CHAPTER 5

DIAGNOSIS OF PHENOLIC COMPOUNDS IMPLICATED IN GRAFT INCOMPATIBILITY OF *UAPACA KIRKIANA* Müell Arg. TREES

5.1 Abstract

The objective of the trial was to determine the influence of phenolics on the graft union of *U. kirkiana* trees. Stem diameters were measured before sample collection. Soluble phenols were extracted with methanol-acetone-water followed by Folin-Ciocalteau reagent procedure and quantity assessed spectrophotometrically. Cell wall bound phenols were extracted and the precipitate was resuspended in 80% methanol before HPLC analysis. Thin (10 µm) layer sections were viewed under UV, blue and white light with or without Vanillin-HCl staining using fluorescence microscopy. Results showed significant differences in stem diameters. The unions were thicker than the scions and stocks. Both soluble and insoluble phenols at the unions were significantly high and homospecific combinations showed a high quantity of soluble phenols above the union. Fluorescence microscopy indicated the presence of flavonoids, anthocyanins or their derivatives above the union. HPLC indicated ferulic acid as the major component responsible for wood discolouration. For a less compatible combination (MW12/12), high peaks of *para*-coumaric acid were found above the union, and hence *para*-coumaric acid, flavonoids or polymers were implicated in graft incompatibility of *U. kirkiana* tree species.

5.2 Introduction

Grafted fruit trees perform better than sexually propagated fruit trees and some desirable attributes include precocious fruiting, improved fruit quality (fruit size) and tree dwarfness. Furthermore, dwarf trees are easier and cheaper to manage than large trees (Usenik & Štampar, 2001; Webster, 2001). However, graft incompatibility has been reported in some fruit trees and this has a negative effect on orchard productivity (Simons, 1987; Facteau *et al*., 1996; Errea *et al*., 2001).

Graft incompatibility has been attributed to many factors which include growth regulators and peroxidases, lack of formation of plasmodesmata and vascular tissue connection and inherent incompatibility at cellular level (Usenik *et al*., 2006). Micromolecules such as proteins, RNA and IAA transport are also involved (Pina & Errea, 2005). For *U. kirkiana*, successful grafting (80% graft take) has been achieved with skilled grafters at Makoka in Malawi (Akinnifesi *et al*., 2004). However, this does not mean long term scion/stock compatibility and field observations have revealed signs of graft incompatibility (Thomson Chilanga, personal communication). Similar observations have been made in *Eucalyptus* trees (Decooman *et al*., 1996). Recent assessment of the field survival of *U. kirkiana* grafted trees showed a decline from 100% after 6 months down to 67% after 13 months of establishment (Akinnifesi *et al*., in preparation). Usenik & Štampar (2001) reported that reduced graft compatibility is manifested by differences in the quantity of phenols at the graft union and the concentration of phenols is often high above the unions of heterografts.

Phenols have been implicated in graft incompatibility (Errea, 1998). Histological study of *U. kirkiana* tree (Chapter 4) revealed the presence of deposits and death of tissues above the union as the main causes of graft incompatibility. Differences in quantity or specific phenols at the union could play a role for reduced graft compatibility. However, when the wounding stress is over, many soluble phenols could be polymerised to other tannin-like compounds (Swain, 1979) and then deposited in the cell walls. Du Plooy (2006) who studied lenticels on mango fruits reported that cell wall bound phenols formed red colouration and that soluble phenols were polymerised into cell walls. Therefore, evaluation of cell wall bound phenols in grafted trees would be interesting, especially when the wounding is complete. Moreover, many studies on graft incompatibility have not focused on cell wall bound phenols, which might play an important role in graft incompatibility. The objective of this study was to determine the influence of phenolic compounds at the graft union of *U. kirkiana* trees.

5.3 Materials and methods

5.3.1 Plant material

Three-year old grafted *U. kirkiana* tree samples were collected at Makoka (see section 2.3.2 for site description) in February 2006. The trees were growing normally in the field and some were already fruiting. Scion, stock and union diameters of forty trees were measured using a pair of digital callipers (Mitutoyo, OE7343) before sample collection. The trees were propagated by skilled grafters using splice grafting method. Table 5.1 shows identification (ID) of the ten *U. kirkiana* tree species sampled. They were placed in cooler

box containing ice blocks and then transported within two days to the University of Pretoria for phenol analysis.

Samples were freeze-dried (Edwards, Modulyo, Pirani 10) to prevent oxidation and then dissected into segments from the union towards either the scion or stock (Figure 5.1). The segment at the union was 1 cm while the other segments were about 0.5 cm. The bark samples comprising the periderm, phloem and the vascular cambium, were ground using a mortar and pestle. The wood samples were ground using a motor grinder (Willey mill, 20 mesh). The fine powder (0.05 g) per sample was placed in Eppendorf tubes for extraction of phenol.

5.3.2 Phenol extraction

Samples were extracted with 1 ml of methanol-acetone-water solution (7:7:1 v/v/v) three times and then evaporated. The mixture was made up to 1 ml with deionised water and mixed in ultrasonic cleaner VWR™ (USC900TH, VWR International bvba/spri, B-3001 Leuven) for 4 min. Samples were centrifuged fro 4 minutes with a bench centrifuge (Combi-spin, type: FVL-2400N, Rochelle Chemicals & lab equipment, Germany). The supernatants were mixed and 1 ml was collected. Since the concentration was high, 100 µl supernatant was added to 900 µl deionised water for soluble phenol quantification.

5.3.3 Quantification of total soluble phenol

Folin-Ciocalteau reagent (Sigma), based on reduction of phospho-molybdene/phospho-tungstate (de Ascensao & Dubery, 2003), was used. Deionised water (175µl) was dispensed in ELISA plate wells, then the sample extract (5 µl), followed by Folin-Ciocalteau reagent

(25 µl). Sodium carbonate (50 µl of 20% w/v) was added and a blank in which water replaced the sample was used as a control. Four wells (replicates) were used per sample and incubated at 40 ^{o}C for 30 minutes. Absorbance was read using Multiskan Ascent spectroscopy (V1.24 345-00007T) set at 690 nm. Gallic acid was used as a phenol standard to construct a standard curve. The concentration of phenols in the extracts was calculated from the standard curve, y = 1.3527x - 0.0109 and multiplied by 10 since the initial dilution was in the ratio of 1: 9. The total soluble phenol quantity (mg) was expressed as gallic acid equivalent per g dry weight (DW) of sample. Data analysis was done using GenStat 4.24DE (Rothamsted Experimental Station).

5.3.4 Fluorescence microscopy

Four samples (MW12/12, MW26/26, MW32/28 and MW7/10) were used and these samples were fixed in formalin acetic acid (i.e. 5% formalin, 5% acetic acid and 90% ethanol). Thin (10 µm) layer sections were dissected at a right angle to the graft union using a sliding microtome (Leitz Wetzlar, 17815). The sections were fixed onto the microscope slides using glycerol and viewed with a Zeiss Axiovert 200 (Zeiss, Göttingen, Germany) microscope fitted with a sensys camera. Digital images of emission at 397 nm, 515 nm and 565 nm were captured with Nikon ACT-1 version 2. The sections were viewed under UV, white and blue light then stained with Vanillin-HCl (1% of concentrated HCl) and viewed again under UV, white and then blue light. Vanillin used was purchased from Sigma Aldrich Chemie (Steinheim, Germany). Blue light did not provide a good contrast between compatible and incompatible combinations. Therefore, the results under blue light will not be presented.

5.3.5 Phenol analysis using HPLC

Cell wall bound phenols were extracted from MW26/26, MW7/10 and MW12/12 samples according to De Ascensao & Dubery (2003) methodology. The reason why these three graft combinations were chosen was because MW26/26 graft combination was partially compatible, MW12/12 showed reduced compatibility (Chapter 4) and MW7/10 was an intermediate. Precipitates, obtained after total soluble phenol extraction, were dried and 0.5 M NaOH added to 0.01 g of precipitate. The mixture was suspended in a water bath (Julabo V, Labotec Pty Ltd, model 101) for 1 hour at 96 ^{o}C. The supernatant was acidified with HCl to a pH of 2.6, centrifuged fro 10 minutes and then extracted with 1 ml anhydrous diethyl ether. The mixture was dried and the precipitates suspended in 200 µl 80% aqueous methanol (MeOH). Folin-Ciocalteau reagent was used to determine the total cell wall-esterified phenolic acids and the remaining solution was separated using a reverse phase high performance liquid chromatograph (RP-HPLC).

The RP-HPLC (Hewlett Packard Agilent 1100 Series) was equipped with 20 µl loop injection value, DAD detection (diode array detector, 280, 325, 340 nm), Luna 3u C-18 (Phenomenex®) reverse phase column (150 mm by 2 mm, µm particle size, Ref. 550) and fitted with solvent deggaser (G1322A). A gradient elution was performed with HPLC water acidified with orthophosphoric acid (pH = 2.6) and acetonitrile (ACN) as follows: 0 min, 7% ACN; 0 - 20 min, 20% ACN; 20 - 28 min, 23% ACN; 28 - 40 min, 27% ACN; 40 – 45 min, 29% ACN; 45 - 47 min, 33% ACN. The flow rate was 0.6 ml min^{-1}. After each sample, the column was stabilized for 5 minutes and 40 µl of each sample was injected through a 20 µl loop. The UV detector was set at 280 nm but this is not optimal for ferulic

acid and its derivatives (Du Plooy, 2006). Identification of phenols was carried out by comparing their retention times (t_R) and UV apex spectrum to those of authentic standards purchased from Sigma Chemical Company, USA.

5.4 Results and discussion

5.4.1 Diameters of scion, stock and graft union

There were significant differences ($P \leq 0.05$) in mean diameters (Figure 5.2) amongst the scions, stocks and the graft union. The graft unions showed thick diameters compared to the scions and stocks. Selection of matching *U. kirkiana* scions and stocks is always difficult because scions are usually thicker than the stocks (Akinnifesi *et al*., 2006). In this experiment, stocks (18 mm) were thicker than scions (15 mm) after grafting (Figure 5.2). This could be attributed to vegetative growth restriction imposed by the stocks (dwarfing stock effect). According to Andrews and Marquez (1993) cytokinins are produced by root tips and acropetally translocated to promote the scion growth. The thin scions observed after grafting could be an indication of problems at the union. Generally, differences in stem diameters impose poor scion/stock alignment (Figure 5.3A). Consequently, wart-like projections of the wood cause poor continuity of the bark, and hence many cracks along the union.

Wood discoloration (Figure 5.3B) and swelling of the union (Figure 5.3C) are attributed to accumulation and oxidation of phenols. With such growth irregularities in the wood, overbearing in the early years might impose stress at the union and the ultimate survival (graft failure risk) of the trees. The lacuna areas in the wood can adversely affect the

support and water movement in the xylem as the trees grow (i.e., increase in size and mass). Presence of lacuna layers and wood discoloration above and at the union indicated that graft incompatibility was intense at these positions.

5.4.2 Total soluble phenol quantity in the wood

Table 5.2 shows total soluble phenols for individual grafted *U. kirkiana* samples and at different positions of the union in the wood. Forty percent of both homografts (MW32/32 and MW26/26) and heterografts (MW80/82 and MW84/57) had similar total soluble phenol above and below the union. The small variations within the homografts are attributed to genetic differences since the rootstocks were raised from seed. Different pollen sources in *U. kirkiana* trees are possible as they are dioecious, and hence mixed pollen on one female tree may occur. MW32/32 (Figure 5.4A) shows graft compatibility while MW32/28 (Figure 5.4B) shows signs of incompatibility in the wood. Pina & Errea (2005) reported that heterograft combinations often show graft incompatibility. There are lacuna or holes and wood discoloration, especially above the graft union. Despite these growth irregularities, all the trees were growing normally in the field.

There was no clear relationship between soluble phenol quantity and the graft union morphology. This could be attributed to the fact that phenols could be modified or polymerised into different forms. They might be deposited in the cell walls (cell wall bound phenols), and hence Folin reagent procedure could not quantify phenols which were bound in the cell walls. According to Pina & Errea (2005), small quantities of phenols could limit cell functions at the union and quantitative differences in phenols between the graft partners could cause metabolic problems. Furthermore, phenols play vital physiological roles during

the early stages of graft establishment. Therefore, effective proliferation of parenchymatous cells during the early stages to break up accumulated phenols is important for vascular continuity.

5.4.3 Total soluble phenol quantity in the bark

Table 5.3 shows total soluble phenols at different positions of the graft union in the bark of the individual graft combinations. Collectively, the graft unions showed significantly high accumulation of soluble phenols. There were no significant differences ($P \leq 0.05$) between above and below the union of 80% of the homograft combinations (MW32/32, MW13/13, MW12/12 and MW76/76). Furthermore, 60% of the heterograft combinations (MW7/10, MW32/28 and MW84/57) showed no significant difference between below and above the union. Gebhardt & Feucht (1982) and Usenik & Štampar (2001) reported that homografts show similar amounts of phenols above and below the union. In this experiment, it is suspected that isolated vascular continuity in the bark enabled tree growth of incompatible combinations. However, poor continuity in wood could adversely affect tree survival as the tree matures.

Table 5.4 shows soluble phenols in the bark and wood. There are significant differences ($P \leq 0.05$) between positions of the union and sample section (bark and wood), but no significant interaction. The data show significantly higher soluble phenol in the bark (87.9 mg g^{-1}) than in the wood (66.0 mg g^{-1}). This is because phenols are produced in the bark (phloem) and stored in the cell vacuoles. They are released in response to wounding or other stimuli. Total soluble phenol was significantly high at the union (85.4 mg g^{-1}) and low both below and above the union (75.2 mg g^{-1}).

5.4.4 Cell wall bound phenols

Figure 5.5 shows the quantity of cell wall bound phenols (mg equivalent of gallic acid per g of dry weight) at different positions (above, below and at the union) in the bark. There were significant differences ($P \leq 0.05$) between positions for MW12/12 and MW26/26. However, MW26/26 shows no significant differences in the quantity of phenol above and below the union. Morphologically, this graft combination showed partial compatibility. MW12/12 shows a higher quantity of cell wall bound phenols in the bark at all the positions compared to MW26/26 and MW7/10. Morphologically, this graft combination (MW12/12) showed incompatibility above, below and at the union. Therefore, this incompatibility is attributed to high quantity of cell wall bound phenols. De Ascensao & Dubery (2003) reported that cell wall bound phenols increased more than other phenols after 36 hours of *Fusarium* elicitor interaction.

Phenol deposition such as lignin and tannins occurs in plants and the former contributes to the stiffening and rigidity of cell walls and other internal structures while the latter helps in inhibiting microbial attack (Swain, 1979). Plants are also able to modify phenols deposited at the wounded site. De Ascensao & Dubery (2005) reported an increase in cell wall bound phenolics at the wounded site. In this trial, the death of a large portion of bark above the union of MW12/12 affected cell activities such as phenol polymerization and modification. This possibly, resulted in more phenol conversion into cell walls below the union (11.02 mg g^{-1}) than above the union (9.11 mg g^{-1}) of the bark.

There were no significant differences ($P \leq 0.05$) between the different positions of the union in the wood for MW7/10 and MW26/26 (Figure 5.6), but there were significant differences

for MW12/12 combination. MW12/12 also shows a significantly higher quantity of phenols above the union (4.40 mg g^{-1}) than below the union (2.54 mg g^{-1}). The same trend was obtained in Table 5.2 for this graft combination. Morphologically, MW26/26 and MW7/10 combinations had good vascular continuity in the wood below the union and showed better compatibility than MW12/12. Lack of continuity in wood tissues can be severe set-back because the wood provides the support. Absence of wood continuity at the graft union interface can cause graft failure.

5.4.5 Fluorescence microscopy

Phenols in the *U. kirkiana* tissues were detected using Vanillin-HCl as a fluorescent dye and Table 5.5 shows the colour characteristics of some phenolics. Fluorescence was observed in both unstained and stained sections (Figures 5.7 - 5.10). A blue staining was pronounced, especially below the union, indicating the presence of ferulic acid (Figures 5.7- 5.10). The darkened and blackened parts show cell death due to crushed cells during grafting. Cell death is pronounced above the union in all the graft combinations and this is attributed to accumulation of phenols. Brown or red staining under the white light for MW32/28 (Figure 5.7A-F) combination indicates presence of flavonoids (quinones). There were also zones of purple fluorescence and this indicates the presence of anthocyanins or terpenoids. Traces of cell wall bound red fluorescence were also present.

Visible observations indicated that MW7/10 and MW26/26 were partially compatible, but MW32/28 and MW12/12 were incompatible. Red or purple staining indicates the presence of flavonoids (Dixon & Paiva, 1995) or anthocyanins (Kangatharalingam *et al*., 2002). Presence of flavonoids has been implicated in graft incompatibility (Errea *et al*., 1994b)

and, from the results presented here, can also be implicated in *U. kirkiana* graft incompatibility. According to Errea *et al*. (1994b), a high amount of flavanoids (red) was found in the phloem of apricot as a response to graft incompatibility. Phenols can be oxidized to quinones which are toxic due to chemical reaction disruption (Errea, 1998).

MW32/28 (Figure 5.7A-F) shows that the cells fluoresced red though not very bright with an orange to brown colour under white light and above the union. This indicates the deposition of polymeric phenolics. According to Du Plooy (2006), polymeric phenolics often disguise the flavonoids (red). There are traces of yellow spots and bright blue at the union, under the UV and white light. There were blue colour lines below the union when *U. kirkiana* sections were viewed under UV and white light (Figure 5.7C-F), indicating ferulic aids. MW7/10 (Figure 5.8A-F) showed a pale brown colour (flavonoid or its derivatives) above the union, but a predominant blue colour (ferulic acid) below the union. Darkened (dead tissues) and brown areas were present at the union. MW26/26 sections (Figure 5.9A-F) show faint purple colour above the union, but a blue staining or colour is predominant below the union. MW12/12 sections (Figure 5.10A-F) show a purple colour (polymers or terpenoids) which is predominant below the union when viewed under the white light. Under the UV light, there are many dead cells (black or dark areas) above the union, purple colour (Figure 5.10E-F) and a few white spots in certain parts of the section. Therefore, cell death and presence of flavonoid or its derivatives could be implicated in graft incompatibility for this combination. Presence of dead tissues, holes and phenols could have prevented vascular continuity formation, caused cell damage and altered phloem cambium around the union.

5.4.6 Phenol analysis using RP-HPLC

Bark: RP-HPLC results indicated that ferulic acid was prominent in all the samples and at all the union positions (Figures 5.11 - 5.13). This also agrees with the fluorescence microscopy results in that ferulic acid was abundant in many sections. Ferulic acids eluted from the column within 14 minutes (t_R = 14) for MW12/12 (Figure 5.11A-B) at all the positions of the union. There were higher peaks (directly related to higher concentrations) of *para*-coumaric acids (t_R = 12.5) above the union for MW12/12 (Figure 5.11A), but low peaks for MW26/26 (Figure 5.12A-B). According to Méndez *et al.* (1968), *para*-coumaric acids strongly hinder cell elongation. Ramina & Masia (1982) reported that *para*-coumaric acids, isolated in peach fruits, were inhibitory to cell elongation and cress seed germination. Furthermore, cell wall bound *para*-coumaric acids were related to pit hardening in peach fruits. In this study, the presence of *para*-coumaric acids at the union might have adversely affected graft compatibility since it is possible that cell elongation and plasticity were inhibited. Generally, higher peaks were obtained below than above the graft unions in the in all the combinations (Figures 5.11 - 5.13). This could be attributed to the presence of many living tissues below the union that were able to carry out cell metabolism unlike the non-functional tissues presence above the union.

There was an unknown phenol (t_R = 44) indicated as phenol 44 (Figures 5.11A-B and 5.12A-B). The peaks for phenol 44 were high for MW12/12 compared to MW26/26 combinations, but this unknown phenol cannot be implicated in graft incompatibility for *U. kirkiana*. This is because it has higher peaks below the union of both MW12/12 and MW26/26 combinations. Morphologically, MW26/26 showed compatibility below the union despite the presence of a high peak of this unknown phenol (Figure 5.12B). This

indicates that its presence has no adverse effects on graft compatibility. Moreover, this unknown phenol was absent in MW7/10 combination (5.13A-B). Therefore, the presence of this phenol is dependent on the tree provenance or the source of a tree provenance.

Figure 5.11 shows the presence of 3, 4 dihydroxybenzoic acid (t_R = 3) only below the graft unions of MW12/12 (Figure 5.11B) and MW7/10 (Figure 5.13B). Its influence on graft compatibility is not adverse since it occurred where the union showed compatibility. For MW7/10 combination, another unknown phenol (t_R = 47) (Figure 5.13A-B) was obtained. This phenol shows also high peak below the union where there was more continuity in the bark than above the union. Furthermore, phenol 44 was absent in the other combinations. Therefore, it is assumed that this phenol had no impact on graft compatibility.

Wood: Generally, there were low peaks for all phenols in the wood and this made identification of peaks difficult (Figures 5.14 - 5.16). Ferulic acid was predominant and this indicates that ferulic acids were bound to the cell walls (Du Plooy, 2006). Vanillin (t_R = 7) was present for M12/12 combinations (Figure 5.14A-B). Phenol 44 was also obtained in the wood of MW12/12 with higher peaks above the union. Comparatively, small peaks were obtained from MW26/26 (Figure 5.15A-B). Low peaks in the wood indicate that many phenols were produced in the epidermal cells of the phloem (bark). Therefore, phenol analysis of the bark tissues (phloem vascular tissues) provided a clearer indication of the available phenols in *U. kirkiana* tree species. Although the quantities (mg g^{-1}) of phenols might be higher below than above the union, differences in accumulation of water could dilute the water-soluble phenols. This might reduce adverse effects on graft compatibility.

RP-HPLC results indicate that wood discoloration observed in all the graft combinations under a light microscope was largely due to ferulic acids bound to cell walls. High concentrations of *para*-coumaric acid above the union of MW12/12 in the bark could be implicated in graft incompatibility. DeCooman *et al*. (1996) reported accumulation of *para*-coumaric acid in less compatible *Eucalyptus gunnii* and Usenik *et al*. (2006) found *para*-coumaric acids to be high above the union of all the Betinka/stock combinations in apricot fruit trees. Betinka/stock was found to be less compatible even within homospecific combinations (Usenik *et al*., 2006). The predominance of ferulic acids in all of the samples provides evidence that ferulic acids were bound to the cell wall structures. More incompatibility problems can be expected with MW12 in heterospecific combinations. However, this needs to be verified since there is seasonality for phenol production.

5.5 Conclusion

Graft incompatibility in *U. kirkiana* is attributed to the presence of *para*-coumaric acids, flavonoids and polymers. MW12/12 was incompatible, while MW26/26 was partially compatible. Wood discoloration was due to ferulic acid which was abundant in all the combinations. Soluble phenols peaked at the union in many combinations in wood and bark. Flavonoids and anthocyanins were observed above the union under the fluorescence microscope on MW12/12, and hence implicated in graft incompatibility. Cell wall bound phenol analysis showed significant concentrations below the union of a less compatible combination (MW12/12). There were also high peaks of *para*-coumaric acids for MW12/12. Therefore, cell wall bound phenol analysis from the bark tissues provides useful information, especially when the wounding stress is complete.

Tables

Table 5.1 Tree identification (ID) of *Uapaca kirkiana* graft combinations collected at Makoka Agricultural Research Station in Malawi

Tree ID	Scion accession name	Tree ID	Stock accession name
MW76	ICR02NkhumbaMW76	MW76	ICR02NkhumbaMW76
MW7	ICR02MalemiaMW7	MW10	ICR02MalemiaMW10
MW12	ICR02SitolaMW12	MW12	ICR02SitolaMW12
MW13	ICR02SitolaMW13	MW13	ICR02SitolaMW13
MW26	ICR02HardwickMW26	MW26	ICR02HardwickMW26
MW32	ICR02YesayaMW32	MW28	ICR02HamiyoniMW28
MW56	ICR02NkhumbaMW56	MW49	ICR02NkhumbaMW49
MW32	ICR02YesayaMW32	MW32	ICR02YesayaMW32
MW84	ICR02NazombeMW84	MW57	ICR02NazombeMW57
MW80	ICR02NazombeMW80	MW82	ICR02NazombeMW82

Table 5.2 Total soluble phenol quantity (mg gallic acid equivalent per g of dry weight) in the wood of ten grafted *Uapaca kirkiana* trees measured above, below and at the union

Graft combinations	Gallic acid equivalent of dry weight (mg g^{-1})			CV (%)
	above the union	at the union	below the union	
Homografts				
MW26/26	61.5[b]	73.9[a]	67.3[ab]	7.1
MW12/12	66.0[a]	67.5[a]	52.7[b]	11.6
MW13/13	30.0[c]	62.0[a]	36.9[b]	6.9
MW76/76	80.9[a]	70.3[b]	58.6[c]	4.4
MW32/32	82.0[a]	78.6[a]	81.5[a]	25.5
Heterografts				
MW84/57	52.5[b]	65.6[a]	51.4[b]	34.7
MW56/49	64.3[b]	83.7[a]	40.5[c]	14.0
MW32/28	71.0[a]	49.0[b]	40.1[b]	20.3
MW80/82	90.8[a]	87.0[a]	77.9[a]	15.9
MW7/10	57.5[b]	95.0[a]	83.7[a]	10.7

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

Table 5.3 Total soluble phenol quantity (mg gallic acid equivalent per g of dry weight) in the bark of *Uapaca kirkiana* grafted trees measured above, below and at the unions

	Gallic acid equivalent of dry weight (mg g^{-1})			
Graft combinations	above the union	at the union	below the union	CV (%)
Homografts				
MW26/26	97.0^{b}	159.4^{a}	159.3^{a}	13.0
MW12/12	79.7^{b}	105.2^{a}	81.5^{b}	15.1
MW13/13	71.4^{a}	71.0^{a}	84.3^{a}	37.0
MW76/76	110.0^{a}	90.0^{a}	109.9^{a}	31.8
MW32/32	79.2^{a}	91.2^{a}	84.0^{a}	26.2
Heterografts				
MW84/57	70.8^{ab}	93.8^{a}	64.3^{b}	17.1
MW56/49	25.2^{b}	65.9^{a}	62.3^{a}	38.1
MW32/28	62.7^{b}	105.3^{a}	90.4^{ab}	15.0
MW80/82	59.9^{b}	100.2^{a}	90.8^{a}	19.5
MW7/10	94.0^{a}	93.1^{a}	85.5^{a}	21.4

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

Table 5.4 Total soluble phenol quantity (mg gallic acid equivalent per g of dry weight) at different positions of *Uapaca kirkiana* graft unions in the wood and bark. Data calculated with standard errors

Position of the graft union interface	Sample sections		
	bark	wood	Mean
Above the union	75.0	65.6	70.3 ± 4.6^{b}
At the union	97.5	73.3	85.4 ± 5.2^{a}
Below the union	91.2	59.1	75.2 ± 6.2^{ab}
Mean	87.9 ± 4.8^{a}	66.0 ± 3.1^{b}	
LSD (P≤0.05)	Part of the graft union		11.1*
	Sample section		9.1*
	Interaction		15.8^{ns}
CV (%)			22.7

* = significantly different, ns = not significantly different at P≤0.05

Table 5.5 Table of fluorescence colours and the associated phenol groups (Regnier & Macheix, 1996; Du Plooy, 2006)

Fluorescence colour	**Group of Phenols**
Strong blue	Ferulic acid
White	Gallic acid/Caffeic acid
Blue-white	Coumaric acid
Light Blue	Hydroxycinnamic acid and Ferulic acid derivatives
Red	Flavonoids
Brown	Quinone
Deep blue / Purple	Not phenolic compounds – polymer, Terpenoids

Figures

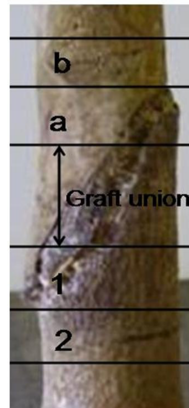

Figure 5.1 Stem segments dissected from a three-year old *Uapaca kirkiana* grafted tree (1 and 2 = segments towards the stock, a & b = segments towards the scion)

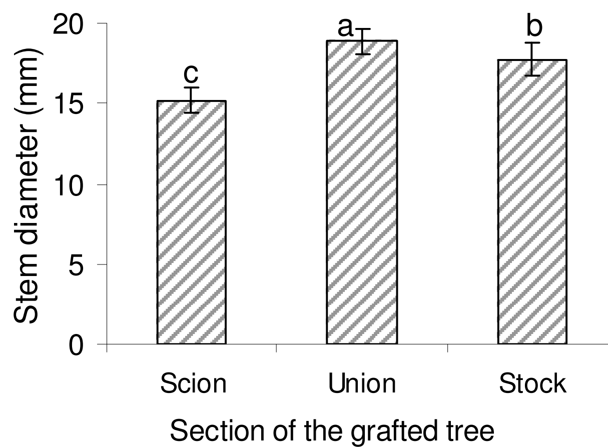

Figure 5.2 Diameters of scions, stocks and graft unions of *Uapaca kirkiana* trees (three years old after grafting) pooled together and measured approximately 5 mm above and below the union area

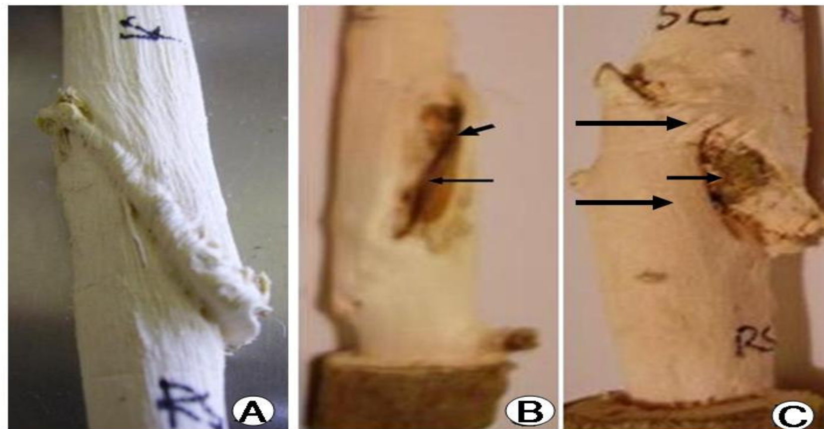

Figure 5.3 Morphological growth structure of *Uapaca kirkiana* wood at the union (A) MW80/82 showing wart-like projections at the union; (B) lacuna or holes (thin arrows) at the wood pith; (C) a swollen graft union (thick arrows) with some projections (Rs = rootstock and Sc = scion)

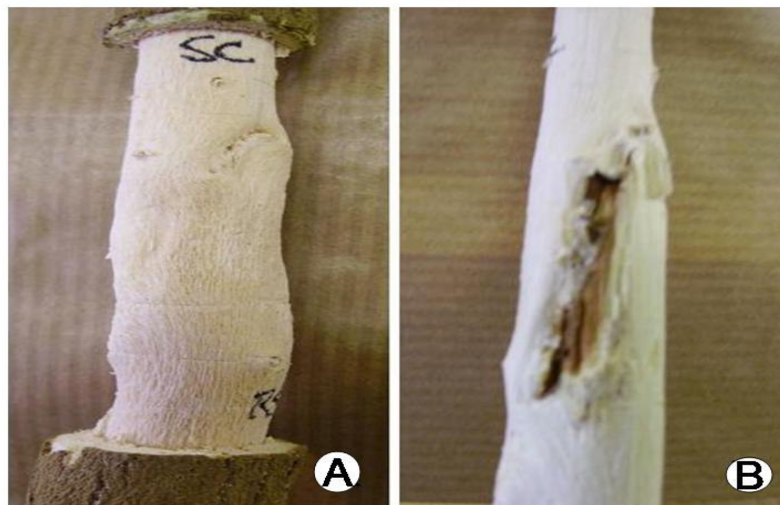

Figure 5.4 Morphology of MW32 *Uapaca kirkiana* scions on two different stocks (A) MW32/32 clonal homograft without wood discoloration or holes; (B) MW32/28 heterograft with lacuna in wood

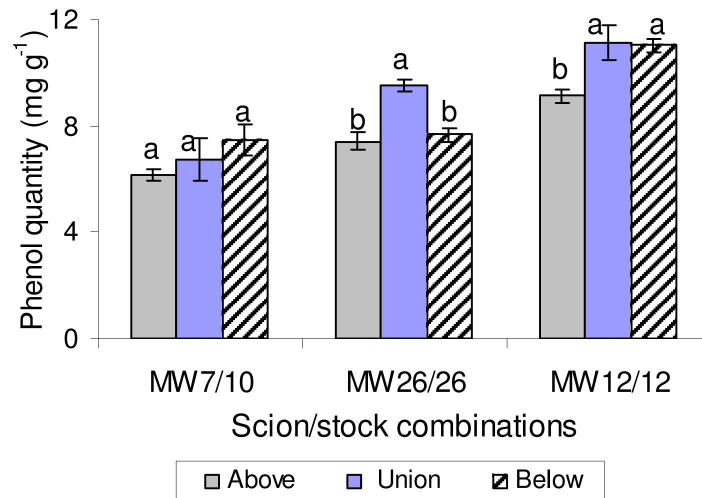

Figure 5.5 Cell wall bound phenol quantity (mg gallic acid equivalent per g of dry weight, DW) in the bark measured above, below and at the union (bars with the same letters within a column are not significantly different at P≤0.05)

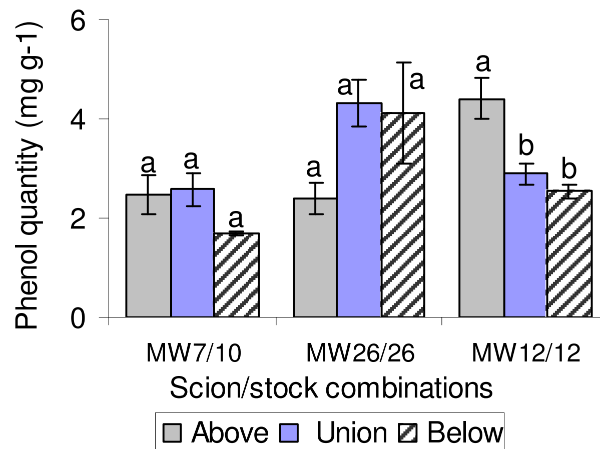

Figure 5.6 Cell wall bound phenol quantity (mg gallic acid equivalent per g of dry weight, DW) in the wood measured above, below and at the union (bars with the same letters within a column are not significantly different at P≤0.05)

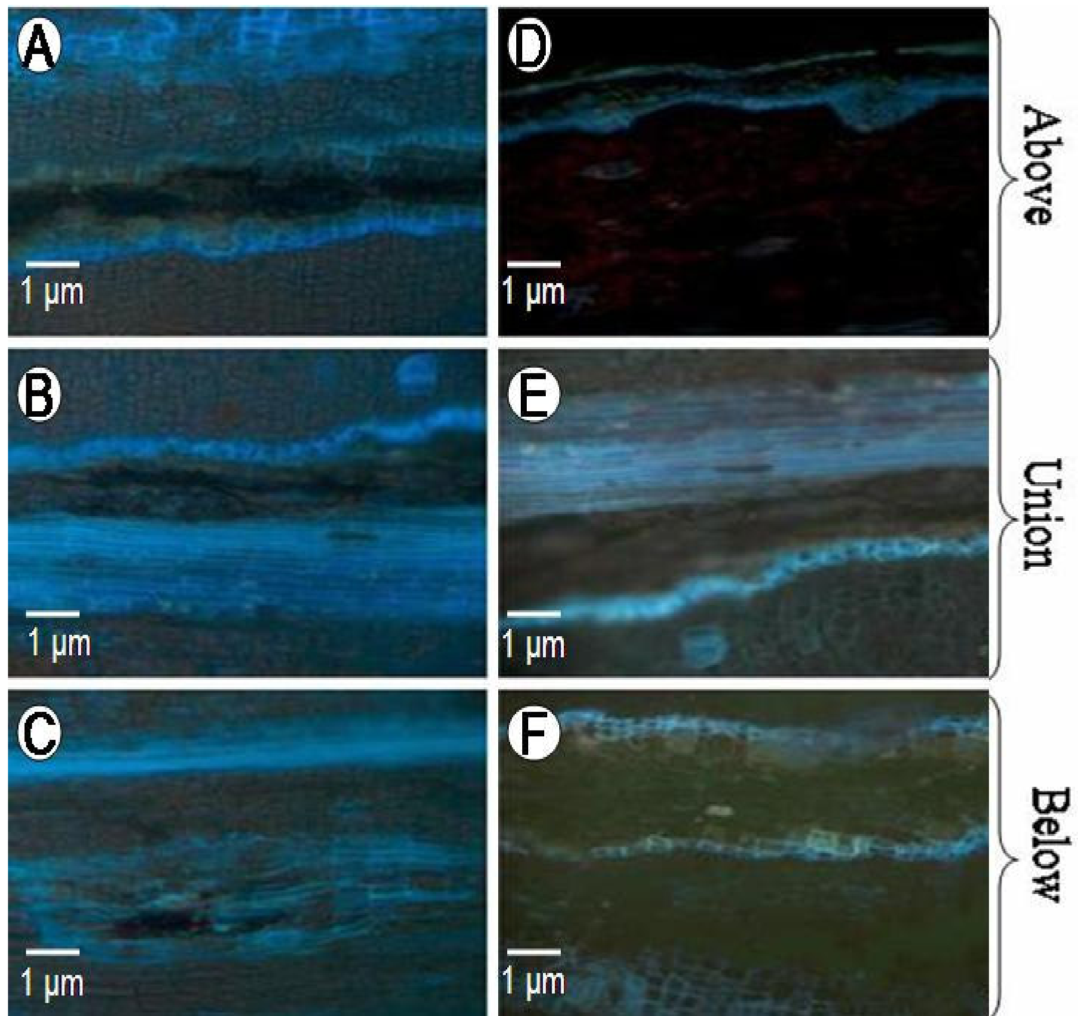

Figure 5.7 MW32/28 *Uapaca kirkiana* sections (above, below and at the union) viewed under a fluorescence microscope using (ABC) UV light and (DEF) Vanillin-HCl and white light

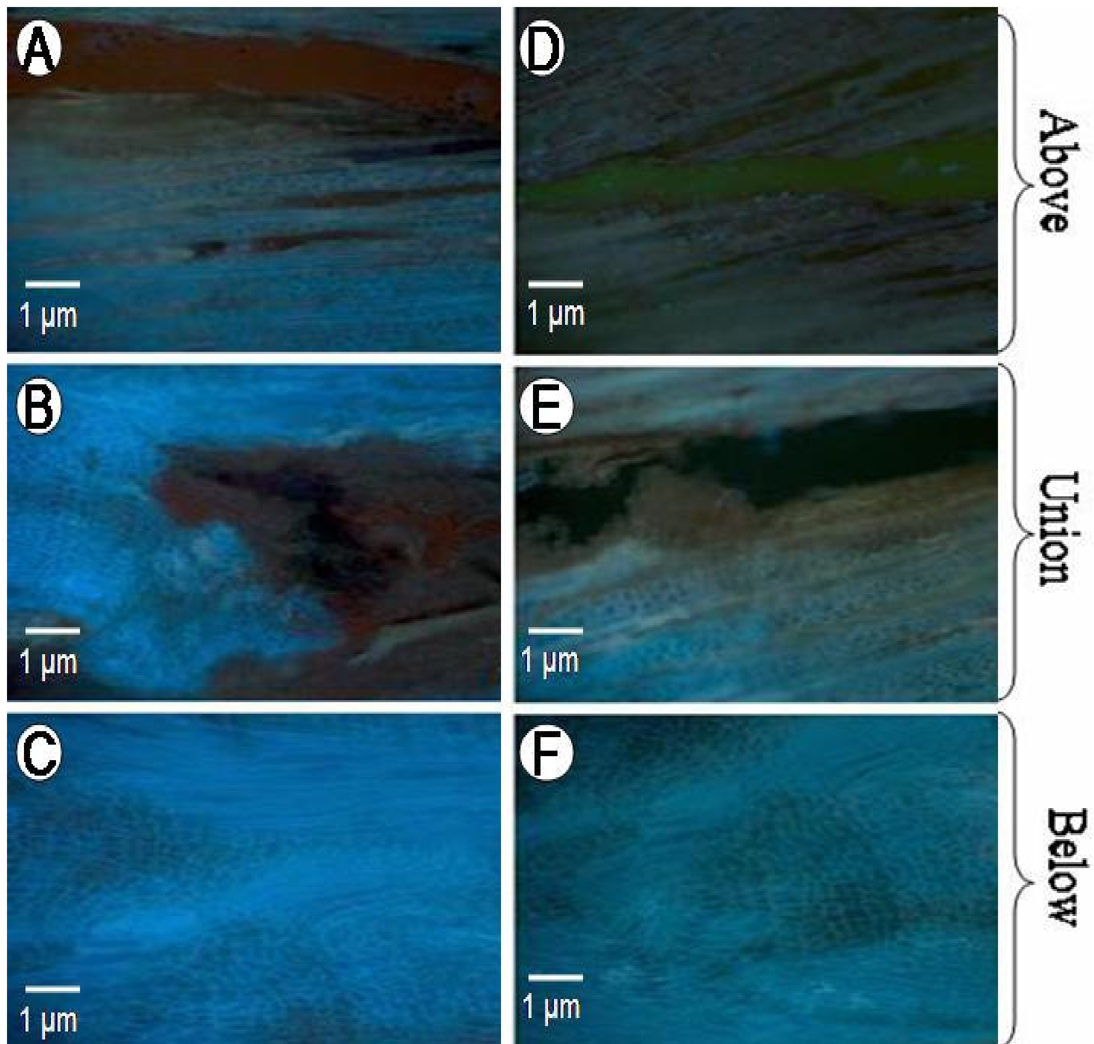

Figure 5.8 MW7/10 *Uapaca kirkiana* sections (above, below and at the union) viewed under fluorescence microscope using (ABC) UV light and (DEF) Vanillin-HCl and white light

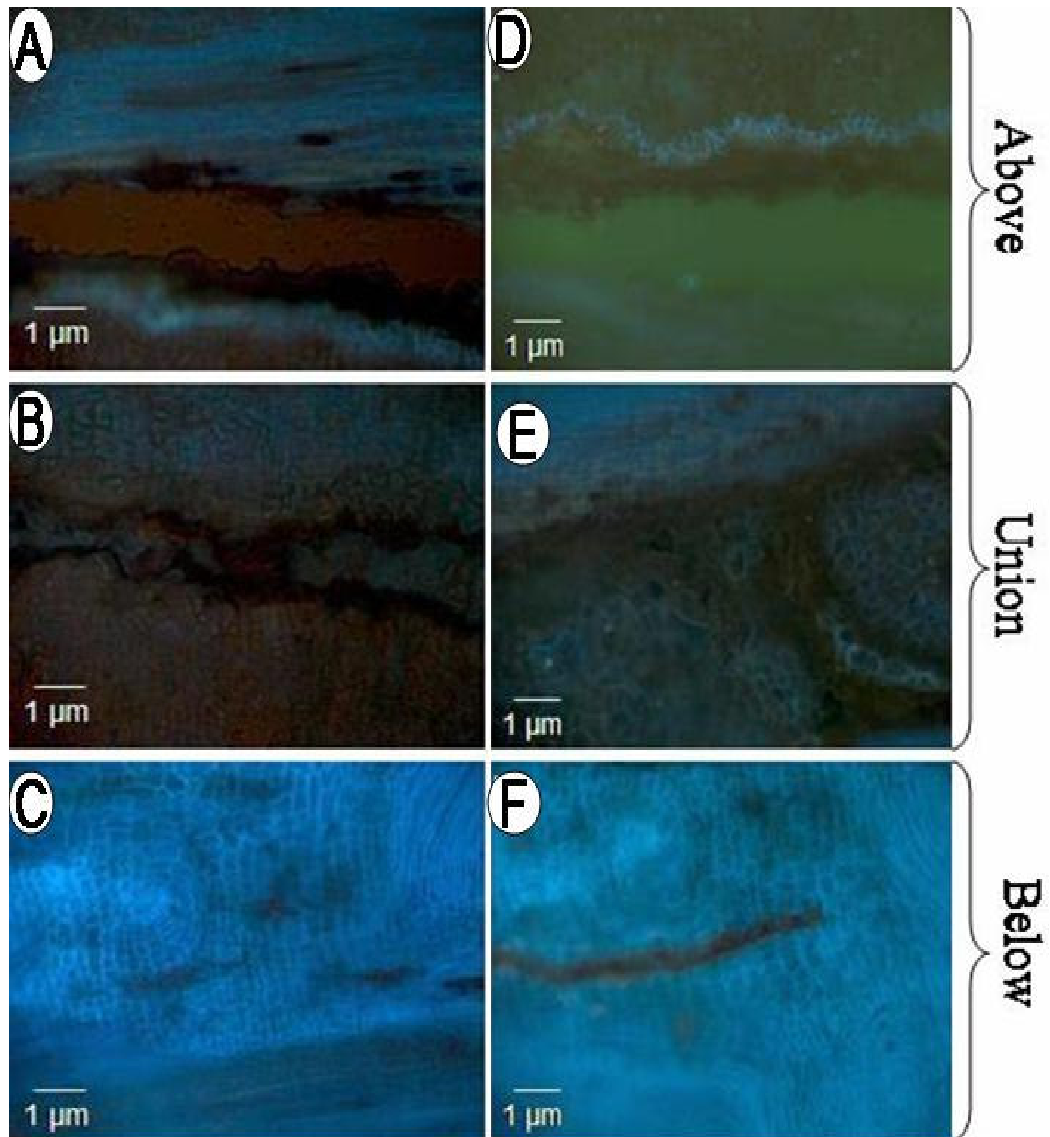

Figure 5.9 MW26/26 *Uapaca kirkiana* sections (above, below and at the graft union) viewed under a fluorescence microscope using (ABC) UV light and (DEF) Vanillin-HCl and white light

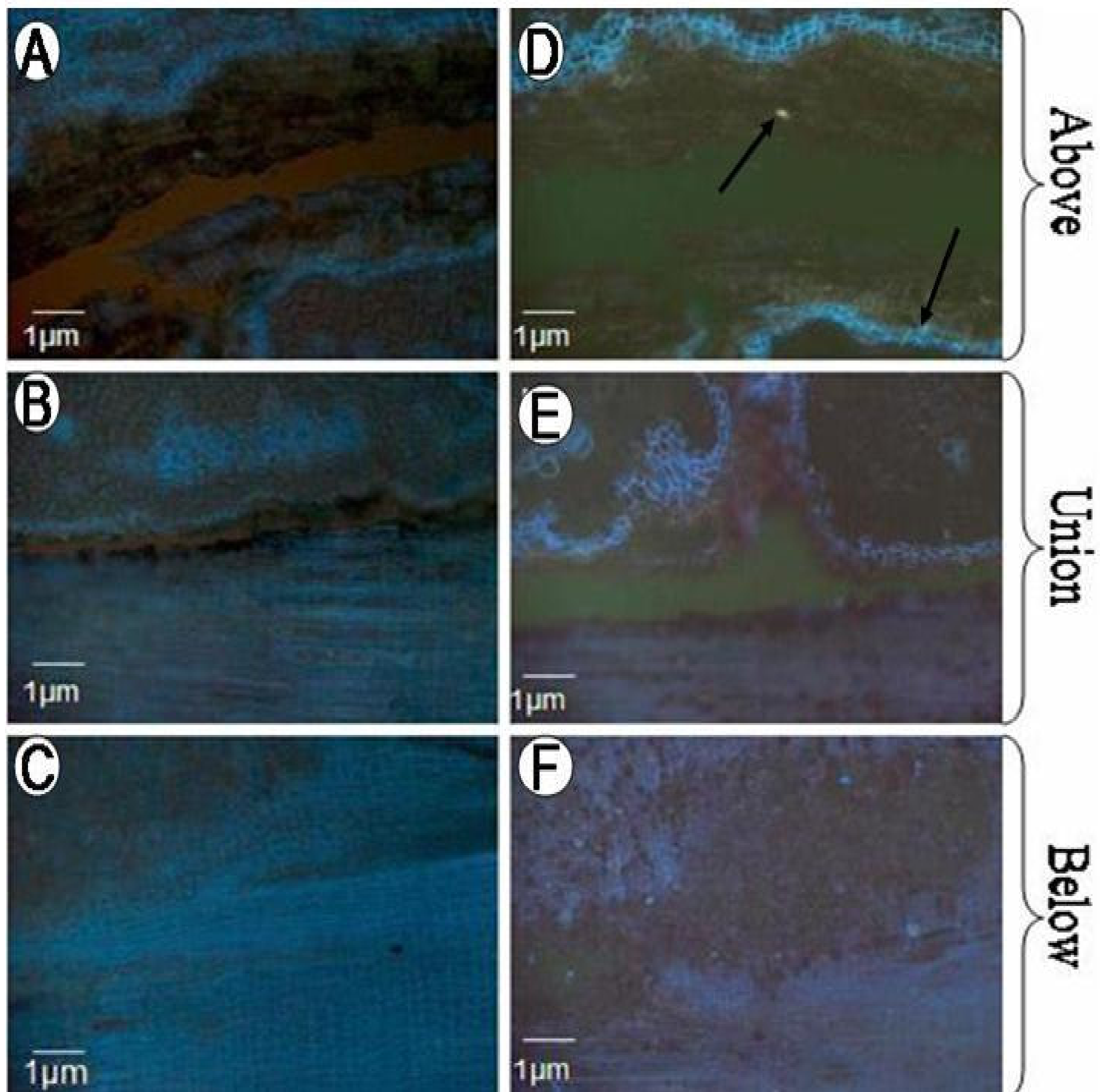

Figure 5.10 MW12/12 *Uapaca kirkiana* sections (above the union, at the union and below the union) viewed under fluorescence microscope using (ABC) UV light and (DEF) Vanillin-HCl and white light (arrows show isolated white spots)

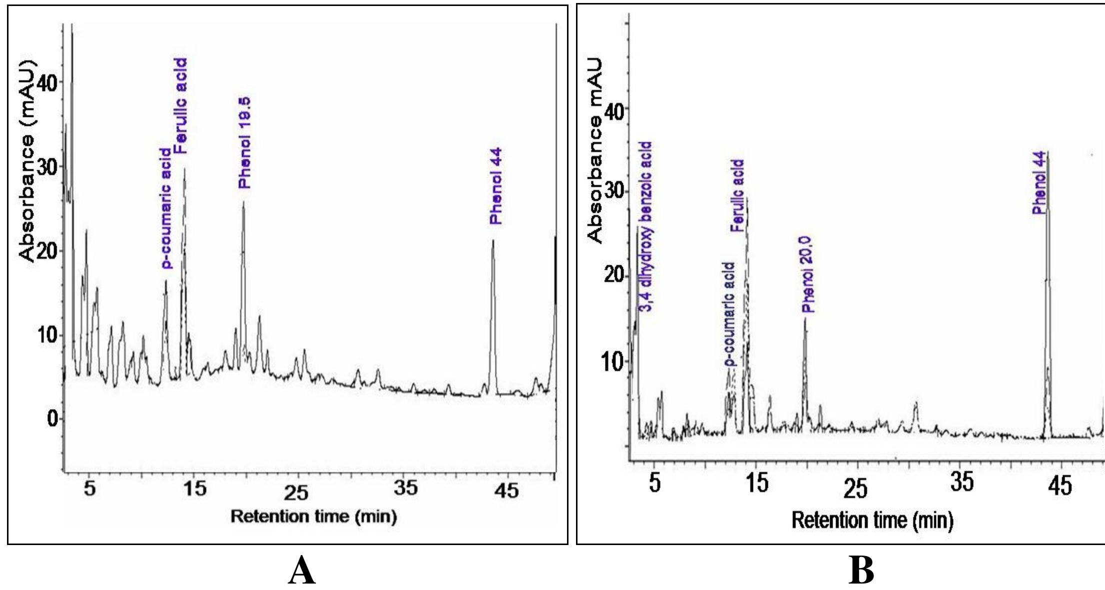

Figure 5.11 Chromatograms of cell wall bound phenols extracted from MW12/12 *Uapaca kirkiana* in the bark (A) above the union and (B) below the graft union

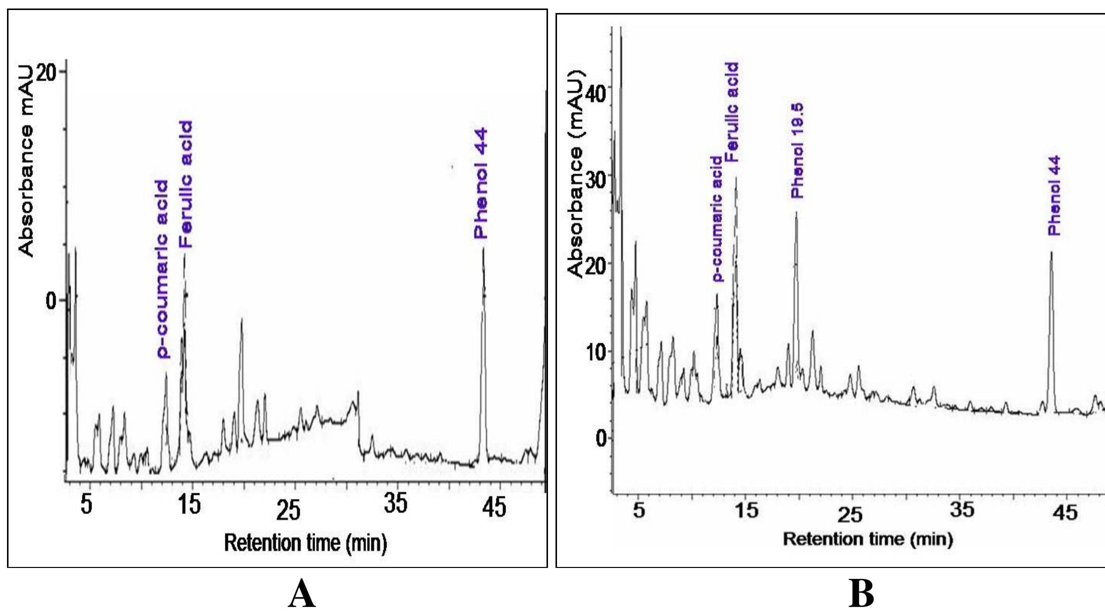

Figure 5.12 Chromatograms of cell wall bound phenols extracted from the bark of MW26/26 *Uapaca kirkiana* combination (A) above the union and (B) below the graft union

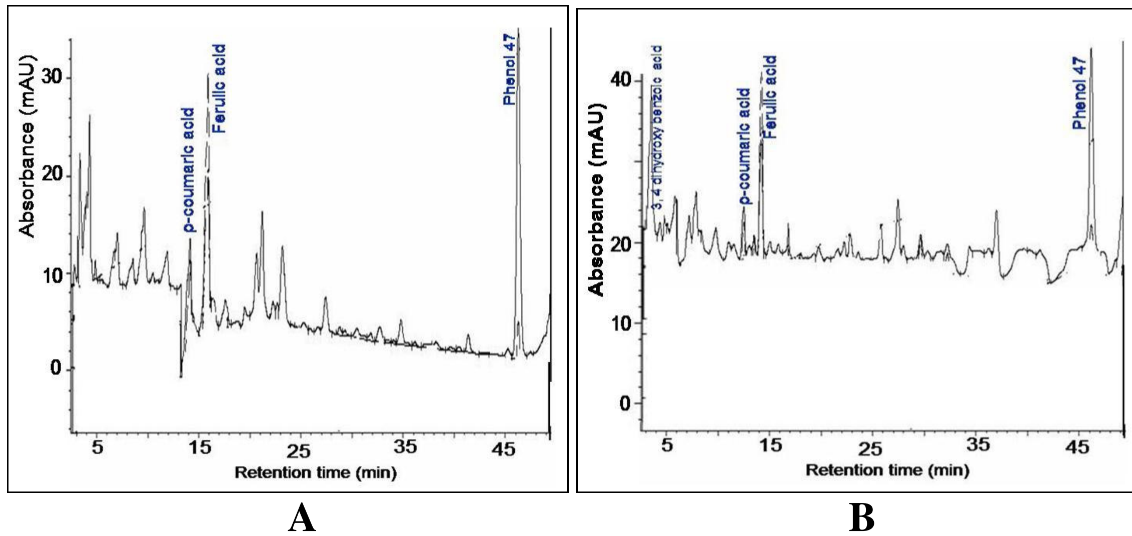

Figure 5.13 Chromatograms of cell wall bound phenols extracted from the bark of MW7/10 *Uapaca kirkiana* combination (A) above the union and (B) below the graft union

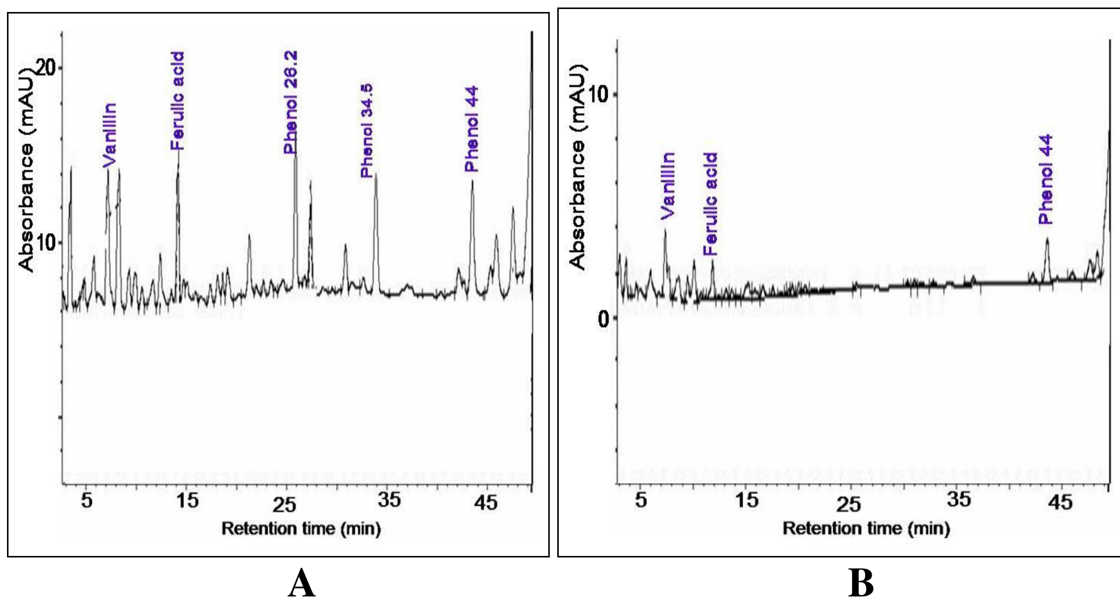

Figure 5.14 Chromatograms of cell wall bound phenols extracted from MW12/12 *Uapaca kirkiana* in the wood (A) above the union and (B) below the graft union

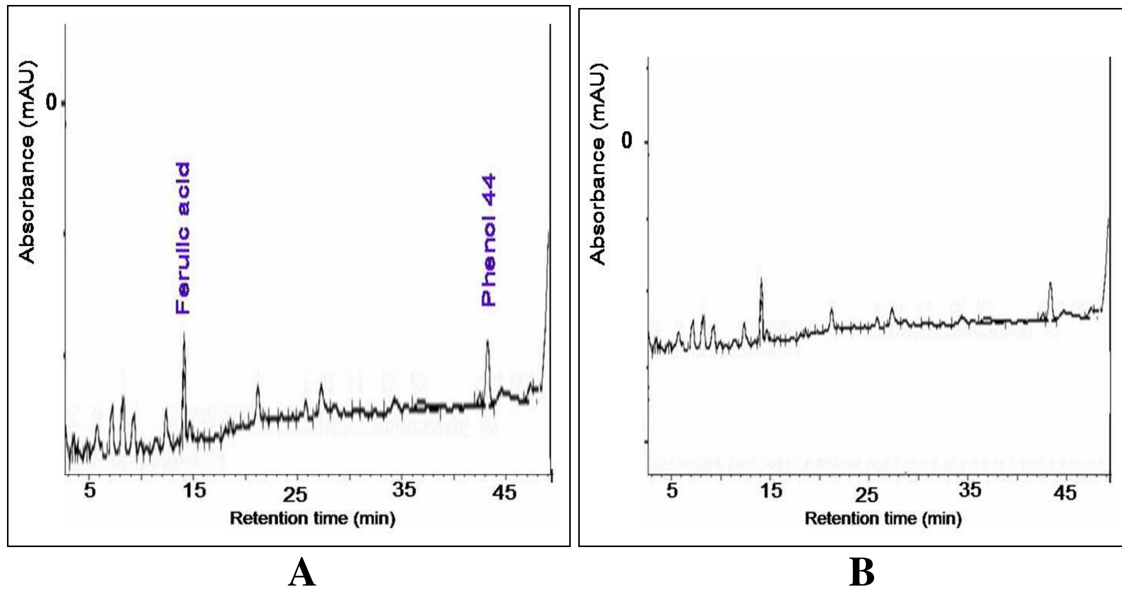

Figure 5.15 Chromatograms of cell wall bound phenol extracts from the wood of MW26/26 *Uapaca kirkiana* combination (A) above the union and (B) below the graft union

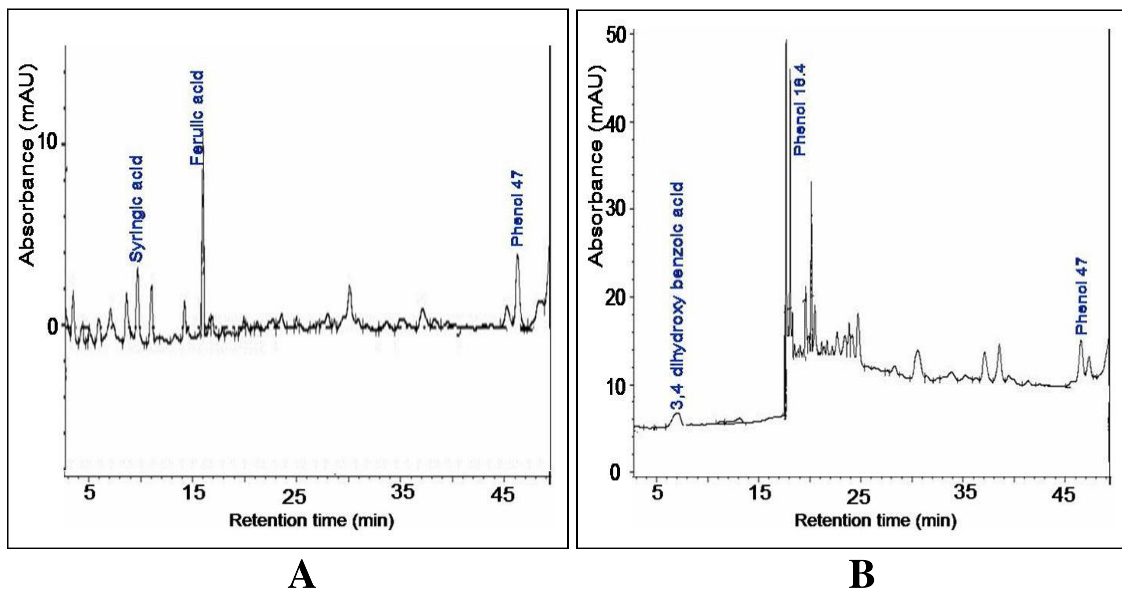

Figure 5.16 Chromatograms of cell wall bound phenol extracts from the wood of MW7/10 *Uapaca kirkiana* combination (A) above the graft union and (B) below the union