CHAPTER 9

GENERAL DISCUSSION AND CONCLUSIONS

9.1 Propagation of woody perennial trees

Different propagation techniques for *U. kirkiana* and *P. capensis* tree species have been the main focus of the thesis. This is because mass multiplication, maintenance and conservation of superior provenances of these plant species depend on reliable propagation methods. Domesticating or managing these trees successfully in their natural habitats hinges on better propagation methods. Generally, many wild fruit trees are sexually propagated (i.e., from seeds), but a few have been successfully propagated by vegetative methods.

Results of this study (Chapter 2) demonstrate that mature *U. kirkiana* tree species are amenable to micro-propagation. The objective of the study was achieved, and hence, precocious fruiting can be achieved using the developed micro-propagation protocol. However, there were still low multiplication rates compared to micro-propagation protocols developed for *P. capensis* tree species. Effect of seasonality on rooting capacity of *U. kirkiana* micro-cuttings needs further investigation to optimise the developed protocol. Furthermore, improvement in survival of plantlets when potted or planted needs to be evaluated.

Results presented in Chapter 2 show that proper stock plant manipulations, and the use of rejuvenated and pre-conditioned stock plants, were necessary for *in vitro* propagation of

mature *U. kirkiana* tree species. Manipulation of stock plants before collection of explants and use of an effective sterilant such as $HgCl_2$ can overcome *in vitro* contamination, rejuvenation problem of explants and phenol accumulation which are the major barriers to micro-propagation of *U. kirkiana* tree species. From this study, lateral shoots from pre-conditioned stock plants are ideal for *in vitro* propagation of *U. kirkiana* tree species.

Results in Chapters 3 to 6 demonstrate that phenol accumulation at the graft union could be implicated in graft incompatibility. However, phenol production is known to be seasonal, and hence timing of macro-grafting can minimise excessive exudates (phenol deposits) which have adverse effects on graft compatibility. Furthermore, quantification of phenol deposits in different seasons would improve our understanding on the adverse effects of these phenols on graft compatibility in different seasons.

The results (Chapters 3 - 6) also demonstrate that poor callus formation, presence of *p*-coumaric acids and cell necrosis were the causes of graft incompatibility in *U. kirkiana* scion and rootstock combinations. From this study, some breakthroughs have been achieved and Figure 9.1 shows a schematic diagram which elucidates problems and possible solutions for successful grafting of *U. kirkiana* trees as described in Chapters 5 - 6.

Plant macro-propagation: this includes rooting stem cuttings, air layering, budding and grafting. Some tree crops are easily propagated by macro-propagation while others are difficult. In this study, rooting of *P. capensis* stem cuttings (Chapter 7) was difficult even with the juvenile plant materials. Therefore, macro-propagation constraints for many woody plants include low multiplication rate and scion/rootstock graft incompatibility

(Chapter 3). There has been no information on the success of grafting, air layering and budding of *P. capensis* tree species, but a low rooting percentage (11%) was obtained with epicormic shoot cuttings.

Micro-propagation techniques increase the multiplication rates of many planting materials (George, 1993; Read & Preece, 2001). Many improved micro-propagation techniques for woody plants have been established. However, there are other important tree crops that are still recalcitrant to micro-propagation techniques (Read & Preece, 2001). Micro-propagation protocol developed for a specific tree crop does not often work for all tree crops, and hence there is no recipe for all tree crops. Each tree species is unique in its requirements for successful propagation and these must be established. *In vitro* propagation protocols for *U. kirkiana* (Chapter 2) and *P. capensis* (Chapters 7and 8) tree crops reported in this study show differences in the way they responded to different growth medium formulations. Mature stock plants (*U. kirkiana*) exhibited rejuvenation problem unlike juvenile stock plants (*P. capensis*). Therefore, an efficient propagation protocol must be developed for each tree crop.

Results from different chapters have been harnessed into a schematic diagram (Figure 9.2) to summarise *in vitro* propagation problems often encountered and the possible solutions for the successful field survival and establishment of *U. kirkiana* planting material. This schematic diagram is to guide potential *U. kirkiana* tree domesticators, nurserymen and growers on the best ways to propagate and manage *U. kirkiana* planting material from the nursery to the early stages of field establishment. This is with the ultimate goal of optimising orchard productivity.

The results obtained in Chapter 8 indicate successful somatic embryogenesis for *P. capensis*. This protocol can be used for genetic improvement either through deliberate introduction of genes with superior traits or desirable somaclonal variation. *U. kirkiana* explants were also amenable to high amount of callusing, and hence high risks of somaclonal variants. This could defeat the efforts involved in selecting superior provenances from the wild. Assessing somaclonal variations would help before mass multiplication of *U. kirkiana* plantlets through indirect somatic embryogenesis was adopted.

U. kirkiana tree species accumulate secondary metabolites and this makes *in vitro* propagation difficult. These metabolites are mainly produced in response to wounding during subculturing and grafting as a healing mechanism. These compounds might reduce graft compatibility as shown in grafted *U. kirkiana* trees (Chapters 4 - 5). Histological studies (Chapter 3), *in vitro* callus fusion technique (Chapter 4), and phenol analysis (Chapter 5) have been used to provide new information about graft compatibility in *U. kirkiana*. Collectively, such studies remain useful to screen out incompatible combinations. Further studies on phenol production associated with seasonality are needed to ascertain whether or not incompatibility can be minimised at different times of the year.

The study has shown that *P. capensis* tree crops can be successfully propagated using seeds (Chapter 6), organogenesis (Chapter 7) and somatic embryogenesis (Chapter 8). The results in Chapter 6 suggest that *P. capensis* seeds exhibit intermediate germination behaviour (i.e. they are neither true recalcitrant nor orthodox). Furthermore, the results suggest that a reasonable seed storage period (six months or more) is possible with cold storage. The

study has demonstrated that organogenesis and embryogenesis are reliable propagation methods for *P. capensis* species to achieve mass multiplication of planting material.

The study has shown that *in vitro* propagation of *P. capensis* was not as difficult as with *U. kirkiana* plants, since explants from the former tree were excised from juvenile stock plants. Shoot multiplication on Murashige and Skoog (Murashige & Skoog, 1962) medium supplemented with 2 mg l^{-1} BAP was an optimal medium formulation and root regeneration was optimal on MS with 0.5 mg l^{-1} IBA (Chapter 7). Frequent subculture of *P. capensis* micro-shoots and addition of adjuvants (casein hydrolysate) minimised shoot tip necrosis. For somatic embryogenesis, continuous exposure to thidiazuron with reduced concentrations at different stages was necessary for somatic embryo induction and maturation. Therefore, reproducible propagation methods were developed for *P. capensis* trees that enable mass multiplication of planting material. Figure 9.3 is an illustration of propagation methods described in this research study for *P. capensis* tree species. This schematic illustration is aimed at guiding potential *P. capensis* tree propagators.

Each plant is unique in its regeneration requirements, and hence better propagation protocols must be identified depending upon the objective, cost and economic value of the crop plant in question. Somatic embryogenesis is more efficient in increasing the number of plantlets regenerated from a single plant.

9.2 Suggestions for future investigations

Based on the results of the present study, some areas that need further studies have been identified. *In vitro U. kirkiana* root regeneration and growth of micro-cuttings (Chapter 2) were not satisfactory and this is suspected to be due to lack of sufficient rejuvenation of stock plants and explants. Therefore, experiments on different methods to rejuvenate stock plants would bring a better insight of *in vitro* growth capacity of *U. kirkiana* tree species. In addition, *in vitro* propagation of *U. kirkiana* using mature stock plants is challenging due to heavy contamination and failure to rejuvenate explants. Furthermore, old shoot explants from grafted trees did not respond to different MS medium supplements. This suggests that there was a rejuvenation problem and possible adverse effects of phenol deposits, but this warrants further studies.

The study has outlined a method to achieve culture asepsis from mature *U. kirkiana* trees (Chapter 2). With this effective decontamination method and rejuvenated lateral shoots, it would be important to investigate micro-grafting of *U. kirkiana*. This is likely to overcome the rejuvenation problem. Moreover, use of lateral shoots will avoid the mismatching scion/stock problem for micro-grafts.

Accumulation of phenols at the union inhibited graft compatibility in *U. kirkiana* grafts. However, other factors are equally important such as activities of enzymes, proteins, starch and auxins (Andrews & Marquez, 1993). These factors have been also implicated in graft incompatibility of many fruit trees (Errea *et al*., 1994b; Ermel *et al*., 1997), but these factors have not been studied in *U. kirkiana* graft combinations. Despite the fact that

phenolics play a role in graft incompatibility of *U. kirkiana* scion and stock combinations, the main cause of graft incompatibility is still complex and requires further research.

Grafting is still a potential propagation method due to dwarfing effects imposed by the stocks. However, graft incompatibility is a threatening problem, and hence selection of compatible scion/stock combinations is needed at an early stage for stable orchard productivity. Furthermore, graft incompatibility is a complex process and phenols are part of the complex mechanism. In the present study, histological experiment provided useful information so too was callus fusion technique. However, there is need for field evaluations for scion/stock combinations that show compatibility using histological, phenol analysis and callus fusion techniques. This will cross-examine if the present graft compatibility assessment techniques simulate field observations.

Scientific research studies on different macro-propagation (vegetative propagation) methods for *P. capensis* tree species have not yet been done. There is need to evaluate air layering, budding, grafting and micro-grafting as potential vegetative propagation methods for *P. capensis* tree species. Furthermore, field survival assessment of air layers and budded or grafted *P. capensis* trees will be required. This is because *U. kirkiana* air layers and budded trees have shown poor field survival despite good rooting capacity or graft take (Akinnifesi *et al*., 2007). Therefore, such research studies will assist propagators in selecting a feasible and reproducible propagation method for *P. capensis* tree species.

Shoot tip necrosis problem for *P. capensis* micro-shoots (Chapter 7) was not completely eliminated despite frequent subculturing and adding casein hydrolysate to the culture

medium. Although the shoot tip necrosis was not very serious, it is good to eliminate it in order to improve shoot multiplication. Therefore, evaluation of other adjuvants or procedures to completely eliminate shoot tip necrosis in *P. capensis* micro-shoots would be required so that to improve production of multiple micro-shoots.

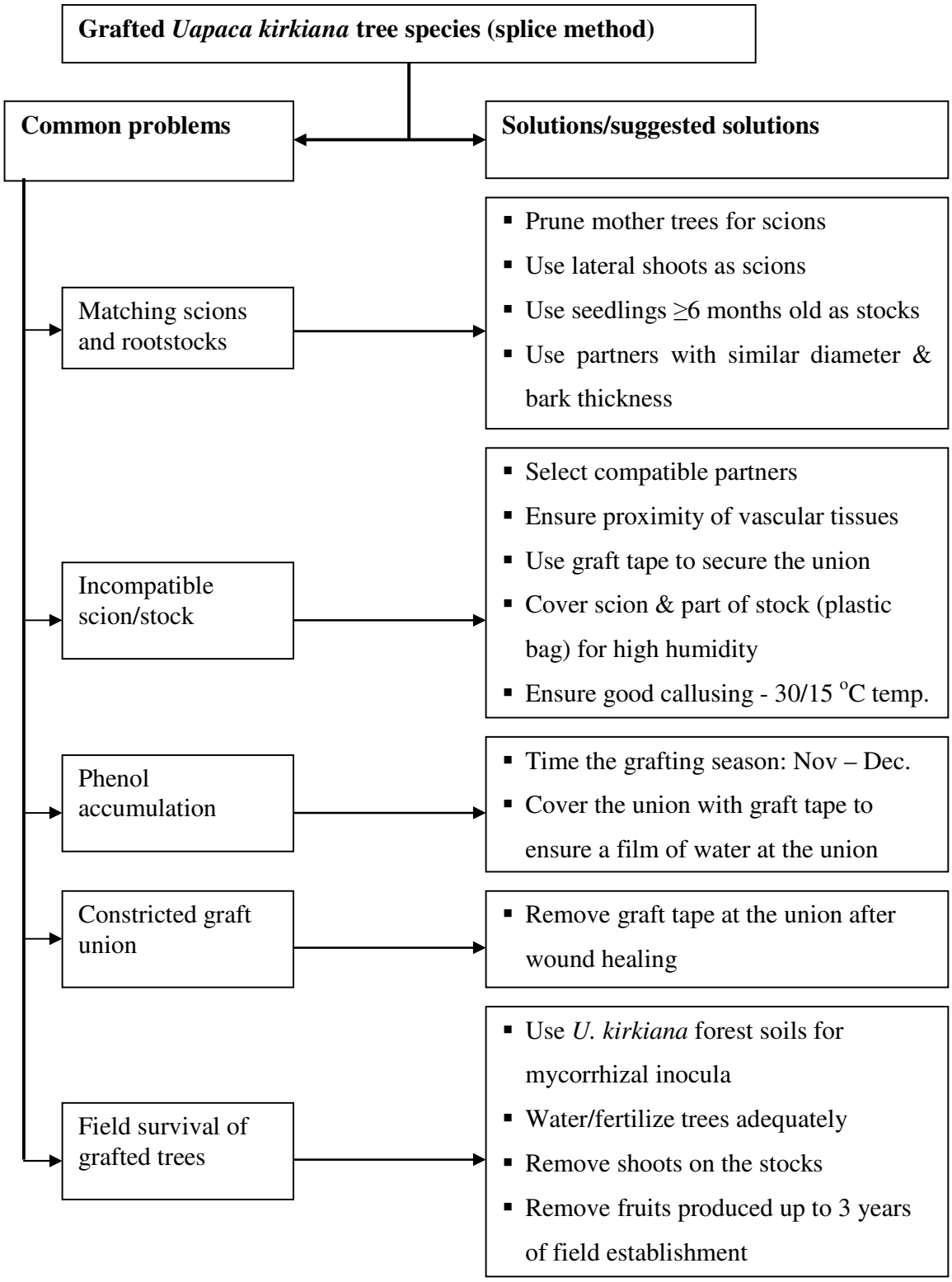

Figure 9.1 A schematic diagram for grafted (splice method) *Uapaca kirkiana* tree species

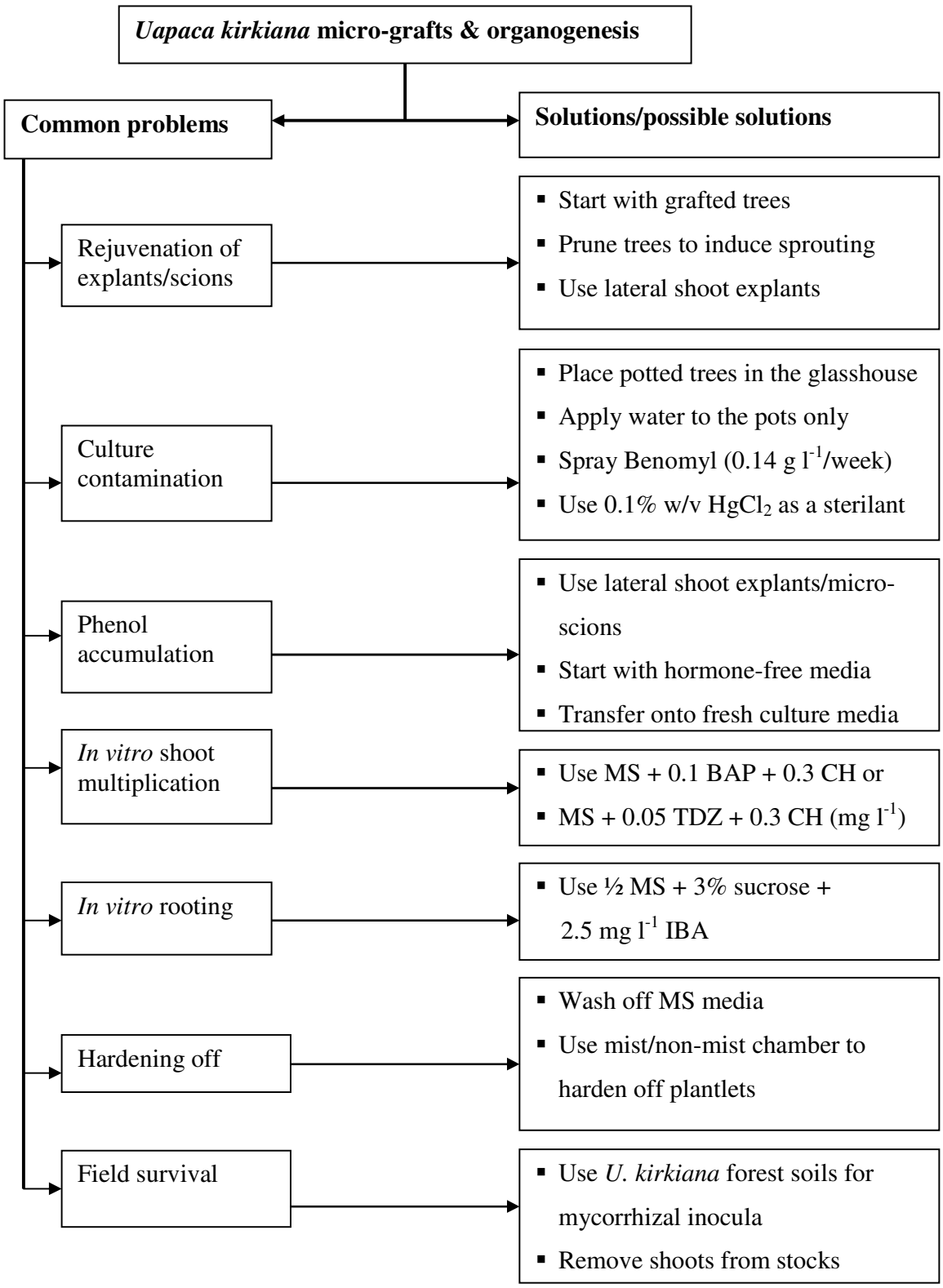

Figure 9.2 A schematic diagram for *in vitro* propagation of *Uapaca kirkiana* tree species

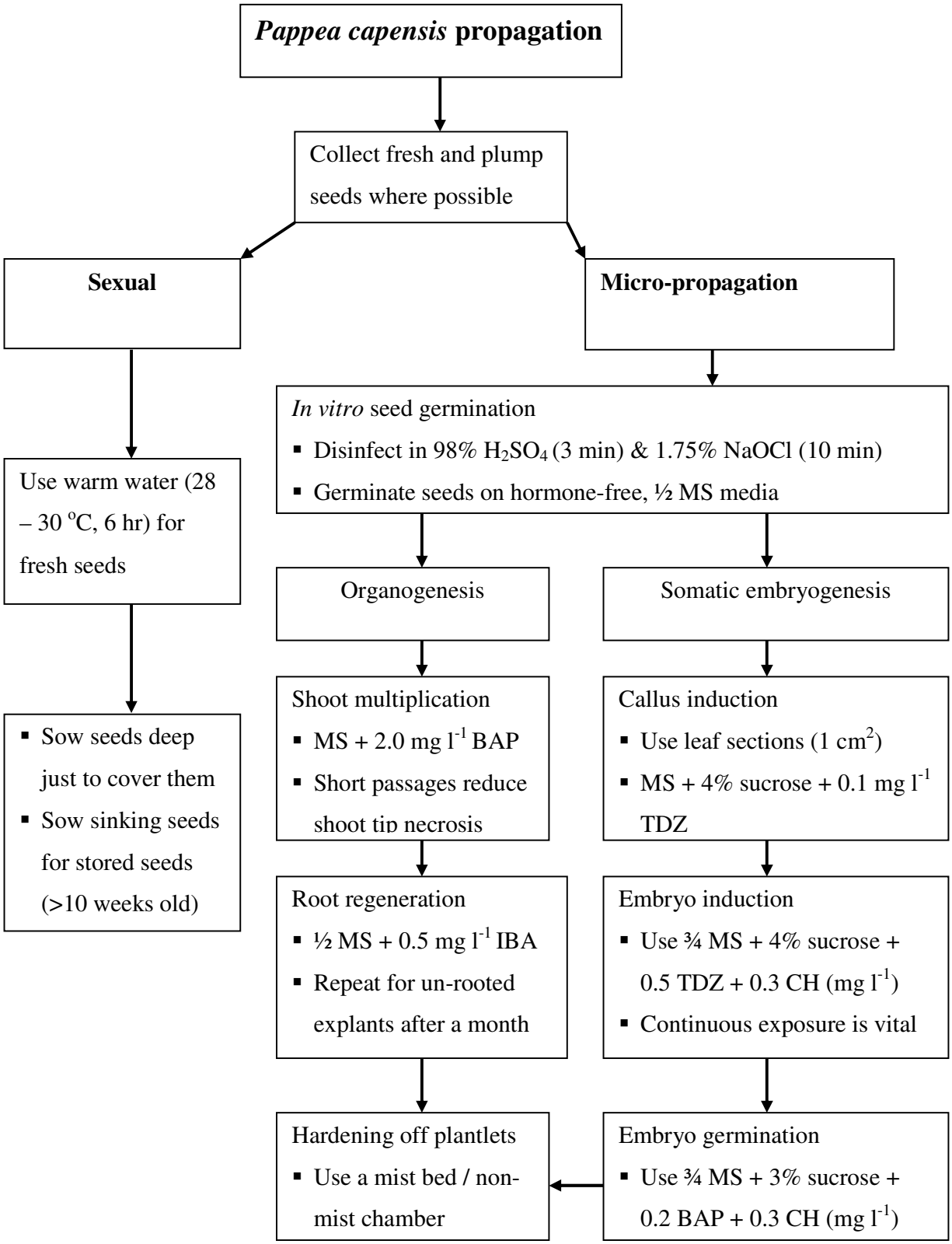

Figure 9.3 A schematic diagram for propagation methods of *Pappea capensis* tree species

SUMMARY

Uapaca kirkiana and *Pappea capensis* tree species are potential sources of income to the rural community dwellers of southern Africa. Therefore, domestication or managing these tree species in their natural habitats is warranted. Domestication, tree improvement and affordable silvicultural management of these renewal resources hinge on reliable propagation protocols. The present study was carried out with the following main objectives:

- To develop propagation protocols that enable rapid and mass production of *Uapaca kirkiana* and *Pappea capensis* planting materials

- To evaluate the graft compatibility within *Uapaca kirkiana* tree clones, provenances and species

A series of experiments were carried out and the results of the study are useful for tree improvement, domestication, conservation and to farmers and nurserymen. Significant findings from the study are as follows:

- A successful decontamination of hard-to-disinfect *U. kirkiana* explants was achieved through pre-conditioning of grafted trees and use of an effective sterilant ($HgCl_2$). Stock plant rejuvenation was achieved through pruning the trees and use of lateral shoots excised from grafted trees. A positive growth response from lateral

shoot explants was obtained on MS medium supplemented with 0.1 mg l^{-1} BAP, 0.04 mg l^{-1} NAA and 0.3 mg l^{-1} CH. Rooting was obtained on MS medium with 2.5 mg l^{-1} IBA (Chapter 2). This reproducible micro-propagation procedure enables rapid clonal multiplication of mature *U. kirkiana* tree species, and hence a major breakthrough in micro-propagation of *U. kirkiana* using mature stock plants.

- Early diagnosis of graft incompatibility is important to reduce losses due to graft incompatibility which might occur several years after a successful orchard establishment. The study (Chapter 3 and 4) has identified poor callus proliferation, cell necrosis and accumulation of phenols at the graft union as indicators of graft incompatibility. Therefore, these parameters can be assessed during early life of the young grafted trees to determine potential graft compatibility. This will enable selection of compatible scions and rootstock, and hence stable orchard establishment.

- Isolation of *para*-coumaric acid (with high peaks) and presence flavonoid derivatives such as quinones (Chapter 5) at the union of less compatible *U. kirkiana* graft combinations improved our understanding on the adverse effects of accumulation of these secondary metabolites on graft compatibility. Therefore, the choice of *U. kirkiana* scion/stocks combination must be done using compatible partners that do not show high accumulation of para-coumaric acid and flavonoid derivatives which hinder cell elongation or callus proliferation, and hence brings about graft incompatibility.

- The study has determined an affordable and easy technique for improved *P. capensis* seed germination (Chapter 6). Use of water floatation method enables farmers to identify the viable seeds after seed storage, and hence ensures good seed germination.

- The study (Chapter 7 and 8) has established new innovations in clonal propagation of *P. capensis*. The developed somatic embryogenesis protocol offers an option for tree improvement through rapid and mass multiplication of planting materials, which can be subjected to selection pressure for superior traits.

Based on the results obtained from different experiments conducted in this study, new knowledge on the causes of incompatibility in grafted scions and rootstocks; and *in vitro* shoot multiplication and root regeneration of *U. kirkiana* and *in vitro* propagation potential of *P. capensis* has been generated. This will contribute to our understanding of different trees species responses to manipulation. Also, the knowledge generated will be useful in advancing propagation and domestication of the tree species.