

Delayed rooting hormone application and stem cutting collection time improved *Flacourtia indica* rooting ability and root growth

Simon Mng'omba (✉ SMngomba@luanar.ac.mw)

Lilongwe University of Agriculture and Natural Resources

Research Article

Keywords: Carbohydrates, indole-3-butyric acid, ortets, phenology, root length

Posted Date: January 12th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-2459173/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Flacourtia indica is an important fruit and medicinal tree in southern Africa but has a low seed germination. Assessing rooting ability of mature (fruit bearing) *F. indica* stem cuttings is vital to attain early fruiting. The objectives were to assess responses of mature stem cuttings to different application time of a rooting hormone (Seradix® No. 2) and stem cutting collection time and to identify important pheno-phases of in *F. indica* yearly tree growth cycle. A significantly higher rooting percentage ($P < 0.001$) was obtained with delayed Seradix® No. 2 application than immediate application. Stem cuttings collected at flowering and leaf fall seasons were responsive to Seradix® No. 2 application, but all stem cuttings collected at fruiting time failed to produce roots. Stem cuttings significantly ($P = 0.0482$) produced more roots with immediate Seradix® No. 2 application than those subjected to delayed Seradix® No. 2 application for stem cuttings collected at flowering period. The failure to produce roots for cuttings collected at fruiting time could be due to low sugars (carbohydrates) in the stem cuttings. It is concluded that the best time to collect mature stem cuttings for rooting is either at flowering or leaf fall period and that delayed Seradix® No. 2 application improves rooting of mature *F. indica* stem cuttings.

1. Introduction

Flacourtia indica is one of the important indigenous fruit tree species in southern Africa for its edible fruits and medicinal value (Akinnifesi et al., 2008; Ruffo et al., 2002). It is ranked sixth and ninth in Malawi and Zambia respectively (Franzel et al., 2008) in terms of preference for its fruits. According to Amritha and Santhosh (2021) *F. indica* is an important medicinal plant against malaria, diabetic and several other heart diseases. This fruit tree species is yet to be cultivated on farmland and all fruit harvest is still from the wild. There has been a wide diversity of *F. indica* tree species in the wild, but no or limited efforts for selection and conservation have been undertaken. A decrease in forest areas in southern Africa threatens diversity and abundance of such important wild fruit and medicinal trees in their natural habitat (Elevitch and Francis, 2006).

Currently, a known propagation method for *F. indica* has been the use of seed, but Prins and Maghembe (1994) reported poor seed germination (20%). They attributed this poor seed germination to either absence of seed embryo or the presence of a hard seed coat. They further reported a problem of seed embryo damage when seed pretreatments (nicking seed and removal of seed coat) are used to improve seed germination. To our knowledge, there has been no efforts to improve *F. indica* seed germination in the recent times.

Vegetative propagation methods such as grafting and marcotting often used for many native and exotic fruit tree species have not been reported for *F. indica*. In any case, such vegetative propagation methods seem difficult for *F. indica* due to its sharp spines or piercing thorns (Fig. 1). A potential propagation method would be the use of stem cuttings. The use of rooted stem cuttings is important as these can perpetuate superior fruit traits considering wide variations in fruit size and taste traits that exist among

many wild tropical fruit trees. Furthermore, the use of stem cuttings could fix desirable fruit traits which may easily be lost with sexual propagation (Akinnifesi et al., 2008; Mng'omba et al., 2009).

Kabir et al. (2017) reported the highest (80.5%) rooting of *F. indica* cuttings with 0.4% indole-3-butyric acid (IBA) solution, but the lowest rooting (62.3%) was from those without IBA exposure (treatment). From their findings it is not clear whether those rooted stem cuttings were derived from juvenile or mature (fruit bearing) trees. It is likely that those stem cuttings were collected from juvenile trees considering the high rooting ability reported even without a rooting hormone (62.3%). Rooting stem cuttings derived from mature trees is desirable due to early fruiting (precocity), but stem cuttings from such mature trees have poor or low rooting ability. There are several factors affecting rooting ability of stem cuttings especially those from mature trees. Augé (1995) and Mng'omba et al. (2007) reported a specific period (seasonality) when stem cuttings become responsive to rooting hormones. They further indicated that the levels of endogenous hormones in the cuttings could influence rooting ability.

Collection time (season) of stem cuttings is critical as it affects rooting ability of cuttings (Auge, 1995). To our knowledge, no research studies have been undertaken to assess seasonality (time of the year) in rooting ability of *F. indica* stem cuttings derived from mature trees and the effect of rooting hormone application time on rooting ability. The objectives of this research study were to (1) identify important pheno-phases (biological processes) in *F. indica* yearly tree growth cycle (phenology), (2) assess rooting ability of mature *F. indica* stem cuttings collected in different times (seasons) of the year, (3) assess the effect of rooting hormone application time on rooting ability and root growth.

2. Materials And Methods

2.1 Plant material

Flacourtia indica stem cuttings from mature (30 individual fruit bearing) trees were collected from Makoka forest in Zomba district of Malawi. These stem cuttings were not from previous year's shoot growth but somehow old as shown in Fig. 1b (semi-hardwood though some were close to be hardwood). In the first year (2010), *F. indica* tree growth phenology data were collected to isolate distinct or noticeable seasons for stem cutting collection. This is because *F. indica* tree growth phenology (for instance, flowering, fruiting, fruit ripening times) were not well established to easily collect stem cuttings from a specific pheno-phase.

Semi-hardwood stem cuttings were collected from mature *F. indica* trees during a specified pheno-phase. They were wrapped in a moist newspaper, placed in a cooler box and transported to Makoka Research Station nursery. They were planted on the same day of collection.

2.2 Stem cutting propagation

Flacourtia indica stem cuttings were cut to a length between 10 and 15 cm and planted in a propagation bed containing sterilized fine sandy soils. The propagation bed had a black shade net about 4 m above it

and a fence covered with a black shade net. A small cut (incision) was made at the base of each stem cutting with a sharp knife before dipping in Seradix® No. 2 (rooting hormone) to promote side rooting (Elevitch and Francis, 2006). Seradix® No. 2 was applied in a powder form and contained 0.3% indole-3-butyric acid, IBA (Bayer, Pretoria, South Africa).

2.3 Tree growth phenology

Flacourtia indica tree growth phenology (pheno-phases) data were collected for a year (2010). This involved recording onset and end of flowering, fruiting, fruit ripening and leaf fall periods and this was to collect stem cuttings from individual trees when 50% or more of tree branches reached a certain pheno-phase (i.e. flowering, fruiting and leaf fall). Thirty (30) individual trees growing within the same forest area were used in this study.

2.4 Stem cutting collection

2.4.1 Flowering pheno-phase

For the first experiment, stem cuttings were collected and planted in October 2010 when *F. indica* trees were in flowers. Five rooting hormone (Seradix® No. 2) application times formed treatments and they were as follows:

- i. Immediate (0 days after planting)
- ii. Three (3) days after planting
- iii. Six (6) days after planting
- iv. Ten (10) days after planting
- v. Twelve (12) days after planting

Except the first treatment (0 days after planting), the rest (referred to as delayed Seradix® No. 2 application) involved removing stem cuttings from a propagation bed after a specified time to apply the rooting hormone. These stem cuttings were rinsed in water to remove the sand sticking to their bases and a fresh small cut (incision) was made at the base of each stem cutting. They were dipped in Seradix® No. 2 powder for a few seconds and then shaken to remove excessive Seradix® No. 2 powder. They were then planted in their original positions in the propagation bed. A total of 800 stem cuttings (40 stem cuttings per treatment) were planted.

For the second experiment, stem cuttings were planted in October 2012 (flowering period) and a total of 420 stem cuttings (20 stem cuttings per treatment) were planted. Seradix® No. 2 application times (treatments) were modified (reducing time of the next application) for this experiment and the subsequent experiments. Seradix® No. 2 application times were as follows:

- i. Immediate (0 days after planting)
- ii. Two (2) days after planting

- iii. Four (4) days after planting
- iv. Six (6) days after planting
- v. Ten (10) days after planting
- vi. Twelve (12) days after planting

2.4.2 Fruiting pheno-phase

For the third experiment, stem cuttings were collected and planted (420 stem cuttings and 20 stem cutting per treatment) during *F. indica* fruiting time (February 2013). Seradix® No. 2 application times (treatments) were maintained as indicated in the second experiment.

2.4.3 Leaf fall pheno-phase

A fourth experiment was carried out when *F. indica* trees were losing their leaves (leaf fall) in August 2013 and a total of 420 stem cuttings were planted. Seradix® No. 2 was applied as described in the second experiment.

2.5. *Experimental design*

All the experiments were laid out in a Randomized Complete Block Design (RCBD). The first experiment had four (4) replications, while the rest had three (3) replications. All stem cuttings were irrigated whenever necessary. All the four rooting stem cutting experiments were allowed to stand for a period three months on the propagation bed for further observations.

2.6. *Data collection*

Data collected included number of rooted stem cuttings, number of roots, root length (longest roots) and shoot number after a month.

2.7. *Statistical analyses*

All data collected were subjected to analysis of variance (ANOVA) using Generalized Linear Model (GLM) of the SAS package (SAS, 2003, Release 9.1). Data for each experiment were analyzed separate because they were collected and planted in different pheno-phases (seasons). Mean separation was done using least significant differences (LSD) at 5% level of probability.

3. Results And Discussion

3.1 Tree growth phenology

Several *F. indica* tree pheno-phases (phenology) from January to December 2010 were identified but there were variations among mature *F. indica* trees. Three main distinct pheno-phases were selected for stem

cutting collection for the four (4) experiments. These were the flowering, fruiting and leaf fall pheno-phases. Figure 2 shows these three main distinct tree pheno-phases.

Flacourtia indica tree flowering period started as early as August and more than 70% of the mature trees were in flowers by October (Fig. 1), but there were still variations among trees. Some trees produced flowers earlier than others within the same forest area and even variations within the same tree. Some tree branches had flowers while others with small fruits. Fruit production was high (> 70%) from March and both fruiting and fruit ripening pheno-phases covered a long period of time. Also, fruit ripening for some mature trees started earlier than others. This shows wide variations among *F. indica* trees and an overlap of pheno-phases within the same tree. Some tree branches were already producing fruits while others were still in flowers hence stem cutting collection was based on 50% or more to determine transition from flowering to fruiting pheno-phase.

Leaf fall pheno-phase was conspicuous and very short and more than 70% of *F. indica* trees dropped their old leaves by August. New leaves quickly replaced the old leaves by October, but some trees did not completely lose all their leaves. Fruits started ripening between February and March.

3.2 Stem cutting collection time

3.2.1 Cuttings collected at flowering (October 2010)

There were significant differences ($P < 0.001$; $N = 800$) between treatments with respect to stem cuttings that produced roots (Fig. 3a) and number of roots per stem cutting ($P = 0.0482$) (Fig. 3b). A few stem cuttings produced roots when subjected to immediate Seradix® No. 2 rooting hormone application. There was a significant increase in rooting of stem cuttings with delayed Seradix® No. 2 application. Luckman and Menary (2002) also reported increased root initiation with delayed auxin application for *Eucalyptus nites* cuttings derived from three months old seedlings. In our study, delayed Seradix® No. 2 application reduced number of roots per cutting (Fig. 3b). This could be that IBA inhibited formation of many roots when applied at a later stage than at root initiation period. A few stem cuttings (< 20%) produced roots in this study and this low rooting ability could be attributed to maturity as stem cuttings derived from mature trees are difficult to root.

3.3 Cuttings collected at flowering (October 2012)

There were significant differences ($P = 0.0258$; $N = 420$) between treatments with respect to rooting percentages (Fig. 4a) and number of roots ($P < 0.001$) for cuttings collected at flowering time (Fig. 4b). Improved rooting of stem cuttings was obtained with delayed Seradix® No. 2 application of a rooting hormone but that negatively affected root number. There was an improvement in number of rooted cuttings and this improvement in rooting of stem cuttings compared to the first experiment (October 2010) could be attributed to tree rejuvenation as the same *F. indica* trees were the sources of stem cuttings for the first experiment. They were pruned to make collection of stem cuttings easy and collecting desirable stem cuttings. Pruning rejuvenated trees and stimulated new tree growth.

The two experiments carried out in the same flowering seasons in two different years confirm that stem cuttings are responsive to Seradix® No. 2 rooting hormone when collected at flowering time. Tree rejuvenation before stem cutting collection could improve rooting ability of stem cuttings derived from mature trees.

From the two experiments carried out at flowering times, delayed Seradix® No. 2 rooting hormone application improved rooting of stem cuttings though reduced the number of roots per stem cutting (Figs. 3 and 4).

3.4 Cuttings collected at fruiting (June 2012)

No single stem cuttings collected and planted at fruiting season (out of 420 stem cuttings) produced any root. Five stem cuttings produced callus only at the base, but no roots were eventually produced even after four months after planting. This indicates that seasonality has a strong influence on rooting of mature *F. indica* stem cuttings. Our findings agree with what Auger (1995) reported that there is a specific time when stem cuttings become responsive to rooting hormones. In this case, *F. indica* stem cuttings responded to Seradix® No. 2 rooting hormone when applied to stem cuttings were collected at flowering time and not at fruiting time. Auger (1995) further indicated that endogenous hormone levels in the cuttings could influence rooting ability of stem cuttings.

Hamoooh (2014) reported higher rooting response (44%) for Olive stem cuttings collected in January than when collected in March (19%). Hamoooh (2014) attributed this to seasonal changes in the carbohydrates especially sugars in both reproductive and vegetative shoots. Furthermore, it was reported that hormonal balance is important to regulate root formation and elongation. An interaction of plant nutrients (carbohydrates) and rooting hormones play a key role in rooting ability of stem cuttings (Hamoooh, 2014). In this study, it could be that plant nutrients including sugars (carbohydrates) were directed to fruit and seed formation and growth rather than to stem cutting rooting. This could be the reason for no rooting when stem cuttings were collected at fruiting time. This study indicates that sugars (carbohydrates) play a key role in rooting stem cuttings.

3.5 Cuttings collected at leaf fall (August 2013)

There were no significant differences between treatments with respect to number of stem cuttings producing roots. Rooting percentage ranged from 5 to 20%. Significant differences ($P < 0.001$; $N = 420$) between treatments were found with respect to number of roots per cutting (Fig. 5).

There were many roots per stem cutting with delayed Seradix® No. 2 application. This indicates that delayed rooting hormone application improved rooting of stem cuttings collected at leaf fall. Delayed Seradix® No. 2 rooting hormone application reduced number of roots per stem cutting at flowering time (Figs. 3 and 4) but increased root number when collected at leaf fall (Fig. 5).

Figure 6 shows some rooted *F. indica* stem cuttings collected at leaf fall (August 2013). These rooted cuttings were collected from old growth and not previous year's growth. This contributed to low rooting

percentage of the cuttings. In the current experiments it be easy to get high rooting of stem cuttings if previous year's stem cuttings were collected, but those collected and used were slightly lignified hence low rooting ability. Kabir *et al.* (2017) had the highest (80.5%) rooting of *F. indica* cuttings with 0.4% indole-3-butyric acid (IBA). This could be due to the nature (age) of stem cuttings and most likely stem cuttings were collected from juvenile trees and yet to start producing fruits and young (previous year's growth) stem cuttings were used.

3.6 Shoot growth

Stem cuttings collected at flowering time had significantly ($P = 0.0324$; $N = 420$) low shoot number per cutting with delayed Seradix® No. 2 application (Fig. 7a). Mature stem cuttings collected at leaf fall delayed Seradix® No. 2 application significantly increased mean shoot number (Fig. 7b).

4. Conclusions

This study has shown that delayed Seradix® No. 2 (rooting hormone) application improved rooting of mature stem cutting despite low rooting ability. Tree seasonality was an overriding factor in rooting ability of *F. indica* stem cuttings. The stem cuttings did not respond to rooting hormone when applied to stem cuttings were collected and planted during fruiting periods. It can be concluded that establishing tree pheno-phases led to a successful rooting of *F. indica* stem cuttings. In this study, the use of stem cuttings has a potential as a propagation method for mature *F. indica* tree species to achieve early fruiting.

Declarations

Acknowledgements

The authors acknowledge the financial support from Field Genebank.

Supplementary data: There is no supplementary data

Contribution of Author: Simon Mng'omba collected and analyzed the data, and wrote the manuscript

Conflict of interest: there is no conflict of interest

Data availability: data used in this manuscript is available for repository

References

1. Akinnifesi FK, Sileshi G, Mkonda A, Ajayi OC, Mhango J, Chilanga T (2008) Germplasm supply, propagation and nursery management of miombo fruit trees. In: Akinnifesi FK, Leakey RRB, Ajayi OC, Sileshi G, Tchoundjeu Z, Matakala P, Kwesiga FR (eds) *Indigenous Fruit Trees in the Tropics: Domestication, Utilization and Commercialization*. World Agroforestry Centre: Nairobi. CABI Publishing, Wallingford, UK, pp 341–368

2. Amritha MS, Santhosh C (2021) Phytochemical and Pharmacological profiling of Aghori (*Flacourtia indica* (Burm.f.) (Merr) – an exploration of the evidence of a potent folklore medicine. *J Res Indian Med* 16(1):1–8. Accessed on 12.11.2022
3. Augé R (1995) The physiological phenomena related to the realization of cultures in vitro. In: Razdan MK (ed) *vitro Culture and Its Applications in Horticulture*. Science publishers, Lebanon, NH, pp 7–31
4. Elevitch CR, Francis JK (2006) Species profiles for Pacific Island Agroforestry. www.traditionaltree.org
5. Elster C, Perdomo L (1999) Rooting and vegetative propagation in *Laguncularia racemosa*. *Aquat Bot* 63(2):83–93
6. Franzel S, Akinnifesi FK, Ham C (2008) Setting priorities among indigenous fruit tree species in Africa: Examples from Southern, Eastern and Western Africa regions. In: Akinnifesi FK, Leakey RRB, Ajayi OC, Sileshi G, Tchoundjeu Z, Matakala P, Kwesiga FR (eds) *Indigenous Fruit Trees in the Tropics: Domestication, Utilization and Commercialization*. World Agroforestry Centre: Nairobi. CABI Publishing, Wallingford, UK, pp 1–27
7. Hamooh B (2014) The effects of cutting collection time, auxin types and auxin concentrations on rooting of *Olive oleaeuropaea* L. cuttings under arid land conditions. *Int J Eng Res Technol* 3(1):1387–1395
8. Kadir A, Khan HM, Rahman M, Rabiul Alam ATM, Hemayet Jahan SM, Mostafa Zaman AKM, Al Mamun MZ (2017) *Int J Agric Syst (IJAS)* 5(1):42–52
9. Luckman GA, Menary RC (2002) Increased root initiation in cuttings of *Eucalyptus nites* by delayed auxin application. *Plant Growth Regul* 38:31–35
10. Mng'omba SA, Akinnifesi FK, Sileshi G, Ajayi OC, Chakeredza S, Mwase WF (2008) A decision support tool for propagating miombo indigenous fruit trees of southern Africa. *Afr J Biotechnol* 7(25):4677–4686
11. Prins H, Maghembe JA (1994) Germination studies of fruit trees indigenous to Malawi. *For Ecol Manag* 64:111–127
12. Ruffo CK, Birnie A, Tengnas B (2002) *Edible wild plants of Tanzania*. RELMA Technical Handbook Series 27. Nairobi, Kenya. Regional land Management Unit (RELMA), Swedish International Development Cooperation Agency (Sida) pp322–323
13. SAS Institute Inc (2003) *SAS/STAT, Release 9.1*. SAS Institute Inc., Cary, NC

Figures

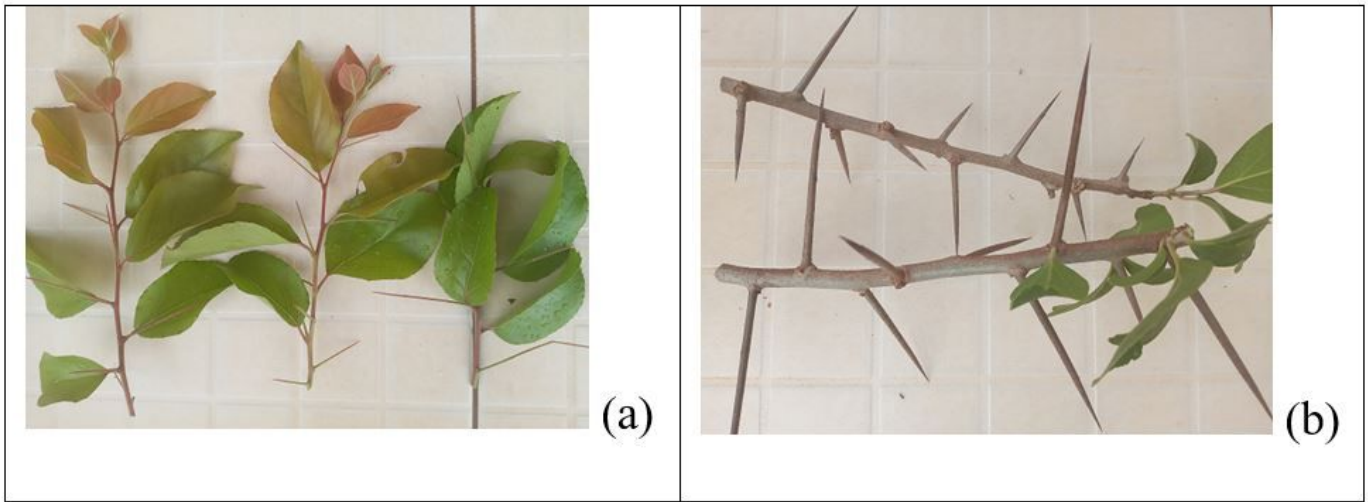


Figure 1

Flacourtia indica stem cuttings showing strong thorns (spines) at different growth stages (a) current season growth and (b) old shoots

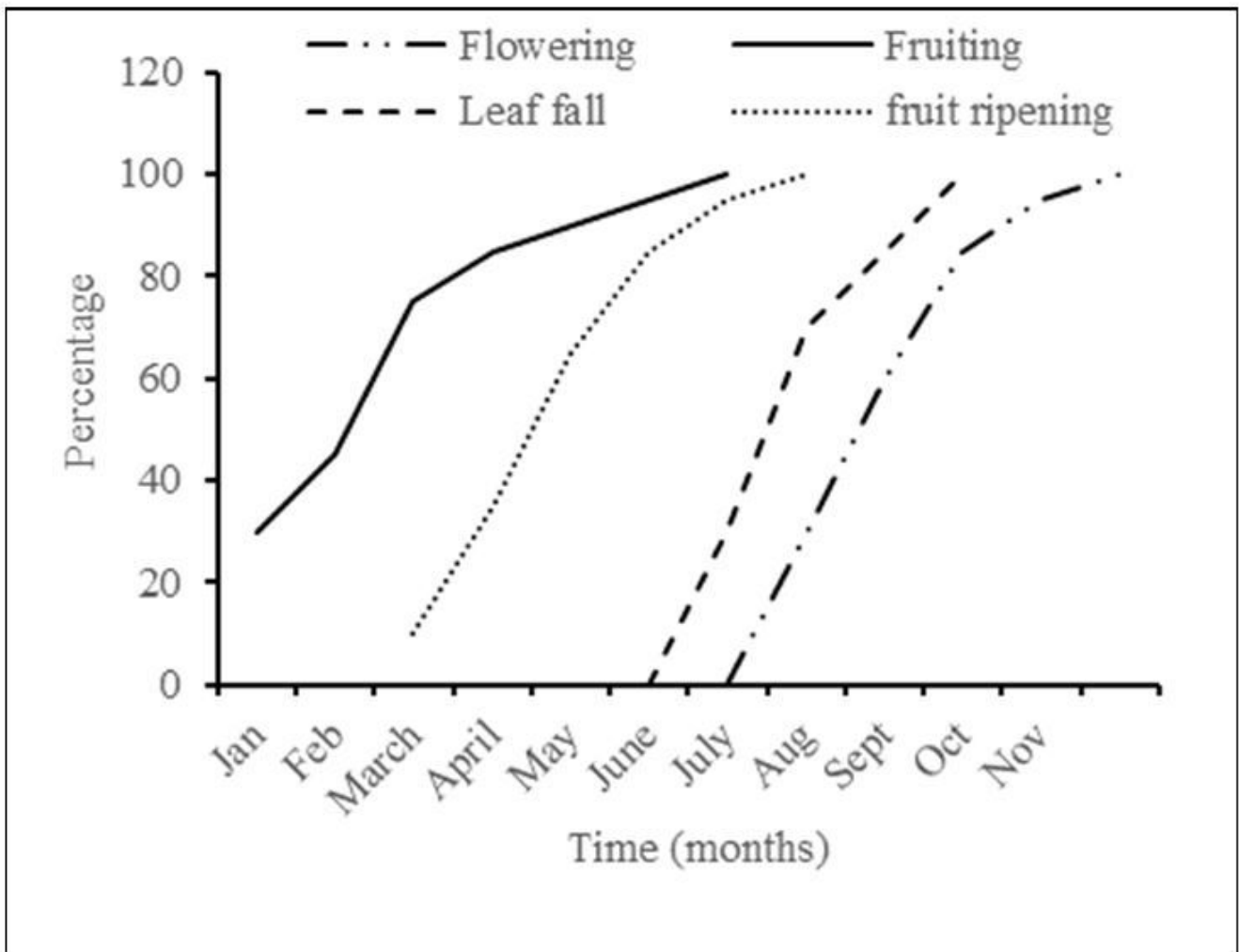


Figure 2

Flacourtia indica pheno-phases during January to December 2010 season at Makoka Research Station

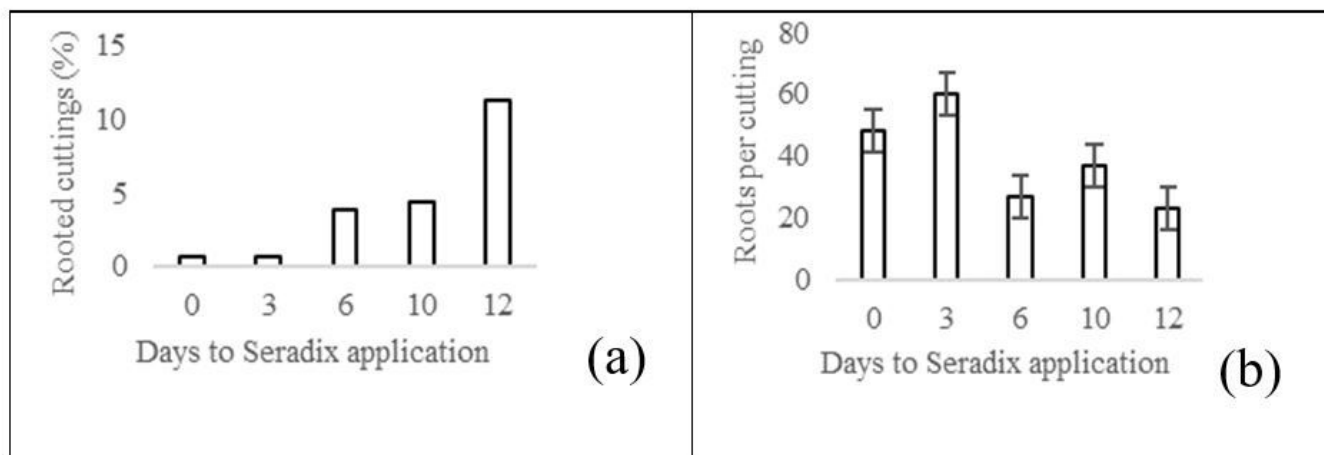


Figure 3

Flacourtia indica stem cuttings collected at flowering showing (a) rooting percentage and (b) number of roots per stem cuttings at Makoka Research Station (October 2010 – February 2011)

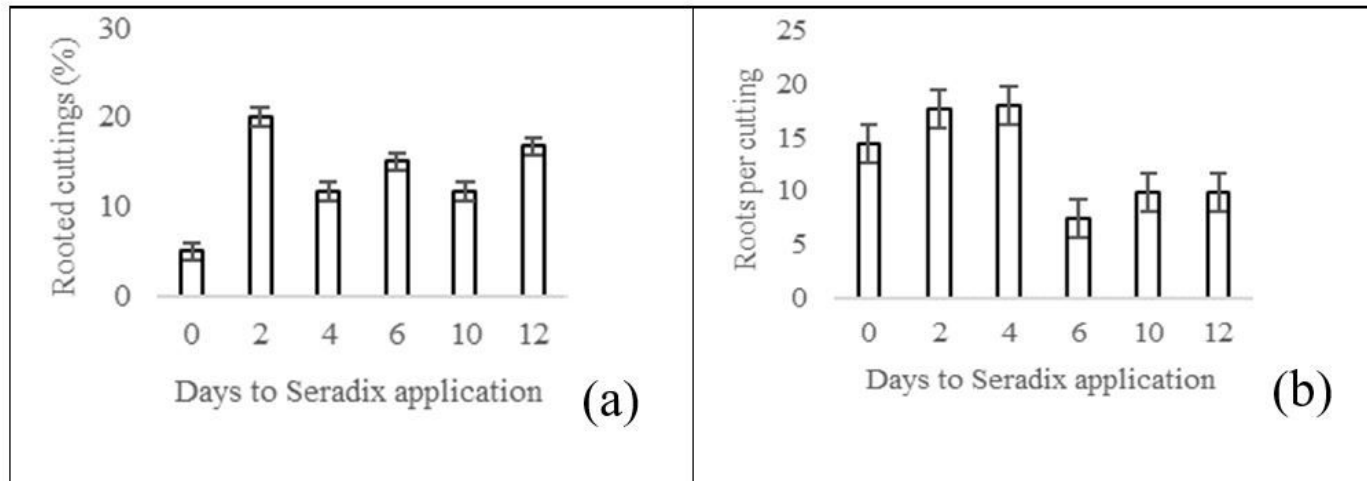


Figure 4

Flacourtia indica stem cuttings collected at flowering showing (a) rooting (%) and number of roots at Makoka Research Station (October 2012 – February 2013).

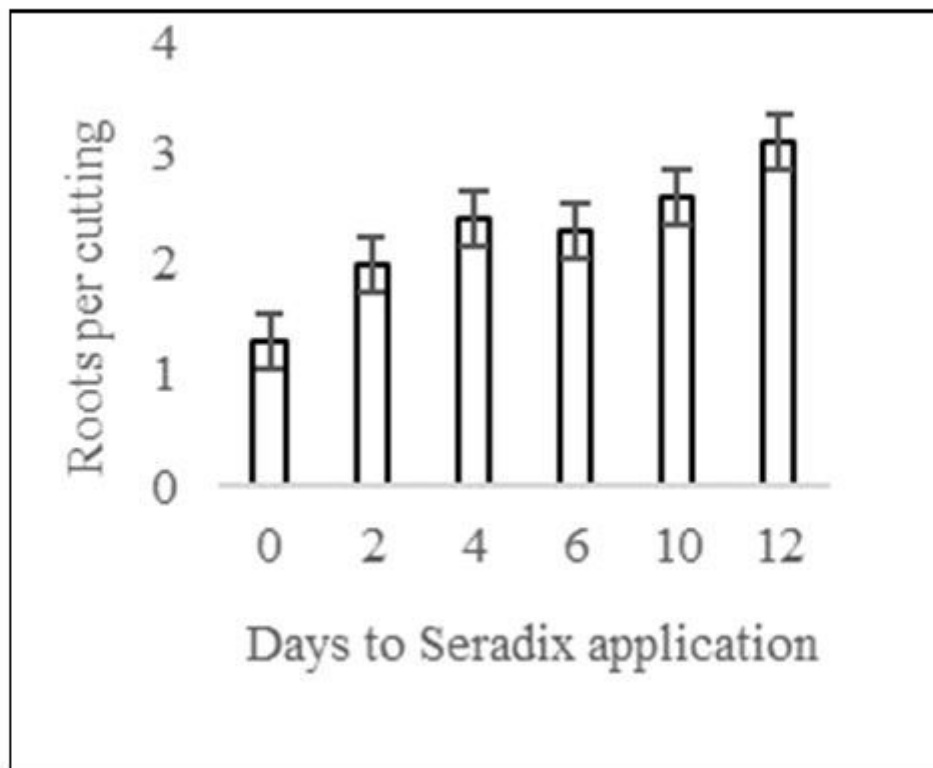


Figure 5

Flacourtia indica root number per stem cuttings collected at leaf fall (August 2013) at Makoka Research Station



Figure 6

Flacourtia indica rooted stem cuttings collected at leaf fall (August 2013) two months after planting at Makoka Research Station

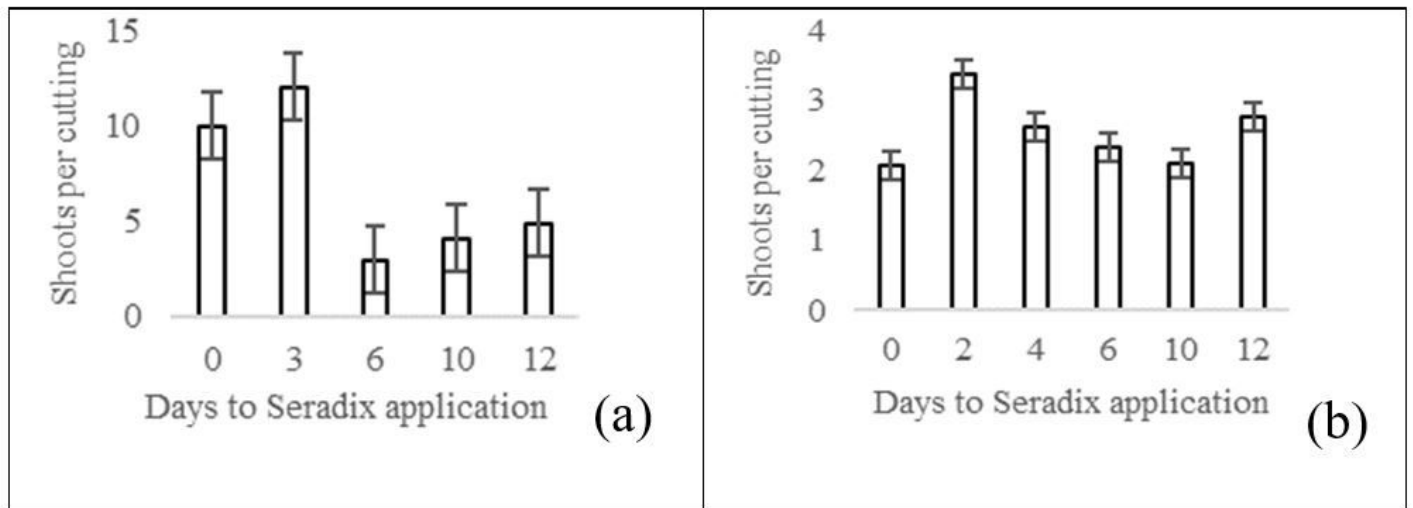


Figure 7

Mean shoot number of *Flacourtia indica* stem cuttings collected at (a) flowering and (b) leaf fall at Makoka Research Station