SEEDLING CHARACTERISTICS OF OPEN-POLLINATED MALAYSIAN CLASS 1 CACAO CLONES

Koey Wen Jun
Faculty of Sustainable Agriculture,
Universiti Malaysia Sabah,
Locked Bag No.3, 90509 Sandakan, Sabah

Azwan Awang
Faculty of Sustainable Agriculture,
Universiti Malaysia Sabah,
Locked Bag No.3, 90509 Sandakan, Sabah
Email: azwang@ums.edu.my

Mohammad Mohd. Lassim
Faculty of Sustainable Agriculture,
Universiti Malaysia Sabah,
Locked Bag No.3, 90509 Sandakan, Sabah

Pang Oi Wei
Faculty of Sustainable Agriculture,
Universiti Malaysia Sabah,
Locked Bag No.3, 90509 Sandakan, Sabah

Boney Muda
Malaysian Cocoa Board,
5, 6 & 7th Floor, Wisma SEDCO,
Locked Bag 211, 88999 Kota Kinabalu, Sabah

Haya Ramba
Malaysian Cocoa Board,
5, 6 & 7th Floor, Wisma SEDCO,
Locked Bag 211, 88999 Kota Kinabalu, Sabah

ABSTRACT

Commercially grown cacao (Theobroma cacao) is generally propagated by grafting. In grafting, the choice of rootstock is important because it affects the growth, vigor, and health of the resultant graft. Class 1 Malaysian commercial cacao clones (MCB C1, MCB C8, KKM 22, PBC 123, and QH 1003) could be proposed as rootstocks due to their superior agronomic traits. However, the growth characteristics of seedlings of Malaysian cacao clones are not known. In this study, the seedling characteristics of open-pollinated class 1 cacao clones and the UIT 1 clone were compared by measuring the number of leaves, hypocotyl length, stem girth, plant height, root length, and dry weight of shoots and roots. The study was conducted in pots under a rain shelter using a Completely Randomized Design with 30 replicates. ANOVA showed significant differences ($p < 0.05$) in all growth parameters among the six cacao clones, except for root length and root dry weight. The results showed that KKM 22 and QH 1003 had better growth performance than the other clones. In terms of shoot dry weight, KKM 22 (3.89 ± 0.24 g) and QH 1003 (3.52 ± 0.21 g) had the highest shoot dry weight but were not significantly different from PBC 123 (3.41 ± 0.03 g) at 70 days after sowing (DAS). In terms of plant height, QH 1003 (28.3 ± 0.92 cm) and KKM 22 (26.4 ± 1.33 cm) also showed the highest plant height but were not significantly different from PBC 123 (26.4 ± 1.16 cm) and UIT 1 (27.7 ± 0.65 cm). In addition, KKM 22 and QH 1003 showed the highest number of leaves of 15 ± 0.45 and 13 ± 0.39, respectively. This might indicate that KKM 22 and QH 1003 were more efficient in nutrient uptake and utilization. Finally, KKM 22 (0.64 ± 0.02 cm) had the largest stem girth, and QH 1003 (8.3 ± 0.20 cm) had the longest hypocotyl length. It has been shown that a large stem girth and a long hypocotyl contribute to grafting success in other plants. Therefore, KKM 22 and QH 1003 may be suggested as potential rootstocks to replace UIT 1, subject to further evaluation such as scion compatibility.

Keywords: *Cocoa, seed, seedlings, growth, rootstock*

INTRODUCTION

Cacao (*Theobroma cacao* L.) is an important source of income for six million smallholder farmers in developing countries of the tropics. It is grown for its seeds, which are used in the food, cosmetics, and pharmaceutical industries. Over the past decade, global demand for cocoa has increased by 35% (ICCO, 2018; ICCO 2021). The increase has been mainly attributed to population increase and economic development in Asian and African countries. The increase in cocoa demand has motivated producing countries to increase productivity by expanding cultivated areas and intensifying production (Tothmihaly et al., 2019).

Yield and production of cacao could be increased by propagating superior planting materials. Generally, cacao trees are grown from seeds or through vegetative propagation (Richards, 2011). Growing cacao from seed is easier and cheaper. However, cacao is a preferentially allogamous and self-incompatible species and therefore produces genetically heterozygous seeds (N'Zi et al., 2017). Seed-derived cacao trees have been reported to have highly variable yields (Maximova et al., 2002), with most being low-yielding (Irrizary and Rivera, 1999). On the other hand, cacao can also be successfully propagated vegetatively by grafting.

Grafting is a technique in which the shoot of a desired plant (scion) is attached to the root system of another plant (rootstock). This technique produces plants that are genetically stable and identical to the original plants. Grafting provides several advantages to the scion, such as shorter maturity time (Daouda et al., 2018), greater flowering (Souza et al., 2018), higher yield (Yin, 2004), better fruit quality (Mijowska et al., 2017), and higher disease tolerance (Ribeiro et al., 2016). In cacao, grafting improved dry bean yield by seven percent compared to propagation by cuttings of plants from somatic embryos (Goenaga et al., 2015).

The choice of rootstock is critical because it affects the growth, vigor, and health of the resultant plant. In general, rootstock selection is based on its resistance to pests and diseases and its compatibility with the scion. Seedlings with robust root systems, vigorous growth, and adaptability to different soil conditions are also among the criteria for rootstock selection. In Malaysia, cacao seedlings of UIT 1 x NA 33 are commonly used as standard rootstocks (Yin, 2004). The hybrid is preferred because the cross produces large seeds and is tolerant to vascular streak dieback but produces relatively low yield. UIT 1 is less cultivated because of the introduction of higher-yielding clones. It is worth noting that most cacao-producing countries still use seedlings from open-pollinated seeds as rootstock (Daouda et al., 2018). In addition, new, better rootstock alternatives are needed as a strategy to improve cacao performance.

Class 1 Malaysian cacao clones (MCB C1, MCB C8, KKM 22, PBC 123, and QH 1003) are elite clones selected by the Malaysian Cocoa Board. These clones produce more than 2.5 tonnes ha^{-1} year^{-1} of dry beans and are tolerant to economically important pests and diseases. Most importantly, they are suitable for cultivation throughout Malaysia (Johnsiul and Awang, 2019). Class 1 Malaysian cacao clones could be proposed as rootstocks due to their superior agronomic traits. In addition, rootstock production from open-pollinated seeds is highly desirable to avoid the hassle of controlled pollination. Farmers could easily produce rootstocks directly on their farms. However, the growth characteristics of seedlings of class 1 Malaysian cacao clones are not known. Therefore, the objective of this study was to compare the seedling growth of five open-pollinated class 1 Malaysia cacao clones and UIT 1 clone.

MATERIALS AND METHODS

Experimental design
The pot experiment was conducted at the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Sandakan, Sabah, from July to November 2018. It was laid out in a completely randomized design (CRD) with six clones replicated 30 times.

Planting materials
Healthy seeds of open-pollinated Malaysian class 1 cacao clones (MCBC 1, MCBC 8, KKM 22, PBC 123, QH 1003) and UIT 1 were provided by the Malaysian Cocoa Board.

Plant establishment and husbandry
Seeds were germinated between two layers of moist jute bags. Germinated seeds were sown in black polybags (7×12 inches) 3/4 filled with topsoil from Quoin Hill, Tawau, Sabah. Seedlings were raised under a rain shelter with a 70% sun protection net. Five grams of NPK Blue (12:12:17:2+ TE) was applied monthly to each seedling as soon as the cotyledons were detached. Seedlings were watered twice daily. Manual weeding and pesticide application were performed as needed.

Observed parameters and statistical analysis
Seedling growth parameters such as plant height, hypocotyl length, number of leaves, and stem girth were recorded every 14 days after sowing (DAS) up to 70 DAS. Plant height was measured from the soil surface to the apical tip of the plant. Hypocotyl length was measured from the root junction to the point where the cotyledon was located. Stem girth was measured with a Vernier caliper around the root junction. Root length and dry weights of shoot & root were determined at harvest after being oven-dried at 70°C for 96 hours. Root length was determined by measuring the longest root. Data were analyzed by analysis of variance (ANOVA) followed by Duncan's multiple range test at $p< 0.05$ when a significant effect was found using Statistical Analysis System (SAS).

RESULTS AND DISCUSSION

Rootstock selection is critical because it affects crop productivity and quality (Mauro et al., 2020; Zhu et al., 2020). In general, rootstocks are selected based on their resistance to soil-borne pests and diseases and their compatibility with scions. In addition, good plant growth could also be used as a selection criterion. In this study, the seedlings' growth characteristics of five Malaysian Class 1 cacao and UIT 1 clone were observed for 70 DAS. The results showed that leaf number, hypocotyl length, stem girth, and plant height increased continuously in the six clones studied. One-way analysis ANOVA revealed significant differences ($p< 0.05$) in all growth parameters among clones except root length and root dry weight at 70 DAS (Table 1).

Table 1: Means of seedlings growth parameters of five open-pollinated Malaysian Class 1 cacao clones and UIT 1 measured at 70 DAS

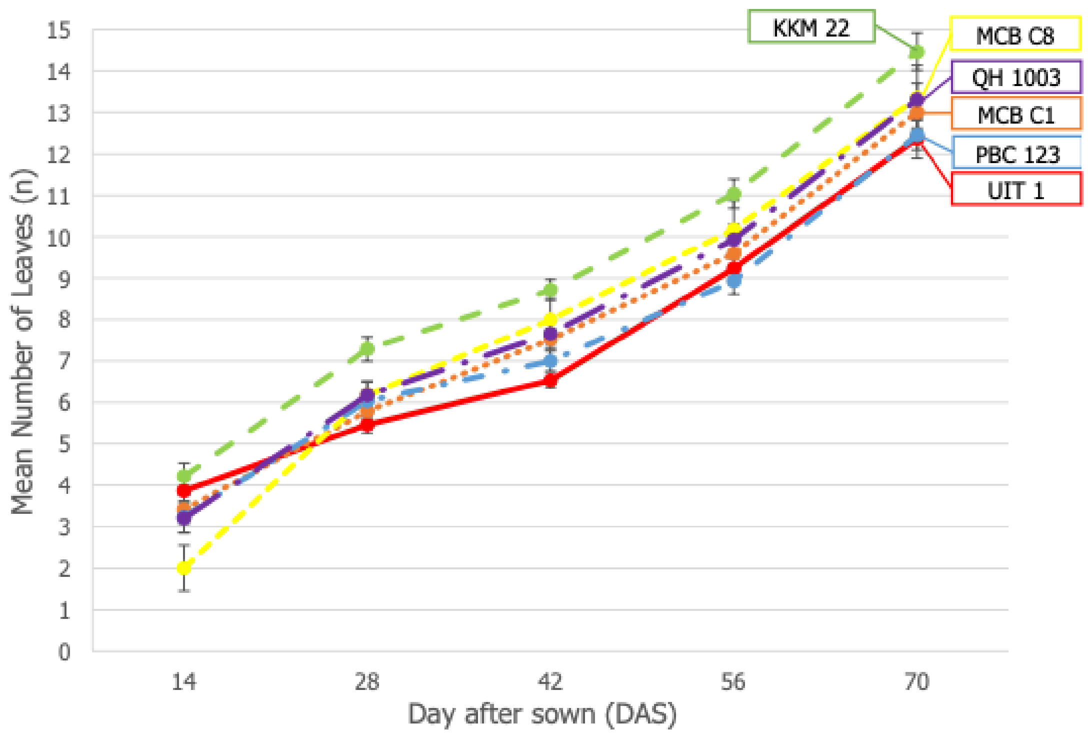
Clones	Leaf Number	Hypocotyl length (cm)	Stem girth (cm)	Plant Height (cm)	Root length (cm)	Shoot Dry Weight (g)	Root Dry weight (g)	Root to Shoot Ratio (g)
UIT 1	12 ±0.48^{b}	7.8 ±0.17^{ab}	0.58 ±0.01^{b}	27.7 ±0.65^{a}	35.9 ±1.62	2.78 ±0.12^{b}	0.98 ±0.20	0.37 ±0.02^{b}
MCB C1	13 ±0.38^{b}	7.2 ±0.19^{c}	0.56 ±0.01^{b}	22.9 ±0.62^{b}	34.9 ±0.91	2.04 ±0.10^{c}	0.96 ±0.05	0.50 ±0.05^{a}
MCB C8	13 ±0.78^{ab}	6.9 ±0.33^{cd}	0.49 ±0.02^{c}	23.1 ±1.11^{b}	32.6 ±1.13	2.49 ±0.22^{bc}	0.78 ±0.09	0.31 ±0.01^{b}
KKM 22	15 ±0.45^{a}	6.5 ±0.20^{d}	0.64 ±0.02^{a}	26.4 ±1.33^{a}	35.6 ±1.25	3.89 ±0.24^{a}	1.08 ±0.08	0.28 ±0.01^{b}
PBC123	12 ±0.35^{b}	7.3 ±0.18^{bc}	0.57 ±0.02^{b}	26.4 ±1.16^{a}	33.9 ±1.73	3.41 ±0.03^{a}	1.07 ±0.09	0.34 ±0.02^{b}
QH 1003	13 ±0.39^{ab}	8.3 ±0.20^{a}	0.56 ±0.01^{b}	28.3 ±0.92^{a}	34.0 ±1.09	3.52 ±0.21^{a}	1.04 ±0.06	0.32 ±0.02^{b}
p-Value	<0.05	<0.001	<0.001	<0.001	0.55^{ns}	<0.001	0.06^{ns}	<0.001

Mean values ± standard errors followed by the same letter are not significantly different, ^{ns} Not significant.

Number of leaves

The average number of leaves produced per plant after 70 DAS is shown in Figure 1. All clones produced leaves rapidly and showed a similar growth pattern. However, the number of leaves differed significantly between clones. KKM 22 consistently showed the highest number of leaves during the study period. This result is consistent with Kasran (1999) and Regazzoni et al. (2014) that KKM 22 showed vigorous canopy formation after only 1.5 years. After 70 DAS, KKM 22 (15 ± 0.45) had the highest leaf number, although it showed no statistical difference from QH 1003 (13 ± 0.39) and MCB C8 (13 ± 0.78). Conversely, the lowest leaf number was observed in UIT 1 (12 ± 0.48) and PBC 123 (12 ± 0.35).

Figure 1: Leaf production of five open-pollinated Malaysian Class 1 cacao clones and UIT 1

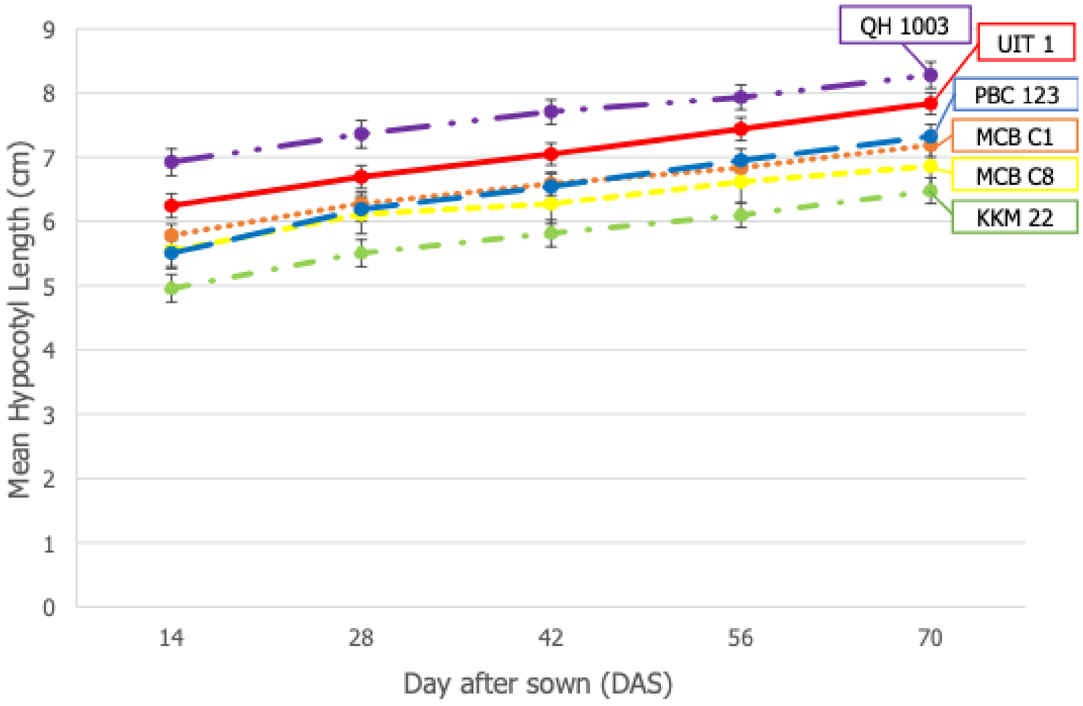


Leaves are the primary organs for performing photosynthesis. The more leaves exposed to sunlight, the higher the food production and the more vigorous the seedling growth. Seedlings with vigorous growth may have a higher capacity for rapid cell elongation and cell division, which is vital for rapid wound healing after grafting (Bose et al., 2019). KKM 22 had 25% more leaves than UIT 1 at 70 DAS. The difference in the number of leaves between the two clones may indicate that KKM 22 has more vigorous growth potential than UIT 1.

Hypocotyl Length

As shown in Figure 2, all clones displayed uniform growth of hypocotyl length. Hypocotyl length was significantly influenced by the different clones. Throughout the study, QH 1003 and UIT 1 had longer hypocotyl lengths, while KKM 22 had the shortest length. At 70 DAS, QH 1003 (8.3 ± 0.28 cm) hypocotyl length was 28% longer than KKM 22 (6.5 ± 0.20 cm). There was no statistical difference between the hypocotyl length of QH 1003 and UIT 1 (7.8 ± 0.17 cm) after 70 DAS.

Figure 2: Increase of hypocotyl length of five open-pollinated Malaysian Class 1 cacao clones and UIT 1



Grafting height can affect scion growth. In apples, it has been shown that higher grafting height can promote lateral branching, earlier fruiting, and higher fruit yield, while tree height decreases (Karlidag and Esitken, 2012). Similar results have been found in other fruit species such as quince, pear, and sweet cherry (Karlidag et al., 2016). Thus, well-branched trees provide desirable tree architecture and influence productivity. On the other hand, short trees are desirable because they are easier to manage, especially during harvest. In another report using Nagpur Mandarin as scion and Rough Lemon seedlings as rootstock, graft height had a positive effect not only on scion growth but also on dry weight, root length, and rootstock diameter. However, the effect depended on the interaction between graft height and graft length (Thokchom et al., 2019).

In addition, a longer hypocotyl length above the soil surface may protect grafted seedlings from disease infection and allow deeper planting while avoiding contact between the graft and the soil (Kleinhenz, 2018). With a longer hypocotyl, grafting can also be done quickly and efficiently (Yetisir and Sari, 2004). Therefore, the length of the hypocotyl is critical in rootstock selection because the scion is usually grafted below the cotyledon scars. Although greater grafting height could improve scion growth and development, it was reported that grafting success was not affected by grafting height (Chalise et al., 2014). Therefore, the result suggests that QH 1003 has an advantage over UIT 1 as a rootstock due to its longer hypocotyl length.

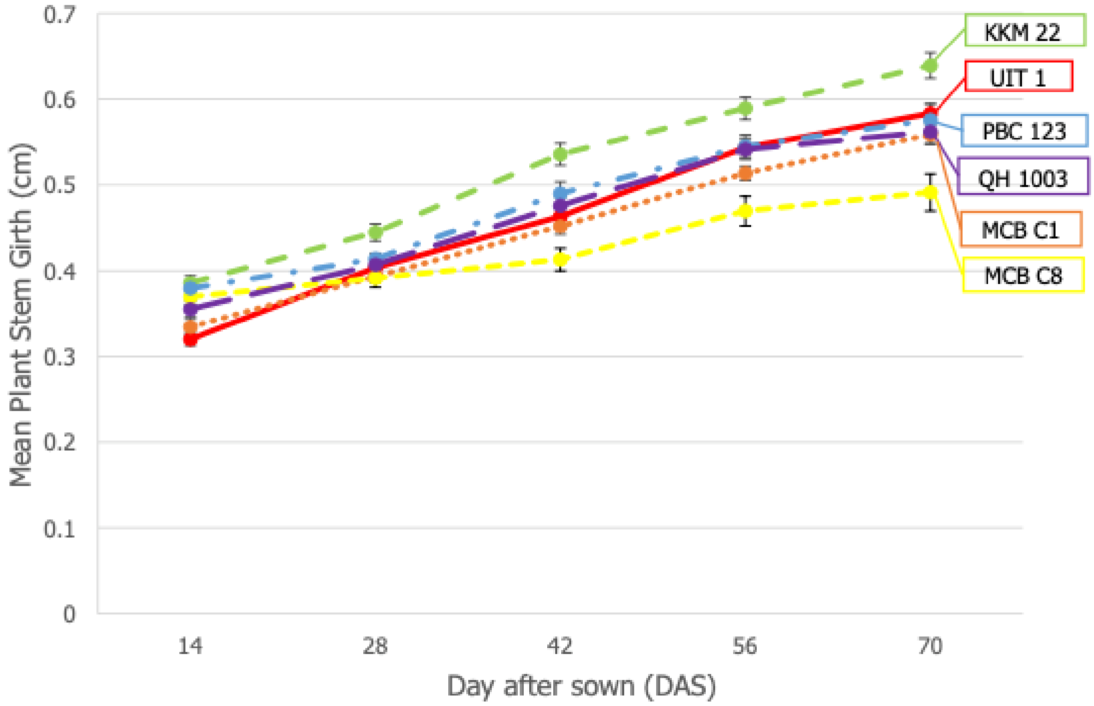
Stem Girth

All six clones showed a gradual increase in stem girth with three different growth patterns (Fig. 3). KKM 22 and MCB C8 showed the highest and lowest stem girth increase, respectively. The other four clones showed intermediate stem girth increase and were not significantly different from each other. Stem girth was significantly influenced by the different clones. A similar result was reported by Li et al. (2013), where genotype significantly affected stem diameter of cacao seedlings. At 70 DAS, KKM 22 (0.64 ± 0.02 cm) had the largest stem girth and followed by UIT 1 (0.58 ± 0.01 cm). The smallest stem girth was measured in MCB C8 (0.49 ± 0.02 cm).

Stem girth is important in rootstock selection because it can influence the success rate and survivable of grafted seedlings. For example, Mng'omba et al. (2010) reported that grafting on mango seedling rootstocks with thicker stem girth at the root collar had higher success and shorter time to graft-take. A similar result was also found in the pistachio seedling study, where it was shown that thicker stem girth improved budding (Ferguson et al., 2005).

In addition, stem girth can be used to determine plant vigor. Plants with vigorous growth have a higher stem girth than plants with less vigorous growth. Moreover, growth traits are positively correlated with yield in cacao (Mustiga et al., 2018). Thus, the result suggests that KKM 22 may be superior to UIT 1 as a rootstock due to its larger stem girth.

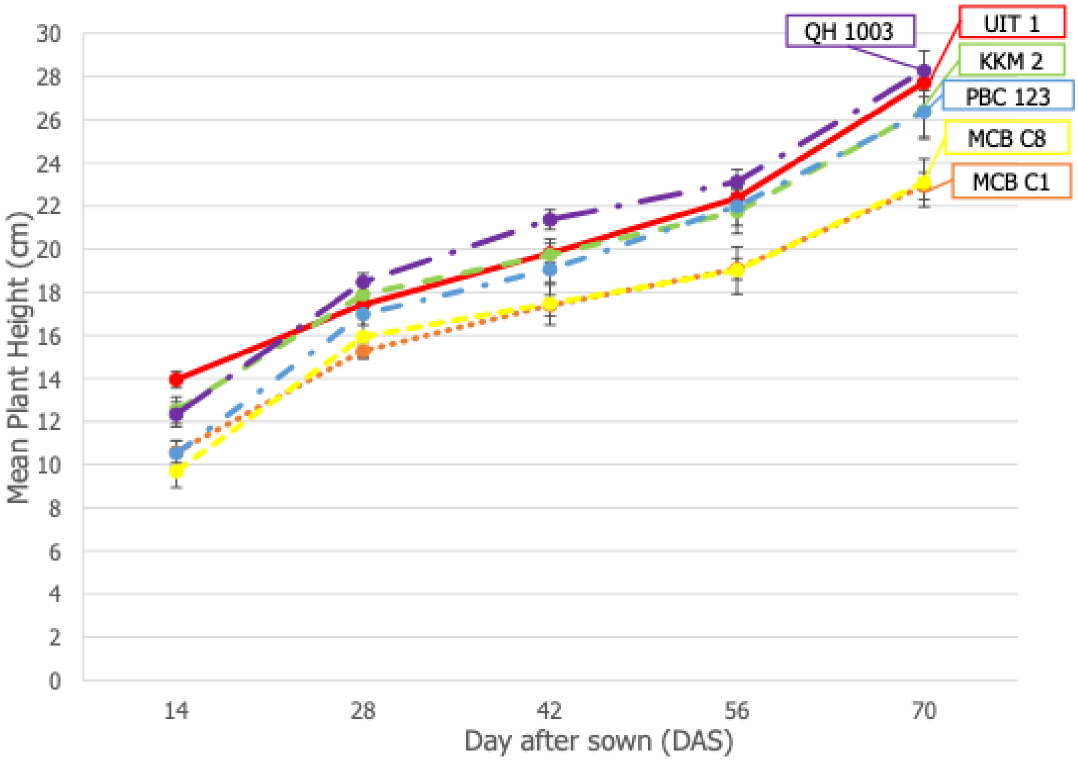
Figure 3: Development of stem grith length of five open-pollinated Malaysian Class 1 cacao clones and UIT 1



Plant Height
Figure 4 shows the increase in plant height over 70 DAS. Plant height was significantly affected by the different clones. QH 1003, UIT 1, KKM 2, and PBC 123 were among the clones that displayed a higher growth rate than the other two clones. At the end of the study, QH 1003 (28.3 ± 0.92 cm) recorded the highest plant height but was not significantly different from UIT 1 (27.7 ± 0.65 cm), KKM 22 (26.4 ± 1.33 cm), and PBC 123 (26.4 ± 1.16 cm). The lowest plant height was measured in MCB C1 (22.9 ± 0.62 cm).

Plant height is closely related to maturity and is a key determinant of a seedling's ability to compete for light (Moles et al., 2009). In several crops, including poplar, maize, and cassava, plant height correlates with high yield (Alcivar et al., 2007). In addition, greater height leads plants to expend more resources on stem development and maintenance (Falster and Westoby, 2003). Therefore, taller seedlings might accumulate more resources in the aboveground parts or use them more efficiently, which could benefit shoot growth and development.

Figure 4: Plant height growth of five open-pollinated Malaysian Class 1 cacao clones and UIT 1



In a study on mango, rootstock height was reported to have a significant effect on grafting success and scion growth (Yadav et al., 2019). It was found that greater rootstock height shortened time to bud break and increased scion uptake and survival. In addition, shoot length and girth and the number of leaves per plant were greater when grafted onto a taller rootstock. Na and Bv (2017) reported a similar result in mango, although the effect of rootstock height on scion growth depended on the length of the grafted scion. Nevertheless, the results found in this work may suggest that KKM 22 has an advantage as a rootstock due to its greater plant height and stem girth.

Shoot Dry Weight

At 70 DAS, the different clones had significant variations in shoot dry weight (Table 1). The highest shoot dry weight was observed in KKM 22 (3.89 ± 0.24 g) but was not significantly different from QH 1003 (3.52 ± 0.21 g) and PBC 123 (3.41 ± 0.03 g). However, these three clones were significantly different from UIT 1 (2.78 ± 0.12 g). On the other hand, MCB C1 (2.04 ± 0.10 g) had the lowest dry shoot weight.

Shoot dry weight indicates the production of biomass by photosynthesis. Plant height and leaf number can affect shoot dry weight. Shoot dry weight can be influenced by genotype and efficiency of nutrient uptake from soil. Ribeiro et al. (2008) claimed that different cacao genotypes have different nitrogen uptake efficiency, which affects shoot dry weight. Accordingly, the highest shoot dry weight of KKM 22 could indicate higher efficiency in nutrient uptake and biomass conversion.

Root Length and Root Dry Weight

Roots play an essential role in absorbing water and nutrients from the soil. A vigorous root system leads to a healthy and productive plant. The longest root length and highest root dry weight were measured for UIT 1 (35.9 ± 1.62 cm) and KKM 22 (1.08 ± 0.08 g), respectively. However, they were not significantly different from the other clones. The result could be due to the limited space for root growth because all seedlings were planted in the same polybag of similar size and the same amount of soil. Seedlings raised in larger polybags could have greater root length and root dry weight. Haldankar et al. (2014) reported that grafted mangoes had longer taproots that were raised in larger bags. They provided more space for root expansion and development and ensured continuous moisture supply for better root growth. A similar result was also obtained in a study with rubber seedlings (Salisu et al., 2018). The polybag size in which each seedling is planted can be a limiting factor.

Root to Shoot Ratio

The ratio of roots to shoots (R:S) indicates the orientation of plant growth, whether the plant distributes its growth to the above or below ground. Appropriate distribution of resources is particularly important for seedling survival (Mašková and Herben, 2018). A low R:S indicates that more biomass is produced in the shoots, while a higher R:S indicates more biomass in the roots. In this study, R:S was calculated based on dry weight at 70 DAS. The highest value was obtained in MCB C1 (0.50 ± 0.05), while KKM 22 (0.28 ± 0.01) had the lowest value. Thus, the difference between the R:S of these two clones was 79%. However, KKM 22 was not significantly different from the other four clones.

Ideal R:S varies between plant species and changes during the life cycle of the plant. In general, R:S decreases as the plant grows, as resources are invested in aboveground structures (Mašková and Herben, 2018). In addition, R:S is also influenced by the environment, such as fertilization and abiotic stress. Famuwagun and Agele (2010) reported that R:S ranged from 0.25 to 0.39 for cacao seedlings raised in a nursery with different plant spacing and seeding methods. In this study, the R:S for all clones were within this range, which could indicate normal growth, except for MCB C1. In addition, a high R:S value also indicates the ability to take up more nutrients from the soil and contribute to the increase in aboveground biomass (Bláha and Argon, 2019). However, MCB C1 did not exhibit this characteristic as it had the lowest shoot dry weight.

CONCLUSION

This study showed that the open-pollinated seedlings of five Malaysian Class 1 cacao clones and UIT 1 clone differed significantly in growth characteristics, except for root length and root dry weight. However, KKM 22 and QH 1003 exceeded or were comparable for all evaluated growth parameters of UIT 1. Seedlings with such growth characteristics theoretically offer superior rootstock characteristics. Therefore, the two clones could be proposed as potential rootstocks to replace UIT 1. Nevertheless, it is strongly recommended to conduct further studies on the compatibility between rootstock and scion and on the growth performance of the grafted plants in the future.

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