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MORPHOLOGICAL CHARACTERISTIC OF VIERUL LOCAL GRAPE (Vitis spp.) CUTTING SEEDS ON PODZOLIC SOIL USING VARIOUS CONCENTRATION OF AUXIN SOAK

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ABSTRACT

The development of grapes (Vitis vinifera) has obstacle among others are limited optimal land and difficult nurseries. Suboptimal land use for grape cuttings can use red yellow podzolic soil (ultisol). Podzolic soil has an acidic pH and low macro and micro nutrient uptake but can still be used as a growing medium. Vierul local grapes are a type of grape that has begun to be developed as root stock in nurseries but needs to be improved for its root success using a synthetic phytohormones bath of napthaline acetic acid (NAA). The purpose of this study was to determine the morphological character of vierul local grape cuttings at various concentrations of NAA soaking in podzolic soil growing medium. This research has been carried out in a screen house from August to December 2021. This study used a non-factorial completely randomized design (CRD) with 3 treatments and 3 replications as block consisting of F_0, F_1, F_2, F_4 following by 0, 2, 4, 6 g L^{-1} respectively. The morphological characteristics observed is number of bud break, time of leaves appear, number of leaves, tendril length and percentage of live cuttings. The results of the study were analyzed using ANOVA at the 5% level and continued using the DMRT at the 5% level. The result showed that soaking of NAA at a concentration of 0 g L^{-1} (F_0) had the best result for the variable number of bud break and number of leaves. Concentration of 6 g L^{-1} (F_3) had the best result for variable length of the tendril while for the variable when the leaves appear, NAA soaking has no significant effect. The percentage of live cuttings is still low because it is influenced by the quality (material) of cuttings, the age of woodstock, growing media and water availability.

Keywords: cutting, grape, NAA, nurseries, phytohormones

INTRODUCTION

The limited amount of optimal land in Indonesia is decreasing, so there is a need to utilize suboptimal land in agricultural cultivation (Sari et al., 2020). One of the suboptimal lands comes from the podzolic soil type (ultisol) with soil physical properties of fine clay texture and clods that affect the absorption of water and nutrients (Abdillah & Aldi, 2021; Dariah & Heryani, 2014). However, podzolik soil can still be used for agricultural cultivation, one of which is used as a planting medium in grape cuttings nurseries (Hartati, 2006).

The development of grapes in Indonesia has several constraints, including optimal soil conditions that are mostly used for food crops and cereals, so the utilization of suboptimal land is quite important (Mansyah & Sutanto, 2020; Choudhury et al., 2017; Servina, 2019). The next obstacle is that imported grape seeds are quite difficult to produce optimal production according to their potential, so it is necessary to develop local grape varieties in Indonesia (Aji & Sugiyatno, 2020) using root stock that is used as a grape rootstock resulting from grafting or grafting from the type of vierul (Amalia et al., 2019), but this type is quite difficult to root in the planting medium so that an auxin soaking treatment is needed.

Auxin is a type of phytohormone in plants that plays a role in the process of plant growth and development, especially for seedling cuttings (Shahzad et al., 2019). However, natural auxin (endogenous) is sometimes not enough to initiate root growth so additional auxin from outside (exogenous) is needed (Astrit & Glenda, 2017). One type of exogenous auxin is naphthalene acetic acid (NAA) which can help in cell division, cell elongation, cell differentiation, and root initiation (Hossain & Urbi, 2016). NAA application can increase the percentage of root formation, encourage shoot growth, plant height, form tendrils, adventitious shoots, number of leaves, and increase the number of leaves (Zahra, 2013; Bhat et al., 2011) and can increase the percentage of root emergence that can be observed by the percentage of live cuttings, the number of leaves, the length of the vine and the time of bud break (Okao et al., 2016). The purpose of this study was to determine the morphological characters and the percentage of living grape cuttings seedlings of local vierul varieties at various concentrations of auxin soaks in podzolic (ultisol) soil media.

RESEARCH METHOD

The research was conducted in an independent screenhouse in Sardonoharjo Village, Ngaglik District, Sleman Regency, Yogyakarta from August to December 2021. The tools used in this research are screenhouse (bamboo and wood frame with 70% shade paranet top and side cover), scissors, cuttings, knives, hoes, bamboo stakes, plastic ropes, shovels, 2.5 × 2.5 mm mesh size soil sieve, paddy, label paper, digital camera, water hose, plastic bucket, meter, ruler, 1000 ml measuring cup, analytical scales, bamboo stirrer and stationery (logbook, pencil, pen, eraser, marker). The materials used in this study were grape cuttings of local vierul

variety (4 years old mother tree) obtained from Rafalfa Green independent vineyard (Tinggarjaya District, Banyumas Regency), yellow red podzolic soil (PMK), 12.5 × 25 cm polybags, manure (goat manure), charcoal burnt husk, sand, well water, synthetic auxin type NAA (powder), fertilizer mix A and B for fruit plants and fungicide (dhitane M-45).

The study was conducted using a non-factorial completely randomized design with 1 factor (NAA concentration) with the first soaking for 2 hours, drained for 1 hour, then soaked again for 4 hours. This study used 4 treatments and repeated 3 times as a block. The treatments consisted of different concentrations of NAA namely F_0, F_1, F_2, F_3 with consecutive concentrations of 0, 2, 4, 6 g of NAA L^{-1}. Maintenance consisted of watering (once a day in the afternoon for the first month, once a day in the afternoon for the last two months) and fertilizing using fertilizer mix A and B for fruit plants (cuttings 1 month after planting). Observations were made once a week for 12 weeks with the observation variables being the number of shoot ruptures (12 weeks after planting/ mst), leaf emergence (week 3), number of leaves (strands) calculated from week 3, vine length (cm) calculated from week 6 and percentage of live cuttings (%) calculated at week 12. The research began with the preparation of the screenhouse, preparation of tools and materials, ordering and selection of grape cuttings, pre-treatment of grape cuttings, making planting media using podzolik/sand/manure fertilizer/burnt husk with a ratio of 1:1:1:1, filling polybags with 1.5 kg of planting media and treating grape cuttings using soaking. At the end of the study, the data obtained were tabulated and analyzed using ANOVA at the 5% level and continued using DMRT analysis at the 5% level. Data were analyzed using statistical product and service solutions (SPSS) 16.0.

RESULT AND DISCUSSION

Based on the analysis, the results showed that NAA soaking at various concentrations had a real effect on the variable number of buds broken, the number of leaves and the length of the vine, while the variable when the leaves appear did not have a real effect (Table 1). The F_0 treatment has the highest value compared to other treatments for the variable number of buds broken, F_1 treatment takes the longest time compared to other treatments for the variable when the leaves

Table 1 The test results of the 12th week of NAA soaking on the morphological characters of grape cuttings seedlings of local vierul varieties

Treatment	Number of buds broken (week after treatment)	Time of leaves appear (week of)	Number of leaves (sheet)	Tendril lenght (cm)
F_0 (0 g L^{-1})	15.67bc	2.00a	12.50bc	11.25b
F_1 (2 g L^{-1})	8.33a	3.00a	9.50a	7.00a
F_2 (4 g L^{-1})	8.67a	2.33a	11.50ab	17.50c
F_3 (6 g L^{-1})	13.00b	2.00a	10.50a	24.45d

Description: numbers followed by the same letter in the treatment show results that are not significantly different according to the 5% DMRT test.

appear, F_0 treatment has the highest value compared to other treatments for the variable number of leaves and F_3 has the highest value compared to other treatments for the variable vine length.

Number of buds broken

The results of the study in Table 1 show that NAA soaking at various concentrations has a significant effect on the number of bud breaks on grape cuttings seedlings with the best number of bud breaks in the F_0 treatment. The results showed that after the eighth week there was no bud break. Endogenous auxin is synthesized in the meristematic tissue of the plant, but in the cuttings seedlings defoliation occursso that endogenous auxin will be transported basipetally to the reduced lateral buds which will initiate the formation of endogenous cytokinin (Kurepa & Smalle, 2022; Schaller et al., 2015). Auxin soaking at the bottom of the stem cuttings has no effect on shoot formation because auxin transport from NAA (exogenous auxin) is used for the initiation of root formation (Rolaniya et al., 2018; Yoon et al., 2021).

Reduced auxin due to defoliation in cuttings can result in the expression of the *isopentenyl-transferase* (IPT) gene (Dierck et al., 2016; Liu et al., 2011). IPT is an enzyme that catalyzes the formation of cytokinin, which causes the lateral buds to break and grow (Frebort et al., 2011; Kieber & Schaller, 2018). When exogenous auxin in the cuttings is used for root initiation, the amount of cytokinin in the lateral buds may increase resulting in differentiation of the transport in the lateral buds (Küplemez & Yildirim, 2020; Ngomuo et al., 2013). Auxin synthesized in the root area of the cuttings is then transported to the lateral buds, so that the higher the auxin that is transported results in the inhibition of cytokinin for the growth of lateral buds (Cline, 1996; Kebrom, 2017). The application of NAA has no real effect on the appear of lateral shoots because cytokinin activity can actually be inhibited (Qiu et al., 2019; Tamba et al., 2020), that the higher concentration of auxin given it can inhibit the growth of lateral buds (Chen et al., 2021; Yulia et al., 2020).

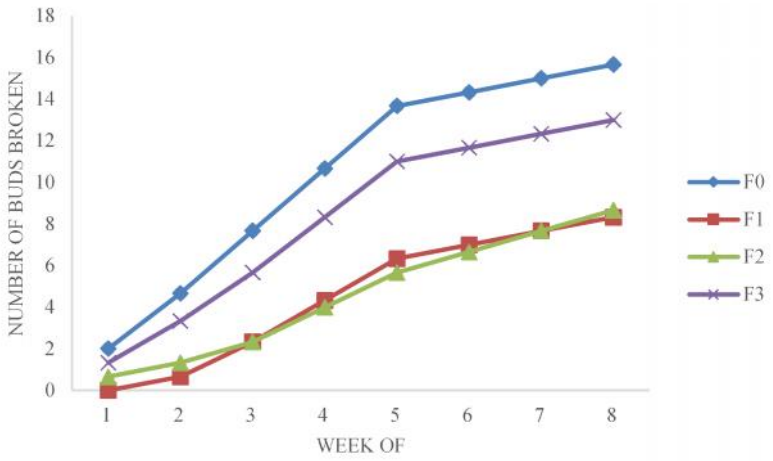

Figure 1 Effect of NAA soaking on the number of bud breaks (12 week after treatment)

Time of leaves appear

The results in Table 1 show that NAA soaking at various concentrations (F_0, F_1, F_2 and F_3) has no significant effect on the time of leaf appear in grape cuttings. The appearance of leaves is influenced by the interaction between auxin and cytokinin contained in the cuttings (Nakhooda et al., 2012; Cabahug et al., 2016). In this study, it is suspected that the root system was only formed in two week so that endogenous auxin had not yet been produced because the uptake of NAA was used by the cuttings for the initiation of root formation. the results showed that grape cuttings seedlings rooting system on planting media can grow after 2 to 4 weeks (Scheiner & King, 2019). Auxin with cytokinin plays a role in cell division in lateral buds for leaf expansion (Benedetto et al., 2015), it is thought that auxin transport from the root system only occurs in the second week.

In addition to being influenced by phytohormones, leaf development is also influenced by the availability of nutrients, water and environmental conditions, this is in accordance with the opinion that Singh & Chauhan (2020) which states that root growth and development in grape seedlings are influenced by environmental conditions, endogenous and exogenous biochemical (phytohormone) content, ontogenic age of cuttings when planted and treatment of cuttings before planting. Nutrients that affect leaf formation are nitrogen, but it is suspected that the application of nitrogen fertilizer in this study was not optimal, thus inhibiting leaf appear. Nitrogen fertilizer given to plants can increase growth, including accelerating the emergence of leaves and increasing the number of leaves (Leghari et al., 2016; Salim, 2022). Appropriate use of nitrogen fertilizer can affect the time of leaf appear and the number of leaves on seedlings derived from cuttings (Evizal et al., 2022; Jiang et al., 2016). Nitrogen uptake in grapevines is also influenced by the availability of water as a solvent for nutrients absorbed by plant roots (Lang et al., 2019). In this study, it is suspected that the availability of water is not optimal due to the media used having a high porosity so that the ability of the media to bind water is low. In accordance with the research result

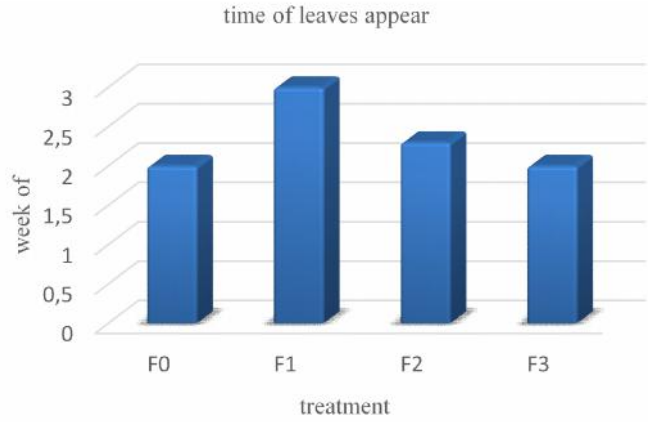

Figure 2 Effect of NAA soaking on leaves appear

Santosa et al., (2020) and Chrysargyris et al., (2020) stated that high porosity nursery media results in lower nitrogen availability compared to phosphate and potassium availability. Lack of water in the vegetative phase of grape seedlings results in slow cell division which can affect leaf formation (Ojeda et al., 2001). Adequate amounts of nitrogen fertilizer but not enough water (as a solvent) will hamper nutrient uptake and transport (Dugo et al., 2010; Plett et al., 2020).

Number of leaves

The results in Table 1 show that NAA soaking at various concentrations has a significant effect on the number of leaves on grape cuttings seedlings with the best number of leaves in the F_0 treatment. In this study, it was found that the treatment without exogenous auxin had more leaves. The number of leaves on cuttings seedlings is influenced by the content and interaction of hormones (auxin and cytokinin), nutrient uptake (nitrogen and potassium) and water availability (Akhtar et al., 2022; Hussain et al., 2021; Kamilo, 2014). Excessive amounts of auxin can suppress the function of cytokinin on leaf formation and expansion (Wu et al., 2021). When the root system has been formed, auxin transport increases so that the expression of IPT genes as cytokinin catalysts decreases (Kurepa & Smalle, 2022). If auxin increases, cell division becomes dominant resulting in more meristematic areas so that it can actually increase the length of the vine on grape cuttings (Jamal Uddin et al., 2020). In this study, it was found that the increase in vine length due to increased auxin was not followed by the increase and expansion of leaves on grape cuttings.

The application of fruit fertilizer mix A and B to grape cuttings is not optimal so that the availability and uptake of important nutrients such as nitrogen and potassium for vegetative development are not optimal. Nitrogen are closely related to seedling growth in cell division and dominant in leaf formation (Hou et al., 2021; Tobing et al., 2018). The application of inorganic nutrient fertilizer is soluble and volatile so that if there is a lack of water, nitrogen is not dissolved and not absorbed by plants while when the temperature is high nitrogen will evaporate so that it is not available to plants (Araus et al., 2020; Shahadha et al., 2021). Lack of nitrogen in the media causes nitrogen compounds in the leaves to undergo autolysis for the transportation of protein compounds to younger, more meristematic tissue, this causes growth and increase in the number of leaves to be inhibited (Verdenal et al., 2021). In addition, it is suspected that soil organisms are not well developed in the planting media so that the process of aminization does not run optimally. Aminization is a biological process carried out by soil organisms such as earthworms. Complex of nitrogen compounds in the planting media will be used by these organisms and released again through excretion in the form of simpler nitrogen compounds, namely ammonium (Isobe & Ohte, 2014). This ammonium will be used by plant seedlings to increase nitrogen

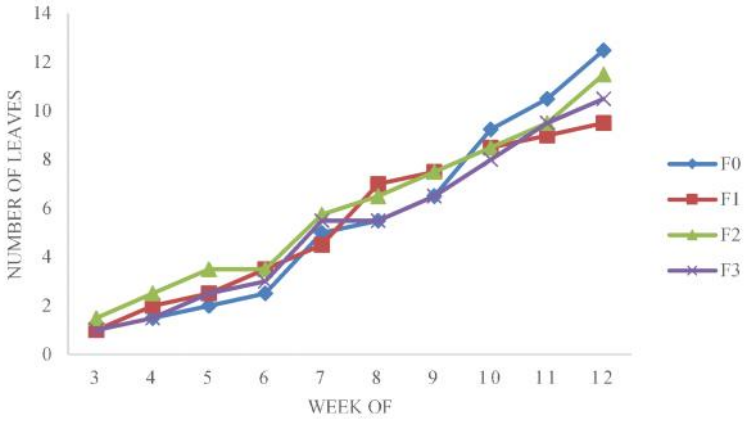

Figure 3 Effect of NAA soaking on leaf number (week of)

uptake in the planting media. This condition is not expected to occur in the planting media for grape cuttings in this study.

Potassium nutrients in the growing medium (inorganic) available to plants and are absorbed in the form of K^+ ions. Ions K^+ affects the photosynthesis process because it regulates the osmotic potential and cell enlargement in the leaf vacuole (Sardans & Peñuelas, 2021). K^+ affects the entry of hydrogen ions (H^+) into the thylakoid membrane so that the pH becomes optimal for the synthesis of *adenosine triphospate* (ATP) as the main energy source in photosynthesis (Szabò & Spetea, 2017). Increased accumulation of K^+ in the vacuole causes the osmotic potential of the vacuole to increase, causing the cells to enlarge, resulting in wider leaves (Kaiser & Scheuring, 2020). Leaf expansion is generally followed by an increase in the number of stomata and chlorophyll as a result photosynthesis will increase so that the results of assimilation can be used optimally for growth and leaf addition (Bielczynski et al., 2017). Medium wich is used in this study is classified as having high porosity so that the soil's ability to store water is low. Lack of water in the vegetative phase of grape cuttings will cause nutrient uptake (nitrogen and potassium) to decrease because water is used as a solvent for these nutrients, as a result cell division and differentiation can be disrupted, affecting leaf formation (Ojeda et al., 2001; Peng et al., 2022). Adequate amounts of nitrogen and potassium nutrients but not enough water can result in a stunted nutrient uptake rate (Dugo et al., 2010; Plett et al., 2020).

Tendril lenght

The results of the study in Table 1 show that NAA soaking at various concentrations has a significant effect on the length of the tendril on grape cuttings with the best tendril length in NAA treatment with a concentration of 6 g L^{-1} (F_3). Growth and elongation of tendrils are influenced by auxin activity and the environment especially light. Auxin can stimulate root growth the more roots the auxin content increases and is distributed to the meristematic tissueof the plant (Vernoux et al., 2010). High auxin can inhibit cytokinin

synthesis so that apical dominance occurs which can result in meristematic tissues such as tendrils can continue to elongate (Balla et al., 2016). Auxin is a type of plant hormone that has the ability to support cell elongation resulting in an increase in tendril length. Physiologically, hormones in plants can support each other the presence of auxin at certain concentrations can encourage changes in the ratio of cytokinin formation in plants (Kurepa & Smalle, 2022). Auxin is involved in cell division while cytokinin together with auxin is involved in cell elongation. The provision of NAA is thought to stimulate cell elongation in the vine because of the stimulation of protein synthesis to activate enzymes that play a role in the synthesis of endogenous hormones (Karunadasa et al., 2020; Skalický et al., 2018). In these conditions there is an increase in plasticity in the cell wall so that it becomes loose, allowing water to enter the cell by osmosis which results in cells experiencing dilation and elongation (Majda & Robert, 2018).

In addition to the effect of NAA, tendril growth is thought to be influenced by light. The screenhouse in this study used has a mesh size of 70%, meaning that the incoming light intensity is 30%, the elongation of tendrils and branches occurs faster in low light intensity conditions, but leaf formation can be inhibited. Auxin at 30% light intensity (low) can work optimally together with cytokinin in cell division and elongation (Niczyporuk et al., 2018; Prerostova et al., 2021; Wimudi & Fuadiyah, 2021). Sufficient light intensity can affect the expression of *proteinase inhibitor* (PIN) genes that play a role in auxin transport in plants (Faizan et al., 2020; Karatas et al., 2010). Auxin plays a role in activating the expression of the *cell division cycle 2* (Cdc2) gene, while endogenous cytokinins activate *cyclin dependent kinase genes* (CDKs) encoded by the Cdc2 gene (Zhang Li, 2021; Wakidah & Rahayu, 2020; Zhang et al., 2005). The activity and expression of the Cdc2 gene is regulated by auxin, CDKs are encoded by Cdc2 so auxin indirectly plays a role in tendril elongation (Tréhin et al., 1998; Umeda, 2000).

Percentage of live

The results of the study in Figure 5 show that NAA soaking at various concentrations does not significantly affect the percentage of live grape cuttings. The results showed

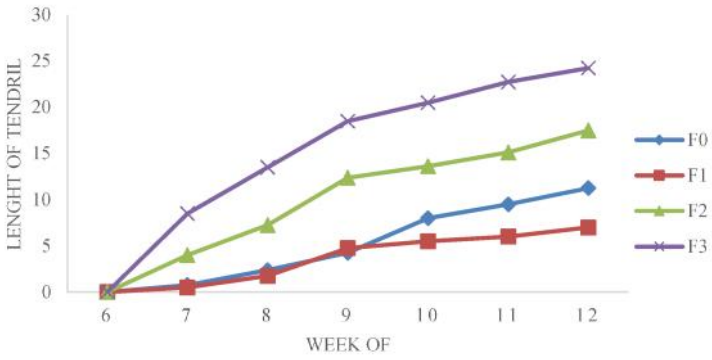

Figure 4 Effect NAA soaking on the length of tendril (week of)

that the percentage of live cuttings was still very low. The low percentage of live cuttings is caused by several things, namely the quality (material) of the cuttings taken, the age of the parent tree and the environment (Bannoud & Bellini, 2021; Bauri et al., 2017; Jaleta, 2019; Shah et al., 2021; Subiakto, 2014). The cuttings used were presumably taken without taking into account the juvenility of the parent tree. The juvenile phase is the time period before the plant produces (flowering), so that in this phase the plant easily forms vegetative organs (nodes, buds, leaves). In this study, cuttings were obtained from stems that had passed the juvenile period so that they had a relatively low ability to root and grow, while cuttings from the juvenile stage were relatively easier to root and grow (Caspa et al., 2010). The closer to the base of the tree the juvenility is better, the higher the tree juvenility is low (Neri et al., 2020). The percentage of cuttings that can grow and develop is also influenced by the age of the mother plant. The older a plant is used as planting material, the more difficult root formation is compared to young plants (Basri et al., 2012; Monder, 2019). In this study, the mother plants used were old plants. In adult phase plants (past juvenile) root formation is influenced by a complex mechanism including cell thickening and sclerenchym tissue and hardening of the cuttings branch tissue which can inhibit root formation (H. Liu et al., 2018; Schneider et al., 2021; Solikin, 2018). However, cuttings from very young parents can also reduce the percentage of live cuttings because they contain of nitrogen still high but low carbohydrates, causing the cuttings to rot when planted(Sylviana et al., 2019; Zerche et al., 2016).

In addition the low percentage of live cuttings is thought to be due to cuttings attacked by fungal pathogens due to the initial treatment of cuttings and planting media that are not sterile. After mixing the planting media, the planting media should be dried first and fungicides should be added (Vuyyuru et al., 2018). In the treatment of cuttings soaking using fungicides is too short so that fungi can still attack the cuttings. Planting media that has high porosity is thought to be a factor in the lack of water availability in the media because the ability to bind water in the media is low. The planting media used consists of a mixture of sand which has a coarse texture, has a large pore space that causes the soil to have a loose texture, as a result it is not optimal in holding water (Franzluebbers, 2020; Li, 2021). This condition causes water infiltration to move downward through the cavity so that the planting medium lacks water. The percentage of live cuttings is thought to have a relationship between the auxin mechanism and the availability of water in the growing medium (Liu & von Wirén, 2022). Auxin plays a role in cell division in the meristematic region by spurring certain proteins in the cell plasma membrane to pump H^+ ions to activate certain enzymes so that it will break several hydrogen cross-links in the cellulose molecular chain that makes up the cell wall, so that water can enter by osmosis for root

initiation (Takahashi et al., 2012; Ulva et al., 2019). However, this condition can be inhibited if there is a lack of water in the planting medium. In addition, NAA is active but quite easy to degrade enzymatically due to the presence of peroxidase enzymes produced by planting material (Pandey et al., 2017; Sharfina et al., 2021) so that the uptake of NAA soak is not optimal in root initiation as a result cuttings can be death.

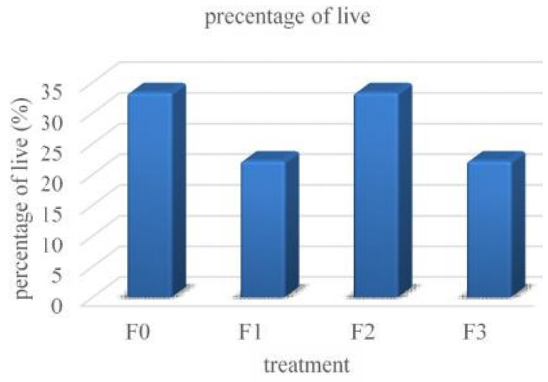

Figure 5 Percentage of live (week of 12th)

CONCLUSION

Based on the results of the study, it can be concluded that NAA soaking at a concentration of 0 g L^{-1} (F_0) has the best results for the variable number of bud break and number of leaves. NAA soaking at a concentration of 6 g L^{-1} (F_3) has the best results for the tendril length variable, while for the NAA soaking variable has no significant effect on the time of leaf appear. The percentage of live cuttings with NAA soaking at various concentrations is still low because it is influenced by several factors including the quality of the cuttings, the age of the parent tree, the planting medium and the availability of water.

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