CHAPTER 4

EARLY RECOGNITION OF GRAFT COMPATIBILITY IN *UAPACA KIRKIANA* Müell Arg. FRUIT TREES

4.1 Abstract

Early signs of graft incompatibility in *Uapaca kirkiana* tree clones and provenances, *U. nitida* and *Jatropha curcas* were studied using *in vitro* callus fusion technique. Calli was induced from leaves explanted on Murashige and Skoog (MS) medium with different supplements. Two pieces of calli were co-cultured on MS medium with 0.1 mg l^{-1} thidiazuron (TDZ) and 0.5 mg l^{-1} α-naphthaleneacetic acid (NAA). Co-cultured calli were embedded in paraffin wax, sections were stained in safranin and fast green and then examined under a light microscope. Results showed that 0.1 mg l^{-1} TDZ in combination with 0.5 mg l^{-1} NAA and 1.0 mg l^{-1} dichlorophenoxyacetic acid (2,4-D) with 0.5 mg l^{-1} NAA were effective in callus induction. There were no necrotic layers at the unions within *U. kirkiana* clones and provenances. Differential growth between *U. kirkiana* and *U. nitida* was observed. Accumulation of phenol deposits in cells was observed in all *U. kirkiana* combinations and more accumulation was found on callus cells from mature explants. Phenols were absent in *J. curcas* callus tissue heterografts but necrotic layers developed on *U. kirkiana* and *J. curcas* unions indicating a symptom of graft incompatibility. This experiment showed that phenol accumulation impeded callus proliferation, and hence inhibited graft compatibility in *U. kirkiana* grafts.

4.2 Introduction

Early signs of graft incompatibility, often only detected after several years in the field, can be identified using *in vitro* callus fusion (Jonard *et al*., 1990; Errea, Garay & Marin, 2001). Formation of a successful graft union involves many processes, but the main mechanism is still not clear. However, cell recognition is the first step in graft compatibility (Pina & Errea, 2005). Callus cells are able to reject partner cells at an early stage and hence bring about an incompatible response. Cell necrosis and vascular connection discontinuity at graft unions are reported to be the main indicators of graft incompatibility (Ermel *et al*., 1997). Cell division and differentiation take place after cell recognition (Considine, 1983). Thereafter, callus cells initiate cell continuity in compatible partners, but necrosis as a result of incompatible partners (Pina & Errea, 2005). Cell necrosis is usually seen when callus tissues grow together for some time (Ermel *et al.,* 1997), but can also occur at any stage, especially during callus proliferation (Moore, 1986). It is clear that graft compatibility is complex and involves a number of processes.

U. kirkiana tree provenances show wide genetic diversity and variations in geographical adaptation (Akinnifesi *et al*., 2004). Random amplified polymorphic DNA (RAPD) has shown genetic variation within *U. kirkiana* provenances (Agufa, 2002). Early fruiting of two to three years has been reported from fruit orchards established from clonal propagation using grafting and marcotting in Malawi and 80% graft take has been achieved with skilled grafters at Makoka in Malawi (Akinnifesi *et al*., 2006). However, as with most fruit trees, this success in graft take may not always result in high field survival due to graft incompatibility (Nito, Han & Katayama, 2005). Furthermore, grafted *U. kirkiana* trees have

not been evaluated for scion/stock compatibility and some trees have shown growth irregularities between the scions and stocks in the nursery and field at Makoka in Malawi. Selection of graft partners based on desirable fruit or stock traits is important, but scion and stock compatibility needs to be evaluated for stable orchard productivity.

Early recognition of graft compatibility is, therefore, important and assessing callus compatibility of *U. kirkiana* tree clones and provenances and of different *Uapaca* species would aid in early diagnosis and selection of compatible scion and stock combinations. The objective was to determine the early signs of callus compatibility within *Uapaca kirkiana* tree clones and provenances, *Uapaca* species and other trees from the same family.

4.3 Materials and methods

4.3.1 Plant material

U. kirkiana leaves from different juvenile tree clones and provenances were collected at Makoka Research Station in Malawi (see section 2.3.2 for site description). Other *U. kirkiana* provenances originally from Tanzania, Zambia and Zimbabwe were also collected at Makoka from a mature provenance field trial. *U. kirkiana* trees from which leaf samples were collected in January and October 2005, included NkhumbaMW49 (MW49), NkhumbaMW57 (MW57), NazombeMW84 (MW84), HardwickMW26 (MW26), YesayaMW32 (MW32), Phalombe and Dedza from Malawi; Chipata and Choma from Zambia; Mapazure, Murewa and Nyamakwaar from Zimbabwe, and Mpwapwa from Tanzania. Since *U. kirkiana*, *U. nitida* and *Jatropha curcas* all belong to the Euphorbiaceae family, graft compatibility within this family was also tested. Leaf samples of *U. nitida*

from Malawi and *J. curcas* from South Africa were collected (Table 4.1). Plant materials were taken to the tissue culture laboratory at the University of Pretoria for callus induction and compatibility evaluations.

4.3.2 Decontamination and callus induction

Leaves were cut into 1 cm^2 sections, stirred for 20 minutes in Benomyl solution (0.14 g l^{-1}) with a few drops of detergent and then washed under running tap water (30 min). They were then stirred in 3.5% sodium hypochlorite (5 min) and washed off under running tap water (1 h). Under the laminar airflow cabinet, explants were sterilized in 0.1% w/v mercuric chloride ($HgCl_2$, 8 min) and rinsed in sterile distilled water for six consecutive times.

Leaf sections were explanted either abaxially or adaxially on 25 ml aliquot of Murashige and Skoog (Murashige & Skoog, 1962) dispensed in 9-cm Petri dishes. The MS medium supplements were: (i) 1.0 mg l^{-1} indole-3-butyric acid (IBA) and 0.1 mg l^{-1} α-naphthaleneacetic acid (NAA); (ii) 0.1 mg l^{-1} thidiazuron (TDZ) and 0.5 mg l^{-1} NAA; (iii) 1.0 mg l^{-1} NAA and 0.5 mg l^{-1} benzylaminopurine (BAP); or (iv) 0.1 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.5 mg l^{-1} NAA.

This experiment was laid out as a completely randomised block design in 4 × 2 factorial arrangement with four treatments (plant growth regulators) and two sources of explants (juvenile and mature tissues). Five Petri dishes per treatment were used and each Petri dish contained eight leaf explants. Petri dishes were covered and sealed with parafilm strips

before incubation (see section 2.3.8 describes incubation conditions). *In vitro* contaminated cultures were discarded and data collected included the number of contaminated callus cultures, a measure of callus induction and proliferation.

4.3.3 Callus co-culture and fixation

Two pieces of calli from different explants were excised and put together in a thermal labile polythene ring and then placed on MS medium supplemented with 0.1 mg l^{-1} TDZ and 0.5 mg l^{-1} NAA. This medium supplement was selected because it was superior in promoting callus proliferation compared to other treatment combinations (refer to section 4.3.2). *U. kirkiana* clonal homografts (grafts from the same mother tree), inter-specific homografts (grafts from different mother trees but of the same kind e.g. MW49/49), heterografts (grafts from different trees in the same population), tree provenances, *U. nitida* and *J. curcas* combinations were evaluated (Table 4.1). All callus co-cultures were maintained on the same medium for at least two months with a culture (with intact ring) transfer interval of three to four weeks onto fresh medium. This was done carefully, for callus combinations with good unions and prolific growth in the rings, to avoid disturbing the combinations.

Callus co-cultures were removed from the rings and placed in polythene tubes containing formalin acetic acid (FAA; 5% formalin, 5% acetic acid and 90% ethanol) for fixation. Samples were placed on a shaker and dehydrated in a series of graded ethanol (30, 50, 70, 100 and again 100%) followed by a series of xylene (30, 50, 70, 100 and again 100%). Xylene was evaporated and then specimens were embedded in paraffin wax using thermolyne sabron equipment (Histo-Center II-N). Specimens were dissected using Stereo Star Zoom autocut 2040 (model: Rechert-jung 0.7x to 4.2x 570) and transversal sections at

a right angle to the callus union were made. They were then dipped in water maintained at 40 ^{o}C in an Electrothermal basin (Model: Cat No. MH8504). Specimens were mounted on microscope slides and staining was done in safranin and dehydrated in a series of graded ethanol (30, 50, 70, 100 and again 100%) and dipped in fast green followed by a series of graded xylene (30, 50, 70, 100 and again 100%). Specimens were viewed under a light microscope (Olympus microscope, ach 1x, SZX7) connected to a digital camera and microphotographs of callus union interfaces were taken.

4.3.4 Statistical analyses

Data on contamination were subjected to analysis of variance (ANOVA) after arcsine transformation (Steel & Torrie, 1980). Visual scoring for the graft union line (scale of 1 to 3: 1 = visibly high, 2 = faint, 3 = absent), necrosis (scale of 1 to 3: 1 = visible, 2 = faint, 3 = absent) and phenolics (scale of 1 to 4: 1 = high, 2 = medium, 3 = low, 4 = absent) was carried out. The scores (percentages) were subjected to correspondence analysis (GenStat, Rothamsted Experimental Station). A perceptual map was drawn to show distribution and association of various callus co-cultures.

4.4 Results and discussion

4.4.1 Decontamination of explants

Although 0.1% $HgCl_2$ is effective in surface decontamination of many explants, *U. kirkiana* culture contamination was high and some explants were completely lost due to this problem. There were significant differences between means ($P \leq 0.05$) with respect to the source of explants, but no significant differences were obtained with respect to the medium

supplement treatments and the interaction between medium supplements and source of explants. Explants excised from mature stock plants were highly contaminated irrespective of the MS medium supplements used (Table 4.2). *In vitro* contamination was observed at any stage, but placing a few explants per Petri dish reduced the risks of losing explants due to contamination. Table 4.2 shows that explants from mature stock plants were difficult to decontaminate (8.9%) unlike those explants excised from the juvenile stock plants (49.5%). Similar observations were also made in Chapter 2 for *U. kirkiana* shoot explants excised from mature trees growing in the forest.

4.4.2 Callus induction

Data in Table 4.3 shows that 0.1 mg l^{-1} TDZ and 0.5 mg l^{-1} NAA, and 0.1 mg l^{-1} 2,4-D and 0.5 mg l^{-1} NAA MS medium supplements were effective in callus induction. Callus was induced on the mid ribs, especially when explants were abaxially placed on the medium and implying that position of explants on the medium was important. No callus was induced on the cut surfaces and some explants curled away from the medium. Sita & Swamy (1993) reported easy callus induction along the cut surfaces of rosewood leaf disc cultures. This could be due to specific differences amongst species. There was rapid callus proliferation on fresh medium supplemented with 0.1 mg l^{-1} TDZ and 0.5 mg l^{-1} NAA, and hence adequate callus mass was achieved by subculturing pieces of calli on this medium. Explants from mature trees resulted in poor callus induction and browning of the MS medium due to exudates. Frequent transferring of excised calli onto fresh medium improved the callus quantity.

4.4.3 Callus union interface between *U. kirkiana* clones

There were some deposits (phenols) at callus union interfaces of *U. kirkiana*. There was no visible union line in the MW32/32 clonal homograft (Figure 4.1A), but a faint union line in the MW49/49 inter-specific homograft (Figure 4.1B). Phenols have been implicated in graft incompatibility (Pina & Errea, 2005), but Errea *et al*. (1994a) reported existence of phenols in both compatible and incompatible *Prunus* tree species. High amounts of phenols have been reported in *U. kirkiana* leaves during DNA extraction (Hamisy, 2004). However, the quantity and nature of phenols at the union might be important factors in determining graft compatibility. Nito *et al*. (2005) found accumulation of deposits (suspected to be phenols) at the callus union of species of the orange subfamily which were not closely related. In the present study, perhaps the nature of plant material used contributed a lot to the presence of phenols even within homografts (Figure 4.1A-B). Furthermore, observations made during transportation of *U kirkiana* planting materials (shoots and leaves) from Malawi (Makoka Station) to South Africa (University of Pretoria) showed accumulation of phenols in the containers used.

Growth irregularities exhibited by some grafted *U. kirkiana* trees in the field might be attributed to accumulation of phenolics which are known to hinder graft union formation and reduce the graft success (Considine, 1983). Browning of MS medium, attributed to phenolic accumulation, was a major problem encountered. This confirms that *U. kirkiana* mature explants release a lot of phenols, so too the juvenile explants. There was no accumulation of phenols at the union lines of the *J. curcas* clonal heterograft (Figure 4.2A), while cells of *U. nitida* heterograft (Figure 4.2B) showed a lot of deposits. Generally, *U.*

kirkiana clonal homografts and inter-specific heterograft had faint union lines, but there were no necrotic layers observed, and hence they were considered to be compatible clones.

4.4.4 Callus union interface within *U. kirkiana* provenances

Some callus combinations showed visible lines at the unions (Figure 4.3A), but these might disappear with time since some areas along the union formed complete cell continuity. The thick arrow in Figure 4.3A (Dedza/Mpwapwa) indicates union areas with complete cell continuity (no line) despite a visible union line in other parts of the culture (thin arrow). For Phalombe/Dedza combination (Figure 4.3B), the union line is very faint while Phalombe/Murewa (Figure 4.3C) provenance combination shows a visible union line and significant deposits (phenol accumulation). Generally, all calli from mature planting materials were stained brown or purple and this indicates a high accumulation of phenols (Figure 4.3C). Dedza/Chipata (Figure 4.3D) combination shows a visible union line and deposits along the union interface. According to Errea *et al*. (1994a), phenols are vital in initiating tissue lignification, an important process resulting in a successful graft union. Therefore, quantification and structural analysis of phenols present at the union would be useful in determining graft incompatibility (Errea *et al.*, 1994b).

Figure 4.4A-B (Dedza/Choma) shows callus from mature explants (Choma) covered in polyphenols, degenerated and dead (MC) while callus from the juvenile plants (JC) was growing profusely. There was no union between the two partners due to a heavy accumulation of phenols which suppressed callus growth of mature explants. In this study, we hypothesise that the use of callus from mature and juvenile trees as a better option to simulate the reality in grafted fruit trees. However, callus co-cultures from juvenile plants

are also used in assessing *in vitro* callus compatibility. Nito *et al*. (2005) used callus from seedlings to assess compatibility among species of the orange subfamily. In practice, grafting of fruit trees is often between scions form mature trees and rootstocks from either young (seedlings) or mature trees, especially to achieve precocious fruiting. Therefore, use of callus from both juvenile partners might mask the effect of phenols on the graft union formation that is normally displayed in grafted trees. Mature woody scions release more phenols than juvenile scions and this might be critical for woody trees that are known to exude a lot of phenols such as *U. kirkiana*. Therefore, in this trial, *U. kirkiana* explants excised from both juvenile and mature planting materials were used.

Calli from both juvenile partners might not capture the effect of deposits (phenols) on graft compatibility in woody trees, but it might be easy to assess cell recognition in the absence of deposits. Dedza/Choma (Figure 4.4B) showed degeneration of callus from mature source (Choma) to an extent that there was no growth of callus from the mature source (MC), and hence no union between the two partners. Consequently, many such partners were weakly joined and easily separated when removed from the rings. This phenomenon depicts the effect of excess phenols on callus growth and union formation. Therefore, excessive phenols can dictate the success of a graft union due to the impact on callus proliferation.

In some provenance combinations, phenols suppressed the growth of the other partner. Figure 4.5A shows that Phalombe/Nyamakwaar callus was brown, possibly due to phenols from the mature partner (Nyamakwaar). There are also callus projections, which are necessary in grafted trees since they interlock tissues of the scion and stock strongly together upon differentiation and thus form a strong graft union. Figure 4.5B shows prolific

and green callus tissues from the juvenile planting materials (Dedza explants) while the mature partner tissues (Chipata) are brown. Calli from the juvenile plant materials have occupied a bigger volume than those from mature plant materials. This observed growth difference could be attributed to high quantities of phenols present in the mature plant tissues. Consequently, the phenols suppressed callus growth as evidenced in Figure 4.5A-B. Furthermore, there was death of callus from the mature planting (MC).

4.4.5 Callus union between *Uapaca kirkiana* and *Uapaca nitida*

There were faint union lines between *U. kirkiana* and *U. nitida* callus combinations (Figure 4.6A) but with some deposits (phenols) accumulated at the union area. This combination seems to be compatible. However, it was observed that there were differential growth irregularities between the two partners. *U. kirkiana* callus was rapidly increasing in volume unlike *U. nitida* and this could be an indicator of a weak combination that might eventually bring poor union. Based on this differential growth, faint union line and accumulation of deposits at the callus union, this exhibits a delayed incompatibility.

4.4.6 Callus unions between *Uapaca kirkiana* and *Jatropha curcas*

There were necrotic layers with dead cells between *U. kirkiana* and *J. curcas* callus combination. Furthermore, accumulation of phenols was seen on *U. kirkiana* but absent on *J. curcas* cells (Figure 4.6B). This indicates cell rejection and hence outright incompatibility. Cell walls of *U. kirkiana* calli had a thicker appearance (Figure 4.6B). This observation agrees with Errea *et al*. (1994a) for incompatible grafts of *Prunus* tree species. We hypothesise that cell recognition is very important when assessing compatibility in trees, especially for those that are distantly related.

Figure 4.7 shows the distribution and association of various callus combinations of *U. kirkiana*, *U. nitida* and *J. curcas* with respect to presence of phenols, union line and cell necrosis attributes. MW84/84 (PP), MW32/32 (DD), *U. nitida* (UN) clonal homografts, *J. curcas* (JJ) and MW49/49 (NK) inter-specific homografts are close together (top right quadrant) indicating a strong association and compatibility. Axis 2, accounting for 29.4% of the total inertia, shows *U. kirkiana*/*J. curcas* (DJ) combination being isolated and closely associated with cell necrosis (bottom left quadrant). Ermel *et al*. (1997) reported that cell necrosis is the main factor for graft incompatibility and our results agree with their finding as this is outright incompatible. *U. kirkiana* and *U. nitida* (NN and PN) combinations are closely associated with each (bottom right quadrant) and display less compatibility. This could be due to the differential growth irregularities that were observed.

It is difficult to declare if provenance combinations are incompatible based on presence of phenols alone because accumulation of phenols in the cells could be seasonal. Success of the grafted partners would also depend on the physiological state of the plants. Moreover, differences in quantity and quality of phenols between grafted partners are known to affect the union and hence the need for structural analysis and recognition of phenols at the onset of graft establishment (Errea, 1998). This observation necessitated quantification and identification of phenolics at the graft union, as presented in subsequent chapter.

4.5 Conclusion

Presence of a visible union line, accumulation of deposits (polyphenols) and cell necrosis were main indicators of graft incompatibility. Due to excessive phenols, compatibility was

not clear-cut within *U. kirkiana* provenances. Callus fusion within *U. kirkiana*, *U. nitida* and *J. curcas* clones were compatible, but compatibility evidence was less so between *U. kirkiana* and *U. nitida*. Callus combinations between *J. curcas* and *U. kirkiana* showed graft incompatibility. Despite accumulation of phenols that suppressed callus growth, callus fusion is a promising technique for assessing early graft compatibility or incompatibility in species that are not closely related.

Tables

Table 4.1 Callus co-cultures of *Uapaca kirkiana*, *Uapaca nitida* and *Jatropha curcas*

No.	Callus combinations	Tree origin	Type of combination
1.	MW84/MW84	Malawi	clonal homograft
2.	MW32/MW32	Malawi	clonal homograft
3.	MW49/MW49	Malawi	inter-specific homograft
4.	MW26/MW57	Malawi	heterograft
5.	*U. nitida*/*U. nitida*	Malawi	heterograft
6.	*J. curcas*/*J. curcas*	South Africa	heterograft
7.	*U. kirkiana*/*U. nitida*	Malawi	species
8.	*U. kirkiana*/*J. curcas*	Malawi/South Africa	family
9.	Dedza/Phalombe	Malawi	provenance
10.	Phalombe/Nyamakwaar	Malawi/Zimbabwe	provenance
11.	Phalombe/Murewa	Malawi/Zimbabwe	provenance
12.	Dedza/Chipata	Malawi/Zambia	provenance
13.	Phalombe/Mapazure	Malawi/Zimbabwe	provenance
14.	Phalombe/Choma	Malawi/Zambia	provenance
15.	Phalombe/Nyamakwaar	Malawi/Zimbabwe	provenance
16.	Dedza/Mpwapwa	Malawi/Tanzania	provenance
17.	Dedza/Murewa	Malawi/Zimbabwe	provenance
18.	Dedza/Chipata	Malawi/Zambia	provenance
19.	Dedza/Choma	Malawi/Zambia	provenance

Table 4.2 Percentage contamination of young and old *Uapaca kirkiana* leaves explanted on different Murashige and Skoog medium supplemented with benzylaminopurine (BAP), indole-3-butyric acid (IBA), α-naphthaleneacetic acid (NAA), 2, 4-dichlorophenoxyacetic acid (2, 4-D) and thidiazuron (TDZ). Means are calculated with standard deviations.

MS medium	Culture asepsis (%)		
supplements (mg l^{-1})	Young leaves	Old leaves	Mean
1.0 IBA + 0.1NAA	12.5	48.5	30.5 ± 8.8[a]
0.1 TDZ + 0.5 NAA	6.9	52.4	29.7 ± 12.3[a]
0.5 BAP + 1.0 NAA	7.6	47.1	27.4 ± 9.3[a]
0.1 2, 4-D + 0.5 NAA	8.3	50.0	29.2 ± 12.1[a]
Mean	8.9 ± 1.5[b]	49.5 ± 5.3[a]	

CV (%) = 48.6

LSD (5%) Medium supplements (M) = 17.6[ns]

Leaves (L) = 12.4*

M × L = 24.9[ns]

* Means with different letters are significantly different at P≤0.05

ns = no significant difference at P≤0.05

Table 4.3 Callus induction of *Uapaca kirkiana* explants on Murashige and Skoog (MS) medium supplemented with benzylaminopurine (BAP), indole-3-butyric acid (IBA), α-naphthaleneacetic acid (NAA), 2, 4-dichlorophenoxyacetic acid (2, 4-D) and thidiazuron (TDZ). Means are calculated with standard errors

MS medium supplements (mg l^{-1})	Callus formation (%)
1.0 IBA + 0.1NAA	21.7 ± 6.0^{b}
0.1 TDZ + 0.5 NAA	58.3 ± 4.4^{a}
0.5 BAP + 1.0 NAA	0.9 ± 0.6^{c}
0.1 2, 4-D + 0.5 NAA	53.3 ± 4.4^{a}
CV (%)	22.4
$LSD_{0.05}$	15.9

Means with the same letters within a column are not significantly different at $P \leq 0.05$

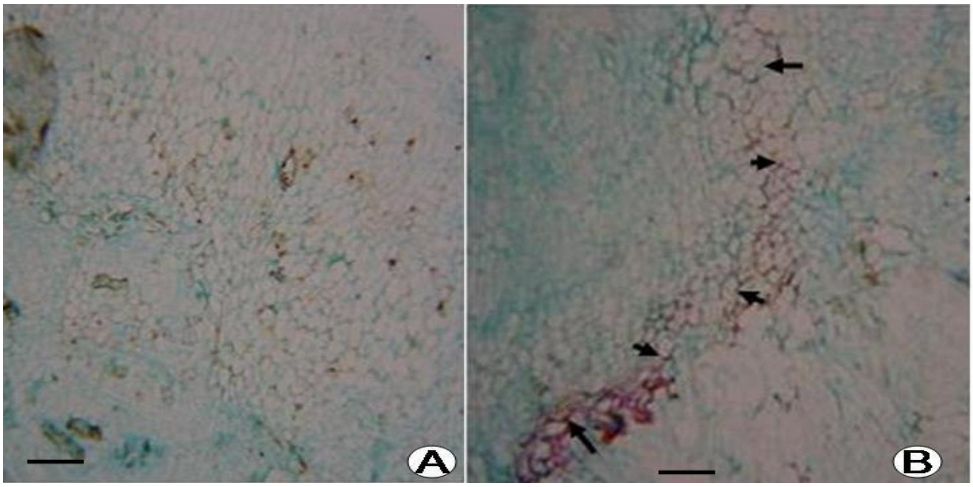

Figure 4.1 Callus union interfaces within *Uapaca kirkiana* clones (A) MW32/32 clonal homograft showing no visible union line; (B) MW49/49 inter-specific homograft. The brown stains on the cells are due to deposits (polyphenols) and arrows show faint union line for MW49/49 inter-specific homograft (bars = 10 µm)

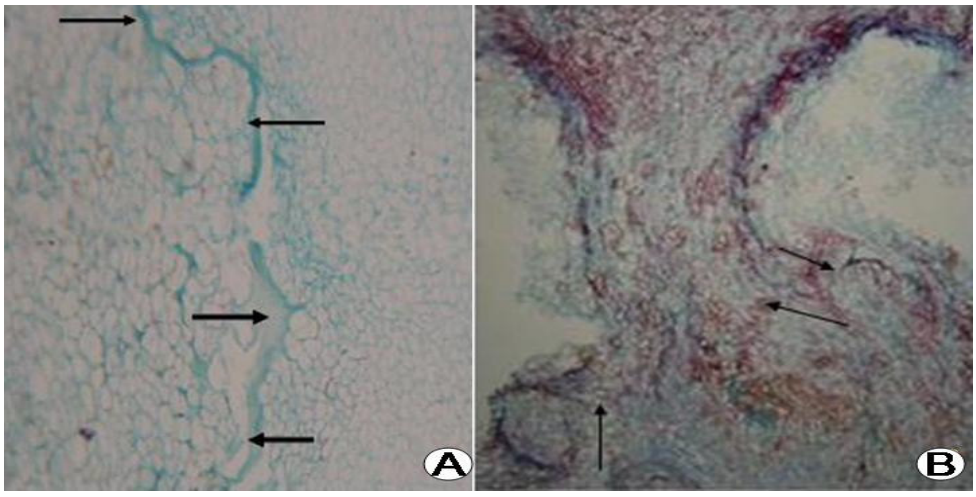

Figure 4.2 Sections of callus union interfaces of (A) *Jatropha curcas* heterograft; (B) *Uapaca nitida* heterograft (arrows indicate the union interfaces). No major phenolic compound staining was present in *J. curcas* cells, but it was present in *U. nitida* cells

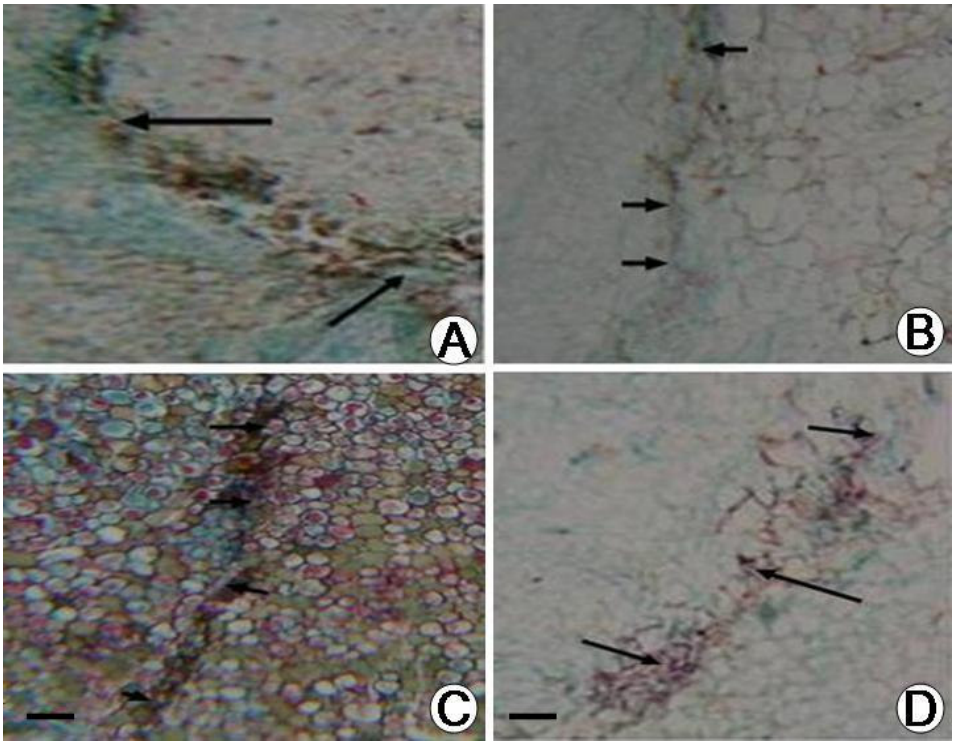

Figure 4.3 Callus union interfaces of *Uapaca kirkiana* tree provenances (A) Dedza/Mpwapwa; (B) Phalombe/Dedza; (C) Phalombe/Murewa; (D) Dedza/Chipata (Arrows show the union line, bars = 10 µm)

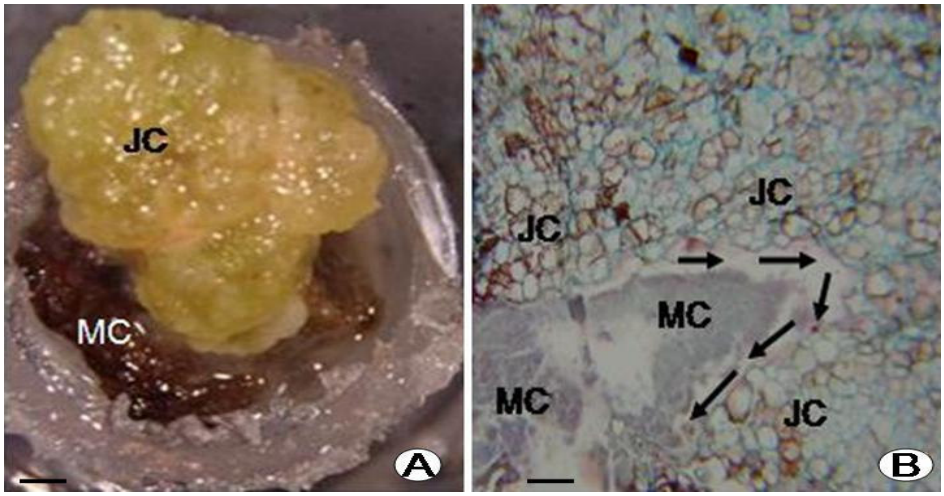

Figure 4.4 Callus union interface of *Uapaca kirkiana* tree provenance (A) a surface view of Dedza (juvenile) and Choma (mature) callus union in a polythene ring; (B) same callus combination section showing callus degeneration at the union (JC = callus from juvenile planting materials, MC = callus from mature planting materials). Arrows indicate union area between calli from mature and juvenile stock plants (bar = 10 µm)

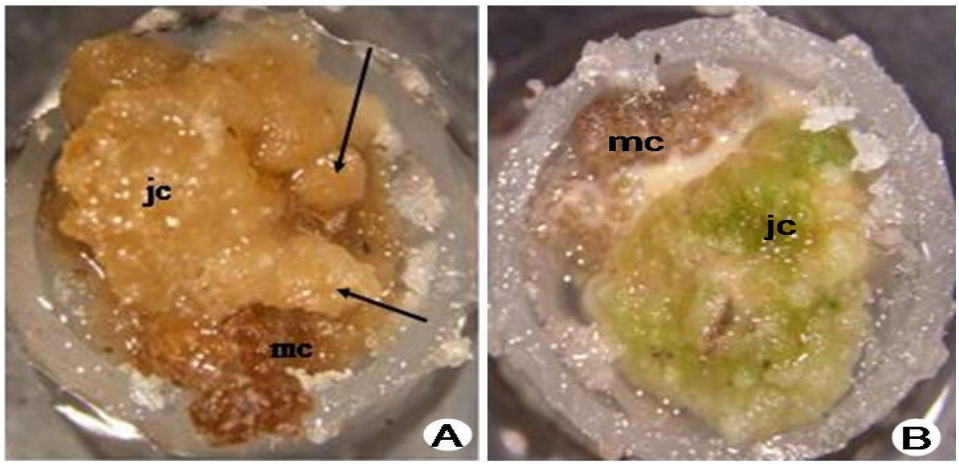

Figure 4.5 Callus co-cultures of *Uapaca kirkiana* tree provenances in thermal labile rings (A) Phalombe/Nyamakwaar; (B) Dedza/Murewa (jc = callus from juvenile tissues, mc = callus from mature tissues). Arrows show bead-like projections

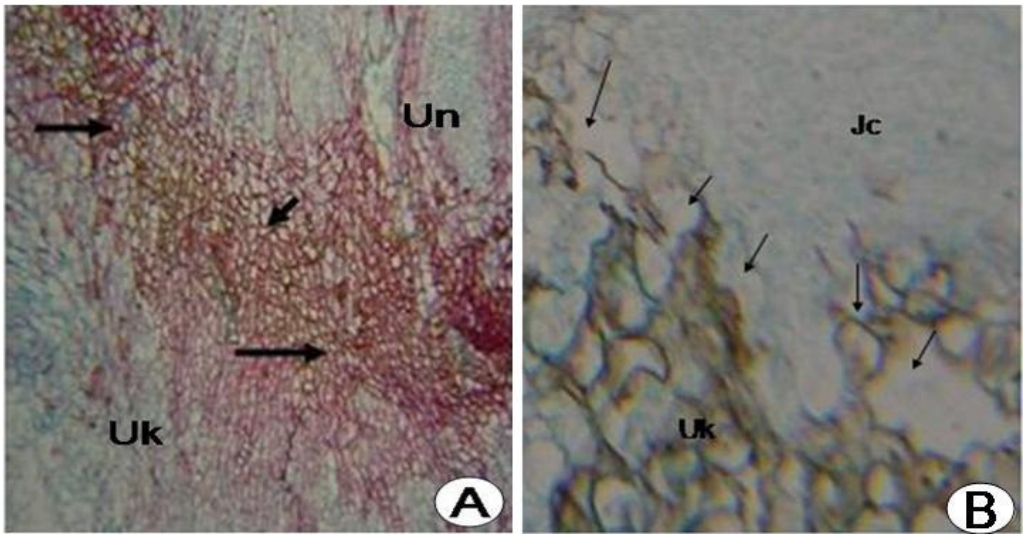

Figure 4.6 Callus union interface sections between: (A) *Uapaca kirkiana* (Uk) and *Uapaca nitida* (Un); (B) *Uapaca kirkiana* (Uk) and *Jatropha curcas* (Jc) showing phenol accumulation on the *U. kirkiana* cells. Arrows show callus union

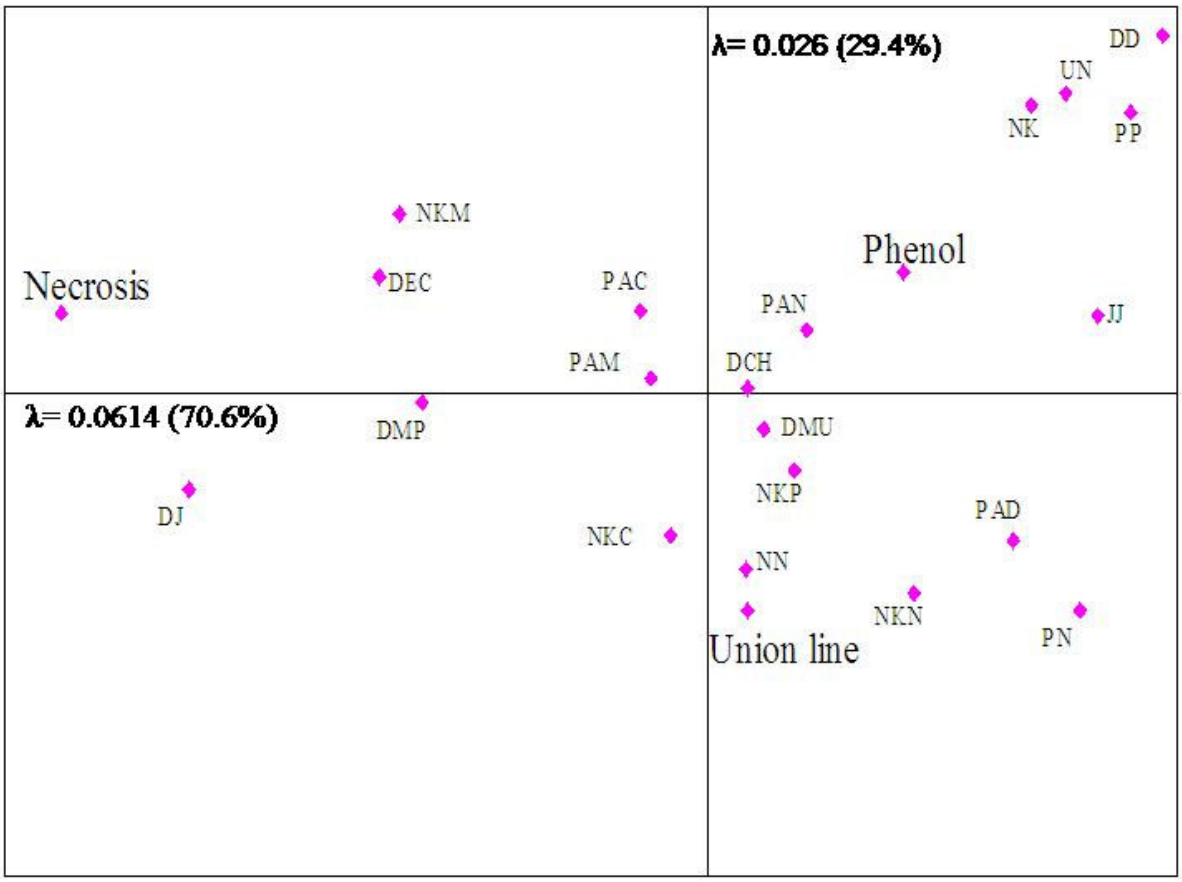

Figure 4.7 Distribution and association of *Uapaca kirkiana* tree clones and provenances, *Uapaca nitida* and *Jatropha curcas* callus combinations with respect to phenol, union line and cell necrosis attributes from correspondence analysis output (PP = MW84/84, DD = MW32/32, NK = MW49/49 inter-specific homograft, PAN = MW26/57, NN = *U. nitida* heterograft, JJ = *J. curcas* heterograft, NKP = MW57/26, PN = *U. kirkiana*/*U. nitida*, DJ = *U. kirkiana* (Dedza)/*J. curcas*, PAM = Phalombe/Murewa, PAC = Phalombe/Chipata, PAD = Phalombe/Dedza, NKM = MW57/Murewa, NKC = Phalombe/Chipata, NKN = Phalombe/Nyamakwaar, DMP = Dedza/Mpwapwa, DMU = Dedza/Murewa, DCH = Dedza/Chipata, DEC = Dedza/Choma) (λ = inertias)