Early recognition of graft compatibility in *Uapaca kirkiana* Müell Arg. clones, provenances and species

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Abstract

Examination of callus micro-grafts in *Uapaca kirkiana* Müell Arg. was carried out with the objective of determining early signs of graft compatibility. Leaves from *U. kirkiana*, *U. nitida* and *Jatropha curcas* trees were used for callus induction. Two pieces of callus were co-cultured on Murashige and Skoog (MS) medium with different supplements. Co-cultured calli were embedded in paraffin wax and dissected. The specimens were stained in safranin and fast green before viewing under a light microscope. Results showed that MS medium with 0.1 mg l^{-1} thidiazuron (TDZ) and 0.5 mg l^{-1} naphthaleneacetic acid (NAA) or 1.0 mg l^{-1} dichlorophenoxyacetic acid (2,4-D) and 0.5 mg l^{-1} NAA was effective for callus induction. There were no necrotic layers at the unions within *U. kirkiana* clones and provenances, but a differential growth (irregularity) between *U. kirkiana* and *U. nitida* co-cultured calli. Phenol deposits were observed at the union interfaces of *U. kirkiana* combinations and were high on calli derived from mature trees. Phenol deposits were absent at the union of *J. curcas* heterografts. Necrotic layers developed at the unions of *U. kirkiana* and *J. curcas* micro-grafts and indicating an outright graft incompatibility. Accumulation of phenol deposits at the union interfaces inhibited graft compatibility in many *U. kirkiana* combinations. Callus fusion technique

can be used to identify partners with an outright graft incompatibility, especially for distant related plant species.

Introduction

Fruits from *Uapaca kirkiana* Müell Arg. trees are widely consumed and traded as an important income source to rural community dwellers in southern Africa (Ramadhani *2002*; Ham et al, *2007*). Recent findings have shown that indigenous fruit trees of the Miombo ecosystem are capable of reducing vulnerability to poverty by more than 30% (Mithöfer et al. *2006*). Other edible, but less popular *Uapaca* species in the Miombo ecosystem include *U. zanzibarica*, *U. banguelensis* and *U. nitida*. *U. kirkiana* is the most preferred indigenous fruit tree earmarked for wider cultivation in Malawi, Tanzania, Zambia and Zimbabwe (Maghembe et al. *1998*). It is widely utilized as food during seasonal food shortages (Maghembe and Seyani, *1991*; Akinnifesi et al. *2004*, *2006)*. However, lack of improved planting materials (propagule sources) which result in low fruiting ability, inferior fruit load and fruit traits and limited propagation skills have been major bottlenecks to spontaneous cultivation by smallholder farmers in southern Africa (Akinnifesi et al. *2004*). Early fruiting in *U. kirkiana* has been achieved through grafting and high graft take (80%) has been reported at Makoka in Malawi (Akinnifesi et al. *2006*). However, as with most fruit trees, success in graft take may not always result in high field survival due to graft incompatibility among other constraints (Nito et al. *2005*). Recently, Akinnifesi et al. (*2007*) have indicated that field survival of grafted *U. kirkiana* declined from 98% at 12 months after establishment (MAP) to 67% at 33 MAP at Makoka regardless of management conditions. The probability of survival at this early growth period was 75% at 33 MAP (Akinnifesi et al. *2007*) and could be attributed to reasons other than tree management.

Signs of graft incompatibility often detected after several years in the field can be identified early using in vitro callus fusion technique (Jonard et al. *1990*; Errea et al. *2001*). Formation of a successful graft union involves many processes and cell recognition is the first step in graft compatibility (Pina and Errea *2005*). Callus cells are able to reject partner cells at an early stage, and hence bring about incompatible response.

Cell necrosis and vascular connection discontinuity at the unions are reported to be the main indicators of graft incompatibility (Ermel et al. *1997*). Cell differentiation takes place after cell recognition (Considine *1983*) and callus cells initiate cell continuity in compatible partners, but necrosis in the incompatible partners (Pina and Errea *2005*). Cell necrosis is often 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*). Therefore, graft compatibility is complex and involves a number of processes. The germplasm of 16 *U. kirkiana* tree provenances have been collected, characterised and established in multilocational field trials in five countries of southern Africa, namely Malawi, Tanzania, Zambia, Zimbabwe and Mozambique (Kwesiga et al. *2000*). These provenances show wide genetic diversity and variations in geographical adaptation (Akinnifesi et al. *2004*). The random amplified polymorphic DNA (RAPD) has also shown genetic variation within *U. kirkiana* provenances (Agufa *2002*). Early fruiting of two to three years has been reported from orchards established from clonal propagation using grafting and marcotting in Malawi (Akinnifesi et al. *2006*). However, these grafted *U. kirkiana* trees were not evaluated for scion/stock compatibility and yet a few trees have shown growth irregularities between the scions and stocks in the nursery and field at Makoka in Malawi.

Callus micro-graft technique has been used in some fruit trees to elucidate the early graft compatibility phenomenon. Although grafted *Uapaca* trees are known to release a lot of exudates upon wounding, no evidence is available on their link to graft incompatibility. The objective of the study was to determine early signs of graft compatibility in *U. kirkiana* tree clones, provenances and within species using callus fusion technique, by assessing callus that would aid early diagnosis and selection of compatible combinations.

Materials and methods

Plant material

Uapaca kirkiana leaves from different juvenile and mature tree clones and provenances, and other mature *U. kirkiana* provenances originally from Tanzania, Zambia and Zimbabwe were collected at Makoka Research Station in Malawi (altitude of 1029 m

a.s.l., 15°30′S, 35°15′E). The total annual rainfall ranges from 560 to 1600 mm, with a ten year-mean of 930 mm (Akinnifesi et al. *2004*). The rainfall is unimodal with most of the rains falling between November and April, and temperature varies between 16 and 32°C. *Uapaca kirkiana* trees from which leaf samples were collected in January and October 2005 included NkhumbaMW49 (MW49), NkhumbaMW57 (MW57), NazombeMW84 (MW84), AlenaHardwickMW26 (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* 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. Plant materials were taken to the tissue culture laboratory at the University of Pretoria for callus compatibility evaluations.

Surface decontamination of explants

Leaves were cut into 1 cm^2 sections and washed in Benomyl (0.15 mg l^{-1}) with a few drops of detergent (20 min) and in running tap water (30 min). They were washed in 3.5% sodium hypochlorite (5 min) and in running tap water (1 h). Under the laminar air flow hood, explants were sterilized in 0.1% w/v mercuric chloride ($HgCl_2$, 10 min) and rinsed in sterile distilled water for six consecutive times.

Callus induction

Leaf sections were explanted either abaxially or adaxially on 25 ml aliquot of Murashige and Skoog (Murashige and Skoog *1962*) dispensed in 9-cm Petri dishes. The Murashige and Skoog (MS) medium supplements were either (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.

Treatment means for all the juvenile tree clones, provenances and species were calculated and pooled together, and so too the treatment means for the mature tree provenances. This is because the main interests for this experiment were the effects of medium

supplements and the ages of explants on culture contamination. Moreover, with so many tree clones and provenances, the interpretation of treatment interactions could be difficult. The experiment was a completely randomised block design in a factorial arrangement. Five Petri dishes per treatment were used and each Petri dish contained 6–8 leaf explants. Petri dishes sealed and incubated under 12 h of daily illumination with 60 μmol m^{-2} s^{-1} PAR using two cool white fluorescent tubes per shelf. Temperatures were maintained at 23 ± 2°C. Contaminated cultures were discarded and data collected included number of contaminated cultures, callus induction and proliferation.

In vitro callus combinations

Two pieces of callus 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. The reason why this medium supplement was selected was because it was superior in promoting callus proliferation. *U. kirkiana* clonal homografts (grafts from the same mother tree), clonal heterografts (grafts from different trees in the same population, half sib), tree provenances, *U. nitida* and *J. curcas* combinations are presented in Table 1. Callus co-cultures were maintained on the same medium for at least two months with culture transfer interval of two to three weeks onto a fresh culture medium.

Table 1 Callus combinations from *Uapaca kirkiana*, *Uapaca nitida* and *Jatropha curcas* trees

No.	Callus combinations	Tree origin	Type of combination
1.	MW84/84	Malawi	clonal homograft
2.	MW32/32	Malawi	clonal homograft
3.	MW49/49	Malawi	clonal homograft
4.	MW26/57	Malawi	heterograft
5.	*U. nitida*/*U. nitida*	Malawi	heterograft
6.	*J. curcas*/*J. curcas*	South Africa	heterograft
7.	*U. kirkiana*/*U. nitida*	Malawi	species

No.	Callus combinations	Tree origin	Type of combination
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

Callus co-culture fixation and staining

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) and placed on a shaker (500 rpm). Samples were dehydrated in a series of graded ethanol (30%, 50%, 70%, 100% and 100%) followed by graded xylene. Specimens were embedded in paraffin wax using thermolyne sabron equipment (Histo-Center II-N). They were dissected using Stereo Star Zoom autocut 2040 (model: Rechert-jung 0.7 × to 4.2 × 570) at a right angle to the callus union and then dipped in water maintained at 40 °C in an Electrothermal basin (Model: Cat No. MH8504). Specimens were mounted on microscope slides and staining was done in safranin. They were dehydrated in a series of graded ethanol and dipped in fast green followed by a series of graded xylene. 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.

Statistical analyses

Data on culture contamination were subjected to analysis of variance (ANOVA) using GenStat *2005* (Rothamsted Experimental Station). Visual scoring was done for the micro-graft union line where 1 = visibly high, 2 = faint, 3 = absent), necrotic layer where 1 = visible, 2 = faint, 3 = absent) and phenol where 1 = high, 2 = medium, 3 = low, 4 = absent). The scores were converted into percentages and then subjected to correspondence analysis. A perceptual map was drawn to show distribution and association of different callus co-cultures with respect to the presence of necrotic layer, phenol deposit and the union line.

Results

Surface decontamination of explants

There were significant differences ($P \leq 0.05$) between the ages of explants with respect to culture contamination (Table 2). Explants derived from mature trees were highly contaminated (49.5%) irrespective of the MS medium supplements used. Furthermore, explants derived from seedlings (juvenile plants) were less contaminated (8.9%). Apart from a thorough external decontamination that was done, significant level of contamination was also noted at the later stages in some treatments, and hence placing a few explants per Petri dish reduced risks of losing many explants to culture contamination. There were no significant differences ($P \leq 0.05$) among treatment means for the medium supplements and the interaction between medium supplements and the ages of explants (Table 2).

Table 2 Percentage contamination of *Uapaca kirkiana* leaves derived from juvenile and mature trees and cultured 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 deviations

MS medium supplement (mg l^{-1})	Contamination (%) of leaf explants		Mean
	Juvenile	Mature	
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}

Ages (A) = 12.4*

M × A = 24.9^{ns}

* Means with different letters are significantly different at $P \leq 0.05$

ns = no significant difference at $P \leq 0.05$

Callus induction

Results showed that MS medium supplemented with 0.1 mg l^{-1} TDZ and 0.5 mg l^{-1} NAA (58.3%) or 0.1 mg l^{-1} 2, 4-D and 0.5 mg l^{-1} NAA (53.3%) was effective in callus induction (Table 3). Callus was formed on mid ribs, especially when explants were abaxially placed on the culture medium and this implies that the position of explants on the culture medium was equally important. There was rapid callus growth when calli were subcultured onto fresh medium supplemented with 0.1 mg l^{-1} TDZ and 0.5 mg l^{-1} NAA and adequate callus mass was obtained by frequent subculturing pieces of callus. Generally, explants from mature trees resulted in poor callus formation and there was

browning of the culture medium. Frequent transferring of excised pieces of callus onto fresh culture medium slightly improved the callus quantity.

Table 3 Callus formation (%) of *Uapaca kirkiana* leaves derived from juvenile trees and cultured 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). Figures are calculated with standard mean errors

MS supplements (mg l^{-1})	Callus formation (%)
1.0 IBA + 0.1NAA	$21.7^{b} \pm 0.06$
0.1 TDZ + 0.5 NAA	$58.3^{a} \pm 0.04$
1.0 NAA + 0.5 BAP	$0.9^{c} \pm 0.01$
0.1 2,4-D + 0.5 NAA	$53.3^{a} \pm 0.04$
CV (%)	22.4

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

Callus union interfaces between *U. kirkiana* clones

There was little phenol deposit accumulation at the union interfaces of *U. kirkiana* clones. This made the union lines faint as shown in MW84/84 and MW32/32 clonal homografts (Fig. 1a) and MW49/49 homograft (Fig. 1b). *U. kirkiana* plant materials released a lot of exudates (phenols) even for the homografts (Fig. 2a,b), and hence browning of the culture medium was a major problem. There was no accumulation of phenol deposits at the union of *J. curcas* heterografts (Fig. 2a), while the cells of *U. nitida* heterograft (Fig. 2b) showed a lot of phenol deposits. Although *U. kirkiana* clonal homografts and heterograft had faint union lines, there were no necrotic layers observed.

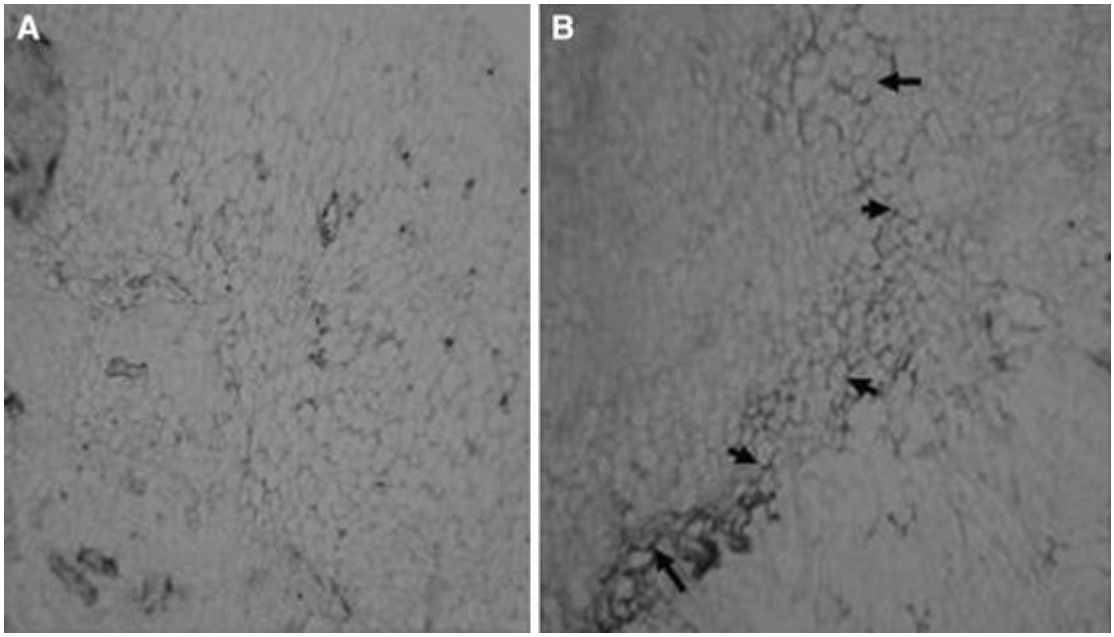

Fig. 1 Callus union interfaces within *Uapaca kirkiana* clones (**a**) MW32/32 homograft, (**b**) MW49/49 homograft. The brown stains on the cells are due to phenol deposits and arrows show faint union line for MW49/49 homograft

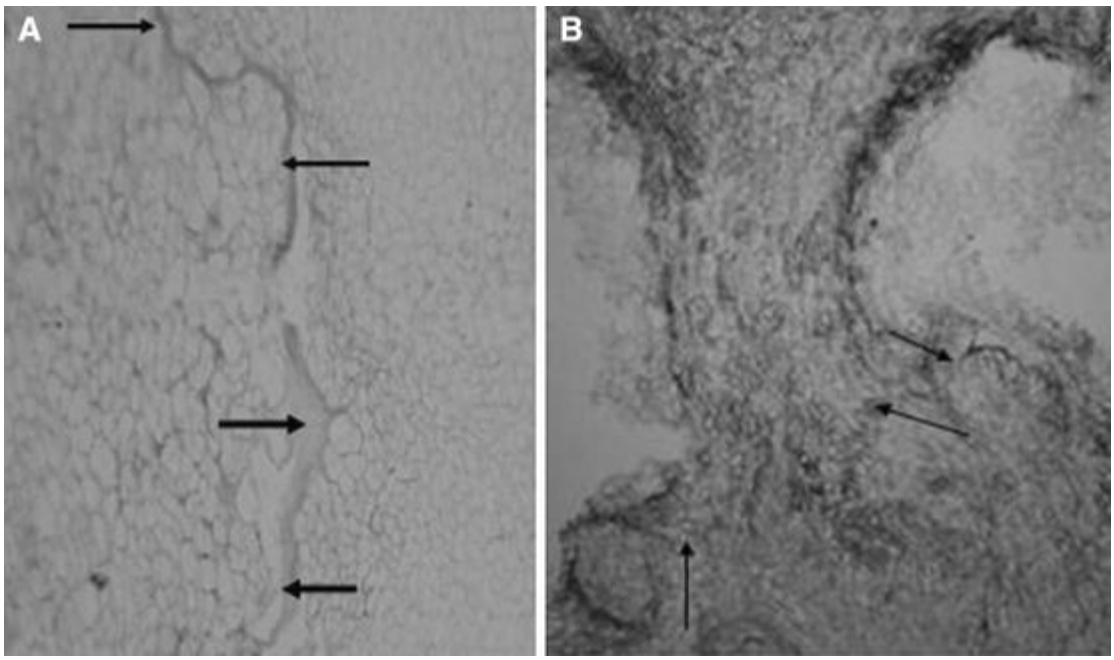

Fig. 2 Sections of callus union interfaces of (**a**) *Jatropha curcas* heterograft (**b**) *Uapaca nitida* heterograft (arrows indicate the union interfaces) *J. curcas* cells show no phenolic compound staining, but present in *U. nitida* cells

Callus union interfaces within *U. kirkiana* provenances

Some callus combinations (sections) showed visible lines at the unions (Fig. 3a). Arrows in Fig. 3a (Dedza/Mpwapwa) indicate union areas with complete cell union despite a visible union line in certain parts of the section. For Phalombe/Dedza combination (Fig. 3b), the union line is very faint, while Phalombe/Murewa provenance combination (Fig. 3c) shows visible union line and a lot of phenol staining, especially along the union interface. Dedza/Chipata callus combination (Fig. 3d) also shows a visible union line and phenol deposits along the union interface.

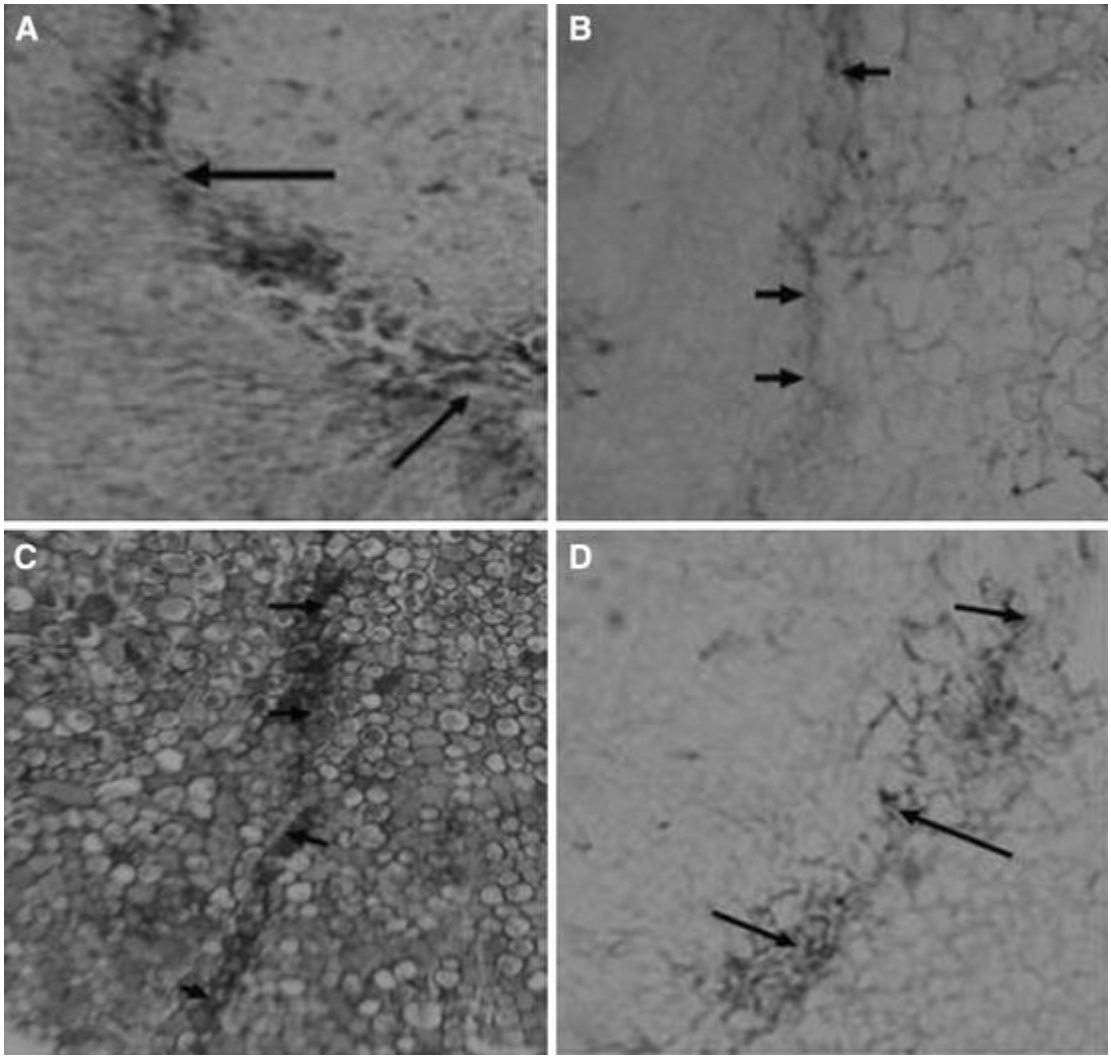

Fig. 3 Callus union interfaces of *Uapaca kirkiana* tree provenances (**a**) Dedza/Mpwapwa; (**b**) Phalombe/Dedza; (**c**) Phalombe/Murewa; (**d**) Dedza/Chipata (arrows show the union line)

Figure 4a (Dedza/Choma) shows callus derived from mature tree (Choma) covered in phenols and appeared degenerated and dead, while callus from the juvenile plants was profusely growing. There was no union between the two partners (Fig. 4a) due to heavy production of phenols which might have suppressed growth of calli derived from mature trees. Dedza/Choma (Fig. 4b) showed degeneration of callus derived from mature trees (Choma) to an extent that there was no growth of callus and hence no or little union between the two partners. Consequently, many such partners were weakly joined and easily separated when removed from the rings.

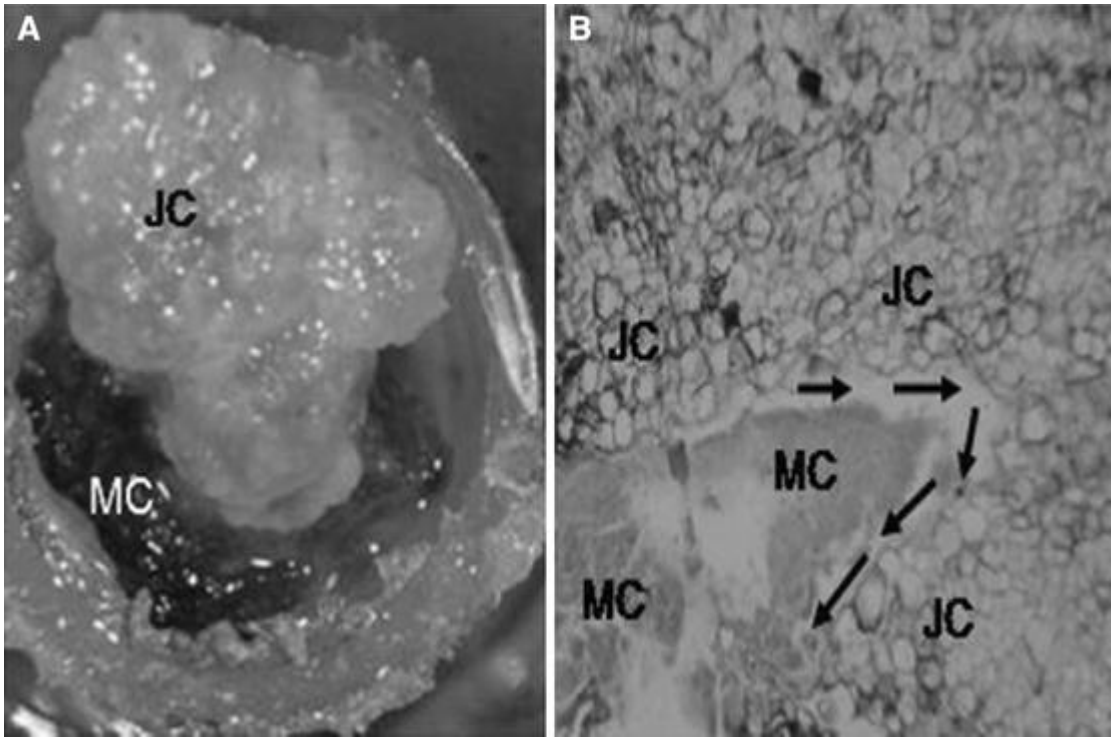

Fig. 4 Callus union interfaces of *Uapaca kirkiana* tree provenances (**a**) Dedza (juvenile) and Choma (mature) callus union in a polythene ring, (**b**) same callus combination showing callus degeneration at the union and staining in safranin and fast green (JC = callus from juvenile plant tissues, MC = callus from mature plant tissues). Arrows indicate union area between callus from mature and juvenile plant tissues

Figure 5a shows that the green colour of Phalombe callus was lost due to phenol deposits from the mature partner (Nyamakwaar). Despite the loss of green colour, beads-like projections were present and these are necessary in grafted trees. Fig. 5B shows the green callus and beads-like projections were formed from Dedza callus despite the presence of phenol deposits from the mature partner.

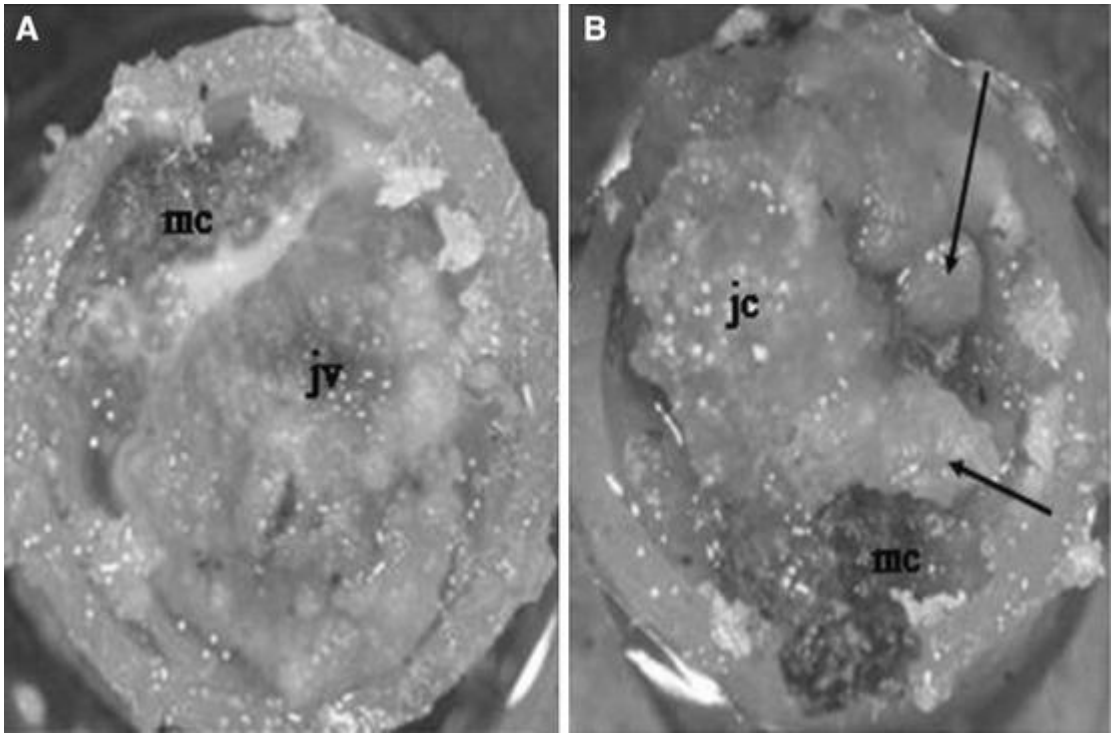

Fig. 5 Callus co-cultures of *Uapaca kirkiana* tree provenances in thermal labile rings (**a**) Phalombe/Nyamakwaar, (**b**) Dedza/Murewa (jc = callus derived from juvenile trees, mc = callus derived from mature trees). Arrows show bead like projections

Callus unions between *U. kirkiana* and *U. nitida*

There were faint union line between *U. kirkiana* and *U. nitida* (Fig. 6a), but with deposits (phenols) accumulating at the union area. This callus combination (section) seems to be compatible although there were differential growth irregularities between the two partners. It was observed that *U. kirkiana* calli were rapidly increasing in volume unlike *U. nitida* calli.

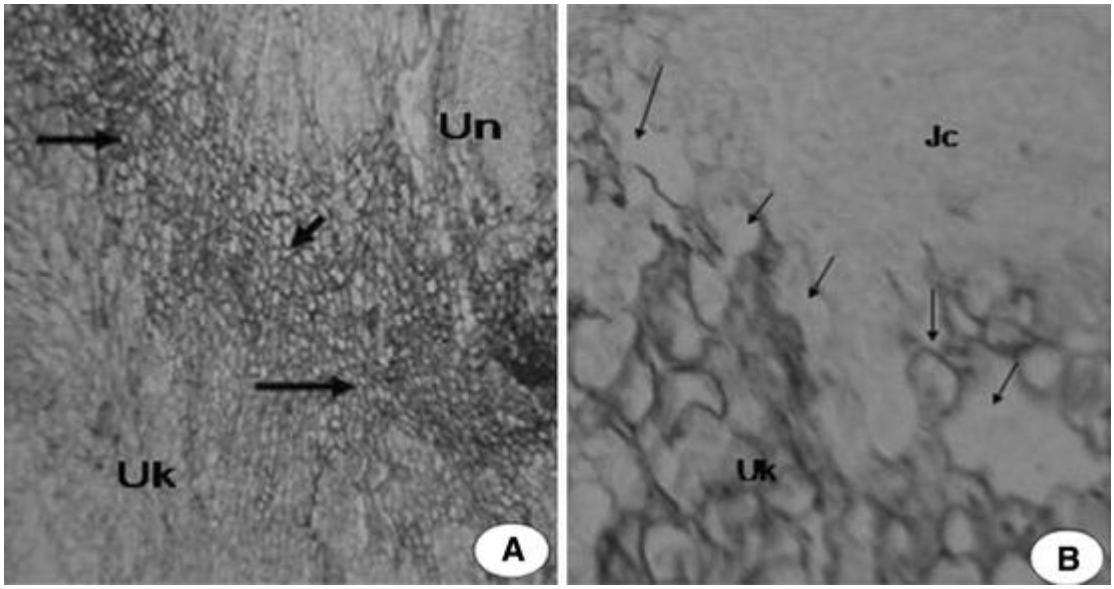

Fig. 6 Callus union interfaces between (**a**) *Uapaca nitida* (Un) and *Uapaca kirkiana* (Uk) showing accumulation of phenol deposits at the union; (**b**) *Uapaca kirkiana* and *Jatropha curcas* (Jc) showing accumulation of phenol deposits and enlarged *U. kirkiana* cells. Arrows indicate the union areas

Callus unions between *U. kirkiana* and *J. curcas*

There were necrotic layers between *U. kirkiana* and *J. curcas* callus combinations (Fig. 6b). Phenol deposits were seen on *U. kirkiana* cells, but absent on *J. curcas* cells (Fig. 6b). Furthermore, cell walls of *U. kirkiana* callus have thicker appearance, while *J. curcas* callus shows small or fine cells (Fig. 6b).

Figure 7 shows the distribution and association of different callus combinations of *U. kirkiana*, *U. nitida* and *J. curcas* with respect to the presence of phenol deposit, union line and cell necrosis attributes. MW84/84 (PP), MW32/32 (DD), *U. nitida* (UN) clonal homograft, *J. curcas* (JJ) and MW49/49 (NK) homograft are close together (top right quadrant) and indicating a strong association and compatibility. Axis 2, accounting for 29.4% of the total inertia, shows *U. kirkiana*/*J. curcas* (DJ) callus combination being isolated and closely associated with cell necrosis (top left quadrant). *U. kirkiana*/*U. nitida* (NN and PN) combinations are closely associated with each other (bottom right quadrant) and display less compatibility.

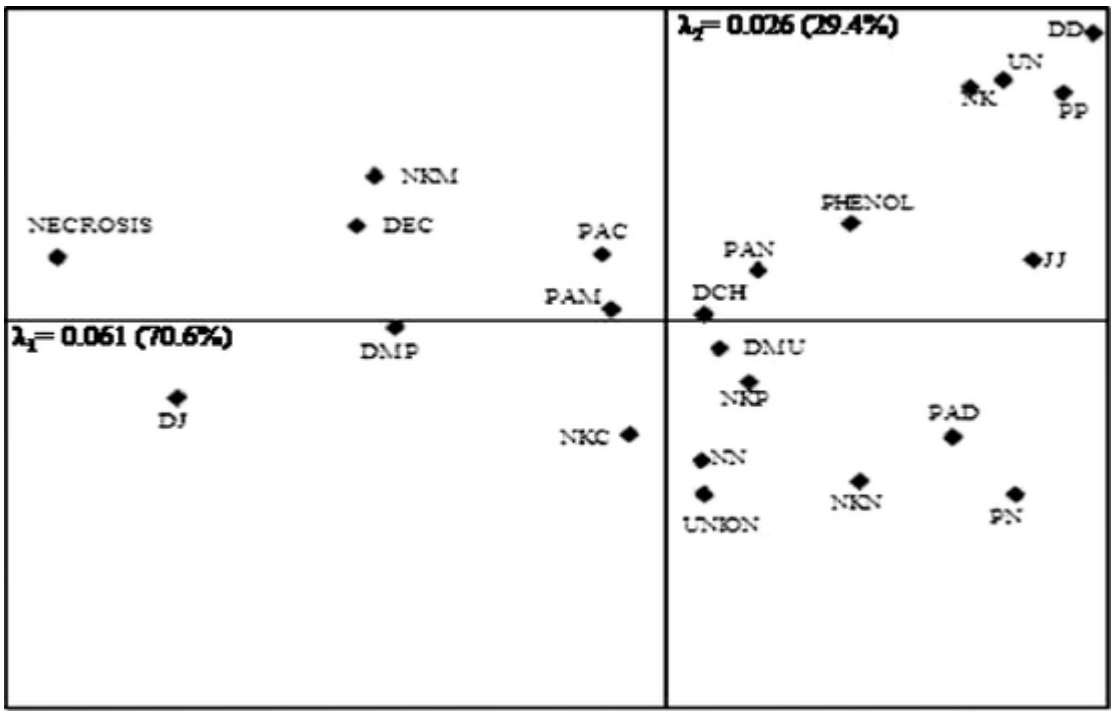

Fig. 7 Distribution and association of *Uapaca kirkiana* tree clone and provenance, *Uapaca nitida* and *Jatropha curcas* callus combinations with respect to phenol, union line and cell necrosis attributes from correspondence analysis output (PP = MW84/84, DD = MW32/32, NK = MW49/49, PAN = MW26/57, NN = *U. nitida* heterograft, JJ = *J. curcas* heterograft, NKP = MW57/MW26, 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)

Discussion

Although $HgCl_2$ is known to be effective in surface decontaminating many explants, in vitro culture contamination for *U. kirkiana* explants was high and some explants were completely lost due to this problem. This high in vitro contamination could be attributed to the presence of endogenous fungal contaminants in the explants derived from mature trees. Generally, juvenile plant materials are less exposed to pathogens (in vitro contaminants), and hence they are likely to be free from in vitro contamination.

For aseptic cultures, there was no callus formation on the cut surfaces and some explants curled away from the culture medium. Sita and Raghava-Swamy (*1993*) reported easy callus induction along the cut surfaces of rosewood leaf disc cultures. However, this could be due to specific differences in plant species.

Phenol deposits were observed on almost all *U. kirkiana* callus combinations. This is an indication that *U. kirkiana* plants exude a lot of secondary metabolites (phenols). High levels of phenols have been reported in *U. kirkiana* leaves during DNA extraction (Hamisy *2004*). Therefore, the quantity and nature of phenols deposited at the union might be important factors in determining graft compatibility. Phenols have been implicated in graft incompatibility (Pina and Errea *2005*), but Errea et al. (*1994*) reported existence of phenol deposits on both compatible and incompatible *Prunus* tree species. Nito et al. (*2005*) found accumulation of deposits (suspected to be phenols) at the callus union of species of orange subfamily which were not closely related. However, the presence of visible union line was the major factor attributed to graft incompatibility in orange species. The main difference could be that *Uapaca* plant materials release more phenols than the orange plant materials. Therefore, this might not be seen as an important factor responsible for graft incompatibility, especially for plants that do not exude a lot of phenols. In the present study, accumulation of phenol deposits at the unions could be responsible for graft incompatibility.

Growth irregularities exhibited by some grafted *U. kirkiana* trees in the field might be attributed to accumulation of phenols which are known to hinder graft union formation and reduce the graft success (Considine *1983*). However, the presence of phenol deposits alone at the union might not be a strong evidence of graft incompatibility. According to Errea et al. (*1994*), quantity and structural analyses of phenols present at the union is useful in determining graft incompatibility. The presence of phenol deposits in compatible and incompatible callus combinations has been reported, but phenols are also vital in initiating tissue lignification, an important process resulting in a successful graft union (Errea et al. *1994*). Therefore, this shows that there could be specific phenols responsible for graft incompatibility.

Callus co-cultures derived from juvenile plant materials have been used to assess *in vitro* callus compatibility. Nito et al. (*2005*) used calli derived from seedlings to assess compatibility among species of orange subfamily. In practice, grafting of fruit trees is between the scions from mature trees and stocks from either juvenile or mature trees in order to achieve precocious fruiting. Therefore, the use of calli derived from both juvenile partners might not portray the influence of phenol deposits on the graft union formation which is displayed in grafted trees. Furthermore, 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*. From the present study, we hypothesise the use of calli derived from both mature and juvenile trees as a better option to stimulate the reality for the grafted fruit trees.

Some graft partners in this study were weakly joined and easily separated when removed from the rings. In some provenance combinations, phenols suppressed the growth of the other partner. This phenomenon could be attributed to excess phenols which might have inhibited callus growth. Differential growth, faint union line and accumulation of phenol deposits at the callus union could exhibit a delayed graft incompatibility. These are collective indicators of cell rejection which brings about an outright graft incompatibility. Ermel et al. (*1997*) reported that cell necrosis is the main factor responsible for graft incompatibility and our results agree with their findings as this can bring an outright incompatibility. For instance, *U. kirkiana* and *J. curcas* callus combinations showed an outright graft incompatibility in this study.

From the present study, it is difficult to declare provenance combinations incompatible based on the presence of phenol deposits because accumulation of phenols in the cells could be seasonal. Success of the grafted partners would depend on physiological conditions of 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*). The effects of seasonality on phenol production and graft success need further investigation.

Cell walls of *U. kirkiana* calli had thicker appearance when co-cultured with *J. curcas*. This observation agrees with the findings of Errea et al. (*1994*) 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.

Uapaca. kirkiana clonal homografts and heterograft had faint union lines, but there were no necrotic layers observed. In this study, these graft combinations were confirmed as compatible. Moreover, some areas along the union formed a complete union. There were also formation of bead-like projections which are necessary in grafted trees since they interlock tissues of the scion and stock together strongly upon differentiation and thus forming a strong graft union. Possibly, such factors could be used as important indicators of graft compatibility.

Conclusion

The presence of visible union line, phenol deposits and cell necrosis were main indicators of graft incompatibility. Due to excessive phenol production, compatibility was not clear-cut within *U. kirkiana* provenances. Callus fusion within *U. kirkiana*, *U. nitida* and *J. curcas* clones were compatible, but less between *U. kirkiana* and *U. nitida*. Callus combinations between *J. curcas* and *U. kirkiana* showed an outright incompatibility. Despite the presence of phenol deposits that might have suppressed callus growth, callus fusion is a promising technique for assessing early graft compatibility, especially in species that are not closely related. Further studies to quantify and recognise phenols at the union areas are needed.

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