ノート (Note)

Growth of *Melia volkensii* Gürke saplings propagated by root cuttings

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Key words: clonal propagation, fast-growing tree, *Melia volkensii*, tree breeding

Introduction

Melia volkensii Gürke is a fast-growing tree species native to the drylands of eastern Africa, from Mt. Kenya to southern Tanzania. It is drought-tolerant and produces high-quality timber used for construction and furniture (Muok et al. 2010). In some areas, especially in Kenya, timber production from this species is a growing industry, and the markets and value chains of *M. volkensii* in the timber industry have been reported (Luvanda et al. 2015, Muthike and Gighiomi 2020).

To develop timber production and increase the incomes of local farmers, the Kenya Forestry Research Institute (KEFRI), along with some international cooperation partners, introduced this tree species to local farmers in the 1990s (Muok et al. 2010). In addition to supporting local tree farmers, KEFRI is actively involved in the breeding of *M. volkensii* in collaboration with the Japan International Cooperation Agency (JICA) and Forest Tree Breeding Center of Japan (FTBC) (Kamondo et al. 2016). They selected candidate plus trees and found that the growth of some improved varieties was approximately 17% higher than that of wild trees (Matsushita 2018). This is expected to increase the economic benefits to local communities in Kenya. Saplings of *M. volkensii* used for afforestation are usually grown from seeds (Muok et al. 2010). In order to increase the plantation of this species, more seeds and mother trees are needed. Further, the mother trees should be clones propagated from improved varieties with superior genetic traits.

Clonal trees are propagated from improved varieties through the grafting method, which requires special technical expertise and additional efforts to grow rootstocks (Kamondo et al. 2016). Hanaoka et al. (2016) suggested that root cutting propagation is an alternative, cost-effective method for producing clones of *M. volkensii*. They proposed convenient criteria for root cuttings; according to their study, the formation frequency of adventitious buds was 77% in roots with a cut edge diameter >15 mm and fresh weight >20 g. However, the growth process of saplings propagated by root cuttings remains unclear.

In the present study, we propagated *M. volkensii* saplings by root cutting and investigated the growth process to examine the possibility of production of clonal saplings.

Materials and Methods

We collected the root materials for propagation from *M. volkensii* saplings that were used by Hanaoka et al. (2016) and grown for approximately six years in polyethylene pots in a temperature-controlled greenhouse with natural light in Hitachi, Ibaraki Prefecture, Japan (36° 41′ N, 140° 41′ E). We attempted to collect more than one root material from each donor sapling and obtained 74 root materials from 30 saplings. The length and weight of the root materials were measured before burying them in pumice (Setogahara Kaen, Gunma, Japan) in a way that their proximal ends (5 mm) were exposed above the ground. We conducted root cutting propagation on June 4, 2020, in a wind-protected greenhouse with natural light and temperature at the Iriomote Tropical Tree Breeding Technical Garden on Iriomote Island, Okinawa Prefecture, Japan (24° 19′ N, 123° 54′ E).

After approximately two months, on July 31, 2020, 45 out of the 74 root materials developed adventitious buds. We randomly selected 23 out of these 45 root materials and transplanted them into 9 cm × 30 cm polyethylene pots using a new medium consisting of commercial gardening soil (Oishi Corporation, Fukuoka, Japan) mixed with an equal volume of

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pumice. If the materials had multiple adventitious buds, we removed the excess buds, leaving only one. The transplanted materials were cultivated under a sun-shade tree for seven months. We measured the height of the saplings every month.

To evaluate the growth process, the heights and days after propagation were fitted to the Gompertz model. To examine the relationship between the heights at the seventh month and the fresh weights before propagation, a linear regression mixed-effect model was used. The parameters of the models were estimated by Bayesian analyses, specifying the individual number of materials as a random effect. Statistical analyses were conducted using R ver. 3.6.2. (R Core Team 2019) with the rstan library ver. 2.21.2. (Stan Development Team 2020) and the brms library ver. 2.14.4. (Burkner 2017, Burkner 2018).

Results

The mean length of the root materials was 17.6 cm (SD 5.9), ranging from 8.0–41.0 cm, and the mean fresh weight was 32.9 g (SD 17.1) , ranging from 10.0–86.1 g. During the first two months of root cutting propagation, 3 out of the 74 materials were damaged by slug feeding on the adventitious buds, and one of the three damaged materials failed to develop new buds. Eventually, the formation frequency of adventitious buds was 61% (45 of the 74 materials) . During the subsequent seven months of cultivation, death was recorded in 22% (5 of the 23 materials) of the materials, one material showed cessation of growth when its height reached 2 cm, and 74% of the materials (17 out of 23) showed normal development. The frequency of intact saplings from root cutting propagation was estimated to be 45% (the formation frequency of adventitious buds (61%) × the frequency of normal saplings after cultivation (74%)). The mean height at the end of the seventh month was 24.5 cm (SD 8.2), ranging from 2–35 cm.

Fig. 1 shows the growth of saplings and the fitted curve generated using the Gompertz model. The fitted curve reached an asymptote in the sixth month. Fig. 2 shows the relationship between fresh weight before propagation and the height at the end of the seven-month cultivation period, as well as the regression line. The slope of the regression line was estimated to be 0.07, with 95% Bayesian confidence intervals ranging from − 0.31 to 0.46.

Discussion

In this study, the frequency of adventitious bud development was found to be 61%, which was lower than the 77% reported by Hanaoka et al. (2016). We used the root materials with a fresh weight between 10.0–86.1 g; some of them were less than 20 g in weight and did not match the criteria of Hanaoka et al. (2016). The use of unsuitable root materials might be responsible for the lower frequency of adventitious buds observed in our study.

In propagation by root cuttings, it is known that larger root materials produce adventitious buds more effectively (Del Tredici 1995, Ky-Demble et al. 2010, Snedden et al. 2010, Hanaoka et al. 2016). Additionally, Snedden et al. (2010) reported that root materials of trembling aspen (*Populus tremuloides* Michx.) with larger diameters produce taller saplings compared to those with smaller diameters. On the other hand, in clonal propagation of *Detarium microcarpum* Guill. & Perr. by root cutting, shoot growth of longer root materials was similar to the shorter ones (Ky-Demble et al. 2010). Shoot growth after root cutting propagation may vary depending on the plant species rather than on the size of root material used for propagation. In this study, the slope of the regression line between plant height after seven months of

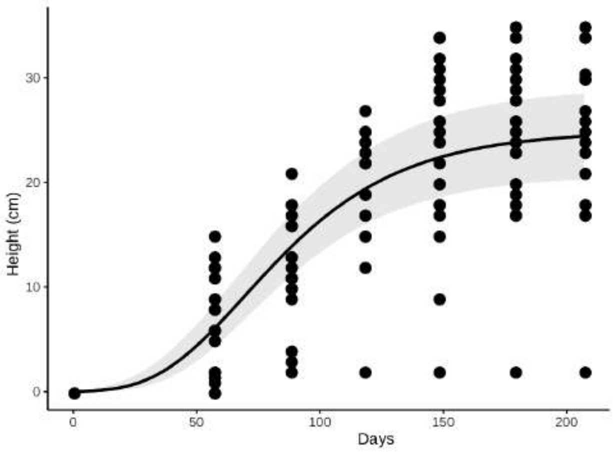

Fig. 1. Growth of *Melia volkensii* saplings propagated by root cuttings. The curve was generated using the Gompertz model. The gray band indicates 95% Bayesian confidence intervals of the fitting curve.

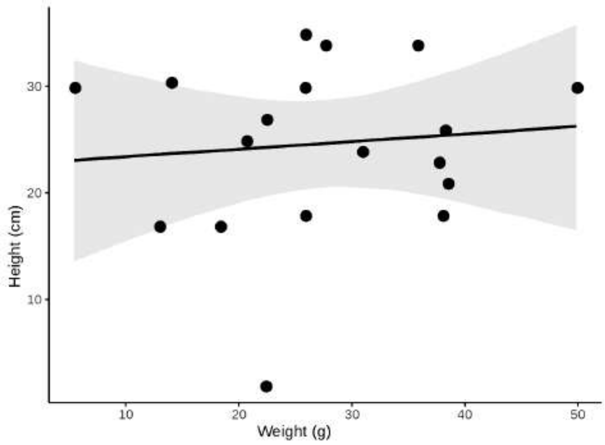

Fig. 2. Relationship between fresh weight before root cutting propagation and height at the end of seven months of cultivation. The line is their regression line. The gray band indicates 95% Bayesian confidence intervals of the regression line.

cultivation and fresh weight before root cutting propagation was estimated to be small (0.07), ranging from negative (− 0.31) to positive values (0.46). We observed that the use of larger root materials of *M. volkensii* did not always result in the production of larger saplings.

In Kenya, *M. volkensii* saplings produced from seeds are used for silviculture and grow up to a height of 30 cm in a period of three to four months (Muok et al. 2010). However, in the present study, a period of at least five months was necessary for the saplings propagated by root cuttings to achieve growth to a size considered adequate for out-planting. The slower growths observed in this study indicated that the environmental conditions on Iriomote Island were less suitable for the growth of *M. volkensii* saplings compared to the conditions in Kenya. The temperature and precipitation on Iriomote Island were higher than those in Kitui, Kenya (1° 22′ S, 38° 1′ E); the nursery in the KEFRI has a mean monthly temperature of 22.7 ℃ and a mean monthly precipitation of 65 mm (Chahilu and Sairinji 1995), whereas the mean monthly temperature and precipitation on Iriomote Island were 26.4 ℃ and 201 mm, respectively (Japan Meteorological Agency 2020). *M. volkensii* is a tree species that shows remarkable adaptation to drylands (Muok et al. 2010, Kamondo et al. 2016). Our results indicated that extremely high rainfall was not suitable for the growth of *M. volkensii* saplings.

In the present study, 45% of the root material achieved growth without being damaged. Even under unsuitable conditions, as seen in Iriomote Island, we could produce a certain number of clonal saplings of *M. volkensii* using the root cutting method. Thus, we expect higher adventitious bud formation frequency and better sapling growth of *M. volkensii* under more suitable conditions. To verify the practical utilization of *M. volkensii* root cutting propagation, studies in areas suitable for the growth of this tree species, such as Kenya, are required.

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