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Development of clonal propagation protocols for *Uapaca kirkiana* and *Pappea capensis*, two southern African trees with economic potential

By

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Submitted in partial fulfilment of the requirements for the Doctor of Philosophy degree (Horticulture)

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Declaration

I hereby declare that the thesis I am submitting for the Doctor of Philosophy degree (Horticulture) at the University of Pretoria is my own work and has not been submitted for a degree at any other institution.

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Simon Alfred Mng'omba Date

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Thanks and Glory to the Almighty God for guiding me through thick and thin. God was always my buckler in this endeavour (Psalms 23: 1- 6)

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BAP	benzylaminopurine
CH	casein hydrolysate
IAA	indole-3-acetic acid
IBA	indole-3-butyric acid
DW	dry weight
GA_3	gibberellic acid
Kin	kinetin
MS	Murashige and Skoog
NAA	naphthaleneacetic acid
PAR	photosynthetically active radiation
RP-HPLC	reverse phase high performance liquid chromatograph
psi	pounds per square inch
TDZ	thidiazuron
t_R	retention time
UV	ultra violet
2,4-D	dichlorophenoxyacetic acid

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Abstract

Experiments were carried out with the objectives of developing propagation protocols for *Uapaca kirkiana* and *Pappea capensis* tree species of southern Africa, and evaluating the graft compatibility within *U. kirkiana* tree clones, provenances and species. Reverse phase high performance liquid chromatography (RP-HPLC), Folin-Ciocalteau reagent, fluorescence microscopy and callus fusion methodologies were used to diagnose graft compatibility. Results indicated that *U. kirkiana* culture asepsis was achieved with 0.1% w/v mercuric chloride ($HgCl_2$) and using pre-conditioned grafted trees. Sodium hypochlorite (NaOCl) improved *P. capensis* seed asepsis and germination, and discarding floating seeds improved germination. Murashige and Skoog (MS) medium with 2.0 mg l^{-1} benzylaminopurine (BAP) and 0.3 mg l^{-1} casein hydrolysate (CH) was superior in shoot multiplication and 0.5 mg l^{-1} indole-3-butyric acid (IBA) for rooting of *P. capensis* micro-shoots. For somatic embryogenesis, three quarter strength MS medium with 0.05 mg l^{-1} thidiazuron (TDZ) and 0.3 mg l^{-1} CH, or 0.2 mg l^{-1} BAP with 0.3 mg l^{-1} CH, were effective in germination of *P. capensis* somatic embryos.

For *U. kirkiana* lateral shoot explants, shoot multiplication was superior on three quarter strength MS medium with 0.1 mg l^{-1} BAP and 0.3 mg l^{-1} CH. Rooting of micro-cuttings (36%) was achieved on ½ MS with 2.5 mg l^{-1} IBA. RP-HPLC, fluorescence microscopy and callus fusion studies showed that phenolic compounds play a major role in *U. kirkiana* graft incompatibility. Less graft compatible combinations showed an increase in phenol deposits above the union and graft incompatibility was more pronounced above the union than below the union. Proliferation of parenchymatous tissues was better below the union than above the union. Fluorescence microscopy showed presence of flavonoids and polymers above the union of less graft compatible combinations. The chromatograms showed that ferulic acid was abundant and responsible for wood discolouration. The chromatograms also isolated *para*-coumaric acids which were predominant above the union of the less compatible combinations. Therefore, *para*-coumaric acids, flavonoids and polymers were implicated in graft incompatibility of *U. kirkiana* trees.

Key words: decontaminants, embryogenesis, graft compatibility, Miombo woodland, organogenesis, phenolics, rejuvenation, seed germination

GENERAL INTRODUCTION

Trees contribute significantly to the livelihood of people in many different ways. They are sources of food, timber, poles and medicines and are grown for ornamental purposes such as for shade and protection. Wild trees have become potential commercial crops because they have multiple uses and imminent value. Edible fruits and oils are sources of food and income, and hence becoming important to many countries. Fruit trees can be utilized in different forms, for example *Uapaca kirkiana* Müell Arg. fruits can be eaten raw and made into refreshing drinks and a variety of wines (Kwesiga & Mwanza, 1995). The fruits have, therefore, become important to people in rural communities, especially in the arid and semi-arid countries of Africa where production of staple food has declined due to erratic rainfall, infertile soils and lack of farm inputs (Akinnifesi, Simons & Kwesiga, 2000a). Consequently, many households are food insecure and suffer from chronic malnutrition, of which children are the most vulnerable group (Akinnifesi *et al*., 2000b). Jacket plum (*Pappea capensis* L.) is another important wild tree which is rich in seed oil (74%) and this oil is usually used to make soap and for oiling guns (Venter & Venter, 1996). This oil is also potential source of bio-diesel (Le Roux, 2004). The tree also produces edible fruits which are made into jelly, vinegar and beverages (Venter & Venter, 1996).

People in rural communities can generate substantial income from wild fruit trees. For example, *U. kirkiana* fruits are gathered from the wild to be sold. They also serve as food reserves during seasonal food shortages (Maghembe & Seyani, 1992; Akinnifesi *et al*., 2006). *U. kirkiana* trees grow in symbiotic association with mushrooms, which is also a cheap but rich source of nutrients (Maghembe, Simons, Kwesiga & Rarieya, 1998). In many rural areas, women and children collect edible mushrooms for food and extra income (Okafor & Lamb, 1992; Saka, Mwendo-Phiri & Akinnifesi, 2002; Akinnifesi *et al*., 2004). Moreover, fresh fruits are good sources of vitamins (e.g. vitamin C). *P. capensis* seed oil can be a viable source of income to the rural communities as it is a potential source of bio-diesel fuel.

Food shortage in arid and semi-arid countries is prevalent but this problem can be reduced through food diversification. Many countries of southern Africa are hit by human immunodeficiency virus (HIV) / acquired immunodeficiency syndrome (AIDS) pandemic, and hence poor nutrition aggravates the plight of HIV/AIDS infected people. Therefore, fruits as nutritional and dietary supplements are vital and would fill in the food shortage gaps as well as being a source of income. The HIV/AIDS pandemic has a serious impact on agriculture as there is a low labour force, and hence loss in productivity (White & Robinson, 2000). Fruit trees are perennial and do not require much labour once they are established unlike annual crops where labour is required annually (Le Roux, 2004).

Incorporating potential wild fruit tree species onto farmland or managing fruit tree species in their natural habitats is important, especially tree species in demand (Simons, 1997). This will reduce continuous harvesting from the wild and allow these trees to be managed as renewable resources. However, incorporating wild fruit trees onto farmland would demand developing reliable propagation methods and protocols that maintain desirable traits of the high valued wild fruit tree species (Akinnifesi *et al*., 2000b).

Major challenges to the rural masses of southern Africa include poverty, poor health and food insecurity and yet the region has some important wild tree species that can provide alternative sources of income and food (Akinnifesi *et al*., 2000b). Availability of markets for *U. kirkiana* and other wild fruits in countries of southern Africa indicates the need for domestication and subsequent commercial production of these wild fruit trees. Many wild tree seeds have valuable oil which can be an alternative source of diesel fuel such as *P. capensis*. These trees can be produced locally and create income-generating activity for the rural communities. Currently, there is lack of knowledge and a shortage of improved planting stocks. Furthermore, cultivation and domestication processes are hampered (Akinnifesi *et al*., 2000b). Rapid propagation would speed up cultivation and commercialisation of potential fruits and reduce food insecurity and poverty prevailing in many rural communities of southern Africa. Therefore, efforts are needed to provide reliable planting materials and adequate information on management of wild trees.

Grafted fruit trees address the fruiting precocity problem and enable the capture of proven superior fruit traits. However, scion/stock incompatibility in certain grafted fruit trees is a major constraint that may cause high losses in planting stock and established trees (Errea, Felipe, Treutter & Feucht, 1994b). Simons (1987) estimated that about half a million grafted peach trees died in southeast USA due to scion/stock incompatibility. Therefore, selection of compatible scion/stock combinations is important to ensure successful orchard establishment and productivity. Such selection of compatible scions and stocks has been neglected previously in many orchards and do not exist in domestication of wild fruit trees. It is against this background that the present study focused on development of propagation protocols for *U. kirkiana* and *P. capensis* tree species. The general objectives for the study are:

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

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

In order to test these general objectives, the following hypotheses were developed:

1. *Pappea capensis* and *Uapaca kirkiana* tree species are amenable to different propagation methods to achieve mass production of planting materials

2. Graft compatibility exists within *Uapaca kirkiana* tree clones, provenances and species

Approach:

Topics are organised into chapter format. A general synopsis of the entire thesis and the two indigenous fruit tree species studied are presented in the general introduction. The literature review (Chapter 1) provides the available information on *P. capensis* and *U. kirkiana* tree species. These include existing propagation methods, graft compatibility and the influence of polyphenols on *U. kirkiana* scion/stock combinations.

Major constraints to the micro-propagation of mature stock plants are rejuvenation and decontamination of plants associated with endophytic or cryptic microbes. Chapter 2 presents methods used to decontaminate *U. kirkiana* explants excised from grafted trees and this is followed by micro-propagation techniques employed (Chapter 3).

There are many unresolved questions on the main cause of graft incompatibility and methodologies to recognize graft incompatibility at an early stage are very limited. Secondary metabolites (phenols) occurring in vascular plants have been implicated in graft incompatibility. The amounts of phenols in plant organs vary with age, developmental state and growth conditions (Muofhe & Dakora, 1999). This provides an opportunity to time the grafts, and hence avoid accumulation of phenols at the graft unions. Chapters 4-6 aim at establishing the role of phenols on graft compatibility and potential methods to identify early scion/stock incompatibility in *U. kirkiana* trees. Histological studies, *in vitro* callus fusion, HPLC, fluorescence microscopy and Folin-Ciocalteau reagent procedures were used to improve our understanding of the role that phenols play in graft compatibility.

P. capensis is an unknown tree crop and is unexploited in terms of its commercial potential. No scientific research has yet been published on *P. capensis* propagation. Therefore, Chapters 7 - 8 focus on different propagation methods of *P. capensis*. Available literature documents that *P. capensis* seed germination is erratic and seedling growth is very slow (Venter & Venter, 1996). Organogenesis and somatic embryogenesis have been achieved in a few tree crops but many are still recalcitrant to *in vitro* propagation. No research has yet been conducted in this field. The aim is, therefore, to improve germination and achieve mass multiplication of different plant sections taken from *P. capensis*.

Chapter 9 provides a general discussion of all the chapters and some general information related to the present study. Literature citation is found at the end of Chapter 9.

From this background information, it is clear that some wild fruit trees need special attention as they contribute significantly to food and income sources for rural community dwellers. Therefore, research is required on propagation techniques of the above mentioned valuable tree species. The results of research presented in various chapters provide the needed information to be utilised for the ultimate aim of improving productivity of the two wild fruit trees of southern Africa.