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Research Article

Optimization of nutmeg (*Myristica fragrans*) grafting using multiple rootstock and scion types

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ABSTRACT

The rootstock number and scion types can be used to optimize grafting success. This study aimed to determine the effect of rootstock number, scion type, and its interaction on the success of nutmeg grafting. The research was conducted at the Seed Breeding Site in Layeni Village, Central Maluku Regency, Maluku. The research started in November 2022 and ended in August 2023. The experiment used a two-factor completely randomized design. The first factor was the rootstock number, i.e., single, double, and triple rootstocks. The second factor was the scion type, i.e., Myristica fragrans Houtt, Myristica argentea Warb, and Myristica sp. The 9 treatment combinations were replicated six times, resulting in a total of 54 experimental units. The result of the study concluded that grafting success was influenced by the rootstock number and the scion types, and there was no interaction between rootstock and scion. The highest success was achieved by using a single rootstock with a success rate of 60%. The use of Myristica sp. scions gave the highest grafting percentage of 76.66%. Successful nutmeg grafting was characterized by the presence of a fusion between the rootstock and the scion, based on anatomical observations. Failed nutmeg grafting was characterized by the absence of fusion between rootstock and scion. The rootstock number and scion type affected shoot emergence time, linked stem diameter, shoot length, and leaf number, while the scion type affected the shoot number. Double rootstock tended to have higher parameters than single and triple rootstock for shoot emergence and leaf number, but the number of failed grafting was still challenging.

Keywords: *anatomical; double rootstock; compatibility; incompatibility; linked stem diameter*

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INTRODUCTION

The nutmeg plant is called the King of spices and is known as the oldest spice plant. The center of origin of nutmeg is Indonesia. Indonesia is the largest nutmeg producer in the world, supplying nutmeg with a share of 60-75%. The area of nutmeg plantations in Indonesia for 2020 reached 252,322 ha with a total production of 38,150 tons and an average productivity of 447 kg ha^{-1} (Directorate General of Plantation, 2020). The area of nutmeg plantation in Maluku was recorded at 35,621 ha with a production of 5,596 tons and an average productivity of 309 kg ha^{-1} (Directorate General of Plantation, 2020).

Banda nutmeg (*Myristica fragrans* Houtt.) covers large plantation area in the Banda Neira sub-district of Central Maluku district and is a champion species in the international market (Lawalata et al., 2017). Since 2009, nutmeg has been designed as a national cash crop therefore, various efforts have been made to develop nutmeg production (Ministry of Agriculture Republic of Indonesia, 2009). Moreover, the expansion of the production area of nutmeg is also a priority to improve farmers' welfare (Hatusupy et al., 2020). Thus it is important to support more efficient cultivation techniques and provide superior plant material that is more tolerant to biotic and abiotic stress, and productivity (Suryadi, 2017).

The most efficient way of growing nutmeg is by vegetative propagation, i.e., grafting. Grafting is the process of connecting two plant segments, the shoot (upper part) known as the scion, and the root (lower part) known as the rootstock (Goldschmidt, 2014). The success of grafting depends on two important factors: physiological compatibility between the rootstock and scion, and proper alignment of the different tissues of the graft union (Ajamgard et al., 2016). The advantages of plant propagation by grafting include: The resulting seedlings have better characteristics than their parents, produce earlier, have good growth ability, have shorter plants and many branches, and have appropriate sex (Ruhnayat & Martini, 2015). From environmental issues, grafting also saves more water than common seedlings (Santosa et al., 2023). The age of rootstock and scion, the type of rootstock and scion, and the environment affect the success of plant grafting (Karimi & Farahmand, 2011).

There has not been much research on nutmeg grafting. Nutmeg grafting using the growth regulator IBA had a success rate of 37.33% while IAA produced a grafting success rate of 43.03% (Heryana & Supriadi, 2011). Nutmeg grafting using different types of rootstock and scion had a nutmeg grafting success rate of 54.03% (Karmanah, 2020). Nutmeg grafting using rootstocks with different ages, namely 60 days, 90 days, and 120 days of seedling age with a success percentage of 48.95, 33.75, and 32.82% respectively (Sudjud et al., 2022). The problem often encountered in the propagation of nutmeg plants by grafting is the low percentage of grafting success. The low percentage of success is thought to be due to factors that influence the success of grafting that have not been properly addressed. These include using poorly selected planting material, genetic factors of the rootstock and scion, grafting techniques, and climatic conditions. It is therefore important to optimize the success rate of nutmeg.

Studies on the use of more than one rootstock for nutmeg grafting have not been conducted. In general, double or more than one-leg grafting has been practiced and can be applied to all types of perennials that are common in cocoa (Santosa et al., 2023). Double legs gives better results for grafting than single (Fitri & Islahuddin, 2018). The use of different scions is aimed at increasing success and obtaining good scions compatibility in nutmeg grafting. Here, *Myristica fragrans* Houtt, *Myristica argentea* Warb, and *Myristica* sp are used as prospective scions. *Myristica fragrans* Houtt and *Myristica argentea* Warb are superior varieties selected from the result of selection in the area of origin (Bermawie et al., 2018). *Myristica* sp is a non-determined species that is most likely a hybrid nutmeg from the natural cross-pollination of *Myristica fragrans* Houtt and *Myristica argentea* Warb, which has not been studied. The taxonomi proximity of rootstock and scion species is very important for successful grafting (Baron et al., 2019); which might increase the high success of compatibilities. This research aimed to determine the effect of the rootstock number, the scion type, and the interaction between them on the success of nutmeg grafting.

MATERIALS AND METHODS

Research design

The research was conducted at the seed breeding site in Layeni Village, Central Maluku Regency, Maluku. The research started in November 2022 and ended in August 2023. The experiment used a two-factor completely randomized design. The first factor was the number of rootstocks, namely: one, two, and three rootstocks. The second factor was the type of scion, namely, *Myristica fragrans*_Houtt, *Myristica argentea* Warb, and *Myristica* sp. The 9 treatment combinations were replicated six times, resulting in a total of 54 experimental units.

Grafting procedure

The seeds of *Myristica argentea* Warb were prepared for rootstock production. Initially, nutmeg seeds were scarified by cutting the tip of hard shell until the endosperm appeared. Nutmeg seeds were then soaked in water for approximately 30 minutes, then germinated on rice husk media in sacks, and left for 10 days. The germinated seeds were transplanted in to media in polybags and maintained in the nursery for three months. Uniform seedling was selected based on plant height, stem diameter, and seedlings with cotyledons.

As many as 540 scions were collected from mother plants with 180 of each *Myristica fragrans* Houtt, *Myristica argentea* Warb, and *Myristica* sp. Scions were taken from orthotropic branches with a length of 10 cm. Scions were wrapped in moistened paper and then covered with plastic. Scions were placed into a coolbox equipped with cooling gel to maintain freshness.

The grafting process was done by selecting a scion with a size that matches to diameter of the rootstock. Initially, the stem of the rootstock was cut. For single rootstock, the stem was incised to split 1-1.5 cm. For double and triple rootstocks, the stem was cut obliquely or sideways for 1-1.5 cm.

The scion, initially was cut 3 cm at the bottom, leaving 7 cm with bud. For single and double rootstocks, the scion was cut with a wedge-shaped incision about 1-1.5 cm. The scion for triple rootstocks, was cut and grooved at a three-sided wedge 1-1.5 cm in length. Then, the graft connection was wrapped with plastic ties. The seedlings were maintained in plastic houses with grouping based on the treatments.

Observation and data analysis

Data were collected in the form of percentage connection success (MCC) (%) where observations were made 6 weeks after grafting with the formula:

$$\text{Percentage of success} = \frac{\text{Number of living seedlings}}{\text{Number of grafted seedlings}} \, x \, 100$$

Observations of compatibility and incompatibility were carried out by analyzing the anatomy of the tissue to see the area of connection between the scion and the nutmeg grafting rootstock. Anatomical observations of the splice plane tissues were made in the sixth week after grafting. Tissue analysis was performed with the following steps: Making transverse slice preparations; the sample cut with a razor blade with a very thin thickness; and observing the cross-sectional preparations using a microscope with 40x magnification.

Observations of linked stem diameter, shoot emergence time, shoot number, and leaf number were observed once a week for ten weeks. Temperature and humidity were controlled every morning (06.00 WIT- Eastern Indonesia Time), afternoon (12.00), and evening (17.00) on a daily basis for six weeks. Watering was done in the morning and evening regularly.

Data were analyzed using the analysis of variance (ANOVA) and followed by Tukey's honest significant difference test (HSD) at α = 5% level. All data were analyzed using SAS.

RESULTS AND DISCUSSION

Nutmeg grafting success

The rootstock number significantly influenced the success of nutmeg grafting (Table 1). The best grafting success was achieved using one rootstock with 60% success and two rootstocks with 58.88%. The success of grafting can be seen by the presence of a bond and connection between the rootstock and the scion. The union between rootstock and scion is the basic principle of shoot grafting propagation (Rasool et al., 2020). Grafting with the use of one and two rootstocks showed faster healing of incision scars compared to three rootstocks. The surface area of the incision wounds on the triple rootstocks was wider than on the double and single rootstocks therefore, the wound recovery process might be longer, and rapid drying caused tissue death, resulting in a low percentage of success. Callus proliferation was also faster on seedlings with one and two rootstocks than those with three rootstocks. Tissue alignment between rootstock and scion was more aligned in one and two rootstocks than in three rootstocks.

Table 1. Grafting success of nutmeg from different rootstock numbers and scion type

Treatment	Grafting success (%)
Rootstock numbers	
Single rootstock	60.00a
Double rootstock	58.88a
Triple rootstock	40.00b
Scion Types	
M. fragrans Houtt	9.44b
M. argentea Warb	72.77a
Myristica sp	76.66a

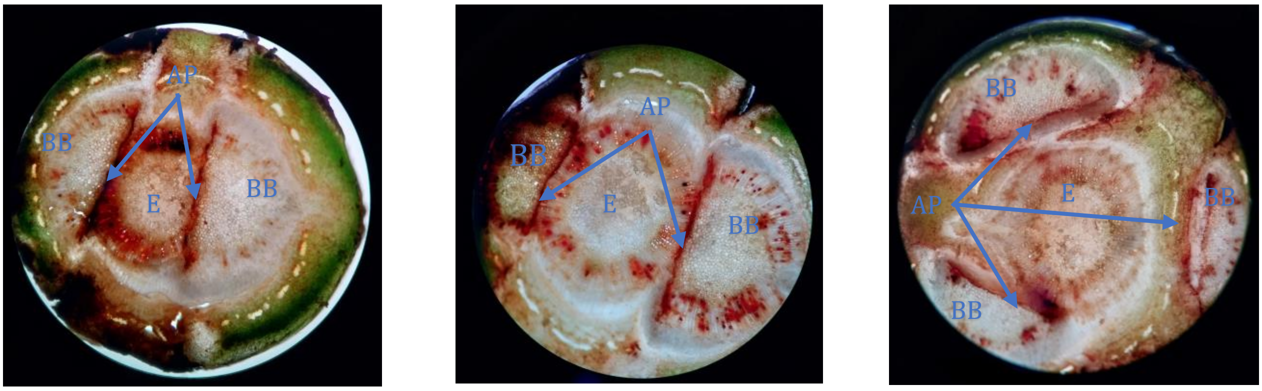
Note: Values followed by the same letter are not significantly different based on the Tukey HSD test at α = 0,05

The scion type had a significant effect on the success of nutmeg grafting (Table 1). The best grafting success was obtained from the use of *Myristica* sp. scion types with a success percentage of 76.66% and *M. argentea* Warb of 72.77%. Grafting with *Myristica* sp. or *M. argentea* Warb scions had a higher percentage of success than *M. fragrans* Houtt. The *M. fragrans* scion, showed slower recovery at the incision wound so callus formation takes longer than the other two scions. The scion diameters were relatively similar, but the smaller size of *M. fragrans* Houtt compared to both the scions and the rootstock might also result in improper tissue alignment.

Successful grafting is characterized by the healing of the incision through a series of molecular and physiological processes at the junction such as cell differentiation, cell recognition, callus bridge formation, removal or clearance of necrotic tissues, and vascular network formation (Adhikari et al., 2022). The formation of callus between the scion and rootstock at the junction is the initial growth stage that determines the success of grafting. Cell proliferation of the grafting callus takes three to four days after grafting to reconnect the phloem, and six to seven days to reconnect the xylem (Fullana-Pericas et al., 2019). However, on average callus formation of nutmeg grafting was six weeks after grafting. The union between the scion and rootstock is due to the union of the vascular cambium of the rootstock with the cambium of the scion (Pina et al., 2017). The union of the vascular cambium is very important for the success of grafting, therefore the greater the union of the vascular cambium, the greater the development of the graft (Balbi et al., 2019). Associated with the results of the cambium union shows that the accumulation of assimilates can stimulate cell division, enlargement, and differentiation, which then promotes the process of union between the scion and rootstock (Roswanjaya et al., 2020). The success of grafting indicates that the scion and rootstock can live and grow together into a whole plant (Gisbert et al., 2011). Successful or compatible grafted plants indicate that translocation of assimilates, water, hormones, and enzymes are smooth and optimized metabolic processes, thus promoting plant growth.

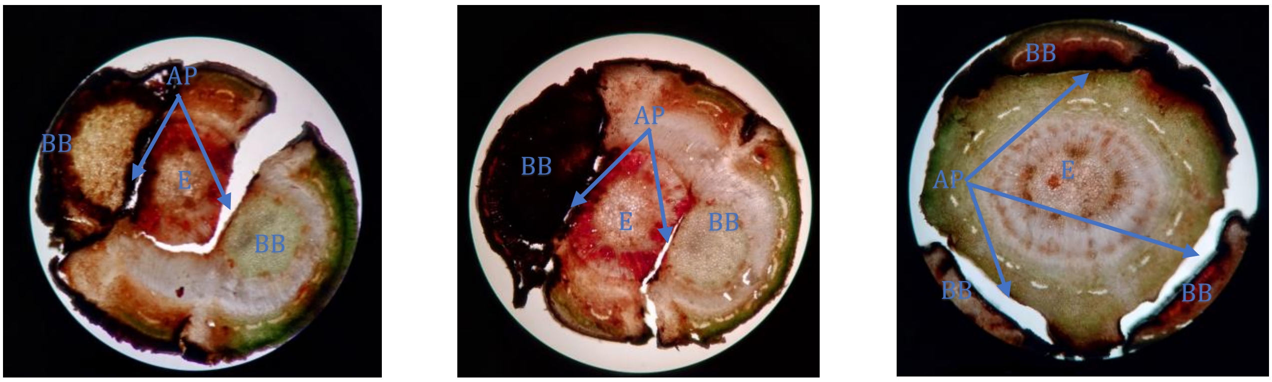
Compatibility and incompatibility

The success rate of plant grafting can be used as an indicator of the level of compatibility, i.e., the higher the grafting success rate, the more compatible the scion and rootstock (Fathan et al., 2017). Compatibility and incompatibility are defined as the success or failure of the graft union between scion and rootstock (Chen et al., 2016). Plant compatibility is difficult to predict, and even among related species in the same family, there are different levels of grafting incompatibility (Li et al., 2021). Plant graft compatibility is influenced by the anatomical, physiological, and genetic variability of plants (Trinchera et al., 2013; Vrsic et al., 2015). The success of grafting is also influenced by microclimatic conditions, the timing of grafting, the cleanliness of the grafting tools, and the skill of the grafters. Variability in compatibility and incompatibility can be observed by anatomical observation of plant tissues, which may show differences between compatible and incompatible grafts. Compatible grafting results were indicated by the fused area between the scion and rootstock (Figure 1). Incompatible grafting results were indicated by the area of the interface that did not appear to be fused (Figure 2).



(Compatibility of one rootstock) (Compatibility of two rootstocks) (Compatibility of three rootstocks)

Figure 1. Success grafting cross-section of the linkage area (AP), rootstock (BB), and scion (E) in nutmeg.



(Incompatibility of one rootstock) (Incompatibility of two rootstocks) (Incompatibility of three rootstocks)

Figure 2. Fail grafting cross-section of the linkage area (AP), rootstock (BB), and scion (E) in nutmeg

Compatible nutmeg grafting is characterized by union rootstock and scion tissues with callus formation. The callus formed will differentiate from a new cambium and unite with the original cambium of the scion, and the rootstock, phloem, and young secondary xylem have been formed so that the physiological process of the plant can take place properly. Compatible grafts also show the formation of new vascular tissue at the graft site and have a light or thin layer of necrosis on the graft. The necrotic layer disappears over time, indicating good tissue compatibility (Melnyk, 2016). Compatible grafts transport and translocate nutrients, carbohydrates, and hormones for normal plant development (Baron et al., 2019).

Incompatible nutmeg grafts are characterized by the absence of rootstock and scion tissues, resulting in callus formation. Incompatibility occurs due to weak tissue attachment and lack of vascular network formation (Melynk & Meyerowitz, 2015). Incompatible grafts have less vascular tissue and more or thicker layers of necrosis. Necrosis may be related to the failure of cell regeneration between the graft surfaces, which inhibits the transfer of water and nutrients from the rootstocks to between the stems and the scion. Some incompatible grafted plants show low vigor and swelling in the area above the graft junction, which are typical symptoms of morphological incompatibility (Li et al., 2021). According to Suharjo (2020), scions and rootstocks that are not fused cause the formation of xylem and phloem bonds so that nutrients and water cannot be transported from the growing medium to the scions and, conversely, the photosynthetic products produced by the leaves of the scions cannot be channeled to the rootstock organs. The main causes of incompatibility include physiological and biochemical factors, cellular and tissue changes at the time of graft integration, and cellular recognition between graft components. Characteristics of incompatible plants include low splicing success, yellowing of leaves on successfully grown plants, bud loss, and death, reduced vegetative growth, and death of spliced seedlings.

Time to shoot emergence

The rootstock numbers and the scion types significantly affected the time to shoot emergence (Table 2). The treatment with two *M. fragrans* Houtt rootstocks showed the longest bud emergence time of all treatments at 57.16 days after grafting, followed by the treatment with one *M. fragrans* Houtt rootstock at 52.5 days after grafting. The one, two, and three rootstock treatments of *M. argentea* Warb and *Myristica* sp. showed a rapid bud emergence time of 49 days after grafting. The three rootstock treatments of *M. fragrans* Houtt had no shoot emergence because all plants in this treatment died or were incompatible. The difference in shoot emergence time is probably due to the different ability of the rootstock to form a grafting linkage. The time of bud burst is related to the buds that are still fresh after grafting to a closed (dormant) state (Sudjud et al., 2022).

The results obtained show that the use of one, two, and three rootstocks did not have a different effect on the formation of new shoots (data not shown). The variation in the number of days required for first shoot emergence may be due to the genetic factors of each type of scion. *M. argentea* Warb and *Myristica* sp. showed a better growth rate than *M. fragrans* houtt. This is consistent with the findings of Bose et al. (2019) on the grafting of mango plants with different cultivars, which showed that cultivars germinate due to genetic differences translocation of food reserves, and changes in cambial activity.

Table 2. Shoot emergence and stem diameter of grafted nutmeg from different rootstock numbers and scion type

Rootstock numbers	Shoot emergence (days)		
	Myristica fragrans Houtt	*Myristica argentea* Warb	*Myristica* sp.
Single rootstock	52.05ab	49b	49b
Double rootstocks	57.16a	49b	49b
Triple rootstocks	-	49b	49b
	Stem diameter (mm)		
Single rootstock	2.60b	4.33a	4.08a
Double rootstock	4.07a	4.95a	4.23a
Triple rootstock	0.00c	5.52a	4.95a

Note: Values followed by the same letter are not significantly different based on the Tukey HSD test at α = 0,05. '-' : no shoot emergence.

Diameter of linked stems

The diameter of the linked stems was significantly influenced by the rootstock numbers and the scion types (Table 2). The graft using three rootstocks of *Myristica argentea* Warb had the largest connected stem diameter of 5.25 mm. Grafting using a *Myristica fragrans* Houtt rootstock had the smallest connected stem diameter of 2.6 mm. Treatment with the number of rootstock and different types of scion in increasing the size of the linked stem diameter showed almost the same effect. This indicates that there has been a union between the scion and the rootstock so cell division activity and cambium activity are causing the stem diameter to increase.

The diameter of the rootstock and scion used was relatively the same, ranging from 1.5-4 mm. Grafting using the same rootstock and scion allows for the alignment of the vascular cambium network (Melynk & Meyerowitz, 2015). Cholid et al. (2014) found that the more similar the diameter of the rootstock and scion, the greater the chance of successful grafting. Similar rootstock and scion diameters result in better tissue alignment. The increase in stem diameter in grafting is caused by phenols or carbohydrates that accumulate as a result of partial cambium continuity at the grafting site (Cholid et al., 2014).

Number of buds

The rootstock numbers used in grafting had the same effect on the number of buds. The number of shoots was significantly influenced by the type of scion (Table 3). Grafting with *Myristica* sp. as scion showed the highest number of shoots with an average shoot number of 20.2. The scion of *M. fragrans* Houtt had the lowest number of shoots with an average of 2.3. This indicates that the *M. argentea* Warb rootstock is more compatible for grafting with *Myristica sp*. than the other scions.

Buds are the result of the development of apical meristems, which develop so that the buds that appear will continue to develop into leaves (Arif et al., 2016). The bud number formed on each type of scion is an indication of the different growth rates of each scion used in grafting and also of the different genetic characteristics of each type of scion. The number of buds that grow can also be influenced by the condition of the scion during grafting and the growth point of each scion. This view is supported by Sudjijo (2009) that there are differences in genetic traits in different varieties that will produce different numbers of buds. Similarly, in grafted plants, the number of buds is the accumulation of the extension of the plant's internodes to form new buds.

Table 3. Number of shoots of grafted nutmeg from different rootstock numbers and scion.

Treatment	Number of shoots
Rootstock numbers	
Single rootstock	13.1a
Double rootstock	14.0a
Triple rootstock	10.7a
Scion type	
M. fragrans Houtt	2.3c
M. argentea Warb	15.3b
Myristica sp	20.2a

Note: Values followed by the same letter are not significantly different based on the Tukey HSD test at α = 0,05.

Shoot length and leaf number

The rootstock numbers and the scion types had no significant effect on shoot length (Table 4). New shoots that appear on nutmeg plants were generally between 1 and 2.5 cm long. Increasing the length of the shoot will increase the number of buds that will emerge. Long shoots have many buds that will emerge. A shoot length of 1.75 cm produces two to three buds per plant. A stem length of 1.21 cm produces one to two buds per plant.

The longer the shoot, the more leaves are produced because the longer the shoot has a greater number of internodes and books where leaves grow (Supriyono et al., 2020). The increase in shoot length continues until the shoots have fully flowered into new leaves. This change is followed by the emergence of new shoots.

The rootstock numbers and the scion types had a significant effect on the number of leaves (Table 4). Grafting with the use of several rootstocks and types of scion showed the number of leaves formed in the treatment with three *Myristica* sp. rootstocks was an average of 24.17 leaves. The lowest number of leaves formed was in the treatment with one *M. fragrans* Houtt rootstock with an average number of leaves of 3.5 stems. The leaf number that grows depends on the number of buds that appear. The number of leaves increases as the number of shoot segments growing leaves increases (Supriyono et al., 2020).

Table 4. Shoot length and leaf number of grafted nutmeg from different rootstock number and scion

Rootstock numbers	Shoot length (cm)		
	Myristica fragrans Houtt	*Myristica argentea* Warb	*Myristica* sp.
Single rootstock	1.21a	1.63a	1.56a
Double rootstocks	1.23a	1.75a	1.73a
Triple rootstocks	0.0b	1.45a	1.63a
	Leaf number		
Single rootstock	3.5d	15.8ab	16.0ab
Double rootstock	4.0cd	14.5b	23.6a
Triple rootstock	-	12.1bc	24.1a

Note: Values followed by the same letter are not significantly different based on the Tukey HSD test at α = 0,05. '-' : no leaf number.

The treatment of three *Myristica* sp. rootstocks showed a large number of leaves corresponding to the produced by the *Myristica* sp. type (Tables 2 and 4). This is because of the ability to grow from the three rootstocks, which are more, so the number of leaves produced is greater. The different entry types show different translocation of nutrients, which also improves the quality of the new leaves (Bose et al., 2019). Firman and Ruskandi (2009) explained that if the quality of the connection is good, the number of leaves will be large because it has fused perfectly. The formation of new leaves will increase the rate of photosynthesis, the faster the rate of photosynthesis, the faster the leaves will be formed (Putri et al., 2016). Leaves can be used as an indicator of healthy seedling growth, including in grafting (Mao et al., 2018).

CONCLUSIONS

Grafting success in nutmeg was influenced by the rootstock numbers and, the scion types, but there was no interaction between rootstock and scion. The highest success was achieved by using a single rootstock with a success rate of 60%, but other growth parameters tended to be higher in the double rootstock. The use of *Myristica* sp scions gave the highest grafting percentage of 76.66%. Successful nutmeg grafting was supported by the presence of perfect morphological fusion. The absence of perfect fusion caused failed grafting. The rootstock numbers and the scion types affected shoot emergence time, linked stem diameter, shoot length, leaf number, and the scion types affected the shoot number.

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