CHAPTER 1

LITERATURE REVIEW

1.1 *Uapaca kirkiana* fruit trees

1.1.1 Botany and ecological distribution

Uapaca kirkiana Müell Arg. is a small to medium sized tree with an evergreen or semi-deciduous growth habit depending on the prevailing environmental growth conditions. In Malawi, it is locally known as 'masuku', belongs to the Euphorbiaceae family and has multiple and spreading branches that form a dense crown. Leaves are simple, large and alternate, leathery, strongly ribbed, dark green and with rounded tips. Young leaves are covered with curly hairs on the under surface. Its wood is light with white sapwood and has reddish brown heartwood (Storrs, 1995). It is dioecious with staminate flowers borne in dense clusters while female flowers are solitary. It is difficult to distinguish male from female trees when they are juvenile. The male and female flowers are greenish-yellow and inconspicuous (Palgrave & Drummond, 1983). Fleshy fruits (3 - 4 cm diameter) contain edible pulp which is rich in dietary nutrients and these fruits ripen towards the end of the dry season, October – December (Ngulube *et al*., 1995). The pulp is yellowish and sweet-tasting (Storrs, 1995). The fruit contains three to five recalcitrant seeds germinating within three months after harvest during the rainy season, January – March (Mwamba, 1995). Recalcitrant seeds are defined as seeds that do not tolerate desiccation injury and have a short storage period. They must be stored at the lowest temperatures that are non-injurious

(Berjak *et al*., 2004). The tree has a long juvenile phase and fruits ripen towards the end of dry season or during the rainy season (Ngulube, Hall & Maghembe, 1995). Figure 1.1 shows *U. kirkiana* tree in fruit (Figure 1.1A); a two-year old grafted *U. kirkiana* tree (Figure 1.1B); *U. kirkiana* tree with a heavy fruit load (Figure 1.1C) and *U. kirkiana* fruits (Figure 1.1D).

U. kirkiana trees are common to Miombo woodland (Figure 1.2). The Miombo eco-region is composed of open canopy and broad-leaved forest trees. It is found on heavily populated plains and the lower mountain slopes and covers about 3.8 million km^2 of the Zambezian phyto-region (Chidumayo, 1997). The area extends over seven countries, namely Angola, Malawi, Mozambique, Tanzania, Zimbabwe, Zambia, Namibia and parts of the Democratic Republic of Congo. *Brachystegia, Julbernardia* and *Isoberlinia* genera dominate the Miombo eco-region (Chidumayo, 1997). The Miombo eco-region has the highest rate of deforestation in the tropics as a result of increasing human population and economical dependence on natural resources (Roy *et al*., 1996).

1.1.2 Importance and commercial potential

Uapaca kirkiana fruit trees contribute significantly to food security, especially to the rural community dwellers. They offer considerable scope for enhancing economic security in the region (Akinnifesi *et al*., 2006). Fruit is available when other foods are scarce (Akinnifesi *et al*., 2004). Consequently, they serve as food reserves during seasonal food shortage periods most of the rainy season, November – February (Maghembe & Seyani, 1992; Saka *et al*., 2002; Ngulube *et al*., 1995; Akinnifesi *et al*., 2004, 2006). The fruits are traded widely, eaten fresh and processed into juice, jam and a variety of wines in the Southern

Africa Development Community (SADC) countries. They are highly valued indigenous fruit trees of the Miombo woodlands and the most preferred fruit by communities in southern Africa (Maghembe *et al*., 1998; Ramadhani, 2002). Surprisingly, there is no known commercial cultivation of *U. kirkiana* and all current market of fruit comes from wild populations. Recent studies in Malawi and Zimbabwe showed that the availability of indigenous fruits, especially *U. kirkiana*, reduces the probability of household poverty by 33% during a seasonal food shortage period (Mithöfer, Waibel & Akinnifesi, 2006).

The *U. kirkiana* trees are also hosts of edible fungi (mushrooms) living in symbiotic association with these trees (Maghembe *et al*., 1998). The tree and its fruits are also sources of income for the rural communities of southern Africa. In Malawi and Zambia, the fruit is used to make wines and gins and is sold along the roadside stalls and in some local markets (Maghembe & Seyani, 1992). The fruit can be eaten raw, made into jams or used to produce fruit juices (Ngulube *et al*., 1995). The wood of *U. kirkiana* trees also has a high market value for making bee hives (Storrs, 1995).

1.1.3 Production and cultivation

The germplasm of 16 *U. kirkiana* tree provenances have been collected, characterised and established in multilocational trials in five of southern African countries, namely Malawi, Mozambique, Tanzania, Zimbabwe and Zambia (Kwesiga *et al*., 2000). This is with the ultimate aim of domesticating *U. kirkiana* trees. Domestication, according to Simons (1997), is defined as a 'human-induced evolution that brings wild plants into wider cultivation through a farmer-driven or market-led process'. Domestication of *U. kirkiana* is required, but a number of processes are needed. These include selecting and breeding

superior tree provenances, developing reliable propagation protocols, multiplying and disseminating germplasm as well as developing orchard management techniques (Akinnifesi *et al*., 2000b). These processes are necessary in order to capture superior germplasm onto farm land. Domesticating *U. kirkiana* fruit trees will benefit subsistence farmers through income generation and improved nutrition.

The wide cultivation of *U. kirkiana* is limited by farmers' lack of knowledge on the biology, ecology, propagation and management of the tree (Maghembe *et al*., 1998; Kwesiga *et al*., 2000). Research on domestication at the World Agroforestry Centre in southern Africa has addressed most of the factors relating to tree selection, establishment and management both on-farm and post harvest and for market development. Propagation has relied on conventional techniques which include seedling, grafting and air-layering (Akinnifesi *et al*., 2004). A need to develop micro-propagation, to allow for mass multiplication of the superior cultivars, has been identified.

1.2 Jacket plum (*Pappea capensis*) tree species

1.2.1 Botany and ecological distribution

Jacket plum (*Pappea capensis* L.) trees belong to the Litchi family (Sapindaceae). *Pappea capensis* is named after Ludwig Pappe (Fivaz & Robbertse, 1993; Venter & Venter, 1996). The tree grows up to 3.9 m high and can be deciduous or evergreen depending upon the climate. It grows tall in areas with heavy rainfall (Anonymous, 1997; van Wyk & Gericke, 2000) and bears alternate leaves forming a rosette at the ends of a small drooping branch. The stem is grey and often lichen-covered in arid areas. According to Fivaz & Robbertse

(1993), jacket plum tree is monoecious. Inflorescences are short and found in leaf axils and the staminate male flowers are borne on a lateral panicle, while the carpellate female flowers are scented and form a raceme (Palmer & Pitman, 1972). The male flowers have 8-10 stamens. Bees are the main pollinators. The trees flower from October to March and set their round to oval fruits in December to May (Anonymous, 1997). Figure 1.3A shows a mature *P. capensis* tree Figure 1.3B shows fruits. *P. capensis* trees are widely distributed throughout southern Africa, only absent in the western Kalahari and northern Namibia. They are common in KwaZulu-Natal, Swaziland, Mpumalanga and tropical Africa and are fairly adapted to a wide range of ecological areas (van Wyk & Gericke, 2000).

1.2.2 Importance and commercial potential

Jacket plum (*P. capensis*) tree bears fleshy fruits, which can be processed into vinegar, jelly and jam (Palmer & Pitman, 1972). The seeds are rich in edible, non-drying and contain fairly viscous oil (about 74%) used for making soap and oiling guns (Palmer & Pitman, 1972; Venter & Venter, 1996; van Wyk & Gericke, 2000). This oil can be exploited as an alternative source of bio-diesel and such diesel-fuels are renewable and emit less greenhouse gasses to the atmosphere (Ramadhas, Jayaraj & Muraleedharan, 2005; Canoira *et al*., 2006). Moreover, vegetable oil yielding trees that are grown locally and would contribute to lower amounts of net greenhouse gasses to the atmosphere than fossil diesel does (Bouaid *et al*., 2005).

1.2.3 Production and cultivation

P. capensis tree species are still growing in the wild and the known method of propagation is by seeds. However, seedling growth is extremely slow (Palmer & Pitman, 1972;

Anonymous, 1997). There has been no scientific research done on seed germination and vegetative propagation of jacket plum trees. Developing a reliable propagation protocol for mature *P. capensis* tree species would be challenging. Managing wild tree species in their natural habitat or on farm land requires reliable propagation knowledge to achieve domestication and optimal productivity.

1.3 Tree domestication process

Germplasm collection and evaluation for tree crop improvement, product quality and market research of *U. kirkiana* fruit trees have been carried out with the ultimate goal of domestication. However, domestication hinges on availability of good quality planting materials that result in precocious fruiting (Akinnifesi *et al*., 2006). Tree domestication involves a number of processes as outlined in Figure 1.4. For *P. capensis*, germplasm collection and improvement, and product quality enhancement are yet to be done since this tree species has been recently identified as a potential tree crop for bio-diesel fuel. Although germplasm collection of *P. capensis* tree species has not been carried out, it is still important to develop efficient and reproducible propagation protocols in order to have adequate germplasm for evaluation and selection.

1.4 Propagation methods

1.4.1 Sexual propagation

P. capensis and *U. kirkiana* tree species are mainly propagated by seeds. Available literature indicates that seed germination is not a problem for *U. kirkiana* and 95% seed germination has been achieved with seeds from fresh fruit (Maliro, 1997). For *P. capensis*,

slow seedling growth has been singled out as the main problem (Venter & Venter, 1996). Even though *P. capensis* seed coats seem to be relatively weak, it appears that they can still impede water imbibition, and hence lead to poor seed germination is obtained (Mng'omba & du Toit, 2006).

1.4.2 Vegetative propagation

Rooting mature *U. kirkiana* stem cuttings is not feasible (Akinnifesi *et al*., 2004) and this is a typical characteristic of many tropical woody trees (Kwapata *et al*., 1999). Some successes in grafting (80% graft take) and marcotting (63%) of *U. kirkiana* trees were achieved at Makoka Research Station in Malawi, but there was poor graft survival and slow growth in the field (Akinnifesi *et al*., 2006). Grafting of fruit trees offers a viable option to propagate mature plants, but stock selection is important, especially for graft compatibility. To my knowledge, there has been no scientific research done on *U. kirkiana* scion/stock combinations, and hence compatibility phenomenon is not well understood in grafted *U. kirkiana* trees. Graft incompatibility has an impact on orchard productivity (Simons, 1997). Therefore, a major challenge is to develop methodologies that diagnose early signs of graft incompatibility. This will allow selection of compatible scion/stock combinations for stable orchard productivity.

(a) Propagation by tissue culture

Tissue culture techniques enable regeneration of plants through organogenesis or embryogenesis. The latter method is useful for crop improvement through gene transfer techniques. Furthermore, somatic embryogenesis enables synthetic seeds to be developed, breeding cycles to be shortened and genetic transformation to be achieved (Singh & Chand,

2003). Somatic embryogenesis has been reported in a number of woody trees (Singh & Chand, 2003; Robichaud, Lessard & Merkle, 2004), but major constraints encountered in somatic embryogenesis include embryo maturation, germination and conversion to plantlets (Robichaud *et al*., 2004). Medium components such as sugars, plant growth regulators, agar and other treatments have been manipulated to regenerate plants through somatic embryogenesis. Unfortunately, failures in somatic embryogenesis are not reported.

The advantage of embryogenesis over organogenesis is the ability for the embryos to develop functional roots within a short period. The shoot multiplication and root regeneration can occur simultaneously and this enables rapid regeneration of emblings (Bajaj, 1986), which is often not possible when plantlets are regenerated through organogenesis. Indirect embryogenesis occurs when embryos are regenerated through callus and according to Bajaj (1986), the number of plantlets that can be regenerated through embryogenesis surpasses those regenerated through organogenesis.

Reliable propagation protocols for mass production and precocious fruiting of *U. kirkiana* fruit trees are needed. *U. kirkiana* trees have long juvenile phase when propagated sexually and this frustrates many potential fruit tree growers. According to Parfitt & Arulsekar (1987), micro-propagation of mature trees is preferred over the embryos or seedlings since it is not always possible to determine if the embryos or seedlings have the genetic potential to develop the desired qualities later in their development. Furthermore, micro-propagation of mature trees is preferred when the gender of the trees needs to be assured. This is important for *U. kirkiana* fruit trees since they are dioecious (i.e. male and female flowers

are found on separate individual trees), and hence the proportion of female and male trees in an orchard plays a major role in terms of orchard productivity.

1.4.3 Culture contamination

Culture contamination is a problem to *in vitro* propagation due to rapid proliferation of pathogens (Enjalric, Carron & Lardet, 1998). With the exception of cryptic contaminants, many are visible at primary initiation. Generally, axenic cultures are preferred at any stage, and hence contaminated cultures are often discarded (George, 1993). Contaminants cause death of explants by exuding toxins or overgrowing the explants. Consequently, contaminants out compete and many adversely affect the growth of explants. Many plants are associated with symbiotic microbes that are contaminants in the growth media. Endogenous or endophytic microbes are often difficult to decontaminate although some are beneficial for the growth of explants (Herman, 1990). According to Cassells (1991), culture asepsis is important in any micro-propagation protocol.

Studies on *U. kirkiana* micro-propagation have been carried out (Maliro, 1997; Chishimba *et al*., 2000; Nkanaunena, 2002), but without success where explants have been excised from adult stock plants partly due to high contamination. *In vitro* propagation protocols of *U. kirkiana* plants were only developed using seedlings (juvenile plant materials) as stock plants (Maliro, 1997; Chishimba *et al*., 2000; Nkanaunena, 2002). However, as indicated above, it is not possible to ascertain the gender and future characters of *U. kirkiana* plantlets regenerated from the seedlings. There have been no scientific results available for micro-propagation of *P. capensis* tree species and successful protocols are yet to be developed.

P. capensis trees are monoecious (Fivaz & Robbertse, 1993), and hence determining the gender of the seedlings or embryos is not as critical as for *U. kirkiana* tree species. Consequently, use of seeds or embryos as planting materials for *P. capensis* will not affect the gender of the trees.

1.5 Mycorrhizae

Many tree species of the Miombo woodlands host mycorrhizal fungi, therefore, introducing them onto farmland is often difficult. This is because mycorrhizal flora is eliminated through continuous cropping and other land uses (Högberg, 1982). Mwamba (1995) reported vigorous growth of *U. kirkiana* seedlings when inoculated with symbiotic fungi. It was observed that the fungal hyphae formed mycelia and increased the volume of soil exploited. This increased nutrient and water uptake. Seven fungal species were isolated from the cultures of *U. kirkiana* seedlings. However, individual isolates were not identified. Högberg (1982) reported that *U. kirkiana* trees host both ecto- and endomycorrhizal fungi apart from other 'specialised' parasites belonging to different fungal groups. Multiple infections on a single *U. kirkiana* host were associated with both symbiotic and parasitic fungi depending on prevailing conditions (Mwamba, 1995). He further reported a significant growth of new roots when the fungal isolates were inoculated on fresh seedlings.

1.6 Effects of phenolics on graft compatibility

Phenolics are secondary metabolites which occur in vascular plants, but quantities vary with plant age, developmental stage and growth conditions (Muofhe & Dakora, 1999;

Wink, 1999). Phenols have aromatic ring structures with one or more hydroxyl (OH) bonds (Fry, 1988; Waterman & Mole, 1994) and polyphenols are compounds with many phenolic substitutes. Furthermore, the composition of phenols depends on the genetic constitution of the plant species, and hence some plants accumulate more than others. Phenols have multiple functions which include plant-insect, plant-pathogen and plant-plant (allelopathy) interactions (Waterman & Mole, 1994; Wink, 1999). Some plants store water-soluble phenols in large amounts (200 – 500 mM) to deter the feeding of herbivores (Wink & Schimmer, 1999). Plants are able to convert some phenolic compounds into different forms. A simplified shikimate pathway for phenolic compounds is shown in Figure 1.5. According to Neish (1964) cinnamic acid derivatives especially, *para*-coumaric, caffeic and ferulic acids are found in many plants. Furthermore, these are involved in biosynthesis of lignin and flavonoids. Plants use *para*-coumaric acids are precursor to lignin, but this biosynthetic reaction is irreversible (Neish, 1964).

Plants release phenolics in response to wounding as a defensive mechanism against pathogen attack (Waterman & Mole, 1994). Wounding occurs during grafting of trees and numerous reports indicate that phenols are implicated in graft incompatibility (Errea *et al*., 1994b; Errea, 1998; Pina & Errea, 2005). Cell walls of some plants contain phenolic compounds including lignin and non-growing cells such as wood cells contain up to 30% lignin (Fry, 1988). Phenolics such as *para*-coumaric acids (Figure 1.6A) are esterified while ferulic acids (Figure 1.6B) are etherised to lignin in the cell walls (Xu *et al*., 2005). These authors further reported that both *para*-coumaric acid and ferulic acid are the precursors of lignin, anthocyanin, phytoalexins and flavonoids. According to Méndez *et al*. (1968), *para*-coumaric acids are inhibitors of cell elongation, and hence they can reduce

parenchymatous cell growth. Consequently, graft compatibility can be reduced due to the actions of *para*-coumaric acids. Usenik *et al*. (2006) isolated *para*-coumaric acid in incompatible scion/stock combinations of apricot. Therefore, presence of this compound at the graft union can indicate a graft combination problem.

Phenols are important in lignification and protein binding (Pina & Errea, 2005), but have been implicated in graft incompatibility. Differences in quantity and the presence of specific phenols above and below the graft union area reduce graft compatibility (Facteau, Chestnut & Rowe, 1996; Usenik *et al*., 2006). When the wounding stress is over, many soluble phenols could be polymerised and deposited in cell walls (Swain, 1979) and might play an important role in graft compatibility.

1.6.1 Methods of separation for phenolic compounds

There are a number of methods used to separate, quantify and identify various phenolics from plant samples. The methods include fluorescence microscopy, Folin-Ciocateau reagent and high performance liquid chromatography (HPLC), among many others. The Folin Ciocateau method provides an estimate of the total phenols in a sample. Fluorescence microscopy technique is based on colour dye associated with specific phenols. However, it only provides an indication that different phenols are present in a sample but the method is not quantitative. HPLC is used to separate and quantify the types of phenolic compounds, and hence a reliable method to quantify phenolic compounds in plant samples.

1.7 Summary

From the literature review, it is clear that *U. kirkiana* and *P. capensis* tree species of southern Africa make significant contributions to food (fruits and edible oil) and income sources. However, there has been limited research done to develop reliable propagation protocols for these tree species. Therefore, there is an urgent need to develop such methods and new management protocols for accelerating domestication of these wild fruit trees to improve production and utilization. Research work is needed to solve these problems for conservation and on farm management of these wild tree species. The various chapters presented in this thesis add new knowledge and improve our understanding of the ways in which these trees can be successfully propagated.

Figures

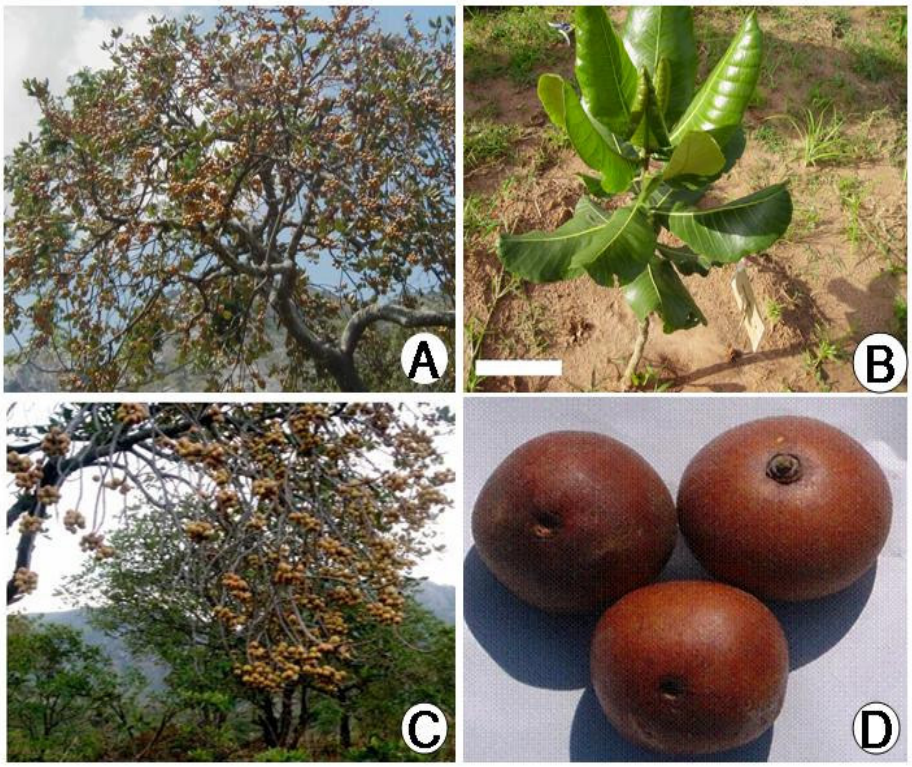


Figure 1.1 *Uapaca kirkiana* (A) tree in fruit; (B) grafted tree growing at Makoka Research Station in Malawi (two years old after grafting); (C) a tree with heavy fruit load; (D) mature fruits

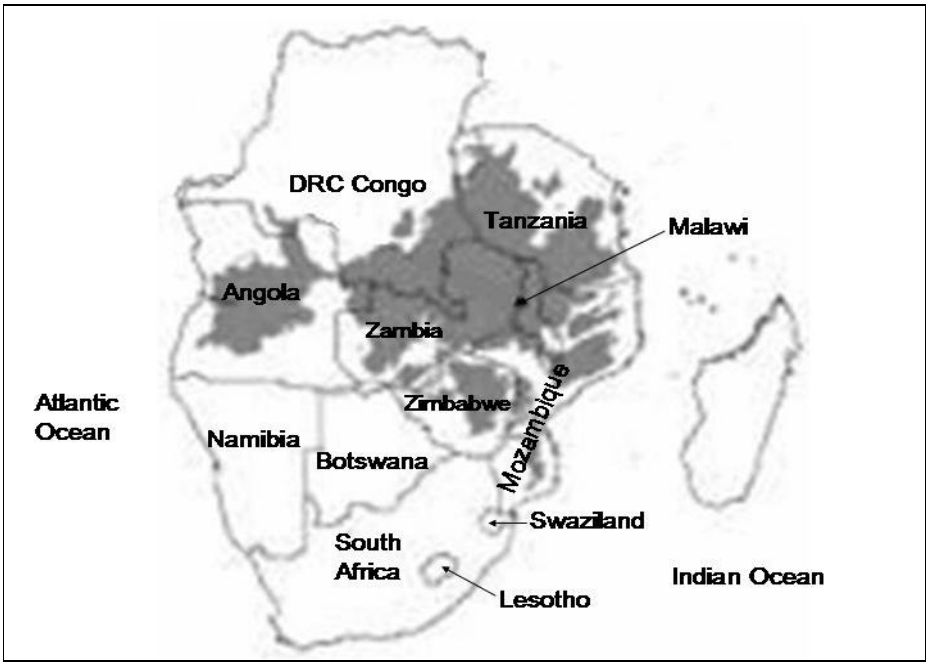


Figure 1.2 Map of southern Africa showing the distribution of Miombo woodlands (shaded areas) (adapted from White, 1983)

Figure 1.3 Jacket plum (*Pappea capensis*) (A) adult tree; (B) mature fruits and a few shattered pods

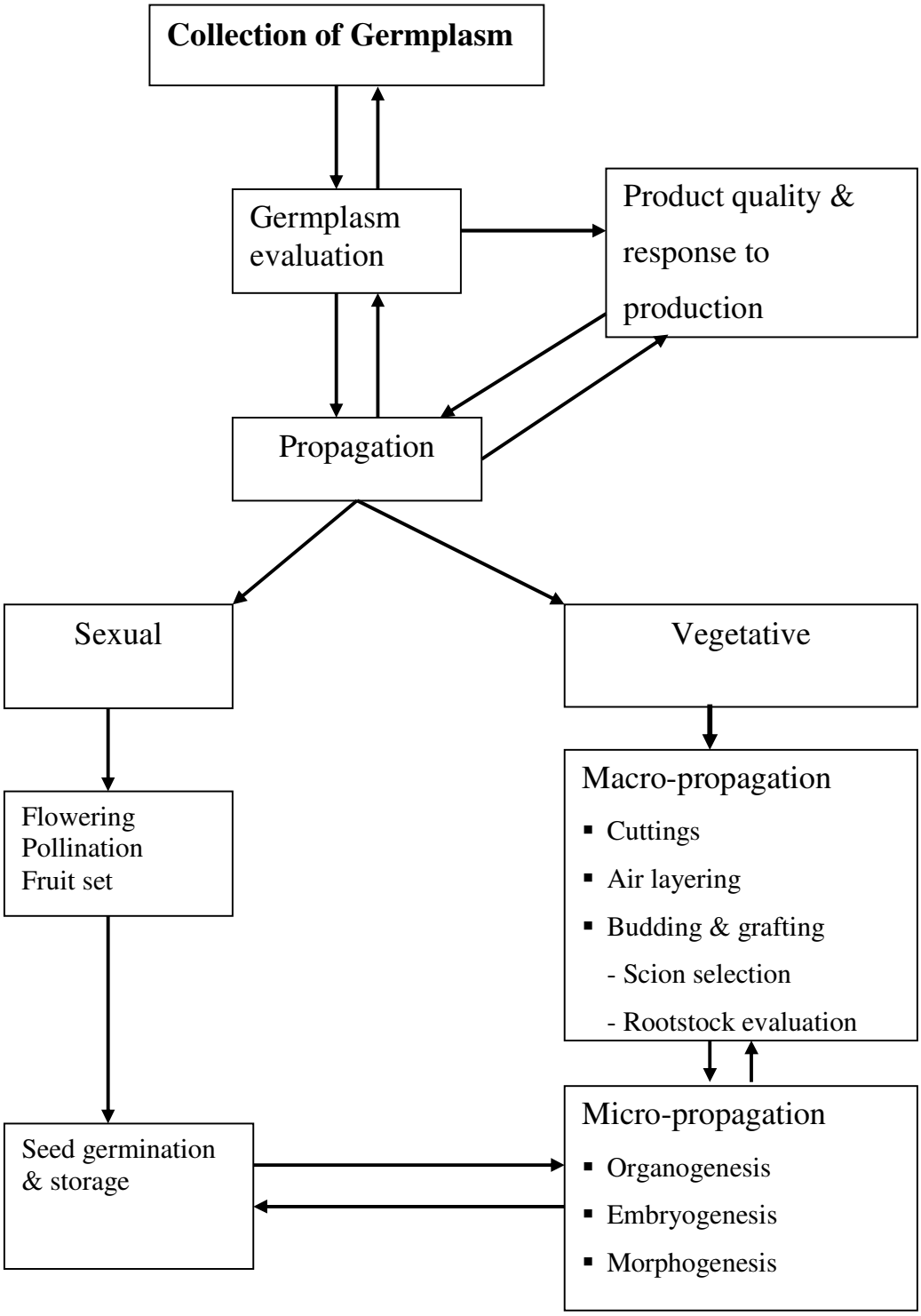


Figure 1.4 A schematic diagram for domestication of wild tree species

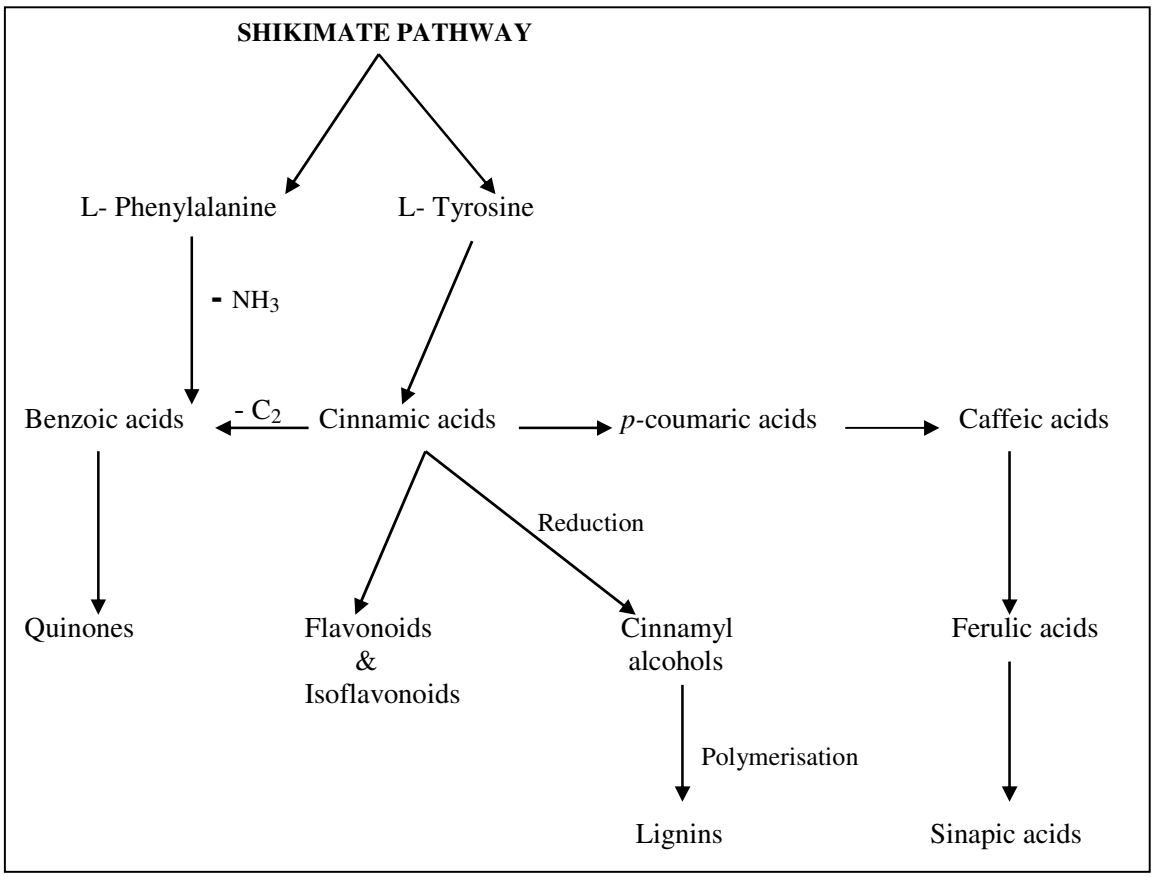
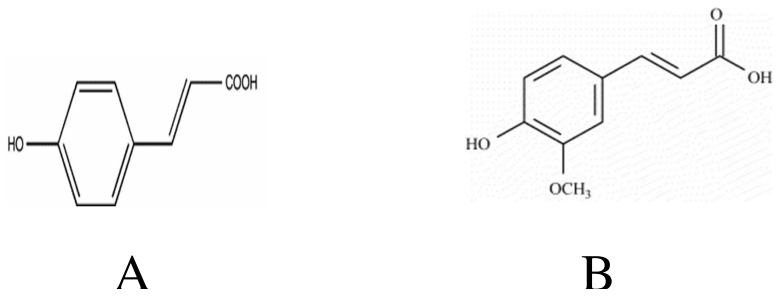


Figure 1.5 Formation of cinnamic acid derivatives from phenylalanine and tyrosine in plants (Neish, 1964; Harborne, 1989) (Only key intermediates and products of interest are shown).



A B

Figure 1.6 Structure of (A) *para*-coumaric acid and (B) ferulic acid (Liu, 2006)