for plants, activating induced systemic resistance, and synthesizing antimicrobial chemicals and plant hormones (Earl *et al*. 2018).

Several studies have shown that endophytic bacteria can improve banana plant growth. Sun *et al*. (2021) demonstrated that applying endophytic *Bacillus subtilis* increased resistance to *Fusarium oxysporum* and promoted root growth in bananas. Biopriming of banana plantlets with endophytic *Bacilus velenzis* has been demonstrated to improve resistance to Fusarium wilt (Xiang *et al*. 2023). Endophytic bacteria may also manufacture the phytohormone IAA and various enzymes, which encourage root growth and make them a viable biocontrol agent (Gusmaini and Kartikawati 2029; Khan *et al*. 2020; Sriwati *et al*. 2023).

While endophytic bacteria can provide benefits such as increased nutrient uptake, disease resistance, and stress tolerance, there is a danger of introducing strains that are incompatible with the host plant or disrupting the balance of the plant's microbiome. Such perturbations could have a negative impact on plant growth and overall output (Berg and Hallmann 2016). As a result, further research is needed to determine the influence of individual or specific consortia of endophytic bacteria on host plants. The purpose of this study was to investigate the influence of an endophytic bacterial consortia on banana plant growth as well as the impact of nutrient

Department of Biology, Faculty of Sains and Technology, Al-Azhar University of Indonesia, Jakarta Selatan 12110, Indonesia
* Corresponding Author: Email: effendiy@uai.ac.id

uptake-related gene expression in *Musa acuminata* var. barangan.

METHODS

Preparation of Lurian-Bertani Broth Culture Media

The primary medium used in this study is Luria-Bertani, which consists of tryptone, yeast extract, NaCl, bacteriological agar, and distilled water. Each ingredient was measured using a digital balance to determine the proper composition for a one-liter media solution. The medium was then autoclaved at 15 atm and 121 °C for 15 minutes (Hadi *et al*. 2021).

Preparation of Bacteria Consortium

Bacterial rejuvenation was done by transferring one dosage of aged bacterial culture onto a Luria-Bertani (LB) agar slant by the streak method. The bacteria (*Cytobacillus depressus*, *Bacillus stratophericus*, and *B. mycoides*) were grown in an incubator at 32 °C for 24 hours. The consortium culture was then transferred from LB agar slant media to 150 mL of LB broth medium in a 250 mL Erlenmeyer flask. Incubation was carried out at 32 °C for 24 hours, with an incubator shaker set at 120 rpm. Following that, each 2 mL of culture was transferred to a 2 mL microtube and centrifuged at 13,300 rpm for 1 minute. The supernatant was discarded, and the pellet was reconstituted with sterile 0.9% NaCl until it reached the capacity of the 2 mL containers. The bacterial consortium suspension was then homogenized using a vortex mixer, and the bacterial density was measured using a spectrophotometer (Hadi *et al*. 2021). Bacterial density was set at 10^8 CFU/mL using the McFarland 0.5 standard.

Inoculation of Bacterial Consortium to Plant Roots

The plants were inoculated with the endophytic bacterial consortium using the method described by Rat *et al*. (2021) with modifications. The suspended endophytic bacteria consortium was dissolved in 500 mL of sterile distilled water for 10 banana plantlets in each treatment and control group, then left to stand for 2 hours. Before inoculation, the plants were properly rinsed with tap water to remove any soil. Afterward, they were soaked in a 70% ethanol solution for 5 minutes. The roots were then immersed in the endophytic bacterial solution for 30 minutes and covered with plastic bags. Instead of an endophytic bacterial solution, control plants received distilled water. Following inoculation, the plants grew into a polybag with sterile Lembang soil and were incubated at room temperature for 24 hours before injecting 1 mL of bacteria consortium into the pseudo stem, roots, and upper half of the banana weevil with a 1 mL syringe.

Morphological and Physiological Data Collection

Morphological data were collected by measuring plant growth characteristics such as leaf width, leaf length, leaf height, and stem diameter at 5-day intervals throughout a 6-week period. Data collection began on the day of inoculation and lasted through day 40. Physiological data were collected by measuring chlorophyll concentration in leaves with the atLeaf CHL Plus Chlorophyll Meter. Data were gathered at 5-day intervals from day 0 to day 40, with three leaves harvested from each plant. Following the 6-week period, banana leaves from both control and treatment plants were freeze-dried every 7 days with liquid nitrogen and stored at −40°C.

RNA isolation

RNA extraction was carried out in accordance with Effendi *et al*. (2017) method, with some modifications. Total RNA was collected from each leaf sample on days 7, 14, and 28 of control and treatment. The leaf sample, which weighed between 200 and 500 mg, was crushed in liquid nitrogen and transferred to a 2-mL tube. Then, added 800 µL of modified CTAB buffer (Li *et al*. 2013) and 16 µL of β-mercaptoethanol, and vortexed. The sample was incubated at 65 °C for 20 minutes, then purified using 480 µL chloroform and centrifuged at 13,000 g for 10 minutes. The supernatant was purified using 480 µL chloroform and an equal volume of chloroform, followed by precipitation with cold isopropanol overnight at −40 °C. After centrifugation at 13,000 g for 10 minutes, the supernatant was discarded, and the pellet was dried and resuspended in 100 µL Nuclease Free Water. Next, add 800 µL GENEzolTM Reagent and 160 µL chloroform, mixed for 10 seconds, and centrifuged at 13,000 g for 15 min. The supernatant was collected, mixed with isopropanol, kept at room temperature for 10 minutes, and centrifuged at 13,000 g for 10 minutes. The pellet was washed with GENEzolTM Reagent, chloroform, and 70% ethanol. Finally, It was air-dried, centrifuged, and resuspended in 40 µL Nuclease Free Water.

RNA Visualization

To prepare a 1% agarose gel, combined 40 mL of 1x TAE buffer, DEPC-treated water, and 1.5 µL of Redsafe gel coloring. The resultant mixture was transferred to the electrophoresis gel's well mold. The agar gel was submerged in a 1x TAE buffer solution that included DEPC-treated water. The electrophoresis gel was loaded with up to 5 µL RNA and 1 µL of loading dye. An electric current of 70 V was delivered to the

electrophoresis process for 45 minutes. The UV illuminator was utilized to see which RNA molecules had successfully moved (Effendi *et al*. 2017).

cDNA Synthesis and PCR Amplification

A BioDrop Duo UVI/VIS Spectrophotometer was used to quantify the total RNA in 50 µL ddH_2O solution. 1 µg of quantified RNA was used to synthesize cDNA with the BioLine Tetro cDNA Synthesis Kit. PCR amplification was performed using 1 µL cDNA, 1 µL primer, 8.5 µL ddH2O, and 12.5 µL Redmix PCR Kit from BioLine for 30 cycles. Denaturation conditions were 99 °C for 2 minutes, annealing with optimization for 15 seconds, elongation at 72°C for 15 seconds, and post-elongation at 72 °C for 10 minutes. Electrophoresis was used to visualize the amplification results, with 30 mL of 1% agarose and 1 µL of Redsafe gel coloring, followed by 35 minutes of running in 1x TAE 70V buffer.

RESULTS AND DISCUSSION

The results obtained from the observations carried out on banana plants suggest that the utilization of *C. depressus, B. stratophericus*, and *B. mycoides* bacteria at the root level leads to a growth rate that closely approximates the dimensions of leaves, including length, width, and overall plant height. The study findings indicate that the application of endophytic bacteria significantly improved leaf length, width, and height compared to the control plants, as depicted in Figure 1. According to Murthi *et al.* (2015), it can be inferred that the utilization of endophytic bacteria has the potential to augment the accessibility of nutrients and promote the synthesis of growth hormones.

The banana plants exhibited increased growth by day 40 (Figure 2). However, on day 30, drought led to leaf yellowing and slight browning, attributed to severe environmental conditions like drought, high temperatures, and excessive sunlight. After a few days, the leaves from day 30 showed signs of desiccation, requiring pruning to prevent plant mortality. By day 40, plant health significantly improved, with no necrotic leaves visible, due to consistent moisture monitoring preventing drought-induced stress. Water scarcity can stress plants, disrupting physiological processes and causing stomatal closure, hindering photosynthesis and carbon dioxide transport (Anggraeni *et al.* 2016).

The introduction of endophytic bacteria resulted in an increase in chlorophyll content in banana plants, though this difference did not achieve statistical significance compared to the control plants (Figure 3). However, both measurements revealed a slight rise in chlorophyll concentration in banana plants from day 0 to day 40.

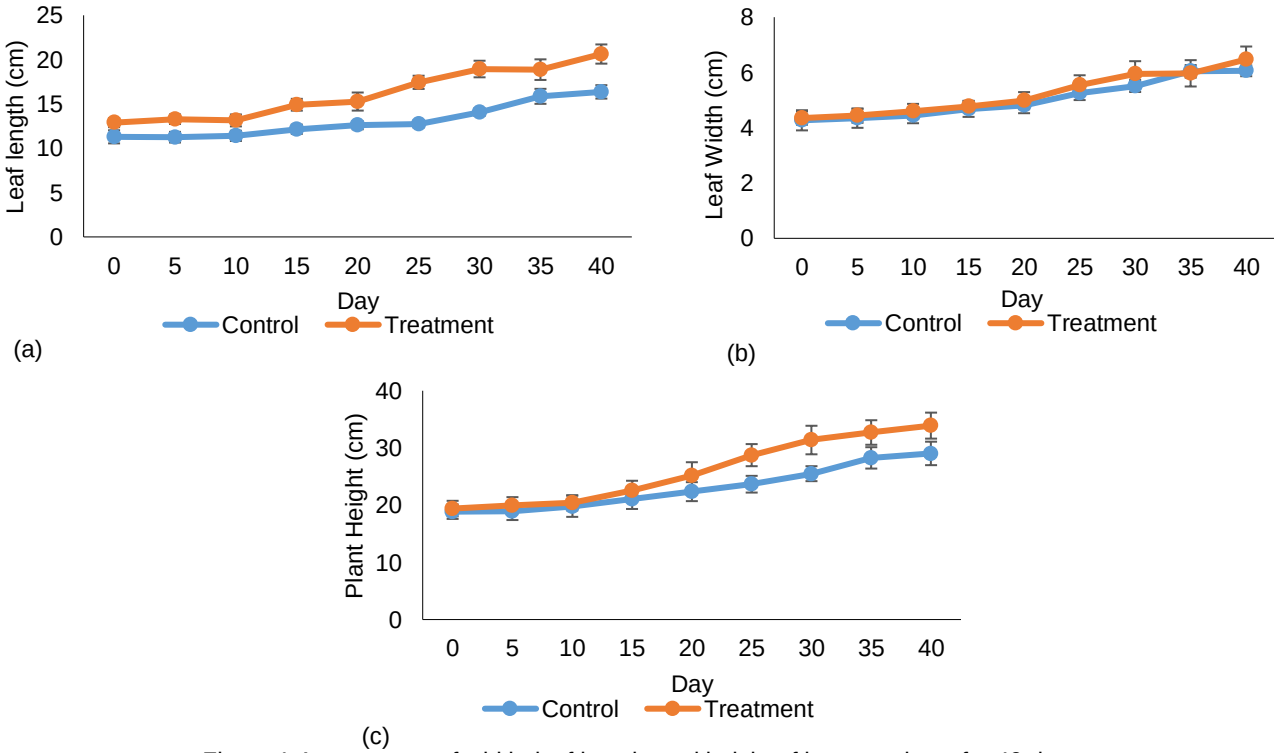

Figure 1 Avera eaf width, leaf length, and height of banana plants for 40 days.

Figure 2 Observations of control and treated banana barangan plants day 30 and day 40.The first two images above (control and treatment) of 30-day-old plants, the next two images (control and treatment) of 40-day-old plants

From day 10 onwards, plants treated with bacterial treatment showed a modest advantage over control plants. The observed low chlorophyll levels in the leaves of both control and treatment plants can be attributed to reduced solar exposure experienced by banana plants. Treatment leaves exhibited a lower chlorophyll concentration compared to control leaves, likely due to nutrients derived from symbiotic mutualism between endophytic bacteria and plants, allowing treatment leaves to absorb more chlorophyll. However, as noted by Lawendatu *et al.* (2019), excessive solar intensity can potentially harm chlorophyll. This finding is consistent with previous research indicating a decrease in chlorophyll levels in various crop species due to elevated temperature stress, which may be exacerbated by increased light intensity (Sharma *et al.* 2016; Patriyawaty *et al.* 2018).

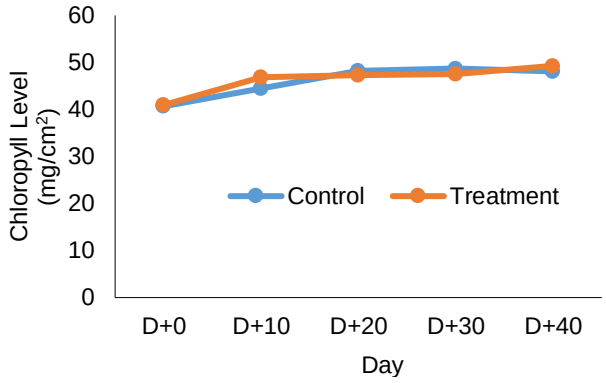

Figure 3 Chlorophyll content of banana plants.

The interaction between plants and bacteria can lead to many mechanisms that promote plant growth. These mechanisms include improved nutrient uptake, nitrogen fixation, phytohormone synthesis, and indirect inhibition of phytopathogens (Berg 2009). Therefore, some genes associated with nutrient uptake were examined to measure their expression after being exposed to the endophytic bacteria consortium. Eight genes, namely *Ma01_g01890, Ma03_g2660, Ma04_g36790, PHO1-2, WRKY11, WRKY26, WRKY31,* and *WRKY33*, were examined to determine their expression levels. The data indicated that the expression levels of all those genes in the treatment plants were greater than the control (Figure 4, 5). In the treated plants, the genes *WRKY33, Ma03_92660, Ma01_901890, Ma04_936790,* and *Pho-1,2* exhibited the highest level of gene expression, with fold changes of 12, 10, 8, 7, and 6, respectively. Simultaneously, the expression levels of the same genes were similarly elevated, but at a smaller magnitude than the treated plants. A similar study by Xu *et al.* (2022) also demonstrated the impact of endophytic bacteria on the regulation of plant growth-related gene expression.

The *WRKY31* and *WRKY11* genes exhibit similar expression levels, with a slight increase observed on days 14 and 28, although not as pronounced as on day 7. Conversely, the *WRKY26* gene showed low expression on day 7, followed by an increase on day 14 and a decrease on day 28. These genes, *WRKY31, WRKY11,* and *WRKY26*, are frequently involved in interactions with hormones and other signaling pathways in plants. A decrease in the expression of these three genes on day 28 may impact hormone regulation and signaling interactions, consequently influencing various aspects of plant response and development. Reduced expression of these genes may compromise the plant's ability to combat infections, rendering it more susceptible to disease attacks (Junlin *et al.* 2021).

The *WRKY33* gene is involved in the interplay between various phosphate signal transduction

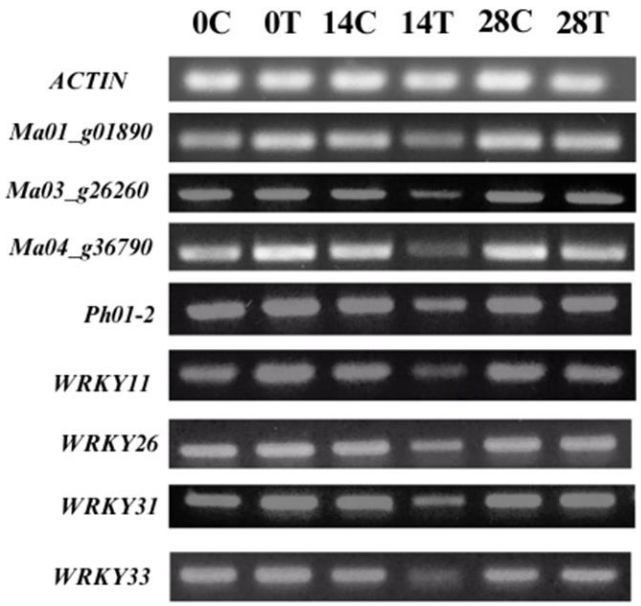

Figure 4 Semi-quantitative PCR electrophoresis results of gene amplification of *Pho-1,2; WRKY11, WRKY26, WRKY31, WRKY33, Ma01_901890, Ma03_92660,* and *Ma04_936790* genes. Level of expression of the genes above were measured at 0, 14, and 28 days post endophytic bacteria application. (C=control, T=Treatment).

pathways in plants, namely in response to drought and salinity stress. The *WRKY33* gene has demonstrated the ability to reinstate plant defense mechanisms in response to diverse stressors. The activation of the *WRKY33* gene augments the ability to withstand oxidative stress and strengthens the immune response against infections. Furthermore, the expression of *WRKY33* has been shown to enhance drought resistance (Junlin *et al.* 2021). The gene known as *Ma03_g26260* exhibits the capacity to mitigate the adverse effects arising from various phosphate deficits in plants, including drought and salinity stress. Consequently, the introduction of bacteria can enhance the ability of plants to withstand abiotic challenges. This is because, as stated by Wang *et al.* (2019), bacteria can offer support by safeguarding plants against different stressors.

In a study conducted by Xiong *et al.* (2018), it was demonstrated that the *Ma01_g01890* gene has enhanced expression in response to phosphate deprivation. This upregulation of cellular metabolism contributes to the maintenance of the antioxidant system, hence mitigating the risk of oxidative damage. In banana plants that undergo growth inhibition, the *Ma04_g36790* gene has been found to have significant expression through the activation of the phosphate transporter pathway (Information National Center for Biotechnology 2019).

The *Pho-1,2* gene can encompass the mechanism of active phosphorus transfer from the surrounding environment to the cells of plant roots. The significance of this matter lies in the fact that phosphorus is typically found in the soil in the form of phosphate ions, which necessitate absorption by the roots and subsequent uptake by the plant for utilization in many biochemical and metabolic mechanisms. When plants encounter phosphorus deprivation, the activation of the *Pho-1,2* gene and other associated genes can play a role in regulating phosphorus homeostasis. This phenomenon facilitates the enhancement of plants' capacity to uptake phosphorus from their immediate surroundings, hence maximizing their nutritional intake and overall growth (Xiong *et al.* 2018).

CONCLUSION

The available data suggests that the application of endophytic bacterial consortiums has the potential to enhance leaf length, leaf width, and plant height. The chlorophyll levels in leaves exhibited a rise over 40 days, with no significant difference observed between the treatment group and the control group. The primers used in this study are *WRKY33, Ma03_92660, Ma01_901890, Ma04_936790,* and *Pho-1,2*. demonstrated enhanced gene expression levels of phosphate metabolism related-genes of banana plants due to endophytic bacteria application.

REFERENCES

Anggraeni N, Faridah NE, Indrioko S. 2016. Pengaruh cekaman kekeringan terhadap perilaku fisiologis dan pertumbuhan bibit black locust (*Robinia pseudoacacia*). *Jurnal Ilmu Kehutanan*. 9: 40–56. https://doi.org/10.22146/jik.10183

Berg G. 2009. Plant–microbe interactions promoting plant growth and health: Perspectives for controlled use of microorganisms in agriculture. *Applied Microbioliogy and Biotechnology.* 84: 11–18. https://doi.org/10.1007/s00253-009-2092-7

Berg G, Hallmann J. 2006. Control of plant pathogenic fungi with bacterial endophytes. *In Microbial Root Endophytes* (pp. 53–69). Heidelberg (GB): Springer. https://doi.org/10.1007/3-540-33526-9_4

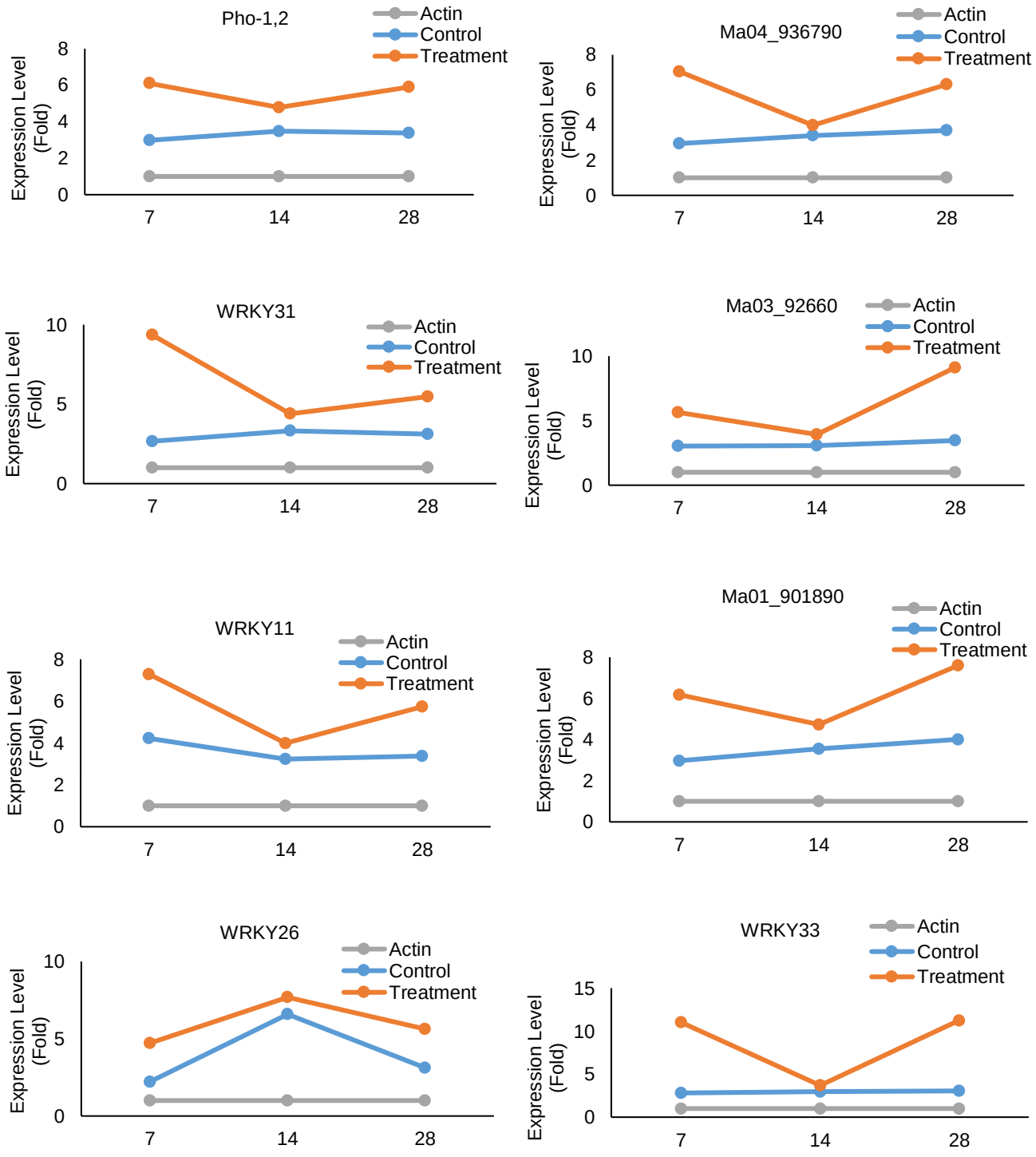

Figure 5 Comparation of gene expression level between treatment and control plants. The data showed almost all genes were up-regulated post application with endophytic bacteria consortium.

Blandina B, Siregar LAM, Setiado H. 2019. Identification phenotypic of the barangan banana (*Musa acuminata* Linn.)**.** in Deli Serdang Regency of North Sumatra. *Jurnal Agroteknologi*. 7(1): 94–105.

Earl AM, Losick R**,** Kolter R. 2008. Ecology and genomics of *Bacillus subtilis*. *Trends in Microbiology*. 16: 269–275. https://doi.org/10.1016/j.tim.2008.03.004

Effendi Y, Pambudi A, Nisrina L. 2017. Analisis ekspresi kelompok gen-gen pertahanan pada tanaman pisang dalam merespons bakteri patogen *Ralstonia solanacearum*. JAI *Seri Sains dan Teknologi*. 4: 44. https://doi.org/10.36722/sst.v4i1.251

Eid AM, Salim SS, Hassan SED, *et al.* 2019. Role of endophytes in plant health and abiotic stress management. *Microbiome Plant Heal Disease*. 119–144. https://doi.org/10.1007/978-981-13-8495-0_6

Gusmaini G, Kartikawati A. 2019. The Evaluation of endophytic bacteria application on nutrient efficiency of black pepper growth in Lampung. *Jurnal Penelitian Tanaman Industri.* 25(2): 100. https://doi.org/10.21082/jlittri.v25n2.2019.100-107

Hadi AE, Khalisha A, Pambudi A, Effendi Y. 2021. Potential of bacteria consortium as growth controller of pathogenic fungi *Fusarium oxysporum* F. sp. *cubense* (Foc). IOP Conference Series: Earth Environmental Science. 637. 120–129. https://doi.org/10.1088/1755-1315/637/1/012029

Junlin Z, Song Y, Chen Y, *et al.* 2021. Genome-wide (ChIP-seq) Identification of target genes regulated by WRKY33 during submergence stress in Arabidopsis. *Journal Genomic Data*. 22(16): 2–10. https://doi.org/10.1186/s12863-021-00972-5

Khan MS, Gao J, Chen X, *et al.* 2020. Isolation and characterization of plant growth-promoting endophytic bacteria *Paenibacillus polymyxa* SK1 from *Lilium lancifolium*. BioMed Research International. 8650957. https://doi.org/10.1155/2020/8650957

Lawendatu OP, Pontoh J, Kamu VS. 2019. Analisis kandungan klorofil pada berbagai posisi daun dan anak daun aren (*Arrenga pinnata*). *Chemistry Progress*. 12(2): 67–72. https://doi.org/10.35799/cp.12.2.2019.27925

Li C, Shao J, Wang Y, *et al.* 2013. Analysis of banana transcriptome and global gene expression profiles in banana roots in response to infection by race 1 and tropical race 4 of *Fusarium oxysporum* f. sp. *cubense*. BMC *Genomics*. 14: 851. https://doi.org/10.1186/1471-2164-14-851

Lin H, Liu C, Peng Z, *et al.* 2022. Distribution pattern of endophytic bacteria and fungi in tea plants. *Front Microbiology*. 13: 872034. https://doi.org/10.3389/fmicb.2022.872034

Murthi S R, Lisnawita, Syahrial,O. 2015. Potensi endofitik dalam meningkatkan pertumbuhuan tanaman tembakau yang terinfeksi nematoda puru akar (*Meloidogyne* spp.). *Jurnal Agroekoteknologi*. 4(1): 1881–1889.

Parniske M. 2018. Uptake of bacteria into living plant cells, the unifying and distinct feature of the nitrogen-fixing root nodule symbiosis. *Current Opinion Plant Biology*. 44:164–174. https://doi.org/10.1016/j.pbi.2018.05.016

Patriyawaty NR, Rachaputi RC, George D. 2018. Physiological mechanisms underpinning tolerance to high temperature stress during reproductive phase in mungbean (*Vigna radiata* (L.) Wilczek). *Environmental and Experimental Botany*. 150: 188–197. https://doi.org/10.1016/j.envexpbot.2018.03.022

Rat A, Naranjo HD, Krigas N, *et al.* 2021. Endophytic bacteria from the roots of the medicinal plant *Alkanna tinctoria* Tausch (Boraginaceae): Exploration of plant growth promoting properties and potential role in the production of plant secondary metabolites. *Frontiers in Microbiology*. 12: 633488. https://doi.org/10.3389/fmicb.2021.633488

Sharma L, Priya M, Bindumadhava H, Nair R, Nayyar H. 2016. Influence of high temperature stress on growth, phenology and yield performance of mungbean [*Vigna radiata* (L.) Wilczek] under managed growth conditions. *Scientia Horticulturae*. 213: 379–391. https://doi.org/10.1016/j.scienta.2016.10.033

Sriwati,R, Maulidia V, Intan N, *et al.* 2023. Endophytic bacteria as biological agents to control fusarium wilt disease and promote tomato plant growth. *Physiological and Molecular Plant Pathology*. 125: 101994. https://doi.org/10.1016/j.pmpp.2023.101994

Sun Y, Huang B, Cheng P, Li C, Chen Y, *et al.* 2021. Endophytic *Bacillus subtilis* TR21 improves banana plant resistance to *Fusarium oxysporum* f. sp. *cubense* and promotes root growth by upregulating the jasmonate and brassinosteroid biosynthesis pathways. *Phytophatology*. 112(2): 219–231. https://doi.org/10.1094/PHYTO-04-21-0159-R

Wang Y, Gao H, He L, *et al.* 2019. The PHOSPHATE1 genes participate in salt and pi signaling pathways and play adaptive roles during soybean evolution. *Journal Plant Biology.* 19: 353. https://doi.org/10.1186/s12870-019-1959-8

Xiang D, Yang X, Liu B, Chu Y, Liu S, Li C. 2023. Bio-priming of banana tissue culture plantlets with endophytic *Bacillus velezensis* EB1 to improve

Fusarium wilt resistance. *Frontiers in Microbiology*. 14: 1146331. https://doi.org/10.3389/fmicb.2023.1146331

Xiong R, Tang H, Xu M, *et al.* 2018. Transcriptomic analysis of banana in response to phosphorus starvation stress. *Agronomy*. 8(8): 141. https://doi.org/10.3390/agronomy8080141

Xu H, Gao J, Portieles R, Du L, Gao X, Borras-Hidalgo O. 2022. Endophytic bacterium *Bacillus aryabhattai* induces novel transcriptomic changes to stimulate plant growth. *PLoS One*. 17(8):e0272500. https://doi.org/10.1371/journal.pone.0272500