Journal of Applied Agricultural Science and Technology
JAAST 8(1): 50-64 (2024)
E-ISSN: 2621-2528
ISSN: 2621-4709

GROWTH OF CUT-GRAFTING ROBUSTA COFFEE SEEDS UTILIZING ORTHOTROPIC AND PLAGIOTROPIC ROOTSTOCKS WITH APPLICATION BACILLUS AND PSEUDOMONAS MIXTURE

Muhammad Ghufron Rosyady*, Larassati, Setiyono, Gatot Subroto, Ketut Anom Wijaya, Distiana Wulanjari, Oria Alit Farisi, Basuki

Faculty of Agriculture, University of Jember, Jember, Indonesia

*Corresponding author
Email: muhammad.ghufron.rosyady@gmail.com

***Abstract**. Vegetative propagation by cut-grafting has the advantage of being able to obtain seeds that inherit two superior traits from two scion clones in a relatively short time. The purpose of this research is to determine the effect of using orthotropic and plagiotropic rootstocks applied by Bacillus and Pseudomonas on the early growth of robusta coffee seedlings from cut grafting. The method used was by using a completely randomized factorial design with 2 factors and was repeated 3 times. The first factor was the use of rootstock cuttings which consisted of 2 levels, which were S_1 (orthotropic stem) and S_2 (plagiotropic stem). The second factor was the concentration of Bacillus and Pseudomonas which consisted of 5 levels, which were B_0 (0 ml/L), B_1 (20 ml/L), B_2 (40 ml/L), B_3 (60 ml/L) and B_4 (80 ml/L). The results showed that (1) there was no interaction between the use of variatic rootstock and the application of the biological agent (2) The use of orthotropic rootstock increased plant growth, that is the number of primary roots and the number of leaves and (3) Application of the biological agent of Bacillus and Pseudomonas with concentrations of B4 increased seedling growth on all observed parameters except the number of shoots. Based on the results of this research, the benefit for coffee planters is to obtain alternative planting materials, namely from orthotropic branches. For further research, the use of orthotropic branch planting material as a scion plant can be investigated.*
***Keywords:** robusta; cut grafting; plagiotropic; Bacillus; Pseudomonas.*

1. Introduction

The coffee commodity is a plantation sub-sector with a high chance of export in Indonesia. The export value of coffee in 2020 reached 379.35 thousand tons, with a total production in 2020 was 762.38 thousand tons (BPS, 2020). Its productivity has increased where in 2019, the productivity of Indonesian coffee plantations was 803 Kg/Ha and in 2020, it increased to 811 Kg/Ha (BPS, 2020). The C. *canephora* is outstanding, usually for presenting neutrality in terms of sweetness and acidity, having a marked aroma of roasted cereals, and standing out for its more pronounced body when compared to arabica coffee. Thus, in recent years there has been a collective effort in the development of studies with the objective of enhancing the quality curve of this species. Among the several possibilities, the fermentation of the beans has been considered promising for the improvement of the sensorial quality, since the process plays an important role in the degradation of the mucilage, conferring several metabolic products precursors of aroma and flavor to the coffee beverage (Lee et *al.*, 2015). Attempt to advance C. *canephora* concerns the

Received July 5, 2023; Accepted November 9, 2023; Published February 24, 2024
https://doi.org/10.55043/jaast.v8i1.189

expansion of cultivation regions, commonly, C. *canephora* is cultivated at low altitudes (100 to 600 m of altitude), adapting well to warmer climates, with average temperatures between 22 and 26 °C and with precipitation above 1200 mm per year (Filete *et al*., 2022).

The problem with robusta coffee nurseries is that they cannot be developed generatively because the pollination type of robusta coffee is cross-pollination. If robusta coffee is developed generatively, the level of segregation is high. Therefore, the provision of robusta coffee seeds must be done by clonal or through vegetative propagation. However, clonal propagation only reduces the superior characteristics of the roodstock. Meanwhile, the demand for coffee seeds in Indonesia is at least to have superior characteristics of resistance to drought stress, resistance to nematodes, and high productivity. These properties cannot be obtained in one robusta coffee clone. Grafting techniques can be used to produce seedlings with two superior properties from scions and rootstocks, but the weakness of the grafting technique is that producing seedlings ready for planting requires a relatively long time. Rootstock not only plays vital roles in improving the resistance to biotic and abiotic stresses of the plants, but also affects the size of caopy, fruit yield and quality even the production of primary and secondary metabolites (Zhu *et al*., 2020). The criteria for rootstocks to be grafted are that they are 10-12 months old or the stem is as thick as a pencil. For this reason, a cut-grafting technique was developed which can combine two clones that each has superior properties in a short time.

The step of cut-grafting is by grafting scion with rootstock first then cutting diagonally at the bottom of rootstock at almost the same time. Propagation with cut-grafting provides seeds more quickly because the formation of the roots of seedlings occurs simultaneously with the recovery connection wound (Irlando *et al*., 2020). Plant propagation by cuttings usually uses orthotropic branches because the growth direction is upright (Rizwan, 2021). Coffe plant exhibits a dimorphic branching behaviour, in which orthotropic (vertical) stems produce plagiotropic (horizontal) branches which in turn produce more plaiotropic branches and coffee fruit. The principal plant stem, or trunk is ortotropic. Ortotropic stems always grow vertically or perpendicular to the soil. Head of series buds on ortotropic stems produce primary plagiotropic shoots or branches. Since plagiotropic branches cannot generate ortotropic stems, cuttings that will be used for planting must originate from ortotropic stems in order to generate a normal, vertically growing tree (Ferreira *et al*., 2019). However, the availability of orthotropic branches is limited. In this research, utilized rootstocks were derived from orthotropic and plagiotropic branches. Plagiotropic branches on shrubs can be used as grafts for cuttings with the result that the plants continue to grow upright. In plants that grow upright, both orthotropic and plagiotropic branches that are used as cuttings will still produce upright stem growth (Anggraini *et al*., 2021).

The successful growth of coffee cuttings apart from the planting material factor also requires the addition of growth hormones. Exogenous auxin application can lead to soccessful graft formation during grafting (Zhai *et al*., 2021).Administration of exogenous auxin is necessary because the endogenous auxin content in plants is very small. Thus, giving the right concentration of growth regulators can stimulate the growth of seedlings, especially roots (Firdaus *et al*., 2023). Bacillus and Pseudomonas, apart from their ability as biopesticides, they also act as PGPR. Its role as PGPR is considered as a suitable tool to support sustainable agriculture due to its use which can replace chemical products that have been used by farmers. Bacillus subtilis and Pseudomonas as plant growth promoting rhizobacteria is capable to preserve constant contact with higher plants and encourage their growth. Direct effects of this bacteria as biofertilizers on plant growth is referred to synthesis of plant growth hormones (Ahmad *et al*., 2019). These two bacteria have three functions, namely as biocontrol through antagonistic effects against soil-borne diseases, as utilization of biofers by increasing the availability of nutrients such as N and P, and as biostimulation through the production of hormones (IAA, gibberellins, cytokinins and, ethylene) (Urgiles-Gómez *et al*., 2021).

Bacillus and Pseudomonas are a type of bacteria that has the potential to become a biological fertilizer and also produces phytohormones. Bacillus and Pseudomonas can produce phytohormones such as IAA (Indole Acetic Acid). IAA is a phytohormone in the group of natural auxin compounds so its function is to support plant growth, specifically as a promoter of cell division, cell differentiation, and protein synthesis which affect plant growth (Istiqomah *et al*., 2018). Bacillus sp. enter into plant tissues through plant roots and other parts of plants such as flowers, lenticels on stems or natural wounds and leaves through stomata. Then, Bacillus sp. that have entered the plant tissue will colonize where it enters or spread to all parts of the plant through the transport network (Husna *et al*., 2019). The leaves of the plant provide a more favorable environment for microbial life, or entrance to the leaves (via the stomata) is much more readily gained than access to the stems (that involves stem or root damage or outer root tissue damage during lateral root development) (Fan *et al*., 2020).

The spectrophotometric results showed a tendency to increase the concentration of tryptophan in the growth medium for rhizobacterium to increase the production of IAA gradually (Kalimuthu *et al*., 2019). Tryptophan is an important amino acid secreted by plants as an exudate and bacteria present in the vicinity of these plants develop a mechanism to utilize this amino acid as a precursor and produce plant hormone IAA by utilizing its own biochemical machinery (Wagi & Ahmed, 2019). When bacteria put in the IAA that they produce, the plant will be stimulated to increase the amount of IAA it has (Patil *et al*., 2011). The concentration of IAA produced by

Bacillus isolate UB-ABS2 was 1.09 ppm and Bacillus isolate UB-ABS6 was 0.69 ppm, while the concentration of IAA produced by Pseudomonas isolate UB-PF5 was 0.93 ppm and Pseudomonas isolate UB- PF6 was 0.84 ppm (Istiqomah *et al.*, 2018). Treatment of cocoa seedlings was applied. Bacillus sp. endophytes at a concentration of 10^{11}-10^{14} cfu/mL had an effect on increasing the growth of seedlings by increasing the number of leaves, stem diameter, seedling height and leaf area of cocoa seedlings (Puspita *et al*., 2018). The treatment of the application of PGPR containing Bacillus with a concentration of 30 ml/L increased the growth of coffee shoot grafting with increased shoot height and shoot diameter (Kafrawi *et al*., 2020). Application of Bacillus with concentrations of 60 gr/L on robusta coffee showed a better growth on plant height, number of leaves, leaf chlorophyll value and leaf area (Rosyady *et al*., 2022). So, in this research, the concentration of the Bacillus sp treatment was increased to find out whether the concentration of 60 ml/L was the best concentration or not.

The purpose of this research is to determine whether there is an interaction between the utilization of orthotropic and plagiotropic branches and the application of concentrations of Bacillus and Pseudomonas in the early growth of robusta coffee seedlings by cuttings. Another objective is to find out whether the growth of Robusta coffee seedlings propagated by grafting using plagiotropic rootstock has the same results or it is not significantly different from those of grafted seedlings using rootstock from orthotropic branches. So, in the future, the plagiotropic branches can be used as planting material for rootstock grafting of robusta coffee cuttings. In addition, this research is aimed to find out the application of the best dose of biological agents of Bacillus and Pseudomonas on the growth of robusta coffee seedlings from grafting cuttings.

2. Methods

2.1. Experimental Design

The research was conducted in Mangaran Village, Ajung District, Jember Regency. The materials needed in this research were robusta coffee clone BP 308 (as rootstock) taken from orthotropic and plagiotropic branches, BP 936 (as scion), and biological agents of Bacillus and Pseudomonas. The method used was by using a completely randomized factorial design with 2 factors and it was done repetitively by 3 times. The first-factor treatment was the use of rootstock cuttings (S) which consisted of two levels, which were rootstock from orthotropic stem (S_1) and plagiotropic stem (S_2). Meanwhile, the second factor was the concentration Bacillus and Pseudomonas, which consisted of 5 levels, which were 0 ml/L Bacillus and 0 ml/L Pseudomonas (B_0), 20 ml/L Bacillus and 20 ml/L Pseudomonas (B_1), 40 ml/L Bacillus and 40 ml/L Pseudomonas (B_2), 60 ml/L Bacillus and 60 ml/L Pseudomonas (B_3), 80 ml/L Bacillus and 80 ml/L Pseudomonas (B_4). Each treatment combination was repeated 3 times so that there were 30 experimental units.

The diameter of the stem used as planting material was uniform, around 1 – 1.3 mm. The scion was cut into three segments and one rootstock was used for a length of approximately 7 cm (3 cm above the nodes and 4 cm below the nodes). The leaves on the scion and rootstock were all cut off. Connecting the upper and rootstocks was done by making a slit at the top of the rootstock with 3-4 cm long with a splicing knife. Then, the lower side of the scion was sliced to form a spire and the size was adjusted to the gap on the rootstock. The next step was to peel all the leaves in the planting material. After that, the connection was carried out by inserting the scion to the rootstock and then the connection was wrapped with clear plastic until the connection was completely closed. After that, the coffee seedlings were applied with a solution rootone-f with the rapid dip method aimed at stimulating root growth. Following that, the seeds were planted in polybags that had been arranged in a lid.

Seedlings were fully opened and ready for treatment after 3 months since planting. The treatment application was carried out based on the experimental design that had been carried out previously. In treatment B_0, plant leaves were sprayed with water. For treatment B_1 (20 ml/L Bacillus and 20 ml/L Pseudomonas), the method was to make a solution where 20 ml of Bacillus and 20 ml of Pseudomonas bacteria were dissolved in 1 L of water, and the same was done in the other treatments. Then, it was applied to the plants by spraying as much as 50 ml/plant using a sprayer. The application was given foliar on the lower surface of the plant leaves in the morning. Foliar was applied so that absorption by plant tissues could be faster and maximized through leaf stomata, in addition to the proper function of bacteria as IAA producers. The application interval for bacteria was once every 2 weeks for 2 months (4x applications). Data collection was carried out 2 weeks after the last bacterial application. Variables observed included the percentage of live cuttings (%), percentage of rooted cuttings (%), number of primary roots/cuttings, length of primary roots/cuttings (cm), number of leaves, number of shoots, shoot diameter (mm), shoot length (cm), plant fresh weight (grams), plant dry weight (grams), and chlorophyll content (SPAD units).

2.2. Data Analysis

The data from the observations were then analyzed using Analysis of Variance. When there was a significant difference between the treatments, a further test was performed using Duncan's multiple range test at the 5% level significant.

3. Results and Discussion

The results of the analysis of variance performed on all observational variables are presented in Table 1. Based on the analysis of variance, it can be seen that there was an interaction between the utilization of rootstock from orthotropic and plagiotropic branches and biological agents of Bacillus and Pseudomonas had no significant effect on all observed variables. The use ofrootstock from orthotropic and plagiotropic branches had a significant different effect on the observed variable number of primary roots and number of leaves, and it had no significant effect on other observed variables. Meanwhile, the main effect of application of Bacillus and Pseudomonas had a significant effect on all observation variables except for the number of buds observation variable. In the observation variable, the percentage of live cuttings and rooted cuttings showed that the overall percentage of treatment was 100%.

Table 1. Recapitulation result of the Analysis of variance (F-count) on all observation variables

No	Observation Variable	Value of F-count		
		S	B	S x B
1	Number of primary roots	7.58*	3.01*	0.54 ns
2	Primary root length (cm)	1.3ns	3.09*	0.79 ns
3	Number of leaves	24.20**	5.44**	0.92 ns
4	Number of buds	0.47 ns	0.50 ns	0.14 ns
5	Bud diameter (mm)	0.01 ns	16.13**	0.14 ns
6	Shoot Length (cm)	0.24 ns	14.03**	0.25 ns
7	Plant fresh weight (gram)	3.48 ns	2.92*	1.95 ns
8	Plant dry weight (gram)	1.71 ns	5.64**	1.40 ns
9	Chlorophyll content (SPAD units)	3.42 ns	10.80**	0.17 ns

3.1. The interaction effect of orthotropic and plagiotropic rootstock utilization with biological agents of Bacillus and Pseudomonas on the growth of robusta coffee seeds from cut-grafting

The results of the analysis of variance on Table 1 show the interaction between the utilization of rootstock derived from orthotropic and plagiotropic branches with Bacillus and Pseudomonas. It can be found that there was no interaction on all observed variables. This is due to the character of rootstock planting material originating from orthotropic and plagiotropic branches having the same response to the application of Bacillus and Pseudomonas. Both orthotropic and plagiotropic branches can be used as planting material and their properties are not different in the growth of seedlings. The use of coffee cuttings planting material on different branch sources did not affect growth parameters. Coffee cuttings using planting material from plagiotropic branches need to consider the diameter of the cutting material used because the diameter of the branch describes a strong meristem. The effect of the size of the branch diameter on the speed of root and shoot formation is due to differences in the amount of carbohydrate content (Tustiyani, 2017).

Figure 1. Final documentation of cut-grafting seedlings of robusta coffee

Figure 1 shows the growth of grafted seedlings of robusta coffee cuttings treated with orthotropic and plagiotropic rootstocks with almost the same concentrations of Bacillus and Pseudomonas. It can be seen that there was no interaction between the use of orthotropic and plagiotropic rootstocks with the application of the same concentration of Bacillus and Pseudomonas.

3.2. The effect of utilizing orthotropic and plagiotropic rootstock on the growth of robusta coffee seedlings from cut-grafting

The results of the analysis of variance on Table 1 showed that the use of orthotropic and plagiotropic rootstock had a significant different effect on the number of primary roots and number of leaves, while the other observational variables showed no significant effect. Based on the Duncan's multiple range test results at 5% level at Table 2, it can be seen that the utilization of rootstock with S_1 (orthotropic) provided a better increase in the number of primary roots and the number of leaves than S_2 (plagiotropic). The plagiotropic branch has a smaller diameter than orthotropic. At the same diameter size, the location of the planting material for the orthotropic branch taken is near the shoot (young branch). Cuttings planting material determined by primordia on younger bark will grow shoots and roots faster. Shoots from older branches have a stronger dormant nature than shoots from younger branches. The older the branches used as planting material, the more energy is needed to grow the shoots and roots of the cuttings (Muningsih *et al*., 2019).

The older the branches used, the higher the production of root inhibiting substances and the phenolic compounds (compounds found in plants) which function as auxin cofactors (non-protein chemical compounds in the auxin hormone) which support the growth of cutting roots. Meanwhile, young branches are characterized by their soft texture and the evaporation process is fast so they wilt easily. The speed of root growth is also determined by the primordia points on the bark of the branch or twig. Primordia located on younger skin will grow roots more quickly than primordia located on older skin. The older the bark of a plant branch, the more energy is needed to grow roots from the branch (Muningsih *et al*., 2019).

In the growth of grafted cuttings that utilize orthotropic branches, vegetative growth is more rapid than plagiotropic branches. This is indicated by the number of primary roots and the number of leaves being greater than the plagiotrope branch treatment. This is thought to be because the food reserves in the plant are sufficient for plant growth so that in the grafting treatment using orthotropic branches, the roots can grow optimally. The number of primary roots is influenced by the hormone auxin and their growth is influenced by food reserves in plant organs until these organs can produce their own food. Root growth can also be controlled by chemical substances in very low concentrations called growth hormones/phytohormones. The hormone that influences root growth is auxin. Auxin regulates processes in the plant body in plant growth. Auxin is a growth hormone whose activation in tissues is related to the balance between synthesis and loss of auxin due to transport and metabolism. Auxin is produced in actively dividing tissue (Muningsih *et al*., 2019).

The largest number of leaves on orthotropic branches. Connecting cuttings that utilize orthotropic branches has leaves that grow perfectly and are in greater numbers so they can play an optimal role in the photosynthesis process so growth can be optimal. In the plagiotrope treatment, the branches already have leaves, but the leaves must provide assimilate results to several plant organs so that a lower value is obtained because the leaf formation is not yet perfect to produce assimilate. Therefore, the growth is hampered. The number of leaves on a plant is influenced by genotype and environment (Muningsih *et al*., 2019).

The position of leaves on a plant (plastochron number) is controlled by the genotype. In coffee plants, the leaves grow opposite each other, in pairs, both on the branches and stems. The pairs of leaves are located in the same plane. Moreover, the rate of leaf emergence is influenced by temperature, light and other factors. Temperature and sunlight can affect the results of plant photosynthesis. The results of leaf photosynthesis when grafting coffee cuttings are in the form of carbohydrates which are then used to form leaves. Young developing leaves require imported assimilation products to grow and develop until they can produce enough assimilation products to meet their own needs. Photosynthesis in leaves will produce photosintat which will be translocated to shoots, stems and roots. Meanwhile, photosynthesis produced in young leaves will not be translocated to other parts. Thus, the number of leaves is influenced by the photosynthesis process in plants (Muningsih *et al*., 2019).

Table 2. Recapitulation of further test results of Duncan's multiple range test at 5% level based on the effect of using otrotropic and plagiotropic rootstocks

Treatment	Number of primary roots	Number of leaves
S_1	5.53[a]	14.2[a]
S_2	3.93[b]	9.8[b]

Based on (Heryanto, 2020), older plant tissues have a reduced content of phenolic compounds resulting in a decreased ability to take root, in which phenolic compounds themselves function as auxin cofactors. In addition, the initial growth of seedlings still utilizes the food reserves in the planting material. Orthotropic branches have more food reserves than plagiotropic branches and the initial growth of coffee shoot grafting using orthotropic branches is better. According to research results (Yusuf *et al*., 2018), the branching of plants is preceded by an orthotropic to plagiotropic branching pattern so that more food reserves are stored in orthotropic branches. Biosynthesis of IAA by bacteria also depends on the availability of tryptophan. Neither the genes nor the enzymes involved in the tryptophan-independent pathway have been identified. However, a number of genes, enzymes and co-factors have been studied in the tryptophan-dependent pathway in (Wijaya *et al*., 2015).

3.3. Effects of utilization of biological agents of Bacillus and Pseudomonas on the growth of robusta coffee seeds from cut-grafting

The results of the analysis of variance on Table 1 state that there was a real influence on the application of biological agents Bacillus and Pseudomonas on the growth of robusta coffee seedlings propagated using cut-grafting techniques. The results of Duncan's multiple range test at 5% level were the main influence of Bacillus and Pseudomonas. In Table 3, it can be seen that the treatment of B_4 (concentration 80 ml/L Bacillus and 80 ml/L Pseudomonas) gave the best effect on the growth variable. In general, the higher the concentration of bacteria Bacillus and Pseudomonas, the population of bacteria that colonize the leaves is also higher so that it increases plant growth faster. In line with (Kalay *et al*., 2020), application of biological fertilizers Bacillus with high concentrations is associated with dense population Bacillus. The denser the population of Bacillus, the production of growth regulators that are put into the plant is also higher. However, it should be noted that the weakness of applying biological fertilizer with too high concentration on the surface of the leaves, apart from being more wasteful, it can also make it difficult for plants to optimally absorb the biological agents contained there, and the more serious consequence is that it can cause poisoning in plants. Concentrations that are too concentrated when foliarly applied can inhibit the photosynthesis process. If the photosynthesis process is hampered, plant growth will be disrupted so that the plant will easily wither and die. Based on Marschner in 1986 in (Wijaya *et al*., 2015), plants have certain limits for absorbing the nutrients they receive. The level of environmental density can influence the permeability of leaf cells and determine the presence of bacterial colonies that can absorb during the fertilization process. There are limitations when using biological agents (Bacillus and Pseudomonas) in high concentrations, namely the limited availability of technology packages for cheap mass breeding which is the main obstacle to use Bacillus and Pseudomonas in high concentrations to support plant growth in the field (Giyanto & Tondok, 2009). Therefore, further research is needed regarding optimal concentrations of Bacillus and Pseudomonas but with satisfactory effects too. The overexposure of plants to the auxin activity in the rhizosphere, when these are inoculated with IAA-producing bacteria, could have negative effects on plant growth and it could explain part of the inconsistency of the inoculation of PGPR in plant (Guerra *et al*., 2023).

Table 3. Recapitulation of further test results of Duncan's multiple range test at 5% level based on the influence of concentration of Bacillus and Pseudomonas

Treat-ment	Number of primary roots	Number of leaves	Shoot height (cm)	Cloro-phyll (SPAD)	Primary root length (cm)	Bud diameter (mm)	Fresh weight (gram)	Dry weight (gram)
B_0	3.17^{b}	8.33^{b}	16.33^{b}	29.67^{c}	17.92^{b}	4.92^{d}	20.67^{d}	5.96^{b}
B_1	4.83^{ab}	11.67^{a}	17.17^{b}	31.26^{b}	19.89^{ab}	5.22^{cd}	23.50^{bc}	7.06^{b}
B_2	4.83^{ab}	12.33^{a}	17.67^{b}	32.17^{b}	23.11^{a}	5.53^{bc}	25.50^{b}	7.60^{b}
B_3	4.50^{ab}	13.00^{a}	21.42^{a}	32.62^{ab}	23.65^{a}	5.80^{b}	22.67^{cd}	7.85^{b}
B_4	6.33^{a}	14.67^{a}	22.30^{a}	33.73^{a}	23.17^{a}	6.37^{a}	30.00^{a}	10.05^{a}

Bacillus sp. and *Pseudomonas* sp.is a endophytes microorganism that reside within plant tissue, and plant survival or death is strongly dependent on these microorganism. Various compounds, including indole-3-acetic acid, gibberellin, cytokinin, siderophore and exopolysaccharides are produced by microbes that aid plant growth (Kaur & Karnwal, 2023). *Bacillus* is one of PGPR that the action increase growth of plant by increase nutrient uptake by the crop mediated by solubilization of insoluble forms, nitrogen fixation, ammonia release from nitrogenous organic matter, also synthesis of plant growth phytohormones (IAA, CKs, GAs) or volatile organic compounds (VOCs) production which regulate plant physiology (Poveda & González-Andrés, 2021). According to research results (Puspita *et al*., 2018), it is stated that endophytic bacteria such as Bacillus and Pseudomonas can produce Indole Acetic Acid (IAA) which works to drive growth. During the carbohydrates synthesis, IAA activates sugar translocation. Some scientists observed that IAA caused increase in shoot length while other reported the decrease, accompanied by expansion in diameter of shoot. However, in both cases, it exerted the affirmative influence on plant growth (Akhtar *et al*., 2012). Treatment B_4 contained the largest bacterial colonies thus providing a higher IAA hormone. IAA produced by these bacteria is incorporated into plant tissue so that plants will be more sensitive to changing their IAA

(Leveau & Lindow, 2005). In line with (Azmi & Handriatni, 2019), the bacteria Bacillus and Pseudomonas produces auxin hormone which can stimulate cell division and enlargement of cells in plant tissues. IAA synthesized by those bacteria can increase the production of root hairs, lateral roots and adventitious roots, leading to enhanced nutrient uptake (Cao *et al*., 2020)

Bacillus and Pseudomonas have the same function to colonize plant root areas and produce plant growth hormones as previously mentioned. The function of these hormones can stimulate cell division, regulate cell enlargement and will stimulate root growth and stimulate the absorption of water and nutrients which have an effect on plant growth. Optimal absorption of nutrients from plants causes plant physiology to run smoothly. One example of a physiological process that occurs is the photosynthesis process which requires chlorophyll to produce carbohydrates which function as an energy source and raw material for the formation of amino acids and other compounds. Amino acids play a role in the formation of proteins, where proteins are the building blocks of cell nuclei and cell division, which means plant growth begins with cell division which requires energy in the form of ATP. Tinendung *et al.* (2014) stated that the growth of a plant is caused by cell division and elongation events. The ongoing division and elongation of plant cells will stimulate growth in the shoots of plant shoots and ultimately will encourage an increase in plant height.

Besides being able to produce IAA hormones, Bacillus and Pseudomonas also play a role in producing cytokinins, gibberellins, and ethylene as well as providing nutrition for plants by dissolving phosphate, fixing nitrogen and other minerals (Khalid *et al*., 2004). The active compound GA_3 comes from the terpedoid group. Biosynthesis has a mevalonic acid pathway in the cytosol or non-mevalonic acid in the plastid which is synthesized from isopentenyl pyrophosphate (IPP). Exogenous administration of GA_3 can increase the supply of IPP to the andrographolide formation pathway which increases the andrographolide content. Exogenous administration of hormones, both GA_3 and IAA, can increase the production and levels of andrographolide and tannin levels. Providing hormones both exogenously and endogenously has the same impact on plants. This indicates that the hormones produced by endophytic bacterial cells have the same impact on increasing the levels and active ingredients produced by plants as with the administration of exogenous hormones (Gusmaini *et al*., 2013).

Bacillus and Pseudomonas have the ability to dissolve phosphate in inorganic form as indicated by the gradual increase in the amount of P dissolved in the media. In addition, these two bacteria can grow on N-free media which proves the ability of Bacillus and Pseudomonas to fixation nitrogen (Abawari *et al*., 2021). These bacteria can fix N_2 from the atmosphere and can release nutrients from organic materials in the soil on decomposition (Din *et al*., 2021). Nitrogen – fixing microorganisms continuously convert atmospheric N_2 into phytoavailable forms such as ammonia and nitrate through the action of a complex enzyme called nitrogenase (Alves *et al*., 2022). In general, the greater the concentration of bacteria applied to the seedlings, the more phosphate and nitrogen solutions are absorbed by the seedlings to support plant growth. Phosphate in seeds plays a role in the formation of cells in several plant tissues such as roots, shoots, and stems (Istiqomah *et al*., 2018). The ability of Bacillus and Pseudomonas which can dissolve bind N is useful for plants for the formation of chlorophyll and chloroplasts in leaves. The high amount of leaf chlorophyll increases the growth of seedlings because chlorophyll has a positive correlation with the rate of photosynthesis. The results of photosynthesis in the form of organic compounds during the respiration process are converted into ATP so that plants can use them in the growth process (Pranatami & Arum, 2017).

4. Conclusions

There was no interaction between the use of orthotropic and plagiotropic rootstocks with the application of Bacillus and Pseudomonas on the initial growth of cut grafted seedlings of robusta coffee. This is due to the character of rootstock planting material originating from orthotropic and plagiotropic branches having the same response to the application of Bacillus and Pseudomonas. Plagiotropic branches can be used as rootstocks for grafting coffee plant cuttings. Application of Bacillus and Pseudomonas on treatment concentration of 80 ml/L Bacillus and 80 ml/L Pseudomonas (B_4) increased seedling growth on all observed parameters except for the number of shoots.

Acknowledgement

The authors gratefully acknowledge the support of the LP2M University of Jember as the research funding provider.

References

Abawari, R. A., Tuji, F. A., & Yadete, D. M. (2021). Multi Traits of Phosphate Solublizing Bacterial and Fungal Isolates and Evaluation of Their Potential as Biofertilizer Agent for Coffee Production. *International Journal of Applied Agricultural Sciences*, *7*(1), 1-15. https://doi.org/10.11648/j.ijaas.20210701.11

Ahmad, A. M., Attia, A. G., Mohamed, M. S., & Elsayed, H. E. (2019). Fermentation, formulation and evaluation of PGPR Bacillus subtilis isolate as a bioagent for reducing occurrence of peanut soil-borne diseases. *Journal of Integrative Agriculture*, 18(9), 2080-2092. https://doi.org/10.1016/S2095-3119(19)62578-5

Akhtar, N., Qureshi., M. A., Iqbal., A., Ahmad, M. J., & Khan, K. H. (2012). Influence of azotobacter and IAA on symbiotic performance of rhizobium and yield parameters of lentil. *Journal of Agricultural Research*, *50*(3), 361-372. https://www.academia.edu/download/30608649/547__361paper-7.pdf

Alves, A. R. A., Yin, Q., Oliveira, R. S., Silva, E. F., & Novo, L. A. B. (2022). Plant growth-promoting bacteria in phytoremediation of metal-polluted soils: Current knowledge and future directions. *Science of the Total Environment*, 838(156432), 1-10.

https://doi.org/10.1016/j.scitotenv.2022.156435
Anggraini, N., Evizal, R., & Septiana, L. M. (2021). Karakteristik Pertumbuhan Melada dan Lada Sambung. *Jurnal Agrotropika*, 20(2), 129–138. https://doi.org/10.23960/ja.v20i2.5322
Azmi, R., & Handriatni, A. (2019). Pengaruh Macam Zat Pengatur Tumbuh Alami terhadap Pertumbuhan Setek Beberapa Klon Kopi Robusta (*Coffea canephora*). *Biofarm : Jurnal Ilmiah Pertanian*, 14(2), 71–81. https://doi.org/10.31941/biofarm.v14i2.794
BPS. (2020). *Statistik Kopi Indonesia 2020*. Badan Pusat Statistik.
Cao, Y., Ni, H., Li, T., Lay, K., Liu, D., He, X., ..., & Qiu, L. (2020). Pseudomonas sp. TK35-L enhances tobacco root development and growth by inducing HRGPnt3 expression in plant lateral root formation. *Journal of Integrative Agriculture*, 19(10), 2549-2560. https://doi.org/10.1016/S2095-3119(20)63266-X
Din, I., Khan, H., Khan, N. A., & Khil A. (2021). Inoculation of nitrogen fixing bacteria in conjugation with integrated nitrogen sources induced changes in phenology, growth, nitrogen assimilation and productivity of wheat crop. *Journal of the Saudi Society of Agricultural*, *20*(7), 459 - 466. https://doi.org/10.1016/j.jssas.2021.05.008
Fan, D., Subramanian, S., & Smith, D. L. (2020). Plant endophytes promote growth and alleviate salt stress in Arabidopsis thaliana. *Scientific Reports*, 10(1), 12740. https://doi.org/10.1038/s41598-020-69713-5.
Ferreira, T., Shuler, J., Guimaraes, R. & Farah, A. (2019). *Coffee : Production, Quality, and Chemistry*. Brazil : Royal Society Chemistry. https://doi.org/10.1039/9781782622437
Filete, C. A., T. R. Moreira., A. R. Santos et al. (2022). The new standpoints for the terroir of coffea canephora from Southwestern Brazil : edaphic and sensorial perspective. *Agronomy*, *12*(8), https://doi.org/10.3390/agronomy12081931
Firdaus, N. K., Oktafiyanto, M. F., Sasmita, K. D., & Pranowo, D. (2023). Efficacy of phytohormones producing isolates on the growth of two stem-cutting robusta coffee (*Coffea canephora*). *IOP Conference Series: Earth and Environmental Science*, *2*(1). 1-8. https://doi.org/10.1088/1755-1315/1172/1/012002
Giyanto, & Tondok, E. T. (2009). Study of liquid organic wastes as mass production media for antagonistic agents of Pseudomonas fluorencens and potency test as bio pesticide. *Jurnal Ilmu Pertanian Indonesia*, *14*(2), 97 - 107. https://journal.ipb.ac.id/index.php/JIPI/article/view/6513
Guerra, M. P., Valero, N. & Ramirez, C.A. (2023). Total auxin level in the soil–plant system as a modulating factor for the effectiveness of PGPR inocula. *Chemical and Biological Technologies in Agriculture*, 10(6). https://chembioagro.springeropen.com/articles/10.1186/s40538-022-00370-8
Gusmaini., Aziz., S. A., Munif, A., Sopandie, D., & Bermawie, N. (2013). Potensi Bakteri Endofit Dalam Upaya Meningkatkan Pertumbuhan, Produksi, dan Kandungan Andrografolid pada Tanaman Sambiloto. *Jurnal Penelitian Tanaman Industri*, *19*(4), 167-177. https://garuda.kemdikbud.go.id/documents/detail/454136
Heryanto, W. (2020). *Pengaruh Pemberian Pupuk cair nasa dan NPK organik Terhadap pertumbuhan dan produksi tanaman Tanaman Sawi Pakcoy* [Skripsi]. Pekanbaru : Fakultas Pertanian Universitas Riau.
Husna, M., Sugiyanta, & Pratiwi, E. (2019). The ability of Bacillus consortium to fix N2, solubilize phosphate and synthesize indole-3- acetic acid fitohormone. *Journal of Soil and Climate*, 43(2), 117–125. https://repository.pertanian.go.id/server/api/core/bitstreams/30e4f9e9-68cc-491c-94bf-f57829ccaf24/content
Irlando, M., Fitriani, D., & Podesta, F. (2020). Pengaruh Pemberian Auksin Alami Terhadap Pertumbuhan Stek Sambung Kopi Robusta (*Coffea canephora* L.). *Jurnal Agriculture*, 15(1), 16–31. https://doi.org/10.36085/agrotek.v14i2.1034
Istiqomah, Aini, L. Q., & Abadi, A. L. (2018). Kemampuan Bacillus subtilis dan Pseudomonas fluorescens Dalam Melarutkan Fosfat dan Memproduksi Hormon IAA (Indole Acetic Acid)

Untuk Meningkatkan Pertumbuhan Tanaman Tomat. *Jurnal Buana Sains*, 17(1), 75–84. https://doi.org/10.33366/bs.v17i1.580

Kafrawi, Arif, R., Kahrir, A. M. S. A., Nildayanti, Kumalawati, Z., & Suriansyah. (2020). Penyiraman Media Tanam Sambung Pucuk Kopi (*Coffea* sp) pada Berbagai Konsentrasi PGPR. *Jurnal Agroplantae*, 9(2), 105–114. https://doi.org/10.51978/agro.v9i2.227

Kalay, A. M., Kesaulya, H., Talahaturuson, A., Rehatta, H., & Hindersah, R. (2020). Aplikasi Pupuk Hayati Konsorsium Strain Bacillus sp dengan Berbeda Konsentrasi dan Cara Pemberian Terhadap Pertumbuhan Bibit Pala (*Myristica fragrans* Houtt). *Agrologia*, *9*(1), 30–38. https://doi.org/10.30598/a.v9i1.1060

Kalimuthu, R., Suresh, P., Varatharaju, G., Balasubramanian, N., Rajasekaran, K. M., & Shanmugaiah, V. (2019). Isolation and characterization of indole acetic acid (IAA) producing tomato rhizobacterium Pseudomonas sp vsmku4050 and its potential for plant growth promotion. *International Journal of Current Microbiology and Applied Sciences*, *8*(06), 443–455. https://doi.org/10.20546/ijcmas.2019.806.050

Kaur, M., & Karnwal, A. (2023). Screening of endophytic Bacteria from stress-tolerating plants for abiotic stress tolerance and plant growth-promoting properties: Identification of potential strains for bioremediation and crop enhancement. *Journal of Agriculture and Food Research*, 14, 1-10. https://doi.org/10.1016/j.jafr.2023.100723

Khalid, A., Arshad, M., & Zahir, Z. A. (2004). Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *Journal of Applied Microbiology*, 96(3), 473–480. https://doi.org/10.1046/j.1365-2672.2003.02161.x

Lee, L.W., Cheong, M.W., Curran, P., Yu, B. & Liu, S.Q. (2015) Coffee fermentation and flavor—An intricate and delicate relationship. *Food Chem*. *185*, 182–191. https://doi.org/10.1016/j.foodchem.2015.03.124

Leveau, J. H. J., & Lindow, S. E. (2005). Utilization of the plant hormone indole-3-acetic acid for growth by Pseudomonas putida strain 1290. *Applied and Environmental Microbiology*, 71(5), 2365–2371. https://doi.org/10.1128/AEM.71.5.2365-2371.2005

Muningsih, R., Putri, L. F. A., & Subantoro, R. (2019). Pertumbuhan Stek Bibit Kopi Dengan Perbedaan Jumlah Ruas Pada Media Tanah-Kompos. *Mediagro*, 14(2), 64–71. https://doi.org/10.31942/md.v14i2.2749

Patil, N. B., Gajbhiye, M., Ahiwale, S. S., Gunjal, A. B., & Kapadnis, B. P. (2011). Optimization of Indole 3-acetic acid (IAA) Production by Acetobacter diazotrophicus L1 Isolated from Sugarcane. *International Journal of Environmental Sciences*, *2*(1), 295–302. https://www.researchgate.net/publication/279804624

Pranatami, D. A., & Arum, S. (2017). Pengaruh Pemberian Dosis dan Frekuensi BIofertilizer terhadap Kadar Klorofil (Paraserianthes falcataria (L .) Nielsen). *Indonesian Journal of Applied Science*, *7*(3), 44–50. https://doi.org/10.24198/ijas.v7i3.15422

Puspita, F., Saputra, S. I., & Merini, J. (2018). Uji Beberapa Konsentrasi Bakteri *Bacillus* sp. Endofit untuk Meningkatkan Pertumbuhan Bibit Kakao (*Theobroma cacao* L.). *Jurnal Agronomi Indonesia*, 46(3), 322–327. https://doi.org/10.24831/jai.v46i3.16342

Poveda, J., & González-Andrés, F. (2021). Bacillus as a source of phytohormones for use in agriculture. *Applied Microbiology and Biotechnology*, *105*(23), 8629–8645. https://doi.org/10.1007/s00253-021-11492-8

Rizwan, M. (2021). *Budidaya Kopi*. Sumatera Barat: Azka Pustaka.

Rosyady, M. G., Subakti, B., Setiyono, & Kusbianto, D. E. (2022). Effects of Bacillus sp. on the growth of immature plants in year 1 robusta coffee clones. *Journal of Soilscape and Agriculture*, 1(1), 7–14. https://doi.org/10.19184/jsa.v1i1.121

Tinendung, R., Puspita, F., & Yoseva, S. (2014). Uji Formulasi Bacillus sp. Sebagai Pemacu Pertumbuhan Tanaman Padi Sawah (Oryza sativa L.). *JOM Faperta*, 1(2), 1-15. https://jom.unri.ac.id/index.php/JOMFAPERTA/article/view/3024

Tustiyani, I. (2017). Pengaruh Pemberian Berbagai Zat Pengatur Tumbuh Alami terhadap

Pertumbuhan Stek Kopi. *Jurnal Pertanian*, *8*(1), 46–50. https://doi.org/10.30997/jp.v8i1.565

Urgiles-Gómez, N., Avila-Salem, M. E., Loján, P., Encalada, M., Hurtado, L., Araujo, S., ..., & Cornejo, P. (2021). Plant growth-promoting microorganisms in coffee production : from isolation to field application. *Agronomy*, *11*(8), 1–12. https://doi.org/10.3390/agronomy11081531

Wagi, S., & Ahmed, A. (2019). Bacillus spp. : potent microfactories of bacterial IAA. *PeerJ*, *2019*(7), 1-14. https://doi.org/10.7717/peerj.7258

Wijaya, A. S., Sangadji, M. N., & Muhardi. (2015). Produksi dan Kualitas Produksi Buah Tomat yang Diberi Berbagai Konsentrasi Pupuk Organik Cair. *e-J. Agrotekbis*, *3*(6), 689-696. http://jurnal.faperta.untad.ac.id/index.php/agrotekbis/article/view/2

Yusuf, H., Sahputra, R., & Sah, R. I. (2018). Pengaruh media tanam dan pemberian pupuk organik cair terhadap pertumbuhan bibit kakao (*Theobroma cacao*, L). *Jurnal PenelitiaN Bionatural*, *5*(1), 1–11. http://jurnal.unsam.ac.id/index.php/jagrs/article/view/850

Zhai, L., Wang, X., Tang, D., Qi, Q., Yer, H., Jiang, X., …, & Li, Y. (2021). Molecular and physiological characterization of the effects of auxin-enriched rootstock on grafting. *Horticulture Research*, *8*(74), https://doi.org/10.1038/s41438-021-00509-y

Zhu, S., Huang, T., Yu, X., Hong, Q., Xiang, J., Zeng, A., Gong, G., & Zhao, X. (2020). The effects of rootstocks on performances of three late-ripening navel orange varieties. *Journal of Integrative Agriculture*, 19(7), 1802–1812. https://doi.org/10.1016/S2095-3119(20)63212-9