Application of Endophytic Bacteria using In vitro Technique to Increase Vigour of Shallots (*Allium cepa* L.) based on Inoculation Time

Aulia Floribunda Harp[2*], *Tia* Setiawati[2], *Alina* Akhdiya[1], *Ali* Husni[2], *Agus* Purwito[3], *Kristianto* Nugroho[1], *Mariana* Susilowati[1], and *Mia* Kosmiatin[1]

[1]National Research and Innovation Agency, Research Center for Horticultural and Estate Crops, Research Organization for Agriculture and Food, 16911 Cibinong, West Java, Indonesia
[2]Padjajaran University, Biology Program, 45363 Jatinangor, West Java, Indonesia
[3]IPB University, Agronomy and Horticulture Program, 16680 Bogor, West Java, Indonesia

Abstract. Shallot is one of the important horticultural commodities in Indonesia. Besides having many benefits, the shallot is also the main ingredient in various traditional dishes in Indonesia. A significant increase in healthy shallot production is needed to meet the increasing demand every year. The aim of this study was to obtain the best strain of endophytic bacteria and inoculation time for increasing shallot vigour through in vitro. This study used a factorial completely randomized design with two factors, the type of bacteria and the time of inoculation which was divided into 3 stages. The first stage consisted of three collections of bacteria (GO53, IB15, S12) with 12 and 24 hours of inoculation. The second stage consisted of two collections bacteria (GO53, IB15) with 4 and 6 hours of inoculation. The third stage consisted of one collection bacteria (GO53) and 4 bacterial strains isolated from shallot (T4(2), T5, T8(1), T8(2)) with 2 and 4 hours of inoculation. The results showed GO53 and T4(2) with 2 hours of inoculation were the best types of endophytic bacteria to increase vigour. The explants in this treatment survived the longest, had more shoots and leaves than the control, and induced root formation more quickly.

1 Introduction

Shallot is an agricultural commodity that is very important in Indonesia because it is widely consumed by the community as a seasoning and traditional medicine. The shallot productivity rate in Indonesia from 2018 to 2020 has increased by 312,007 tons, from 1,503,438 tons to 1,815,445 tons [1]. The increase in shallot productivity figures still does not meet the needs of the community. According to [2], the projected consumption of shallots in Indonesia in 2017-2020 is expected to increase by 4.92% per year, but this figure could increase if population growth is more than the projected figure.

* Corresponding author: auliafloribunda@gmail.com

The fulfillment of shallot needs in Indonesia is still insufficient because production is not optimal due to poor seed conditions, disease attacks, and environmental factors. Farmers in Indonesia still use traditional farming methods by using seeds from previous plantings repeatedly, so the quality is not optimal [3,4].

Endophytic microbes are an alternative that can be used to increase plant vigor and control pests and diseases that are environmentally friendly [5]. The use of microbes in plants that are non-chemical will have a very good impact on the environment because they will not poison and harm. Endophytic microbes live symbiotically with the plant host in the host tissue. This causes endophytic bacteria to increase plant vigor by increasing nutrient acquisition capacity and root architecture, and increasing environmental stress tolerance [4]. Endophytic bacteria, namely Bacillus sp. can increase the vigor of rice plants because these bacteria produce indole-3 acetic acid (IAA) [6]. Endophytic bacteria Pseudomonas sp. and Bacillus sp. also succeeded in increasing plant viability and vigor in tomatoes because they produced IAA [7]. IAA is a natural plant growth regulator that is important for aspects of plant growth that regulate many physiological processes, such as cell division and differentiation and protein synthesis, thus supporting stem elongation by increasing cell size enlargement and division [8]. In addition, endophytic microbes can produce a biological agent that can combat plant diseases, so plants will directly avoid diseases caused by other microbes [9]. Endophytic bacteria from Wakatobi were able to inhibit the growth of Alternaria porri and Fusarium oxsysporum colonies, which cause purple spot and wilt disease in shallots, respectively [10].

Inoculation time is very influential in the application of microorganisms. The right information about inoculation time is needed in the application of bacteria to plants or testing the resistance of a plant to pathogens [11]. This is because inoculation time is closely related to the number of microorganisms that grow [12].

The application of endophytic microbes in plants is currently done by soaking or watering into the planting media, but the application is often constrained by the environment such as rain, wind, heat, etc. [13]. One of the new methods that can be used to overcome these problems is tissue culture. In addition, using tissue culture techniques can also obtain plants that have the same characteristics as the original plant (parent) with tissues that remain young (actively dividing-meristematic) [14].

This study was conducted to obtain the best strain of endophytic bacteria and inoculation time that can increase the vigor of shallot plants. The results of this study are expected to increase knowledge about endophytic bacteria that can increase the vigor of shallot plants. In addition, this research is also expected to be a reference in increasing the vigor of shallot plants by applying endophytic bacteria in vitro.

2 Materials and methods

2.1 Material and equipment

This study was conducted at cell and tissue biology laboratory, Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), West Java from January to December 2023. The main treatment materials were seven strains of endophytic bacteria. Bulb of shallot Bima variety was used as genetic material.

The tools used in this research are a beaker glass, culture bottle, bunsen, petri dish, cutter, Erlenmeyer, measuring cup, hand sprayer, hot plate, Laminar air flow cabinet (LAFC), refrigerator, millimeter block, autoclave, oven, digital pH meter, tweezers, culture rack, tube rack, sieve, scalpel, spatula, test tube, and analytical balance. The plant material used is onion

bulbs onion bulbs of the Bima variety. Other materials used for the media were Murashige and Skoog basic media (MS), casein hydrolysate, gelzan, sugar, kinetin, and naphthalene acetic acid (NAA). The main ingredients treatment were seven strains of endophytic bacteria. Other supporting materials are agrept (bactericide), distilled water, 70% and 96% alcohol, aluminum foil, cefotaxime, dithane M-45 (fungicide), kanamycin, filter paper, kinetin, bleach solution (Clorox), plastic wrap and soap.

This study used a factorial completely randomized design with two factors, the type of bacteria and the time of inoculation which was divided into 3 stages. The first stage consisted of three collections of bacteria (GO53, IB15, S12) with 12 and 24 hours of inoculation. The second stage consisted of two collections bacteria (GO53, IB15) with 4 and 6 hours of inoculation. The third stage consisted of one collection bacteria (GO53) and 4 bacterial strains isolated from shallot (T4(2), T5, T8(1), T8(2)) with 2 and 4 hours of inoculation. The first stage was an experiment on the application of endophytic bacteria from the existing collection at 12 and 24 hours. The second stage was an experiment on the application of endophytic bacteria from the collection that was declared to have potential after stage one with the inoculation time reduced to 4 and 6 hours. Stage three was the application of the most potent endophytic bacteria from stages one and two and the application of four endophytic bacteria isolated from shallot explants with inoculation times of 2 and 4 hours. The use of 12 and 24 hours in this research design refers to previous research on the application of endophytic bacteria in vitro on citrus shoots [15]. The next time (2, 4, 6 hours) is the time that will first be tried on inoculation of shallot explants in vitro. The basis for the use of this time is because shallots are seasonal herbaceous plants that contain more water, so it is known that if the plant is too long in a humid/watery environment it can cause vitrification due to excessive water absorption [16, 17].

2.2 Explant sterilization

Explants in the form of shallot bulbs were sterilized using the technique reported by [18]. The bulbs were peeled off the outer skin and then washed thoroughly using running water and soap for 30 minutes. Furthermore, shallot bulbs were soaked in fungicide in the form of dithane M-45 and bactericide in the form of agrept 3g/L each for two hours, then rinsed with sterile water three times. The sterilization process is then continued in the Laminar air flow cabinet (LAFC). In the LAFC, the tubers were immersed in 96% alcohol for 3 minutes and then burned using a bunsen burner. After the fire was extinguished, the bulbs were soaked in 30% clorox solution for 30 minutes. After soaking is complete, shallot bulbs are rinsed with sterile water 2 times. After that, the bulbs are peeled as much as 1-2 layers and soaked in 20% clorox solution for 30 minutes. After 30 minutes, the shallot bulbs were again rinsed with sterile water 2 times and 1-2 layers of bulb peeling were carried out accompanied by cutting the top of the bulb until a white layer was obtained. After that, the explants were soaked in 10% clorox for 15 minutes. After 15 minutes, the shallot bulbs were rinsed with sterile water 2 times. Explants containing basal plates or discs were then soaked in 5% clorox for 10 minutes before planting into the media.

2.3 Endophytic bacterial inoculation

Sterile explant cultured on MS + Kinetin 2 mg/L + NAA 2 mg/L media + casein hydrolysate 0.5 mg/L, then transferred to inoculation treatment media in the form of MS liquid media + Kinetin 2 mg/L + NAA 2 mg/L + casein hydrolysate 0.5 mg/L with the addition of bacteria 1:1. Each test tube contains 1 explant with 6 repetitions for each type of bacteria so that from each type of bacteria each obtained 42 individuals. Explants were then inoculated for 2, 4, 6,

12, and 24 hours in a room with a temperature of 23-25ºC and irradiation using a lamp every 16 hours/day.

2.4 Subculture of Explants

Explants that have been inoculated with bacteria are then rinsed using antibiotics. Rinsing was done twice with antibiotics in the form of a mixture of cefotaxime and kanamycin at 400 ppm and then rinsed with distilled water once. After the explants are clean from bacteria the explants are dried with filter paper. Dry explants were then cultured on MS + Kinetin 2 mg/L + NAA 2 mg/L + casein hydrolysate 0.5 mg/L culture media in a room with a temperature of 23-25oC and illumination using lights every 16 hours/day.

2.5 Observation

There were four types of parameters observed in this study, namely explant death, number of shoots, leaves, and roots. Observation of explant death was carried out every day and was stopped when there was 100% death in one group of explants that had been soaked in bacterial isolates. The number of shoots was observed starting from the 1st day after planting until there was 100% death in one group of explants. Several leaves were observed starting from the 1st day after planting until there was 100% death in one of the groups of explants that had been soaked in bacterial isolates. The leaves that were counted were leaves that have a cylindrical shape and are green in color. The number of roots was observed starting from day 1 after planting until there was 100% death in one group of explants that had been soaked in bacterial isolates. The roots that were counted were round-tipped roots with a white-brown color.

2.6 Data analysis

The observation results were then processed using the SAS 9.0 program. If the results had a significant effect, then continue with the Duncan Multiple Range Test at the 95% level (α = 0.05).

3 Results and discussion

3.1 Number of shoots

Stage 1 of the experiment, namely for three types of endophytic bacteria collection (IB15, GO53, S12) with a time of 12 and 24 hours, it was found that a significant effect on the third day for the interaction of inoculation time and type of bacteria on the number of shoots. This indicates that there was an interaction between plant tissue and endophytic bacteria in the first experiment.

Table. 1. Average number of shoots at the interaction of bacterial species and inoculation time stage 1 (12 and 24 hours).

Application	Day after planting (DAP)		
	1	2	3
A1B0	1.167 a	0.83 a	0.67 a

A2B0	1 a	1 a	1 a
A3B0	1.167 a	0.67 a	0 b
A4B0	1.167 a	1.167 a	1.167 a
A1B1	1.167 a	1 a	0.83 a
A2B1	1 a	1 a	1 a
A3B1	1.167 a	1 a	0.83 a
A4B1	1 a	1 a	1 a

Notes: Mean values followed by the same letter in the same column are not different in the Duncan Multiple Range Test (DMRT) at a significant level of 5%. DAP = days after planting; A1 = IB15; A2 = GO53; A3 = S12; A4 = Control; B0 = 24 hours; B1 = 12 hours.

In Table 1, bacteria S12 (A3) gave a negative response characterized by a short explant life only until the third day for an inoculation time of 24 hours and the fifth day for an inoculation time of 12 hours. This caused the S12 (A3) bacteria to be eliminated and not used in stage 2. Bacteria S12 (A3) is one of the strains from the Bacillus spp. collection of bacteria. Bacillus sp. is a commonly used bacterium for endophytic bacterial applications on several plants due to its ability to produce indole-3 acetic acid (IAA) [8]. Even though Bacillus spp. has many advantages when applied to other plants when applied to shallot explants, Bacillus spp. bacteria gave a negative response with bacterial overgrowth on the explant media. This indicates that the Bacillus bacteria used in this study did not form a symbiosis with the explant roots. The non-formation of symbiosis can occur because the Bacillus bacteria used are only symbiotic on certain plants but not for shallot plants. The application of Bacillus endophytic bacteria successfully suppressed bacterial leaf blight (HDB) in shallot plants with strains isolated from shallot plants [19]. Based on this, it can be seen that shallot explants can symbiotic with Bacillus bacteria if they come from shallot plant hosts as well. This indicates that shallot plants are specific with several types of Bacillus bacteria to be symbiotic, so that Bacillus spp. used cannot enter the shallot explant tissue because the bacterial strain is not isolated from the shallot plant.

Stage 2, namely for two types of endophytic bacteria collection (IB15 and GO53) with 4 and 6 hours, it is known to have a real effect on the number of shoots only at the time of inoculation. Based on data analysis, inoculation time is significantly different in the number of shoots on the fourth day for 6 hours. Inoculation time is important because it requires the right duration so it becomes one of the factors that determine the success of inoculation [11]. This is because the number of microorganism cells in the media is influenced by time, time is related to the rate of multiplication of microorganisms [12]. So, the longer the microorganisms are left in a medium, the more the number of bacteria in the medium will be. The number of microorganisms whose inoculation time is optimum, not too long or short, will produce an optimum product [12]. With the decrease in the number of shoots at the inoculation time of 6 hours, it is known that 6 hours is not a suitable time for inoculation of the parameter of the number of shoots when compared to the time of 4 hours.

Based on the results of stages 1 and 2, it is known that shallot plants can only interact with endophytic bacteria isolated from shallot plants. This led to the addition of new types of endophytic bacteria in the third stage isolated from shallot plants. The bacteria used were isolated from the same host as the explant material used in this study, shallots of the bima.

Stage 3, which is for 1 type of bacteria collection (GO53) and 4 types of bacteria (T4(2), T5, T8(1), T8(2)) isolated from shallots with inoculation time for 2 and 4 hours, it is known to have an effect only on the type of bacteria on the number of shoots. Bacteria GO53 and T4(2) gave a positive response at inoculation times of 2 hours and 4 hours respectively with the number of shoots still present until the last day of observation. GO53 bacteria gave a positive response for inoculation time of 2 hours which was characterized by an increase in the number of shoots faster than the control on the sixth day and the highest number of living explants. T4(2) bacteria gave a positive response to the inoculation time of 4 hours with an increase in the number of shoots that exceeded the control. GO53 bacteria is one of the strains from the collection of endophytic bacteria Micrococcus endophyticus. Micrococcus endophyticus can produce IAA-like compounds and can fix free nitrogen. The application of Micrococcus endophyticus bacteria on potato plants can increase plant height and the number of shoots [20]. It is also proven that the application of Micrococcus endophyticus in this study can increase the number of shoots on shallots and accelerate the formation of shoots on the sixth day if the inoculation time used is appropriate, namely 2 hours.

3.2 Number of leaves

Stage 1 of the experiment, namely for three types of endophytic bacteria collection (IB15, GO53, S12) with a time of 12 and 24 hours is known to have a real effect on the interaction of time and type of bacteria on the number of leaves. GO53 bacteria gave the best response to the number of leaves for both inoculation times (12 and 24 hours) with a stable average number until the third day. The S12 bacteria gave the worst response by a decrease in the number of leaves and eventually died. Bacteria S12 gave a negative response characterized by a short explant life only until the third day for inoculation time for 24 hours and the fifth day for inoculation time for 12 hours. fifth day for inoculation time of 12 hours. This caused the bacteria S12 to be eliminated and not used again in stage 2. Bacteria S12 (*Bacillus* spp.) are capable of producing indole-3 acetic acid (IAA) which can increase plant growth. However, the response of this bacterial application to the number of leaves is the same as in shoots, which gives a negative response. With the occurrence of a negative response that is also given to the number of leaves, it is clear that the bacteria collection S12 is not a type of endophytic bacteria of *Bacillus* that can be symbiotic with shallots.

Stage 2, which is for two types of endophytic bacteria collection (IB15 and GO53) with a time of 4 and 6 hours is known to have an effect on the number of leaves on the type of bacteria. Bacteria GO53 gave the best response to the number of leaves with an increase in the number of leaves that exceeded the control. IB15 bacteria gave a negative response with the continuous decrease in the number of leaves and dying earlier than GO53. IB15 bacteria is one of one of the strains from the collection of Bacillus sp. bacteria isolated from potato plants. As already mentioned earlier, Bacillus sp. bacteria are very commonly used as endophytic bacteria because they can produce indole-3 acetic acid (IAA) which can increase plant growth [11, 8]. However, the application of IB15, which is a bacterium of Bacillus sp. from potato plants gave a negative response to the number of leaves of shallot plants. This further confirms that Bacillus sp. bacteria applied to shallot cannot be isolated from other plants besides shallot plants.

Based on the results of stages 1 and 2, it is known that shallot plants can only interact with endophytic bacteria isolated from shallot plants. This led to the addition of new types of endophytic bacteria in the third stage isolated from shallot plants. The bacteria used were isolated from the same host as the explant material used in this study, shallots of the bima.

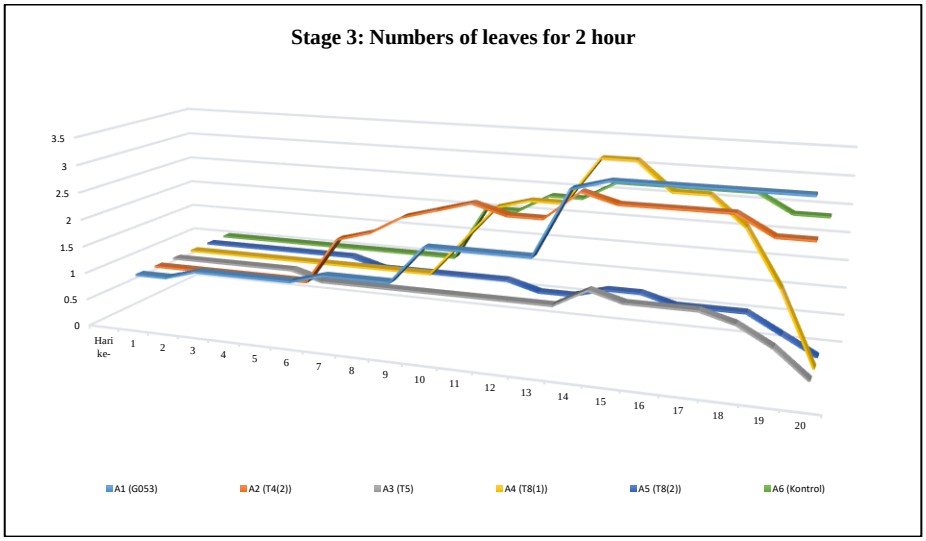

Fig. 1. Number of leaves at 2 hours inoculation time of stage 3

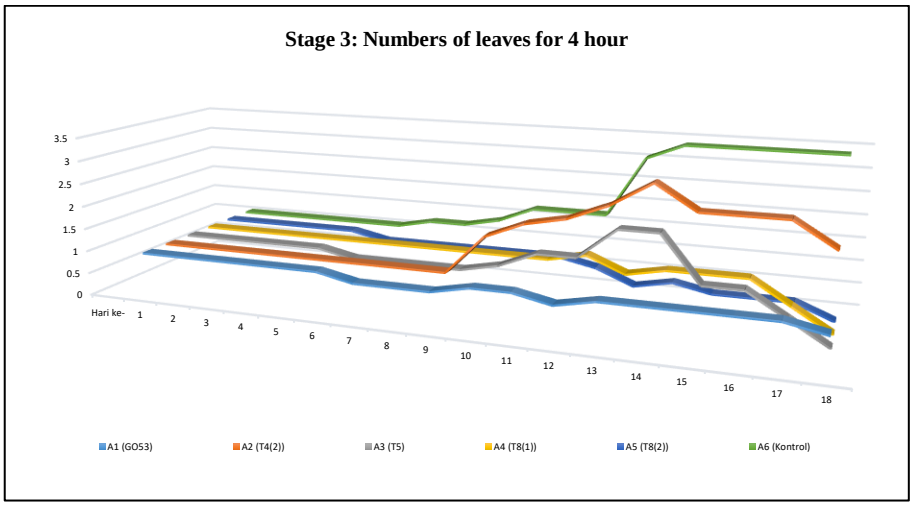

Fig. 2. Number of leaves at 4 hours inoculation time of stage 3

Stage 3, namely for 1 type of bacterial collection (GO53) and 4 types of bacteria (T4(2), T5, T8(1), T8(2)) isolated from shallots, it is known that with a time of 2 and 4 hours, it is known to have a significant effect on the type of bacteria and inoculation time on the number of leaves. The types of bacteria that give a positive response at inoculation times of 2 and 4 hours are GO53 and T4(2) bacteria which are characterized by a greater number of leaves compared to the control (figure 1 and figure 2). GO53 bacteria gave a positive response for inoculation time of 2 hours which was characterized by an increase in the number of shoots faster than the control on the third day and the number of explants that lived until the end of the study was the most. GO53 bacteria are Micrococcus endophyticus bacteria that increase the number of leaves due to the ability of these bacteria to produce IAA-like compounds and can fix free nitrogen [20]. IAA is one type of auxin that is identified as an active auxin in plants produced in active meristematic tissues [21, 22]. Auxin in the meristem tissue spurs cell elongation and division, causing an increase in the number of leaves. The persimmon plants the addition of auxin group IAA gives the best response to the increase in the number

of leaves [23]. The nitrogen fixation ability of these bacteria also affects the increase in the number of leaves. This is because nitrogen, which is a macro element for plants, plays a very important role in vegetative growth so that it can supply nutrients for the increase in plant height, number of leaves, and stem diameter growth [24].

3.3 Number of roots

Stage 1, which is for three types of endophytic bacteria collection (IB15, GO53, S12) with a time of 12 and 24 hours. In this treatment, the three types of bacteria gave a negative response to the number of roots. This can be seen by none of root increase until the last day in each treatment, while in the control there is an increase in the number of roots on the second day. IB15 (*Bacillus* spp.), GO53 (*Micrococcus endophyticus*), and S12 (*Bacillus* sp.) are endophytic bacteria capable of producing IAA. IAA which is an auxin if given at the appropriate concentration can induce root formation in plants [21]. However, in this treatment, all three gave a negative response. The negative response given is thought to be caused by the amount of IAA that is too much so it inhibits root growth. Giving excessive concentrations of auxin in the media will cause inhibition of explant growth due to disruption of the process of division, enlargement, and elongation of plant cells so that plants do not grow well [25, 26]. This is because the addition of exogenous auxin will increase the concentration of endogenous auxin in plant cells and tissues, thus inhibiting cell elongation and division in the cambium tissue which causes root formation to be disrupted [27, 11]. However, if the addition of auxin is accompanied by cytokinin at the appropriate concentration, it will cause cytokinin to suppress auxin to form roots to spur the process of growing and developing tissues faster because of the synergy between the two phytohormones in the elongation and increase in the number of shoots and leaves [28, 29, 30]. The function of cytokinins is to induce cell division in meristematic tissues, organ formation, adventitious bud differentiation, apical dominance, and leaf expansion and increase the mobility rate of elements in plants [25, 26].

Stage 2, which is for two types of endophytic bacteria collection (IB15 and GO53) with 4 and 6 hours, it is known that there is no interaction between the type of bacteria and inoculation time on the number of roots. The coefficient of variation (CV) for the number of roots is quite diverse. The factors that affect the CV value in a study include the heterogeneity of tools, materials, and equipment. In this study, the explants used were not obtained (sterilized) on the same day. The wider the treatment interval used, the CV value will be greater, and vice versa [20]. In addition, measurement of observation variables carried out from outside the culture bottle can also be the cause of the high CV value in this study. When viewed from the number of roots there is an increase in the number of roots from the application of IB15, but the explants from the treatment cannot survive until the end of observation. This explant death occurred because the explants appeared to be vitrified. This can be seen from the explants whose leaves become withered, weak, and small. The cause of vitrification in vitro applications can be caused by the concentration of agar or cytokinin or the water application because it is related to the water potential in plant tissue [31].

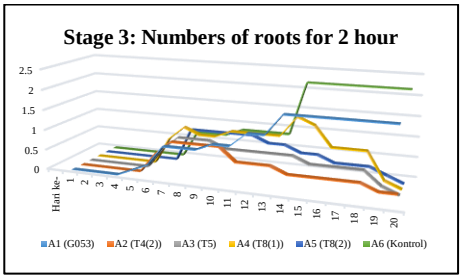

Fig. 3. Number of roots at 2 hours inoculation time of stage 3

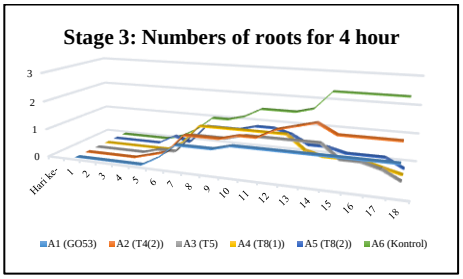

Fig. 4. Number of roots at 4 hours inoculation time of stage 3

Stage 3, for 1 type of bacteria collection (GO53) and 4 types of bacteria (T4(2), T5, T8(1), T8(2)) isolated from shallots with inoculation times of 2 and 4 hours were found to have a significant effect on the number of bacteria isolated from shallots. The types of bacteria that gave a positive response at both inoculation times were the bacteria GO53 and T4(2) which is characterized by an increase in the number of roots faster than the control (fig.3 and fig.4). Bacteria GO53 gave a positive response for inoculation time of 2 hours which was characterized by the increase in the number of roots faster than the control on the fifth day and the number of explants that explants were the most alive. GO53 bacteria are Micrococcus endophyticus bacteria, which are endophytic bacteria capable of producing IAA. The application of Micrococcus endophyticus bacteria on tobacco plants can increase the length of root, shoot, and dry weight [32]. IAA is one type of natural auxin. Auxin has a function to induce the formation of roots, and callus by spurring cell elongation and division in the cambium tissue at the point of shoot growth. [26, 29]. Because of its function, auxin produced by Micrococcus endophyticus can induce root formation in shallots.

4 Conclusion

Based on the data obtained in this study, the application of endophytic bacteria in vitro with different inoculation times is known to increase the vigor of shallot plants. The most effective endophytic bacteria to increase the vigor of shallot plants are GO53 (*Micrococcus endophyticus)* and T4(2), characterized by an increase in the number of shoots, leaves, and roots. The best inoculation time to increase the vigor of shallot plants is 2 hours, with two types of endophytic bacteria that can survive 20 daps, GO53 (*Micrococcus endophyticus)* and T4(2). There is an interaction between inoculation time and the type of endophytic bacteria used in stage 1 treatment for 3 types of bacteria collection with inoculation time for 12 and 24 hours for the parameters of the number of shoots, and leaves.

Acknowledgements

The authors would like to thank BRIN Indonesia's 'Organisasi Riset Pertanian dan Pangan' for funding this research through the 'Rumah Program ORPP' funding scheme for fiscal year 2023.

References

1. Statistics Indonesia. (2020, 15 February). “Vegetable Crop Production 2020”. Statistics Indonesia [Online]. Available: https://www.bps.go.id/indicator/55/61/1/produksi-tanaman-sayuran.html

2. R. Widaningsih, et al. “Food Crops and Horticulture Outlook”, Jakarta: Center for Agriculture Data and Information System Secretariat General - Ministry of Agriculture, 2017.

3. H. A. Aziz, H. E. Andi, dan Bahrudin. “The seed source characterization of onions from the centra regions production in Palu valley”, J. Agrotekbis, vol. 1 pp. 221-227, August 2013. https://www.neliti.com/id/publications/250935/karakterisasi-sumber-benih-bawang-merah-dari-berbagai-daerah-sentra-produksi-di

4. R. Defez, A. Andreozzi, S. Romano, G. Pocsfalvi, I. Fiume, R. Esposito, C. Angelini and C. Bianco. “Bacterial IAA-Delivery into Medicago Root NodulesTriggers a Balanced Stimulation of C and N Metabolism Leading to a Biomass Increase”, Microorganisms, vol. 7, pp. 403, Oct 2019. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6843515/

5. H. Wulandari, Zakiatulyaqin, dan Supriyanto. “Isolation And Antagonistic Test of Endophytic Bacteria from Pepper (Piper nigrum L.) Againt Velvet Blight Pathogen (Septobasidium sp.)”, J. Perkebunan & Lahan Tropika, vol. 2, pp. 23-31, Dec 2012.

6. P. Lestari, Y. Suryadi, D. N. Susilowati, T. P. Priyanto, dan I M. Samudra. “Characterization of Bacteria Producing Indole Acetic Acid and Its Effect on Rice Seed Vigor”, Berita Biologi, vol. 14, pp. 19-28, April 2015.

7. J. La Fua, L. Sabaruddin, L. S. Bande, and S. Leomo, “The Potential of Indigenous Endophyte Bacteria to Promote Local Tomato Growth Isolated from Dry Land in Muna, Southeast Sulawesi, Indonesia”, International Journal of Scientific & Technology Research, vol. 8, pp. 2975-2980, Dec 2019. https://www.ijstr.org/final-print/dec2019/The-Potential-Of-Indigenous-Endophyte-Bacteria-To-Promote-Local-Tomato-Growth-Isolated-From-Dry-Land-In-Muna-Southeast-Sulawesi-Indonesia.pdf.

8. E. E. Idris, D. J. Iglesias, M. Talon, and R. Borriss. “Tryptophan-dependent production of indole-3-acetic acid (IAA) affects level of plant growth promotion by Bacillus amyloliquefaciens FZB42”. Mol. Plant Microbe Interact., vol. 20, pp. 619-626, June 2007.https://apsjournals.apsnet.org/doi/abs/10.1094/MPMI-20-6-0619?url_ver= Z39.88-2003&rfr_id=ori:rid:crossref. org&rfr_dat=cr_pub%20%200pubmed.

9. R. Melliawati, R. S. Suherman, and S. Bambang. “Assessment of Endophytic Molds From Mount Halimun National Park As Glucoamylase Producers”, Berkala Penelitian Hayati, vol. 12, pp. 19-25, Dec 2006.

10. G. A. K. Sutariati, A. Khaeruni, and A. Madiki. “Wakatobi Native Bacteria Inhibit the Colony Growth of Alternaria porri and Fusarium oxyporum of Shallots in in Vitro Study”, Jurnal Fitopatologi Indonesia, vol. 16, pp. 105-111, May 2020.

11. N. Hidayah dan T. Yulianti. “Effect of Inoculation Time and Inoculum Amount on Pathogenicity of Phytophthora nicotianae on Tembaka Seedlings”, Buletin Tanaman Tembakau, Serat & Minyak Industri, vol. 2, pp.75-80, Oct 2010.

12. R. R. Siburian, A. Ahmad, and S. R. Muria. “Effect of Inoculum Inoculation Time in the Preparation of Bioethanol from Palm Fronds Using Saccharomyces cerevisiae”. J. Online Mahasiswa Fakultas Teknik), vol. 2, pp. 1-7, Oct 2015.

13. H. Marwan, Rainiyati, and S. Mulyati. “The Influenceof Endophytic Bacteria Application on the Developmentof Blood Disease (Ralstonia solanacearum Phylotipe IV) on Banana”. Jurnal Budidaya Pertanian, vol. 16, pp. 95-101, March 2020.

14. Santoso, U dan F. Nursandi. “Plant Tissue Culture”, Malang: Universitas Muhammadiyah Press, 2003
15. K. T. Wirakusuma. “In Vitro Germination and Growth of Lime (Citrus aurantifolia) for Selection of Endophytic Bacteria to Control Huanglongbing Disease”, thesis, Dept. Agronomy, IPB Univ., Bogor, 2021.
16. D. Rindang. Plant Tissue Culture. Denpasar: Pelawa Sari, 2002.
17. H. Iwan dan I. Masyarafina. “The Diversity of Undergrowth Species in The Eastern Part of Gunung Papandayan, Garut, West Java”. J. Silvikultur Tropika, vol. 6, pp. 119–125, 2015.
18. B. A. Tri. “Shoot Multiplication and Micro Bulb Formation of Shallot Bima Varieties”, thesis, Dept. Agronomy, IPB Univ., Bogor, 2021.
19. Z. Resti, T. Habazar, D. P. Putra, dan Nasrun. “Characterization Of Endophytic Bacillus Isolated from Shallot Root as Biocontrol of Bacterial Leaf Blight Disease”. J. HPT Tropika. Vol. 18, pp. 31-38, March 2018.
20. K. A. Hanafiah. Research Design. Jakarta (ID): PT. Raja Grafindo Persada, 2010.
21. R. Arimarsetiowati dan F. Ardiyani. “The effects of shooting and rooting of arabica coffee propagation throughembryogenesis somatic auxin uses”, Pelita Perkebunan, vol. 28, pp. 82-90, August 2012.
22. D. Hoesen, S. Hazar, Priyono dan H. Sumarnie. “The role of growth regulators IBA, NAA, and IAA in the propagation of Red Amaryllis (Amaryllidaceae)”. Proceedings of the National Heritage Day Seminar. Lab Treub Balitbang Botani Puslitbang Biologi, Bogor, 2000.
23. E. Setiawan. “Effectiveness of IAA, IBA, NAA and Root-up Application on Persimmon Seedling”, J. Hort. Indonesia, vol. 8, pp. 97-103, August 2017.
24. Havlin, J., L. James, D. Beaton, S. L. Tisdale and W. L. Nelson. Soil Fertility and Fertilizer, 6th ed, Prentice Hall, New Jersey, 1999.
25. I. Mahadi. “Breaking the Dormancy of Kenerak Seeds (Goniothalamus umbrosusu) Using the hormones 2,4-D and BAP by Micropropagation”, Sagu, vol. 10, pp.20-23, March 2011.
26. A. A. Sumiati dan Sutoyo. “Growth Stimulation of Dendrobium sp Using Auxin Hormones Naphtalena Acetic Acid (NAA) and Indole Butyric Acid (IBA)”, Jurnal Buana Sains, vol. 21, pp. 19-28, June 2021.
27. D. Trisnawati, “Application of NAA and kinetin on the growth of dragon fruit (Hylocereus costaricensis) explants through tissue culture techniques in vitro”. Thesis, Dept. Scicence and Mathematics, Riau Univ., Pekanbaru, 2013.
28. A. G. Demissie. “Effect of different combinations of BAP (6-benzyl amino purine) and NAA (Napthalene acetic acid) on multiple shoot proliferation of plantain (Musa spp.) cv. Matoke from meristem derived explant”, International Journal of Plant Research, vol. 1, pp. 36-43, Feb 2015.
29. E. G. Lestari. “The Role of Growth Regulators in Plant Propagation through Tissue Culture”. Jurnal Agro Biogen, vol. 7, pp. 63-68, April 2011.
30. R. L. M. Pierik. In Vitro Culture of Higher Plants. London: Martinus Nijhoff Publisher, l987.

31. C. T. Phan, and P. Hadagus. “Possible Metabolic Basis for The Developmental Anomaly Observed in In Vitro Culture, Called Viteous Plant”. Plant Cell, Tissue and Organ Culture, vol. 6, pp. 83 – 94, Oct 1986.
32. Y. S. Fatma. “The ability of endophytic bacteria Micrococcus endophyticus GO53 to produce IAA-like compound and increase plant growth”, thesis, Dept. Biology, IPB Univ., Bogor, 2014.