EFFECT OF ROOTSTOCK TYPE, SCION SOURCE AND GRAFTING METHODS ON THE HEALING OF *Allanblackia stuhlmannii* GRAFTS UNDER TWO NURSERY CONDITIONS

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Abstract

***Allanblackia stuhlmannii*, a multi-purpose fruit tree, produces edible oil from seeds, is a new commercial species in global food and cosmetic industry. Cultivation of this fruit tree is limited because of lack quality and quantity planting materials due to poor seed germination. Our study therefore aimed at evaluating healing and survival of scions grafted on two types of rootstocks under two nursery conditions to promote cultivation of *A. stuhlmannii* by rural farmers. Scions taken from 2 coppiced (L26 and L62) and 3 un-coppiced (L19, L28 and L63) mother sources were grafted on a 2 year-old rootstocks using top cleft, side-veneer grafting and budding methods. Each treatment per site contained 10 seedlings of two rootstocks type (with/without leaves) arranged in a randomized complete block design with nursery sites as blocks (replicates). The grafts take (survival) was assessed 4 months after grafting. The survival rate of the grafts was generally poor in both nurseries. Top-cleft grafting method with scions from coppiced sources produced the best success rate. There was a significant differences on various grafting methods and using scion from coppiced sources, and the interaction of grafting methods and scion sources, *p* <0.0001. It is presupposed that the low percentile of success (19%) is linked to the physiologic condition of the scions sources, besides less favourable environmental conditions at the time of grafting. The top-cleft grafting method using coppiced scions source proved most effective method in the survival rate of grafts. This method has to be recommended to promote the cultivation of this species.**

Keywords: Plant propagation, vegetative propagation, rootstock, grafting, Usambara

Introduction

Allanblackia stuhlmannii (Engl.) Engl. is an important tree species playing a significant socio-economic role as a source of income for many populations in eastern Arc Mountains, Tanzania. *A. stuhlmannii* is one of nine species belonging to the family *Clusiaceae* (Mangosteen family). The geographical distribution of *genus Allanblackia* ranges from Senegal to the West and Tanzania to the East (Jamnadass *et al*., 2010). *A. stuhlmannii* is an indigenous, tall forest tree of about 35 to 45 m high, restricted to the eastern Arc Mountains, Tanzania (Schulman *et al*., 1998; Jamnadass *et al*., 2010). *Allanblackia* forms one of the dominant tree species of submontane forest canopy where it is associated with other species such as *Cephalosphaera usambarensis, Parinaria excelsa, Albizia gummifera, Beilschmiedia kweo, Diospyros abyssinica, Englerodendron usambarense* and *Drypetes gerrardii* (Mwaura and Munjuga, 2007). *Allanblackia* trees have the largest fruits of all rainforest plants (Jamnadass *et al*., 2010). *A. stuhlmannii* trees fruit and flower from December to March each year. The fruit is brown 15–25 cm long with many berry-like seeds (40–100 seeds per fruit). The seeds contain edible fat, making it valuable to both soap and food industry. Air-dried seeds contain about 50 % oil composing of triglycerides of stearic (45–58 %) and oleic (40–51 %) fatty acids (Munjuga *et al*., 2010, Jamnadass *et al*., 2010). Local communities have long valued this tree for fuelwood, timber, herbal medicine and as edible oil source (Schulman *et al*., 1998; Jamnadass *et al*., 2010).

Recently, there has been increased interest in turning *A. stuhlmannii* into a significant commercial species for edible oil production in global food markets (Amanor *et al*., 2008). To have a sustainable chain supply business, domestication to increase the productivity is necessary as the species is harvested from wild populations. To cultivate *Allanblackia* for commercial production sufficient number of plants must be produced using a successful propagation method. However, very little information on propagation techniques such as seed germination, grafting and air-layering is available for *Allanblackia*. In addition, *Allanblackia* fruit production encounters a number of other difficulties particularly in relation to ageing, slow growth, long juvenile phase, fruit production variability and lack of knowledge on the silviculture of the species (Akinnifesi *et al*., 2008). According to Jamnadass *et al*. (2010), on-farm *Allanbalckia* are characterised by predominance of old trees, a lack of regeneration and has never been planted traditionally. The juvenile phase of the naturally regenerated *Allanblackia* varies between 15 and 20 years. Whilst fruit production may commence at 20 years, full production is only reached after 40 to 50 years (Jamnadass *et al*., 2010). Fruit production varies from one year to another, from place to place and between individual trees as it is influenced by climatic factors, human activities (expansion of agricultural land) and genetic variability (Akinnifesi *et al*., 2008). These factors will be disincentives for farmers to plant Allanblackia in the future unless more productive planting stock can be developed.

Knowledge about propagation of these important indigenous fruit trees species, which provide economic or environmental benefits, is essential in order to produce adequate supply of planting stocks. Such basic information is, however, scant for many African trees that are of high economic potential (Akinnifesi *et al*., 2008). Information on tree improvement techniques such as grafting, rooting cuttings and air-layering that is needed to understand how to promote regeneration and domestication of such species threatened by agricultural expansion and other developments is not available or is poorly documented. Seed germination for *Allanblackia* has been quite difficult (Jamnadass *et al*., 2010). Furthermore, the species is dieocious and germinated seeds may either be male or female (Jamnadass *et al*., 2010; Peprah *et al*., 2009) and the sex ratio cannot be controlled. Again, propagation of *Allanblackia* by the cuttings face many difficulties such plagiotropism, cuttings producing few and weak adventitious roots that are concentrated within a small radius of cutting. On the other hand, propagation by air-layering (marcoting) is also difficulties as the materials takes more time to regenerating roots and produce fewer plants, hindering the horticultural procedures (Abd El-Zaher, 2008). Developing genetically uniform material derived from selected desirable individuals to capture interested traits to enhance the multiplication of trees in the reproductive phase (Hartmann *et al*., 2002), could help to address some of the problems, since grafting has shown to reduce the gestation period in *Allanblackia floribunda* (Hartmann *et al*., 2002; Asaah *et al*., 2011). A two year old *A. parviflora* in Ghana flowered within 2 years after grafting, although they did not bear fruits. It appears that grafting is a method of vegetative propagation that can be employed to produce improved planting materials and also shorten the gestation period in *A. stuhlmannii* as has also been reported by Hartmann *et al*., (2002) and Sanou (2004). The methods of grafting and environmental conditions are however important since grafting has yielded low success rate in many indigenous species tried in the past (Mhango *et al*., 2000; Mannan *et al*., 2006).

Efforts to graft *A. stuhlmannii* wildings in Amani resulted in poor success rate (10%), partly because of rotting of rootstock-scion union, high humidity at the time of grafting and healing, wilding rootstocks condition at the time of grafting, and the abundance of latex prevented contact between cambial cells of the scion and the rootstock (Mugasha, 1980). However, in other *Allanblackia* species, grafting has proven to be very successful. For example, Ofori *et al*., (2008) reported grafting success of 80% and 50% in *A. parviflora* in Ghana using cleft and side veneer grafting respectively. Therefore, the objective of this study was to investigate success of *A. stuhlmannii* grafts as affected by 1) scions origin or mother trees sources, and 2) various methods of grafting.

Methodology

Study site

The study was conducted at Amani Nature Reserve in Muheza district, Tanga region in the North Eastern Tanzania. Kwamkoro is located in the highland at 980 m above sea level (a.s.l.) whereas Longuza is lowland forest at 180 m a.s.l. (50°5'S and 5°14'S latitude and 38°40'E and 38°32'E longitude). This nature reserve is within the East Usambara Mountains, an area renowned for its biological importance in terms of species richness and endemism, and is part of the Eastern Afro-montane Biodiversity Hotspot (Burgess *et al*., 2007). Three main forest types characterize this area: plantation forest at Longuza, secondary forest predominate the Amani community area and primary forest at Kwamkoro (Newmark, 2002). The soils are largely clay and clay-loams usually between 1-5m deep. They are generally red, acidic clay to clay-loams and well drained with pH of 5 at 900m and 4 at 1,050m a.s.l. The rainfall is almost throughout the year with peaks from March to May and from October to December. The mean annual rainfall is 2,262mm while mean annual temperature is 20.6^{o}C (Hamilton and Bensted-Smith, 1989).

Plus-tree selection for scions, scion collection and root stocks

Fruiting healthy looking prolific trees (big fruit, large number of fruits, fruiting each fruiting season and large kernel) were identified as source of scions in the farms. Five trees, 2 coppiced (L26 and L62) and 3 un-coppiced (L19, L28 and L63) mother trees were marked and used as source of scions for this study. Recently flushed, green shoots from the crowns of the selected plus-trees were collected either early in the morning when cool (to minimise physiological stress) and kept in a Snaplock® company bag. They were used to provide the scions or buds for grafting on to the rootstocks depending on the grafting technique being tested. Grafting onto the rootstocks was done on the same day immediately after scion collection (to minimise physiological stress). Three hundred rootstocks for this study per site were selected from seedlings of approximately 2 year-old on the basis vigorous growth and free from diseases. Three methods of grafting were investigated: top cleft, budding and side veneer grafting.

Experimental design

Five scion sources (L19, L26, L28, L62 and L63), three methods (top cleft, budding and side veneer), and two rootstocks pre-treatment methods (rootstocks with/without leaves) were tested under two nursery conditions (Kwamkoro and Longuza) in a randomized complete block design with nursery as blocks (replicates). Ten rootstocks per treatment were arranged in nursery bed and grafted. The standard procedures for grafting was used as described by Hartmann *et al*., (2002) as follows: the scions, budwood and the rootstocks were cleaned with 10% sodium hypochlorite; the scion or bud and rootstock were matched in size, so that the cambial tissues are juxtaposed; after inserting the scions, the scion or bud and the root stock was tied together by wrapping with transparent plastic sheet, and the grafted seedlings was covered with clear polythene bag for 14 days to increase the relative humidity and avoid leaf dehydration by water loss. Each graft was labelled, with the date of grafting and source of scion. The grafting success (survival) was scored as 0 = dead or 1= alive. The assessment was made for four months after grafting for graft success and graft survival percentage.

Prior to statistical analysis, the data was arc-sine transformed (Webster and Oliver, 1990) and analysis of variance (ANOVA) was performed on different parameters using GenStat (GenStat, 2009). The significant differences of the means of treatments were compared by Bonferroni Test.

Results

Effect of grafting methods on grafting survival

Top cleft method achieved highest success rate at both nurseries followed by side cleft (Figure 1). Budding on the other hand did not produce any grafting success in either nursery. There was a significant main effect for grafting methods, $F(2, 57) = 42.54$, $p < 0.0001$, and a significant interaction of grafting methods and scion sources, $F(8, 33) = 7.94$, $p < 0.0001$. However there was no significant differences between interaction of grafting methods, rootstock treatments and scions sources $F(8, 33) = 2.13$, $p = 0.0694$.

Effect of nursery site on grafting survival

The survival rate of the grafts was generally poor with Kwamkoro (highland nursery) achieving a slightly higher survival than Longuza (Figure 2). There was no statistical significant difference between the two nursery sites, $F(1, 58) = 0.74$, $p = 0.3985$, and neither were there significant differences between interaction of nursery sites and grafting methods, $F(2, 57) = 1.98$, $p=0.1590$, nor nursery sites and seedlings treatments, $F(1, 58) = 0.27$, $p=0.6108$.

Effect of scion sources on grafting survival

Scions from coppiced sources tended to have higher success rate than from un-coppiced scions sources (figure 3). There was a significant differences between scion sources, $F(4, 55) = 12.43$, $p < 0.0001$, and a significant interaction of grafting methods and scion sources, $F(8, 33) = 7.94$, $p < 0.0001$. However there was no significant differences between interaction of rootstock treatments, grafting methods and scions sources $F(8, 33) = 2.13$, $p = 0.0694$.

Effect of rootstock treatment on grafting survival

Rootstocks with leaves intact achieved a slightly higher success rate than rootstocks without leaves (Figure 4). There was no significant between rootstock treatments, $F(1, 58) = 0.74$, $p = 0.3985$, and there was no significant differences between interaction of seedlings treatments and nursery sites, $F(1, 58) = 0.27$, $p=0.6108$, grafting methods, $F(2, 57) = 3.27$, $p=0.0540$, scion sources, $F(4, 55) = 1.13$, $p=0.3619$, and seedling treatment and grafting methods, $F(8, 33) = 2.13$, $p=0.0694$.

Discussion

Grafting success of *Allanblackia* (43%) was rather low in both sites. This was attributed by the wet weather condition at the time of grafting rootstocks as earlier reported by Mugasha (1980) and Ofori *et al.*, (2008). The reduced success of grafting during the rainy season may be due to high humidity, which caused mould to grow on the grafts. However, in other species moderate temperature and high relative humidity are major factors related to success of grafts (Yelleshkumar *et al*., 2008). Incidence of disease is reported in the literature to be one of the most common environmental causes of grafting failure (Hartmann *et al*., 2002; Sanou 2004). The low success rate observed in both nurseries could also be as a result of the dry conditions following the grafting time. In many indigenous fruit trees, the best time for grafting is usually during the transition from dry to rainy season when the meristematic activity is starting for the scion-rootstock union to be established quickly (Yelleshkumar *et al*., 2008). In other species like avocado, seasonality effect has been discussed as a contributing factor in the success rate of grafting (Garedew and Tsegaye, 2011). In East Usambara, this can be achieved before the short rainy season i.e. September and during the short rainy season when the trees are flushing flower buds.

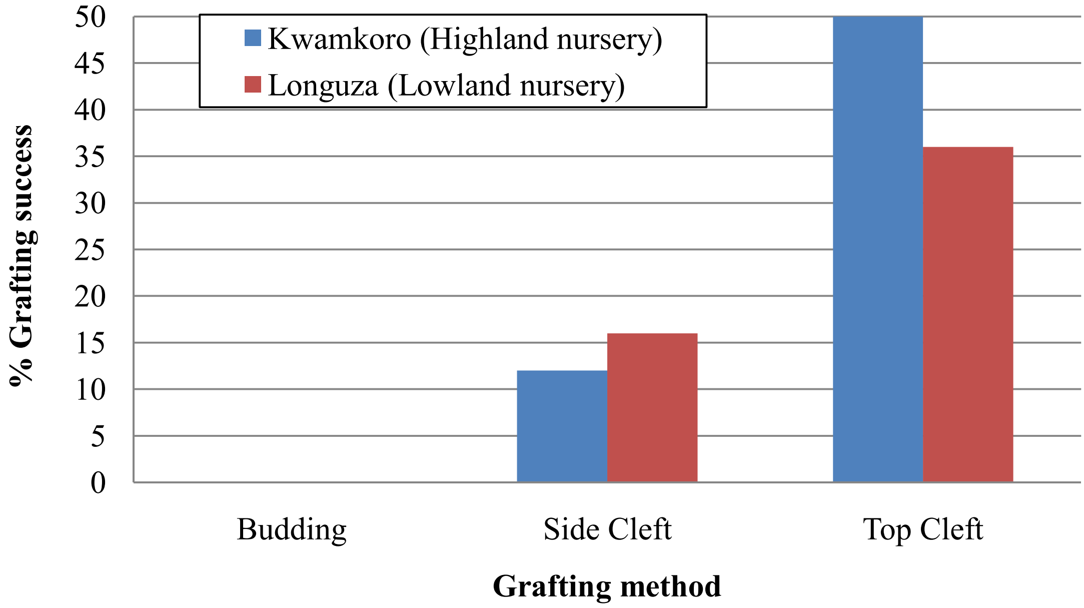

Figure 1: *Effects of grafting methods on grafts survival as influenced by nursery site.*

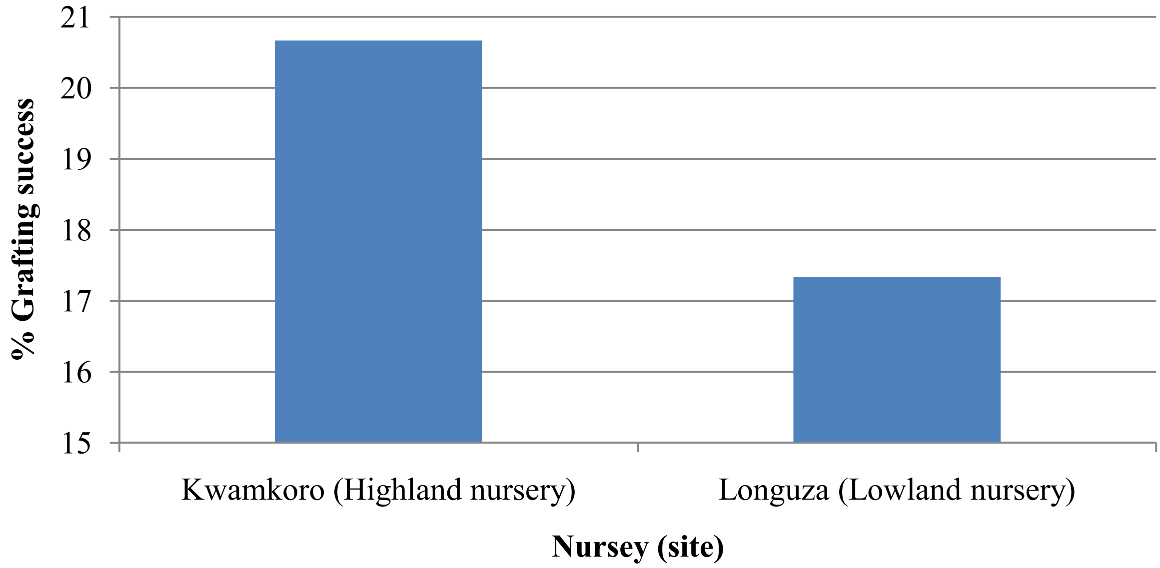

Figure 2: *Effects of nursery location on grafting success.*

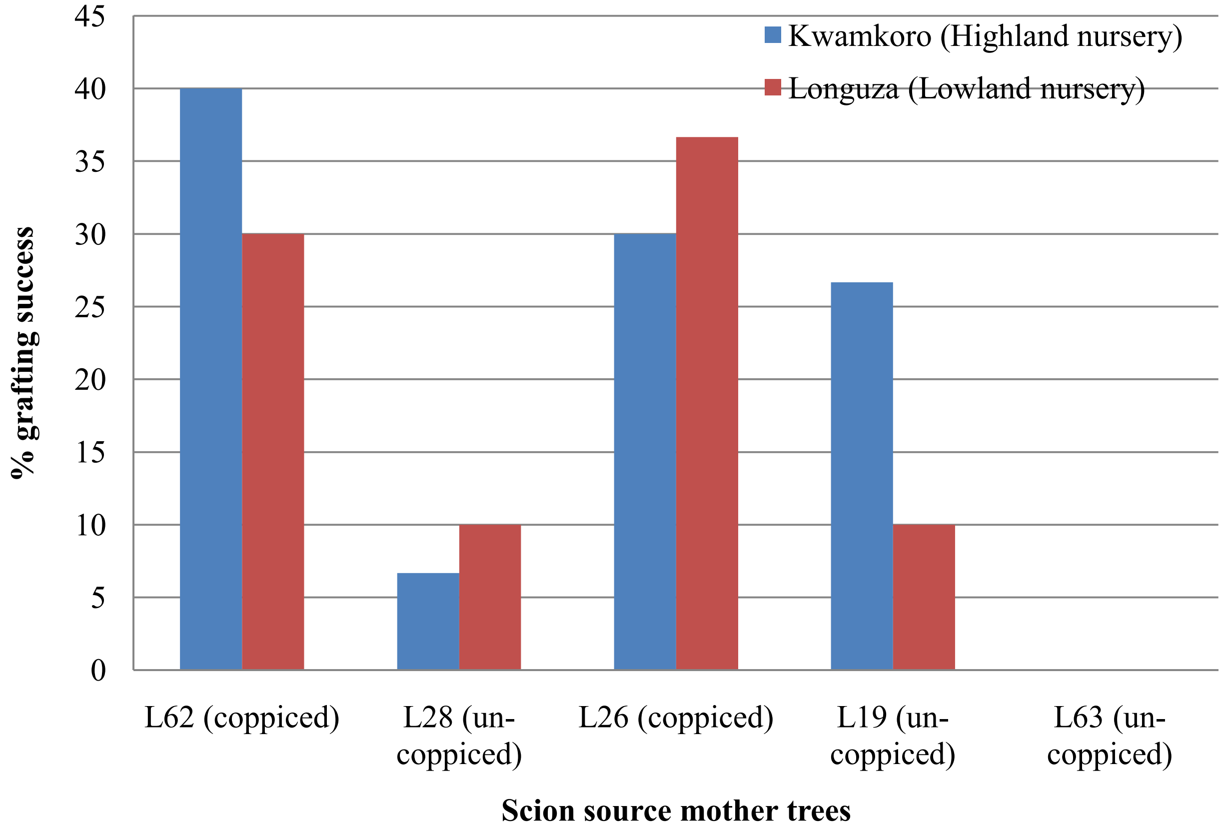

Figure 3: *Effects of scions source on grafts success as influenced by nursery site.*

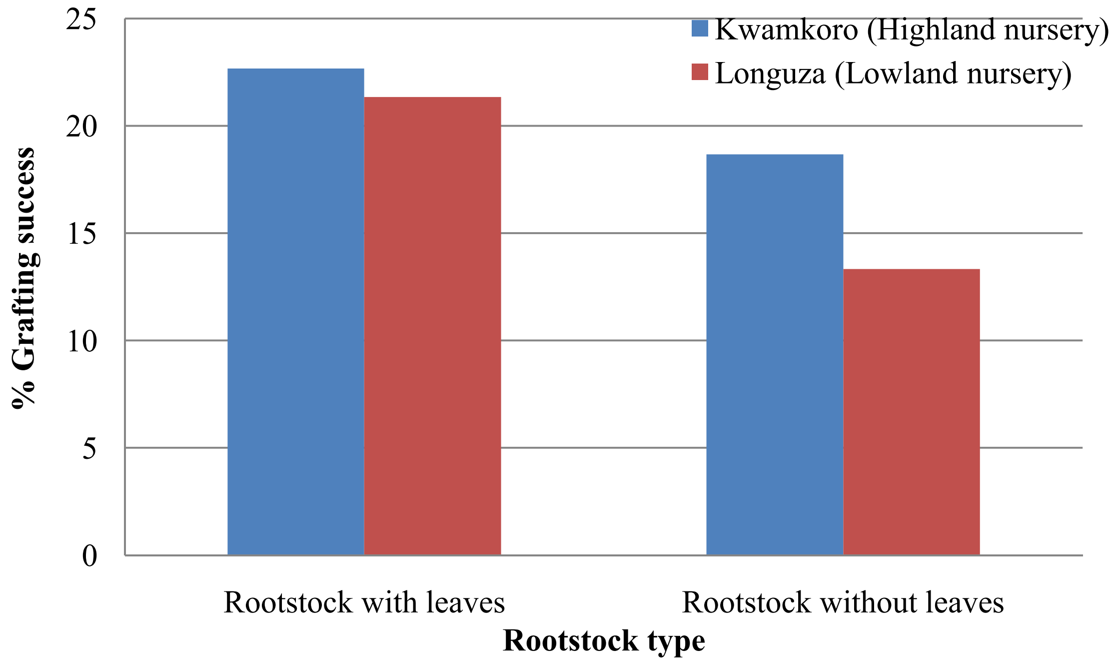

Figure 4: *Effects of rootstock type on grafting success as influenced by nursery site.*

The results indicated that *A. stuhlmannii* can be successfully grafted using the top cleft grafting methods. This has been previously demonstrated in *A. stuhlmannii* with little success (Mugasha, 1980) and similar study has also been conducted on *A. parviflora* in Ghana (Ofori *et. al.,* 2008) with good success (over 70%). In many species, it has been reported that cleft grafting is easier to use (Kulwal and Tayde 1989; Sanou 2004; Mannan *et al*., 2006) and more successful than other methods of grafting (Ram 1997; Hibbert-Frey *et al*., 2010). The ease of using top-cleft method and the grafter experience might be the critical factors in determining the healing success of grafts. The reasons for this are unclear and probably relate to the ease of developing good contact between the cambium in the rootstock and scion, the prevention of desiccation and other factors not examined here. On the other hand, failure of budding might be due to the small size of buds used which made it difficult to position it well on rootstocks. Therefore, top cleft method is a superior grafting technique for this *genus* as confirmed by Ofori *et al*. (2008) and this demonstrates the potential benefit if the technique can be perfected. However, more tests and practice is required to perfect the art.

Significant differences observed between scion source types (coppiced or un-coppiced mother sources) may be due to the vigour of juvenile material from coppiced sources which contribute to active growth period of mother trees with higher level of nutrients in scion shoots. Better survival of grafts from coppiced mother sources can also be correlated to higher cell activity (Hartmann *et al*., 2002) when the flowers are flushing whereas the un-coppiced material has used most of their food supplies from the fruit production of previous season. This in turn may be due to decreased synthesis of endogenous auxin and mobilization of reserved food material promoting healing of the scion-rootstock union (Akinnifesi *et al*., 2008). Poor success of grafts in un-coppiced material may also be attributed to the reduced rate of division of cambial cells, their differentiation and consequent development in healing of stock scion union. Again, the low success rate could also been as a result of scions larger diameter from natural stands grafted on smaller rootstocks. Presence of high concentration of phenolic compounds in its tissues and their oxidation by wounding (Neondo *et al*., 2011) during grafting might provide low rate of callus formation hence low success rate (Gandev, 2007). This therefore created compatibility challenges between the scions and the rootstock.

Pre-treating the rootstock by removing or leaving the leaves intact did not improve the survival of scions. The absence of significant difference between the two methods of pre-treating rootstock suggests that the rootstock without leaves is as good as with leaves. Unlike *A. stuhlmannii*, Medagoda and Weerawardana (2005) reported *Macadamia* species to produces higher grafts success rate when scions are grafted on rootstocks where all the leaves from the rootstocks were removed. 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). Furthermore, proper alignment of scion and rootstock cambium tissues could determine the graft success (Pina and Errea, 2005). The skill of grafters in aligning cambium tissues of rootstock and scions together is important in reducing graft failure. Skills in grafting seedlings are gained through practice, but it is whether this graft success would also depend on the level or duration of training nurserymen needs to be investigated further.

Even though none of the grafted rootstock developed into flowers during the experiment, it is expected the maintenance of ontogenetic age in this species, will drastically reduce the gestation period as observed in other species within the *genus* (Ofori *et al*., 2008; Asaah *et al*., 2011).

Planting grafts in mother blocks as sources of genetic materials for multiplication would also be advantageous in the elimination of frequent trips to the forest or on farms for collection of materials for propagation.

Conclusion

Based on the results of this study, top cleft grafting technique using scions from coppiced mother sources produced the best results. We therefore recommend this technique as a promising propagating technique in *A. stuhlmannii;* however further investigation on top-cleft is necessary to improve on the graft take. It is recommended further investigation are still needed to understand the scion-rootstock union, scion source cambial characteristics and rootstock especially when grafting is applied *in-situ* both on-farm or in natural environment. Further future studies need to examine the matter adequately imparting different shading or covering effects of both stock plants and grafted seedlings, time of grafting or seasons, method of grafting, effects of temperature on grafting take, age of scion and defoliation of scion as it influences the grafts take. Also, further studies are needed to understand the physiological characteristics of mother source trees or stock plants affecting the success of grafting.

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