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Full Length Research Paper

Rootstock growth and development for increased graft success of mango (*Mangifera indica*) in the nursery

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The graft success of seedlings depends on rootstock size and the skills of grafters. A study was carried out to evaluate the effects of rootstock diameter (at root collar), skills and experience of grafters on mango seedling thinning and grafting. Skilled and unskilled grafters were involved in grafting mango plants for at least three consecutive months. Different rootstock sizes based on root collar diameter of mango seedlings were assessed. The results showed significant differences in graft-take among three groups of nurserymen. The skills and experience of grafters had a significant effect on graft success. The late emerging seedlings showed higher increase in height and diameter after thinning. A higher survival (> 75%) and reduced time to graft-take (19 days) were achieved with thicker rootstocks (0.7 cm) than thinner rootstocks (0.3 cm). The early emerging plants produced more leaves than late emerging plants after grafted. It was concluded that a higher graft-take can best be achieved with skilled grafters and thicker rootstocks.

Key words: Apomisis, *Mangifera indica*, nucellar, nurse, polyembryony, zygote.

INTRODUCTION

The supply of good quality and sufficient mango (*Mangifera indica*) planting materials in the tropics hinges on the development of good nursery management practices which include propagation methods. Generally, improvement in the supply of good quality planting materials would ensure good tree survival and establishment in the field. Grafting is a common and preferred vegetative propagation method for mango trees (Bally, 2006). The graft success can be improved when rootstock selection is considered and based on desirable growth attributes of rootstocks (Simons, 1987), the skills and knowledge of grafters (Akinnifesi et al., 2008).

Rootstocks play an important role for tree survival and establishment in the field, tree productivity and dwarfing of grafted fruit trees (Mng'omba et al., 2008). Seedling rootstocks with desirable attributes such as rapid growth (in height and diameter) could reduce the 'waiting period' to grafting time. Furthermore, proper alignment of scion and rootstock cambium tissues could determine the graft success (Pina and Errea, 2005). The skills of grafters in aligning cambium tissues together are important in reducing graft failure.

Mango possesses either polyembryonic (apomictic) or monoembryonic seed (Bally, 2006). A polyembryonic seed contains two or more embryos and they are zygotic (sexual) and nucellar (asexual or maternal) embryos. Monoembryonic seeds contain only a single zygotic embryo (a cross between maternal and paternal parents that is nurse or zygotic seedlings). According to Bally (2006), zygotic and nucellar seedlings are both used as rootstocks. The use of nucellar seedling rootstocks would ensure fruit orchard uniformity unlike nurse or zygotic seedlings. Nucellar seedlings are identical to the parent plant (Xiang and Roose, 1988; Garcia et al., 1999; Ruiz et al., 2000), but differences could be due to somatic variations (Frost and Soost, 1968).

Identification of zygotic seedlings (referred to as off-type) from nucellar seedlings (true to type) is important for maintaining genetic homogeneity which ensures field uniform performance of rootstocks (Rao et al., 2008). However, this has not been easy in many polyembryonic seeds (Frost and Soost, 1968) and this includes mango

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seeds. Identification of the two types of seedlings requires the use of genetic markers, but the use of these markers can be expensive, especially for many local or ordinary nurseries.

The use of nucellar seedlings can increase the availability of rootstocks unlike zygotic seedlings. The polyembryonic seeds produce numerous nucellar seedlings, although distinguishing nucellar from zygotic seedlings has been a setback. At Pedro Sanchez (Snr) research nursery of the World Agroforestry Centre (ICRAF) in Makoka nursery, remarkable differences in mango seedling emergence and growth has been observed. The first or early seedling to emerge per seed has been taller and thicker than late emerging seedlings. It was assumed that most of the late emerging seedlings were nucellar since they were numerous. It is unlikely that a polyembryonic mango seed can produce as many zygotic seedlings as nucellar ones. According to Bally (2006), only twin or triplet zygotic seedlings can be produced from a single mango seed in rare cases.

Graft-take or success in *Uapaca kirkiana* (wild loquat) has been shown to depend on the skills of grafters and the time of the year (Akinnifesi et al., 2008). Skills in grafting trees and thinning seedlings are gained through practice, but it is unclear whether this graft success would also depend on the level or duration of training nurserymen. It was observed that the speed of grafting fruit trees strongly depends on practice, generally known as ‘green finger’.

Apart from the skills of grafters, the effects of different rootstock sizes on graft success remain unclear. According to Hartmann et al. (1997), 10 mm thick (diameter) is an ideal rootstock size for grafting fruit trees. However, this could depend on the species. For *U. kirkiana*, it is often hard to find scions that match the small size of one year old rootstock. Based on present knowledge, the effect of grafting skills, knowledge of nurserymen and rootstock sizes on mango seedlings at grafting have not been assessed. Therefore, the objective of this study was to assess the effects of rootstock sizes and skills of nurserymen on graft success of mango plants.

MATERIALS AND METHODS

Study site description

The study site was Pedro Sanchez (Snr) research nursery of the World Agroforestry Centre (ICRAF) in Makoka, there after referred to as ‘ICRAF-Makoka Nursery’ located at the Makoka Agricultural Research Station in Malawi (latitude 15° 30’ S and longitude 35° 15’ E) and lies 1029 m above sea level. It has an annual unimodal rainfall with an average range of 560 - 1600 mm and temperatures vary between 16 and 32°C (Akinnifesi et al., 2004).

Seed collection and management

Ripe mango fruits were bought from local markets around Zomba in Malawi in December 2007. They were taken to ICRAF-Makoka nursery where they were processed to extract seeds. Disease-free seeds were sown in polythene bags (1 litre-bag size) containing a growing medium which comprised a mixture of top soil, rice husk and fine sand (3:1:1). The experiment was left on the open ground for observations. Watering was done whenever necessary and almost daily during the dry months. Super actellic (Novactellic 500 EC at the rate of 1 ml/3 l) was applied every month. Pot weeding was done by hand throughout the growth period.

Seedling thinning

Each seedling (nurse or nucellar) was thinned into a separate polyethene bag (1 litre-bag size) three months after sowing. They were thinned into polythene bags containing the same growing medium composition used for mango seed germination. The nurserymen were divided into three groups based on the number of years in nursery management practices (experience). These three groups of nurserymen formed a random component drawn from a population of nurserymen and these groups included either (1) more than ten years of experience, (2) up to three years of experience or (3) less than one month experience in nursery management practice. There were at least three individuals per group and the last group was hired a month before the beginning of the experiment and had a quick orientation on many aspects of fruit tree nursery management practices.

The experimental design was a randomized incomplete block with three groups of nurserymen as a random component and four replications. Each group of nurserymen thinned 300 seedlings into new polythene bags per day and water was immediately applied to the seedlings. Growth and survival of seedlings were recorded once per month.

Graft success

Grafting was carried out ten months after sowing and the graft success was evaluated for five months (September, October, November, January and February 2009). The same nurserymen also formed three groups of grafters (random component), namely: (1) more than ten years of experience, (2) up to three years of experience or (3) less than one month experience (unskilled grafters) in grafting. There were four individuals per group and whip or splice grafting technique was used. Disease-free scion wood (previous year’s growth) was collected from actively growing mango trees (Haden cultivar).

The experiment was randomized incomplete block design with four replications. Each individual nurseryman grafted a total of 500 mango plants per month and the experiment was repeated for five months. Graft success per group was recorded monthly.

Rootstock size

Graft success was evaluated using four different rootstock sizes. Rootstocks were selected from early emerging seedlings (probably nurse) and late emerging seedlings (most of nucellar seedlings). For the first group of seedlings (nurse), the rootstock size was either 7, 5, 4, or 3 mm thick at root collar diameter (RCD). For the late emerging seedlings, the sizes were 5, 4.5, 4 or 3.5 mm at RCD. Selection of rootstock sizes was based on the availability of each group of rootstock size adequate for a treatment. Since rootstock size is continuous, it was difficult to obtain uniform rootstock size. For the early emerging seedlings, inclusion of any rootstock into each group was based on ± 0.4 mm departure or difference from the actual group size, while the late emerging seedlings was based on ± 0.2 mm from the actual rootstock size.

Mango scions of similar thickness to the rootstocks were collected from Haden mango cultivar. This was to avoid graft failure problem which could be due to differences in scion and rootstock sizes or differences in cultivars. Grafting was done by skilled grafters only (those with more than ten years of experience). The experimental design was a randomized complete block design with three replicates. There were 100 grafted mango plants per rootstock size. The early emerging seedling rootstocks were grafted in October 2008, while the late emerging seedlings (mostly nucellar seedlings) were grafted in November 2008.

Statistical analyses

Data on seedling height, survival, days to graft-take and number of leaves were collected. Growth increase in root collar diameter and seedling height was calculated by subtracting the initial from final measurements. The data on rootstock sizes for the early emerging seedlings (nurse) and late emerging seedlings were analyzed separately due to differences in grafting time and also rootstock sizes. The data were analyzed using mixed models (SAS package). In this analysis, groups of nurserymen were treated as random component as they were drawn from a large population of nurserymen.

RESULTS

Seedling thinning

There were no significant differences ($P \leq 0.05$) in seedling survival for the early and late emerging seedlings (data not shown) irrespective of skills or experience of nurserymen. More than 90% seedling survival was obtained from early and late emerging seedlings.

Increase in root collar diameter (difference between the initial and final diameter) was not significantly different and also the interaction between the type of seedlings and groups of nurserymen (skilled or unskilled). Significant differences ($P \leq 0.0026$) were obtained between seedling types with respect to an increase in root collar diameter (Figure 1). For nurserymen with more than ten years of experience, a greater increase (0.14 cm) was obtained from nucellar compared to nurse seedlings (0.11 cm).

Significant differences ($P \leq 0.0001$) between seedling types were obtained with respect to increase in height (Figure 2), but no significant differences were obtained among the three groups of nurserymen and the interaction between groups of nurserymen and seedling type. A similar trend showed that increase in height for the late emerging seedlings (2.3 cm) was more than the increase obtained from the early emerging seedlings (1.6 cm). A similar trend was also obtained for the root collar diameter (Figure 1).

Graft success

Significant differences ($P \leq 0.001$) were obtained among the groups of nurserymen, time (months) and their interactions. Skilled grafters showed superiority over unskilled group of nurserymen (one month experienced grafters) across the time (Figure 3). The best graft success was achieved in November where skilled nurserymen with ten years experience had 97% success compared to 72% success for nurserymen with less than one month experience. There was a decline in graft success from December to January (Figure 3). There was an improvement in graft success for unskilled nurserymen in September and also in November (Figure 3).

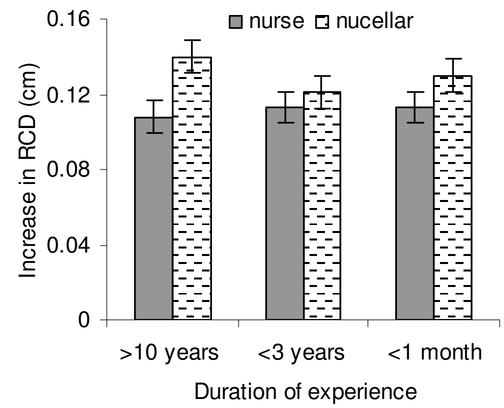

Figure 1. Increase in root collar diameters (RCD) of mango (M. indica) seedlings as influenced by nurserymen's experience in seedling thinning. Bars represent standard errors.

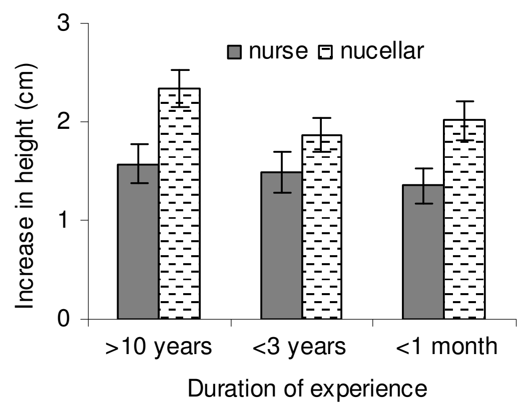

Figure 2. Increase in mango (*M. indica*) seedling height as influenced by skills or experience of nurserymen in thinning seedlings. Bars represent standard errors.

Rootstock size for grafting

Significant differences ($P \leq 0.0001$) were obtained among treatments (rootstock sizes) with respect to time to graft-take (Figure 4). Thicker rootstocks (0.7 cm diameter at root collar) showed early graft-take unlike the thinner

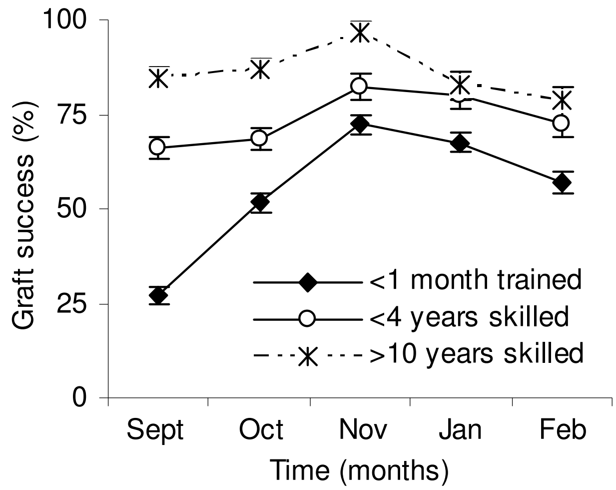

Figure 3. Percentage of successful mango (*M. indica*) grafts by three different groups of nurserymen during five months.

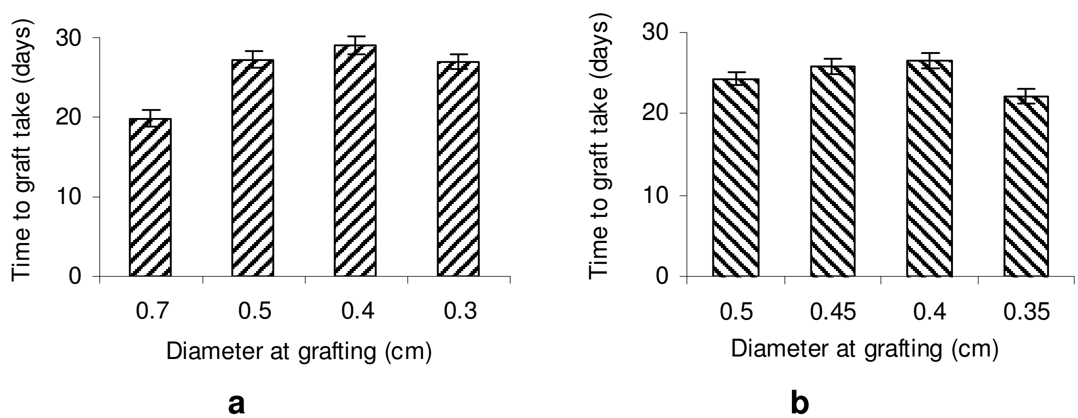

Figure 4. Duration to graft success (graft-take) of grafted mango (*M. indica*) plants with different rootstock sizes of (a) early emerging seedlings and (b) late emerging seedlings.

rootstocks (Figure 4a). There were no significant differences between treatments for the late emerging seedlings (Figure 4b).

There were significant differences ($P \leq 0.0001$) in plant height with respect to rootstock sizes and across time, but their interactions were not significant (Figure 5a). Thicker rootstocks (0.7 cm) were also tall across time compared to the thinner rootstocks (Figure 5a). For the late emerging seedlings, there were significant differences among plant heights ($P = 0.0034$) and across time ($P \leq 0.0001$), but not their interactions (Figure 5b).

Significant differences ($P \leq 0.0001$) were obtained among treatments and time ($P \leq 0.0001$) and the interaction between treatment and time ($P \leq 0.0095$) with respect to the survival of grafted plants. A trend showed that rootstocks with thicker root collar diameters had a higher percentage survival (73%) than the thinner rootstocks (60%) at 90 days after grafting (Figure 6).

Significant differences ($P \leq 0.0001$) among treatments and time ($P \leq 0.0001$) were obtained, but not with the interaction between time and treatment with respect to the number of leaves per grafted plant (Figure 7a). A general trend indicated that plants with thicker root collar diameters at grafting had more leaves (at least ten leaves per plant 90 days after grafting) than the thinner grafted plants(eight leaves per plant). Similarly, early emerging plants with thicker diameters produced more leaves than those with thinner diameters (Figure 7b). A separate data analysis on the final seedling height showed that the early emerging seedlings were significantly different ($P \leq 0.0001$) among the three groups of nurserymen (Figure 8a), but not for the late emerging seedlings (Figure 8b).

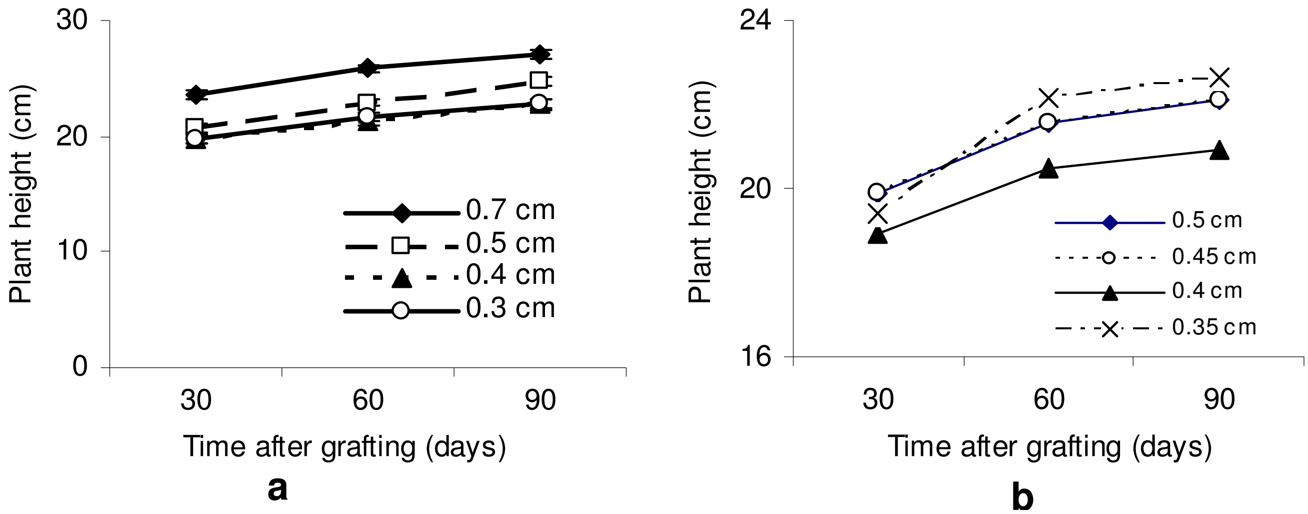

Figure 5. Growth of grafted mango (*M. indica*) plants (a) derived from early emerging seedlings and (b) late emerging seedlings at three different times

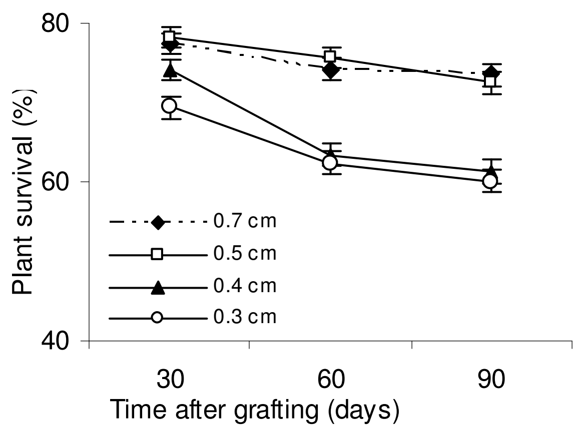

Figure 6. Survival (%) of grafted mango (*M. indica*) plants at 30, 60 and 90 days after grafting

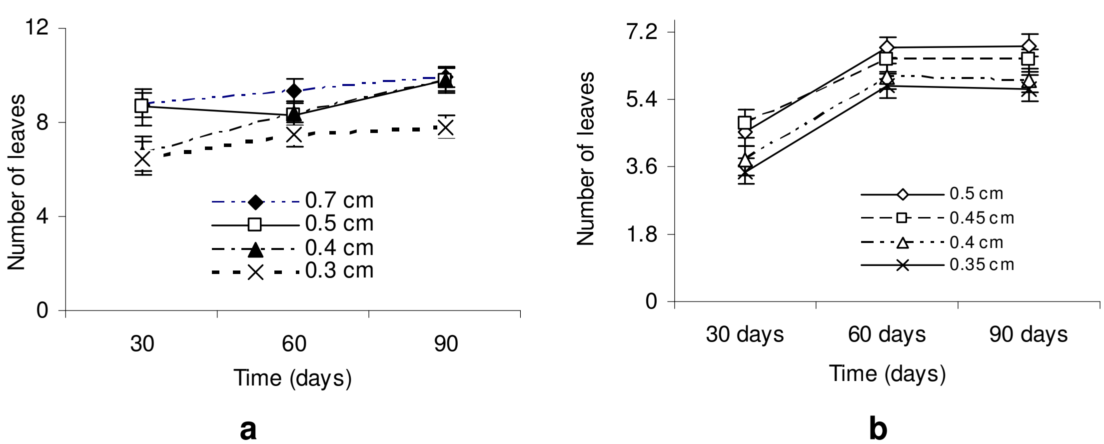

Figure 7. Number of leaves of grafted mango (*M. indica*) plants with different rootstock sizes (root collar diameter) at grafting (a) early emerging seedling (b) late emerging seedling.

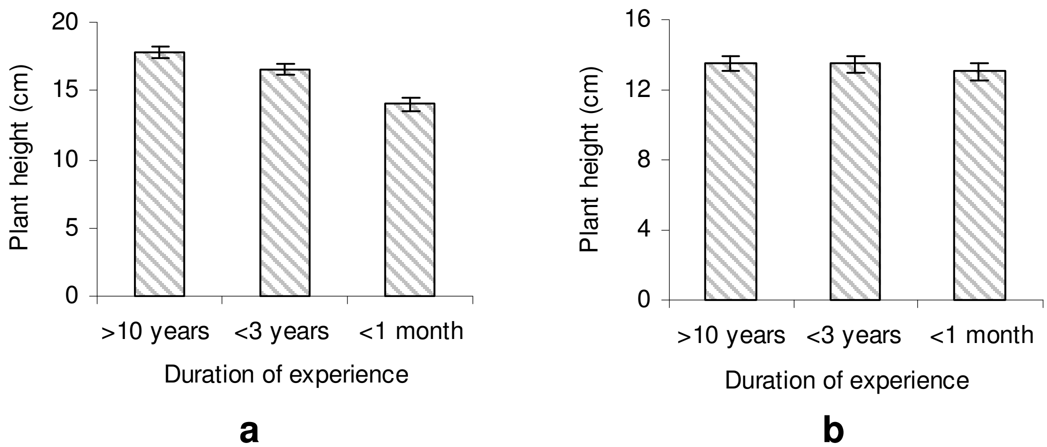

Figure 8. Plant heights of mango (*M. indica*) seedlings ten months after thinning (a) early emerging seedlings (b) late emerging seedlings.

DISCUSSION

A greater increase in plant root collar diameter and height for the late emerging seedlings, particularly for nurserymen with more than ten years, compared to the early emerging seedlings after thinning indicated growth potential of nucellar seedlings. It showed that growth of nucellar seedlings could have been suppressed by the early emerging seedling. Therefore, thinning seedlings is important because it enables late emerging seedling to grow. It was hypothesized that improving the growing medium could further improve growth of nucellar seedlings to attain a desired size for grafting. According to Bally (2006), a desirable rootstock thickness for grafting should be within the range of 6 - 15 mm diameter and 100 - 200 mm long. Hartmann et al. (1997) reported at least 10 mm as a desirable rootstock thickness for grafting fruit trees. In the present study, early emerging seedlings were within this root collar diameter range and this could be attributed to the success in grafting and survival. Furthermore, both seedling types attained desirable heights for grafting.

The seedlings (nurse and nucellar) used in this study were ten months old from sowing and it is likely that the late emerging seedlings could attain the right size (stem diameter) for grafting after more than 12 months. There is a need for an improvement in the growing medium to achieve rapid growth. In this study, fertilizer was not applied as most local nurserymen do not apply fertilizer to boost the growth of rootstocks for grafting. According to Mhango et al. (2008), application of fertilizer to the growing medium improved the growth of *Uapaca kirkiana* seedlings with respect to root collar diameter, but not the heights. Tyree et al. (2009) also reported that fertilization increased seedling foliage and roots. Growth increase is important, especially when nurserymen want to have rootstocks ready for grafting within a year. Mango rootstocks grafted at less than 5 mm thick did not promote the overall survival of grafted plants as well as plant height, especially for the early emerging seedlings. In this study, a general trend showed that grafts with thicker rootstocks retained more leaves that those with thinner rootstocks. It can be assumed that the low photosynthesis due to a few leaves could negatively impact graft survival.

Early emerging seedlings (nurse) had insignificant increase in plant height and root collar diameter after seedling thinning. The insignificant differences in plant height for the late emerging seedlings indicated uniformity in growth and this confirmed that nucellar seedlings could bring tree uniformity if used as rootstocks. Because of a long ‘waiting period’ before rootstocks are grafted, an improvement in growth rate to attain a desirable size, especially stem diameter within a year is important. This is because there is a cost incurred in keeping rootstocks in the nursery for a long time.

Generally, grafted trees planted during the rainy season (planting seasons) have a high chance of early survival and establishment. Based on the present findings, it is easy to achieve desirable plant height within a year, but difficult to achieve desired rootstock thickness, especially for the nucellar seedlings. Achieving a one-year cycle for mango planting materials could be cost effective to the nurserymen. Therefore, it is important to optimize growth of late emerging seedlings as they are numerous compared to the nurse seedlings.

Thinning seedlings is an important step in improving the quality of grafted plants. From this study, early thinning of seedlings improved growth of nucellar seedlings and this is attributed to little or no growth suppression by the early emerging seedling. It is also a stage where coiled and/or twisted seedlings are discarded. This is because the use of such poor rootstocks can adversely affect growth and productivity of the fruit orchard. Thinning seedlings presents an opportunity to examine root and shoot growth structure of seedlings. The present study showed that thinning mango seedlings was not as difficult as grafting apart from identification problem of nucellar and zygotic seedlings. However, careful thinning of seedlings must be observed to avoid damaging the seedlings.

From this study, the skills of nurserymen had less impact on seedling growth after thinning. The nurserymen with less than one month training had minimal seedling damage during thinning. The lack of uniformity for the zygotic (nurse) seedlings was an over-riding factor that caused significant differences in plant height among the three groups of nurserymen after seedling thinning.

The present study showed that January and February were not the right time for grafting mango plants as there was a decline in graft success. This could be attributed to the heavy rainfall received during these months as the experiment was deliberately placed on the open ground. A few nurserymen can afford a greenhouse and this was the reason we observed performance of grafted mango plants under the open ground condition. The poor drainage (wet feet) in the growing medium adversely affected the survival of grafted mango plants.

Although it was difficult to distinguish nurse from nucellar seedlings, the use of nucellar seedlings would be ideal as they are many and ensure tree uniformity as shown in Figure 8b. Identification of nucellar seedlings from zygotic seedlings in many polyembryonic seeds has been difficult. According to Das et al. (2005), orange zygotic embryos are large and occupy the most space, while nucellar embryos are tiny. In this study, it was also observed that the early emerging seedlings occupied most of the space and were bigger than the late emerging seedlings. The number of seedlings produced per polythene bag ranged from two to ten. Although twin or even triplet zygotic seedlings are possible in mango seeds, the bulk of the seedlings are nucellar seedlings.

Though, roguing zygotic seedlings would ensure orchard uniformity, this might lead to difficulty in identifing zygotic twin or triple zygotes (Rao et al., 2008). Based on present knowledge, there are no genetic or morphological studies done to characterize the nucellar and zygotic mango seedlings. The use of genetic markers and morphological observations could be useful to guide nurserymen in selecting nucellar seedlings from zygotic seedlings.

Nurserymen must choose nucellar seedlings for rootstocks because of their uniformity, but discard zygotic seedlings (off-types) because of their variability in characteristics such as internode length, leaf size, shape, color, growth rate, foliage density and branching. Screening the germinating embryos at an early stage on the basis of morphological origin might be useful (Das et al., 2005).

This present study has shown that the days to graft-take decreased as the root collar diameter increases for the early emerging seedlings. However, this trend was unclear for the late emerging seedlings and maximum root collar diameter size attained was 5 mm thick. This suggested that at 5 mm diameter or lower, the time taken for successful graft-take varied. The study also showed that graft success depends on the experience of grafters. The present findings agreed with the findings of Akinnifesi et al. (2008) who reported that experience or skills of grafters are important. This is an indication that grafting is more of an art than science which involves proper aligning of vascular tissues together for a quick graft union formation.

The percentage graft-take for unskilled grafters increased with time as they acquired more experience in grafting. This further confirmed that grafting is an art of connecting scions and rootstocks together for a successful growth and development of the grafts (Nito et al., 2005) and this can be learned and improved with time.

Conclusion

Graft success of mango plants depends on the skills of grafters and time of the year when grafting is done. Success in thinning and survival do not necessarily depend on skills of nurserymen. Seedling rootstocks with thicker diameters are the best for grafting mango plants as the duration to graft take is short. Seedling thinning and correct choice of seedling rootstocks based on desirable diameter and architectural growth will optimize graft success of mango plants. Studies to improve growth of the late emerging seedlings are needed in order to increase graft-take and achieve fruit orchard uniformity.

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