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Short Communication

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Survival of transport-stressed black pepper stem cuttings after glucose, sucrose, fructose, and IBA treatments

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ABSTRACT

This study aimed to determine the survival, sprouting, and rooting performance of black pepper stem cuttings to glucose, sucrose, and fructose at different concentrations. The two-node stem cuttings were prepared from six to seven-node cuttings collected from mother vines of black pepper in the field from far and remote areas in Sarawak, Malaysia. The two-node cuttings were soaked completely for one hour with the following treatments: 1% glucose, 2% glucose, 3% glucose, 1% sucrose, 2% sucrose, 3% sucrose, 1% fructose, 2% fructose, 3% fructose, and 1000 ppm IBA served as a positive control, while filtered water was a negative control. All treated cuttings were sown in the polybags containing soil mix and monitored daily for up to 45 days. The study found that black pepper cuttings treated with a 3% glucose solution exhibited significantly better survival, sprouting, and rooting, total number of roots, and total length of roots at day 45, but they were about the same when soaked in IBA at 1000 ppm. The present study demonstrates that cuttings that have been in transit for a long time require sugar and hormone treatment once they arrive at a certain location to restore their vitality and survival.

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INTRODUCTION

Due to its spicy qualities, black pepper, or its scientific name, *Piper nigrum* L., is a well-known and popular spice. Most food items use the whole peppercorns of *P. nigrum* or its active components (Ahmad *et al.*, 2012). Piperine, a bioactive compound found in black pepper, has pharmacological effects including antitumor, antioxidant, anti-obesity, antimicrobial, anti-aging, and immunomodulatory effects in both lab and living organisms (Haq *et al.*, 2021). Sarawak is the largest region in Malaysia, accounting for about 95% of the planted area that produces black pepper, and most of the farming participants are small farmers. This ranked Malaysia in fourth place among the major black pepper-producing countries in the world (Business Today, 2022).

Black pepper cultivation typically uses stem cuttings as propagating material. Traditionally, farmers typically prune 6-7 nodes of stem cuttings from the mother vines and plant them directly into the soil. However, they also transport some cuttings to distant areas susceptible to stress conditions such as low humidity and high temperature. These stressed cuttings will lose moisture and carbohydrates, which are essential components for root induction and growth (Otiende & Maimba, 2020). Pruning the black pepper stem cutting from the mother plant will lead to its desiccation, placing the cutting under stress. The stem tissue will lose water and carbohydrates, which will limit the energy source for regeneration. As a result, the metabolic activity responsible for root formation experiences suppression (Monder *et al.*, 2020).

According to Sheikh *et al.* (2022), internal and external factors such as light, temperature, hormones, and sugars regulate the initiation and growth of roots in higher plants. Dipping cuttings into sugar solution is one of the methods to improve root induction and proliferation in stem cuttings (Takashi *et al.*, 2003). Sugars are one of the most important factors in the root system's development, not only as an energy source but also for constructing structural components of cells and cell walls (Jarvis, 1986). Sugars act as dual agents in root formation: as metabolic energy sources and structural precursors for cell wall development, aiding in overcoming stress-induced carbohydrate depletion during transit (Bhattacharya & Kundu, 2020). The application of sugar solutions in nursery production is not new for several nursery plant species. Different types of sugars, like sucrose and glucose, have different effects on the roots of *Eucalyptus* spp. (Corrêa *et al.*, 2005), *Casimiroa edulis* (Abo El-Enien & Omar, 2018), and *Punica granatum* (Pratibha *et al.*, 2021). While the application of sugar solutions has been explored in other species like Eucalyptus and pomegranate, this study uniquely investigates its potential in black pepper, a critical cash crop in Malaysia with limited propagation studies.

The low rooting percentage of pepper stem cuttings, especially when transported to other farm areas or far from their origin, poses a major constraint in the nursery cultivation of black pepper, thereby compromising their freshness. The warmer temperature during shipping or transportation causes carbohydrates to lose water in stem cutting, which reduces survival and rooting rate (Jahnke *et al.*, 2019). The rooting ability of pepper stem cutting is critical during transportation from nursery production due to stress conditions, leading to low stem cutting viability and difficulty obtaining healthy runner shoots (Aziz *et al.*, 2015). Commercial practitioners employ several techniques to restore freshness and induce rooting in pepper stem cuttings, including soaking them in a 2% sugar solution. However, a literature search yielded no scientific evidence supporting the use of sugar to enhance the survival and rooting of black pepper stem cuttings. Another technique involves treating the stem cuttings with either rooting hormone or indole butyric acid (IBA). However, farmers rarely apply IBA to promote pepper stem cuttings, particularly in remote areas, and the associated effort is less cost-effective. Therefore, using sugar as a root-promoting substance may be more effective because it is easily available and economical. This study aimed to determine the vegetative response in terms of survival, sprouting, and rooting performance of black pepper stem cuttings to glucose, sucrose, and fructose at different concentrations.

MATERIALS AND METHODS

Black pepper stem cuttings of six to seven nodes of the Semengok Aman variety were obtained from a black pepper farm in Sarikei, Sarawak, Malaysia (1°58'08'' N, 111°30'31'' E). Stem cuttings were kept on the sand bed and watered before use. The two-node stem cuttings were prepared for this study by cutting from six to seven nodes stem cuttings. The experiment was carried out in a shade house (70% sunlight) located at the nursery site of Sarawak Pepper Farm @UPMKB, UPM Bintulu Sarawak Campus (3°12'20'' N, 133°04'44'' E). The distance between the origins of the planting material to the study location at about 293 km. This study was conducted from the early week of May to the early week of June 2022 which was the beginning of the southwest monsoon which starts from May to October and experiences low rainfall. The daytime temperature reaches 35 °C and the night temperature is as low as 23 °C (Malaysian Meteorological Department). The potting mixture was prepared by mixing topsoil, sand, and compost with a ratio of 2:1:1. Before planting, all the black polythene bags (6×9-inch size) were filled with the soil mixture and watered.

The choice of glucose, sucrose, and fructose was based on their known roles in providing metabolic substrates and osmotic balance, with glucose being a primary energy source during stress recovery. Treatments of glucose, sucrose, and fructose (Analytical Grade, Merck) were prepared with 1%, 2%, and 3% concentrations of each type of sugar. A commercial rooting hormone, MAPA IBA Root hormone was used and served as positive control following the manufacturer's instruction during population (200 g/L equivalent to 1000 ppm IBA), and filtered water was served as a negative control. A total of 220 unrooted two-node black pepper stem cuttings (10-15 cm in length) were soaked in the respective treatments for 60 min. In this study, more soaking time was needed to allow more water imbibed in the plant tissues as compared to the previous work at 30 min of soaking time which showed lower survivability (unpublished data). All treated cuttings were sown into polybags filled with soil mix and arranged in a completely randomized design with 20 replicates. All experimental polybags were watered daily until 45 days.

At 45 days after sowing, data on survival rate, sprouting rate, rooting rate, the total number of roots per cutting, and the total length of roots per cutting were recorded. The following is the formula to determine the survival rate, sprouting rate, and rooting rate:

The total number of roots per cutting was determined by counting manually number of roots that emerged from the node. Five cuttings represent one which was randomly selected in each treatment from a total of 20 cuttings. The total length of roots per cutting was conducted by measuring using a ruler to each individual root and then summing all individual root lengths.

Data on survival, sprouting, and rooting rates were presented as descriptive statistics due to the limited sample size to compare variations between treatment groups, this study was only able to summarize and describe the data. Data on the total number of roots per plant, and the total length of roots per plant (mm) were analysed for variance (ANOVA) and the Tukey test at a 5% level of significance was used to compare means. Statistical analysis was performed using SAS version 8.4.

RESULTS AND DISCUSSION

Survival of black pepper cutting after being pruned from the mother plant is one of the important aspects determining the success of establishing black pepper seedling nursery production. After 45 days of sowing, two-node black pepper cuttings received concentrations of glucose at 2% and 3%, and sucrose at 3% survived while other sugar treatments were none (Figure 1). Control data showed no cuttings survived. This was likely to happen due to the weather conditions during the southwest monsoon in the nursery site that experiences low rainfall making the effort to maintain humidity at a critical level. This was also compounded by the condition of the cuttings taken from outside Bintulu of Sarawak and brought overnight usually without special treatment to preserve the freshness of the stem cuttings (Vickers *et al.*, 2019). However, the data shows that IBA

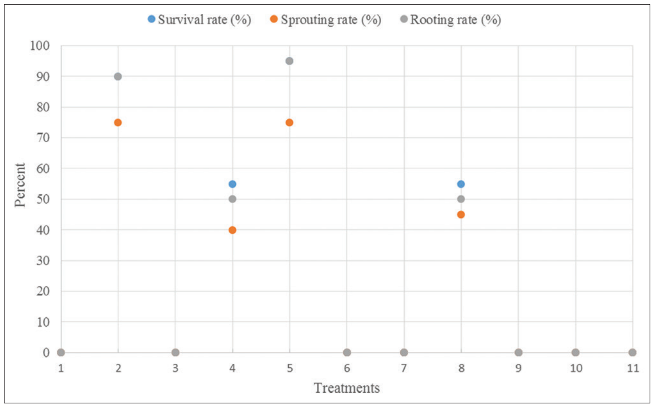

Figure 1: Scatter plot showing the rates of survival, sprouting and rooting of two-node black pepper stem cuttings after 45 days treated with glucose, sucrose, and fructose at different concentrations. Note: List of treatments numbered as 1 to 11. Control 1 (water)=1, Control 2 (IBA)=2, 1% Glucose=3, 2% Glucose=4, 3% Glucose=5, 1% Sucrose=6, 2% Sucrose=7, 3% Sucrose=8, 1% Fructose=9, 2% Fructose=10, and 3% Fructose=11

and some sugar treatments can deal with environmental stress during the study period which recorded a 55 to 95% survival rate (Figure 1).

The surviving cuttings were 2% and 3% glucose, and 3% sucrose solutions. The non-survived black pepper stem cuttings were turned black and dry. Of the sugar-treated-survived cuttings, stem cuttings that received 3% glucose solution had the highest percentage rate of survival at 95% and were better than positive control of IBA at 1000 ppm. Stem cuttings dipped in 2% glucose and 3% sucrose solutions recorded a similar survival rate of 55%. All sugar-treated survived stem cuttings were sprouted and rooted with stem cuttings treated with 3% glucose solution recorded greater sprouting and rooting percentages at 75% and 95%, respectively, and almost comparable with positive control IBA solution. Stem cuttings dipped with 2% sucrose solution and 3% sucrose solution demonstrated lower sprouting and rooting percentages at 40% and 50%, and 45% and 50%, respectively.

Stem cutting when removed from the mother plant which is an original source of its nutrients and water makes it under stress and eventually wilt and die. Applying sugar solution at certain concentrations could provide an immediate source of energy for the tissue of plant stem cutting (Abo El-Enien & Omar, 2018; Malele *et al.*, 2021). Sugar solution could also minimize stresses during ongoing dehydration and may provide enough time for recovery (Agulló-Antón *et al.*, 2014).

However, certain types and concentrations of sugars may not be suitable to help in promoting cutting survival, sprouting, and rooting (Agulló-Antón *et al.*, 2011). The present study found that black pepper stem cuttings treated with fructose at all levels failed to survive, sprout, and root. Based on our literature searches, no record was found on the effect of fructose on black pepper cuttings. However, a similar response was recorded in apple rootstock and found that the fructose was not able to initiate root formation (Bahmani *et al.*, 2009). Based on findings from several reports, the type and concentration of sugar have different effects depending on the type of plant cuttings (Custódio *et al.*, 2004; Corrêa *et al.*, 2005; Bahmani *et al.*, 2009). According to Pawar *et al.* (2020), stored carbohydrates present in black pepper, cuttings may aid in sprouting such as early sprouting over untreated cuttings. However, the present phenomenon may be not the case where the exogenous source of sugar plays an important role in supporting the stored carbohydrates to induce sprouting and rooting processes.

The number of roots indicates the number of points where the new root emerged from the stem node. Among the sugar-treated survived black pepper stem cuttings (Figure 2), the total number of roots of black pepper stem cuttings treated with 3% glucose solution (19.10±0.93) was significantly higher as compared to 3% sucrose solution (15.00±0.90) and 2% glucose solution (10.14±0.94). In different plant species, Takahashi *et al.* (2003), using Arabidopsis seedlings found that sucrose at concentrations of 0.5-2.0% was most effective in inducing adventitious roots, but at a higher level of 5.0% sucrose suppressed the root induction. A positive effect of glucose on cutting rhizogenesis was found if this hexose was supplied during the root induction phase, followed by sucrose in the root formation step, especially for *E. globulus*. The 3% glucose solution likely promoted root formation by replenishing depleted carbohydrates, a critical substrate for respiration and energy-intensive growth processes during the root induction phase (Takahashi *et al.*, 2003). Unlike glucose and sucrose, fructose may not provide the same efficiency in energy transfer or structural utility required for root induction in black pepper (Corrêa *et al.*, 2005). However, the number of roots of black pepper stem cuttings treated with 3% glucose solution was significantly lower than the IBA treatment. Black pepper stem cuttings dipped in IBA at 1000 ppm recorded a significantly higher total number of roots at 22.14±0.94 than black pepper stem cuttings dipped in 3% glucose solution. The primary role of IBA as a rooting hormone was much anticipated in promoting roots of black pepper stem cuttings instead of sugar which mainly functions as a source of energy (Corrêa *et al.*, 2005; Mehta *et al.*, 2018).

The total length of roots refers to the sum of the length of each individual root recovered from a cutting. The effect of the treatment on this parameter will show whether the treatment increases the total root length or decreases the total root length. The total length of the root will reflect the surface area of the root that is available thereby increasing the functional space of the root to absorb water and nutrient solutions or secrete exudates. The present study demonstrated the effect of 2% glucose, 3% glucose, and 3% sucrose solutions on the total length of roots of black pepper stem cuttings 45 days after sowing (Figure 3). Among the survived black pepper stem cuttings, the total length of roots of black pepper stem cuttings treated with 3% glucose solution was significantly greater than other treatments such as IBA (3.43±0.80 mm), 3% sucrose (35.60±0.74 mm), and 2% glucose (30.60±0.74 mm). Figure 4 illustrates the rooting and sprouting responses of black pepper (*Piper nigrum* L.) stem cuttings, where treatments with IBA (1000 ppm) and 3% glucose demonstrated the most robust root and shoot development, while 2% glucose and 3% sucrose

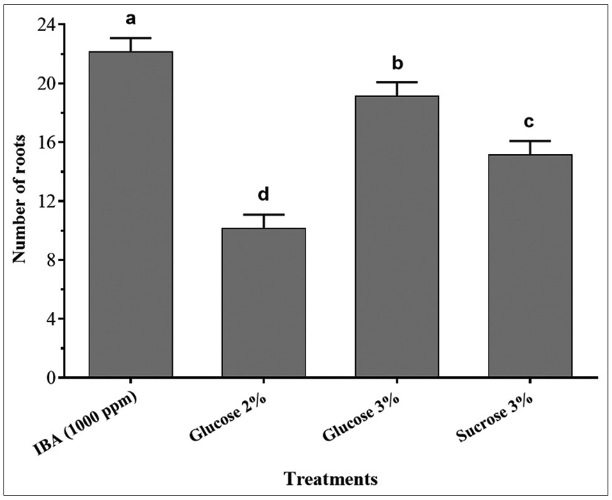

Figure 2: Bars representing the number of roots per plant of black pepper stem cutting after 45 days treated with 3% sucrose, 3% glucose, and 2% glucose, and IBA at 1000 ppm served as a positive control. Means with the same alphabet is not significantly different at P<0.05 (Tukey's test)

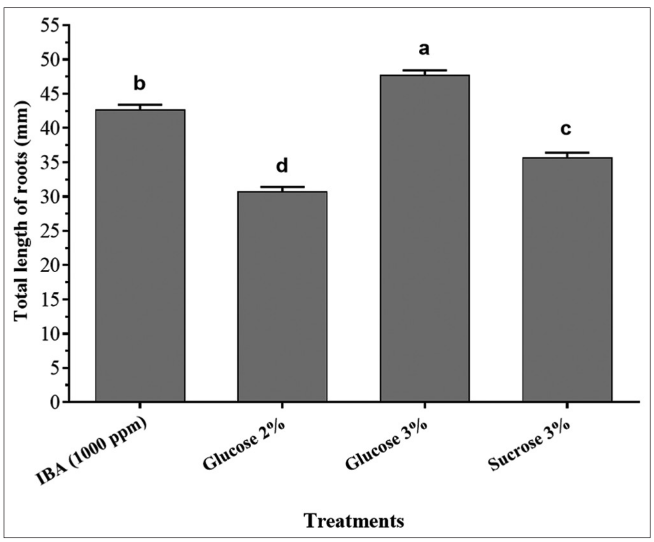

Figure 3: Bars representing the total length of roots (mm) per plant of black pepper stem cuttings after 45 days treated with 3% sucrose, 3% glucose, and 2% glucose, and IBA at 1000 ppm served as a positive control. Means with the same alphabet were not significantly different at P<0.05 (Tukey's Range Test)

resulted in comparatively weaker growth, highlighting the superior effectiveness of higher glucose concentrations and IBA in promoting propagation success.

This study demonstrates the feasibility of using 3% glucose as a cost-effective and accessible alternative to traditional rooting hormones like IBA, which could be particularly beneficial for farmers in remote regions. The use of glucose solution used in this study showed that the sugar has the potential as a cheap and cost-effective source of plant stimulants, especially in terms of providing an additional source of energy to the tissue of pepper stem cuttings to carry out physiological and metabolic activities for the development of the next growth phases such as root and shoot growth (Yaseen *et al.*, 2013).

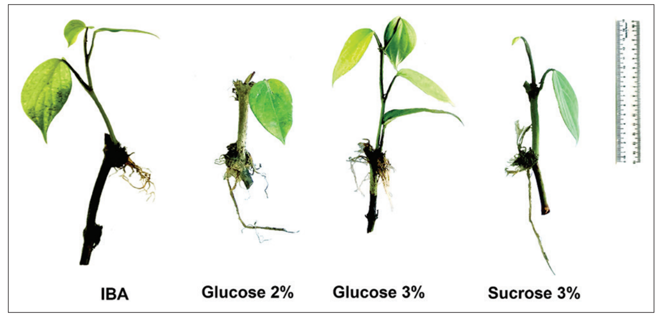

Figure 4: Comparison of rooting and sprouting responses in black pepper (*Piper nigrum* L.) stem cuttings treated with IBA (1000 ppm), glucose solutions (2% and 3%), and sucrose solution (3%). The IBA and 3% glucose treatments showed superior rooting and sprouting, while 2% glucose and 3% sucrose treatments resulted in weaker responses, as indicated by reduced root and shoot development. A ruler is included for scale

CONCLUSION

This study underscores the potential of glucose as a simple, effective treatment to enhance black pepper propagation, offering an innovative approach for nurseries, especially in stress-prone conditions. Notably, the findings of this study indicate that immersing black pepper stem cuttings in a 3% glucose solution led to enhanced outcomes in terms of survival rates, sprouting percentages, rooting percentages, as well as the quantity and overall length of roots. This suggests that the utilization of glucose holds promise as an economical and cost-effective means of providing plant stimulants in the nursery for cultivating black pepper stem cuttings. This discovery may have practical implications for optimizing the cultivation practices of black pepper, offering a more efficient and affordable approach to enhance the vegetative performance of stem cuttings.

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