Histological Evaluation of Early Graft Compatibility in *Uapaca kirkiana* Müell Arg. Scion/Stock Combinations

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Abstract. Compatibility of scion and stock combinations in *Uapaca kirkiana* fruit trees has not been evaluated despite noticeable growth irregularities. The objectives of this study were to determine graft compatibility of scion/stock combinations and possible causes of graft incompatibility. Scion, stock, and graft union diameters were measured. Stem sections comprising the graft unions were immersed in formalin acetic acid and then washed in sterile water. These were transversely dissected across the unions and examined under light microscope. There were considerable growth disorders at the unions, which included significant overgrowth of stocks and unions and constricted unions. There were cracks in the bark across the union in many graft combinations. Anatomic and histological studies showed accumulation of phenol deposits and necrotic tissues, and there was no continuity of vascular tissues above the union. There were also differences in proliferation of callus tissues among grafted partners. Continuity in wood and bark tissues below the unions supported growth of partially compatible partners, whereas isolated parenchymatous tissues at the union supported growth of incompatible partners. There were many necrotic tissues and unfilled areas above the union. Accumulation of phenolic and necrotic cell deposits, poor or a high level of callusing at the union, and possibly specific incompatibility reactions were implicated as the causes of graft incompatibility in *U. kirkiana* trees.

*Uapaca kirkiana*, an indigenous fruit tree of the Miombo ecozone, offers considerable scope for enhancing the nutritional and economic security of rural communities of southern Africa (Akinnifesi et al., 2006). Recent studies in Malawi and Zimbabwe showed that indigenous fruits, especially *Uapaca*, can reduce the probability of household poverty by 33% during a seasonal food shortage (Mithöfer et al., 2006). Moreover, it is widely preferred by small-holder farmers in southern Africa (Maghembe et al., 1998).

Germplasm of *U. kirkiana* trees from 16 provenances has been collected, characterized, and established in multilocal trials in five southern African countries (Kwesiga et al., 2000). These *U. kirkiana* tree provenances show wide genetic diversity and variations in growth and geographical adaptation (Akinnifesi et al., 2004b). Consequently, a participatory clonal selection of *Uapaca* from the wild has been initiated in some southern African countries to identify superior cultivars for multiplication and wider cultivation (Akinnifesi et al., 2006). According to Maghembe et al. (1998) and Akinnifesi et al. (2004a), tree dwarfing and precocious fruiting are desirable characters that potential growers of *U. kirkiana* trees would like to see being addressed in domestication and improvement initiatives.

*Uapaca kirkiana* trees have a long juvenile phase (10–12 years) when propagated sexually (Akinnifesi et al., 2004a), but this has been reduced to 3 to 4 years using vegetative propagation (Akinnifesi et al., 2006). Different vegetative propagation methods such as air layering, budding, rooting stem cuttings and grafting have been evaluated at Southern African Development Community–International Council for Research in Agroforestry (SADC-ICRAF) Makoka Station in Malawi. Budding and rooting stem cuttings have yielded poor results. According to Mhango et al. (2000), air layering was promising, but root development after tree establishment was problematic. Grafting by the splice method has been the most promising method for *U. kirkiana* (Akinnifesi et al., 2004a, 2006). Moreover, rootstocks can impart desirable characters such as tree dwarving, improved fruit traits (fruit sweetness, size, load, and color), and precocious fruiting (Ferree and Carlson, 1987; Usenik and Stampar, 2001; Webster, 2001).

Improved graft take (80%) has been achieved in *U. kirkiana* trees when grafting was done by skilled grafters (Akinnifesi et al., 2004a). However, growth irregularities, possibly as a result of graft incompatibility have been observed in some grafted trees in the nursery and the field, and thus early or late rejection is suspected. Graft incompatibility occurring some years after grafting in normal growing trees constitutes a major concern in many grafted trees (Errea, 1998; Simons, 1987). Recent assessment of field survival of *U. kirkiana* grafted trees showed a declining trend starting from 100% at 6 months after field establishment to 67% at 33 months (Akinnifesi et al., 2007). Therefore, early evaluation of scion/stock combinations is important for successful orchard establishment and productivity. However, there is no scientific research devoted to graft incompatibility in *U. kirkiana* trees to date. The objectives of this study were to determine graft compatibility of different scion and stock combinations, and the possible causes of scion and stock incompatibility in *U. kirkiana* trees.

Materials and Methods

A *Uapaca* nursery stock (1 year after grafting) at SADC-ICRAF Makoka Research Station in Malawi (alt., 1029 m asl; lat., 15°30’S; long., 35°15′E) was used for this study. This site has a total annual rainfall of 560 to 1600 mm, and the temperature varies between 16 and 32 °C (Akinnifesi et al., 2004b). Stem diameters of scions, stocks, and graft unions were measured using a pair of calipers. Bark thickness for both scions and stocks was also measured.

Ten grafts of *U. kirkiana* trees, grafted by the splice method, were randomly selected. Table 1 shows the identity, sources and codes of scions and stocks selected. Samples were collected by cutting 4 to 5 cm below and above the scion/stock union. Stem sections were immediately immersed in formalin acetic acid (5% formalin, 5% acetic acid, and 90% ethanol) and later rinsed in sterile water to remove the acid. Samples were then mounted on a slide microtome stage (model E. Leitz, Wetzlar 17815, Germany) by freezing CO₂ gas. Thin-layer transverse sections (10 μm) were cut at a right angle to the union and then mounted onto microscope slides. Sections were viewed under a light microscope (Olympus microscope model: ach 1x, SZX7, Germany), connected to a digital camera (Olympus, Japan), and microphotographs of the union interfaces were taken.

Visual scoring for graft compatibility included a visible union line in the bark and
wood where 1 is visible, 2 is faint, 3 is very faint, and 4 is absent; browning intensity of deposits at the union interface where 1 is high, 2 is medium, 3 is low, and 4 is absent; and callus proliferation where 1 is high, 2 is medium, 3 is low, and 4 is absent. Visual scores were converted to percentages (0% to 25%, absent/very low; 26% to 50%, very faint/low; 51% to 75%, medium/faint; 76% to 100%, high).

Data on diameters of scions, stocks, and unions, and bark thicknesses of scions and stocks were arranged in a completely randomized design and then subjected to analysis of variance. Data on visual scores were analyzed using correspondence analysis in GenStat (Rothamsted Exp. Sta.). Histological variables (union line, callus proliferation, and deposit intensity) were used to discriminate the compatible from the incompatible combinations [adapted from Ermel et al. (1995)].

Results and Discussion

There was an increase in stem diameter at the unions compared with the scions and stocks. However, there were no significant differences in diameter between the scions and stocks (Table 2). Results agree with the findings of Tshokoeva and Tsonev (1995), who reported marginal differences between scion and stock diameters in grafted apricot trees, but a significant increase in diameter at the union. An increase in stem diameter at the union could be attributed to accumulation of metabolites (presumably phenols and carbohydrates) as a result of partial cambium continuity at the union. Errea (1998) reported that translocation problems caused accumulation of some compounds. Moreover, high levels of calluses forming into the undifferentiated parenchymatous cells could also cause the union to swell. Rootstocks had significantly thicker bark than scions, and this could be attributed to differences in growth and callusing rates after grafting (Table 2).

Fig. 1A shows a good level of callusing and healing at the union of the MW26/22 combination. Fig. 1B (MW1/61) shows an increase in union diameter and cracking of the bark. Generally, many U. kirkiana trees have cracks in bark running almost vertical to the tree axis and this can be attributed to a genotypic trait. However, horizontal cracks across the union could be implicated in graft incompatibility.

Andrews and Marquez (1993) reported that hormonal imbalances between stocks and scions are involved in graft incompatibility. Furthermore, flavonoids (phenolics) are known to inhibit callus growth whereas auxins control callus formation (Andrews and Marquez, 1993; Errea et al., 1994b). DeCooman et al. (1996) reported accumulation of ρ-coumaric acid (phenolic compound) in less compatible Eucalyptus gunnii graft combinations, and Usenik et al. (2006) found high levels of ρ-coumaric acids above the union of incompatible apricot graft combinations.

According to Akinnifesi et al. (2004a), matching the cambial cells between scion and stock has been a challenge in grafting U. kirkiana trees because scions are always thicker than the stocks. Therefore, correct matching depends on selecting scions and stocks with almost similar stem diameter and bark thickness. This improves proximity of vascular tissues of the scions and stocks. In this trial, bark thickness at the union was not measured, but could be a factor contributing to an increase in union diameter. This is because the presence of nonfunctional tissues can increase the union diameter. Simons and Chu (1981) reported an overgrowth of the union resulting from radial growth of vascular tissues.

Fig. 2 illustrates the external view and longitudinal section of the unions. There are variations in the level of callus proliferation and union line visibility. Fig. 2A shows a poor level of callusing (external view) and a visible union line at the union interface. Fig. 2B shows continuity in the bark and wood tissues below the union, and this is termed a “partial” graft union (Unal, 1995.) Graft partners with a partial union showed a good level of callusing at the union (external view and longitudinal section). Therefore, a poor graft union could be associated with a low level of callusing, as shown in Fig. 2A.

Fig. 3 shows incompatible (Fig. 3A) and partially compatible (Fig. 3B) unions between U. kirkiana partners. MW84 (Nazombe) on

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Table 1. Tree identification (ID) of selected Uapaca kirkiana stocks and scions from different districts and locations on landscape (natural forest or cultivated field) in Malawi.

| Tree ID | Accession name | District | Landscape | Fruit trait |
|---------|----------------|----------|-----------|-------------|
| MW1     | ICR02 NkhumbaMW1 | Zomba    | Forest    | Sweetness   |
| MW2     | ICR02 KanyotaMW2 | Zomba    | Forest    | Sweetness   |
| MW7     | ICR02 MalemiaMW7 | Zomba    | Forest    | Sweetness   |
| MW10    | ICR02 MalemiaMW10 | Zomba    | Forest    | Sweetness   |
| MW12    | ICR02 SitolaMW12 | Zomba    | Forest    | Sweetness   |
| MW13    | ICR02 SitolaMW13 | Zomba    | Forest    | Sweetness, size |
| MW22    | ICR02 ElsonMW22 | Dedza    | Forest    | Sweetness   |
| MW26    | ICR02 HardwickMW26 | Dedza    | Field      | Sweetness, load, size |
| MW28    | ICR02 HamiyoniMW28 | Dedza   | Field      | Sweetness   |
| MW32    | ICR02 YesayaMW32 | Dedza    | Forest    | Sweetness, size |
| MW49    | ICR02 NkhumbaMW49 | Phalombe | Forest    | Sweetness, fruit load |
| MW57    | ICR02 NkhumbaMW57 | Phalombe | Forest    | Sweetness, fruit early |
| MW61    | ICR02 MigowiMW61 | Phalombe | Forest    | Sweetness, fruit early |
| MW71    | ICR02 NkhumbaMW71 | Phalombe | Forest    | Sweetness, fruit load |
| MW84    | ICR02 NazombeMW84 | Phalombe | Forest    | Sweetness   |

Table 2. Scion, stock, and graft union diameters, and bark thickness of Uapaca kirkiana fruit trees pooled together (1 year after grafting).

| Plant parts | Stem diameter (cm) | Bark thickness (cm) |
|-------------|--------------------|---------------------|
| Scion       | 1.10 b'             | 0.18 b              |
| Stock       | 1.21 b              | 0.25 a              |
| Unions      | 1.50 a              | —                   |
| Coefficient of variation (%) | 14.3      | 20.5     |

LSD<sub>0.01</sub>  0.22  0.06

*Mean separation by LSD within a column at P ≤ 0.01.

**Not measured.

Measurements were taken ±5 mm below and above the union and the bark.
Prunus transformation and trees. According to Errea species. In this trial, bark tissues U. kirkiana A trees. (Errea species. Errea leaves during DNA extraction. 2007 H c Uapaca kirkiana scion and stock combination illustrates (Fig. 3B). partners (Fig. 3A) than in incompatible partial compatible tissues was smaller in partial compatible necrotic. The area of stained and necrotic above the union were heavily stained and visible (parenchymal) tissues. Errea et al. (1994a) presence of some undifferentiated combinations, whereas those at the right and upper quadrant were incompatible. However, it is difficult to interpret anatomic and histological studies based on longitudinal sections because of variability induced during grafting and variations in incompatibility symptoms (Ermel et al., 1995). Moreover, there are problems in identifying the right statistical procedure to separate compatible combinations from incompatible combinations. Errea et al. (2001) used contingency tables, but no statistical differences were found and there was reduction in validity of the test. Correspondence analysis seems to offer a better procedure to discriminate compatible combinations from incompatible combinations (Ermel et al., 1997). It is based on $\chi^2$ transformation and produces dimensions that represent the $\chi^2$ distances (Lebart et al., 1984). In this trial, the principal inertias ($\lambda$) were 0.06 (79.5%) and 0.14 (18.0%) at one and two dimensions respectively. Therefore, a two-dimensional correspondence analysis was appropriate because it represented 97.5% of the profiles (Fig. 4).

Fig. 5 shows a common trend for callus proliferation at the unions observed using a light microscope. Callus cells were prolific below the union, where a good union was formed. Tissues above the union were necrotic and highly stained, and hence there was no continuity in the bark and wood. Observations showed that U. kirkiana plants exude a lot of secondary metabolites (phenols) in response to wounding. Hamisy (2004) reported high levels of phenols in U. kirkiana leaves during DNA extraction. Therefore, it is suspected that phenols could play a role in graft incompatibility of U. kirkiana trees. According to Errea (1998), phenols have been implicated in union formation processes because they can cause insufficient callus proliferation, cell necrosis, and metabolic interactions. These ultimately cause disorder and damage at the union.

Fig. 5A shows a high deposit accumulation in an incompatible combination. There are many unfilled areas and necrotic layers above the union. Fig. 5B, C shows a high deposit accumulation above and at the union. Errea et al. (1994b) reported that high phenol accumulation occurs at the union of less compatible combinations. Fig. 5C shows that prolific callus cells make the union line narrow. According to Ermel et al. (1997), cell necrosis and discontinuity of vascular connections at the union are the main indicators of incompatibility. Gebhardt and Feucht (1982) reported a high quantity of phenols above the union as a cause of graft incompatibility in Prunus species. Errea (1998) and Considine (1983) reported that some incompatible partners may grow without any external indication of incompatibility, but the presence of phenols accumulating at the union serves as an indicator of problems in graft combinations. However, graft incompatibility has been attributed to other factors, including hormones, peroxidases, lack of plasmodesmata formation and vascular tissue connection, RNA and indole-3-acetic acid (IAA) transport, and inherent incompatibility at the cellular level (Andrews and Marquez, 1993; Usemik et al., 2006).

Fig. 6 shows the presence of phenolic deposits below the union of a compatible combination. Callus tissues were breaking up the deposits from the lower side of the union (Fig. 6A), and small pockets of phenolic deposits were observed. Errea et al. (1994) reported that phenols prevent cambial
Connection continuity. Therefore, the presence of necrotic tissues and phenolic deposits are implicated in graft incompatibility of *U. kirkiana* trees. Fig. 6C shows a narrow line of deposits, and this indicates a gradual increase in quantity of deposits from the lower to the upper part of the union.

Hartmann et al. (1990) reported that maintaining a film of water at the union during grafting is necessary for callus formation. This water could possibly dilute some phenols, especially water-soluble phenols, as they accumulate below the union. This could aid in breaking up deposits (phenols) by the prolific callus cells. Consequently, grafted partners are able to establish cambial continuity. However, such a hypothesis needs to be tested further for *U. kirkiana*.

Graft set in *U. kirkiana* trees during the November to December period (63%) was better than those set in June (30%) (Akinnifesi et al., 2004a), and this suggests seasonality in phenol accumulation. Tree samples used in this trial were grafted during the months of June, August, and early October. Therefore, time of grafting of *U. kirkiana* trees could play a role in graft compatibility.

**Conclusion**

Indicators of graft incompatibility in *U. kirkiana* trees include growth irregularities at the union, presence of necrotic tissues, and phenolic deposits at the union interface. Such findings confirm existence of graft union problems, although these trees were surviving in the nursery. However, phenol quantification and identification are needed to support the role of phenols in graft compatibility. For graft-incompatible partners, portions of parenchymal tissues supported the graft unions. MW26/26, MW26/22, MW7/10 graft combinations were partially compatible in this study.

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