MICROGRAFTING OF SELECTED KENYAN PAPAYA (*CARICA PAPAYA L.*) LINES

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Abstract
Grafting facilitates the exploitation of attributes of two varieties; the scion being responsible for the good quality characteristics of the product and the rootstock providing desirable attributes like tolerance to challenges associated with the medium of anchorage. The aim of this study was to evaluate success of in vitro micrografting method in three selected Kenyan papaya lines. In the study, shoot tips; c. 1.0cm were excised from three month old seedlings, sterilised and cultured on Murashige and Skoog basal media supplemented with 0.1mg/l 6-Benzylaminopurine and 0.05mg/l α Naphthalene Acetic Acid. When the shoots reached a length of c. 2.0 cm, the upper 1.0 cm tips were excised and used as scions, while the remaining portion was used as rootstock. The scions and rootstocks of the same genotype were used as the controls. Twenty eight days after grafting, the proportion of scions that were still alive, number of leaves and scion length of individual combinations were recorded. From all grafting combinations tested, the highest success rate was obtained from papaya line1 and papaya line 2 grafted on their own rootstocks papaya combinations with 75%and 80% grafting success rates respectively. Papaya lines grafted on their own rootstocks gave better results than when grafted on different rootstocks.

Key words: Papaya, Kenya, grafting, *in vitro*

1 Introduction

Papaya (*Carica papaya* L.) is an important horticultural fruit crop in Kenya grown by both small and large scale farmers (Imungi and Wabule, 1990) for local and export markets (HCDA, 2011). The fruit crop has a potential to produce fruits throughout the year. Ripe fruits, which are very rich in vitamins A and C, are popularly used for dessert or processed into jam or wine. Latex from green fruits contains papain, a proteolytic enzyme, which is used in pharmaceutical, cosmetics and garment industries (Nakasone and Paull, 1998). Thus papaya is a good source of nutritious food as well as income for the producers. Despite the above benefits, papaya producers and researchers encounter numerous challenges. These include unreliable methods of picking the required sex of seedlings at planting time, lack of disease-free planting materials, lack of improved varieties and devastating diseases that are difficult to control (Ministry of Agriculture, 2005). Additionally, papaya tree can normally live for 5-10 years, but in commercial plantings they are replanted every 2-3 years because the trees become too tall for economic harvesting.

The major contribution of papaya cloning is the possibility of maintaining the original characteristics of the parent plants which, does not occur in the conventional system of production where seeds are harvested, in the majority of cases, from commercial open pollinated orchards. If the growing of papayas is to remain competitive, it is essential to establish papaya cultivation that have high genetic potential, are precocious, have adequate height, are resistant to pests and diseases, and have high productivity and quality of fruits (Lima and Yamanishi, 2004)

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Grafting is often undertaken as a means of vegetative propagation of plants due to a number of reasons: (1) to impart disease resistance and hardiness, contributed by root stock, (2) to shorten the time taken to first production of fruits by the scion, (3) to dwarf the scion, making both its height and shape more convenient for harvesting and other managerial practices, as with apples (4) to provide the most economic use of scion material, in cases where there is some difficulty with stem cuttings producing roots (Rosa *et al*., 2003). Grafting also ensures wind firmness since grafted plants are shorter and stronger. The advantage also extends the economic period of a fruit tree by enabling production of dwarfed fruit trees which would otherwise be too tall to access the fruits for harvesting.

In papaya, grafting has been attempted in an effort to address the problem of raising plants with the desired sex. Airi *et al.* (1986) successfully cleft-grafted scion shoots from cultivars Co-1 and Honey dew onto uniformly established seedlings. Chong *et al.* (2008) also used cleft grafting to establish clonal hermaphrodite 'Eksotika' plants and reported an initial success of 80%, although this was reduced later due to infection of soft-rot fungi. In Malaysia, some papaya growers have used field grafting to replace female trees of the 'Eksotika' cultivar in the orchard (Cheah *et al.,* 1993). As soon as the sex of the trees can be determined, the female trees are side-cleft grafted with scion shoots harvested from hermaphrodite 'Eksotika' trees. Advantages of grafted papaya trees is that they bears fruits much lower and earlier and are dwarf in stature with longer economic life cycle (Chong *et al.,* 2008). Although grafting in papaya has been done in various places, problems such as bacterial infection of the scions and soft rot fungi infection (Chong *et al*., 2008) have been shown to reduce the success rate.

Micrografting is a technique mostly used for obtaining virus-free plants and other disease free plant materials, separating viruses in infections, breeding specific genotypic combinations and for studying graft incompatibility between scions and rootstocks (Navarro, 1988). Compared to traditional grafting, micrografting has several potential advantages in that it is much more rapid, requires much less space, produces disease free plants and genetically uniform plantlets. By *in vitro* grafting, disease free, early bearing and wind firm seedlings can be raised through which the problems that go with the propagation

of papaya can be addressed. As a result, the main of this study is to evaluate the in vitro grafting ability of selected papaya lines in Kenya.

Materials and Methods

This experiment was conducted in the Tissue Culture Laboratory of Institute for Biotechnology Research, Jomo Kenyatta University of Agriculture and Technology (JKUAT).

2.1 Plant Materials

Fruits of three locally adapted papaya lines from ongoing papaya research project on "commercial and Industrial development of papaya (*Carica papaya* L.), varietal improvement, production and processing technologies" JKUAT, in Juja were selected. The criterion of selection was based on fruit yields, plant height and days to first flowering. Seeds were extracted and stock plants established in a green house. When the seedlings were three month old, 1.0 cm shoot tips were excised and washed in savlon detergent and sterilized in 20% household bleach (jik®) containing 3.85% sodium hypochlorite and 2 drops Tween 20® for 10 minutes. Thereafter, the shoot tips were three times rinsed with sterilized distilled water.

After surface sterilization, the external surfaces of the shoot tips were trimmed to about 0.7 cm long and cultured in Murashige and Skoog (MS) (1962) medium solidified with 2.5 g/l gerlite and enriched with 0.5mg/l 6 Benzyl aminopurine (BAP) combined with 0.1mg/l Naphthalene Acetic Acid (NAA) for shoot multiplication. Consequently, shoots were transferred to MS enriched 0.1 mg/l BAP combined with 0.05mg/l NAA for shoot elongation.

The pH of the medium was adjusted to 5.7-5.8 before autoclaving at 1.1 kg cm^2 and 121^0C for 20 minutes. Cultures were maintained at 25 $\pm 1^0C$ under a 16-hour photoperiod using warm fluorescent lamps. Under aseptic conditions, shoots of uniform length and diameter were selected from *in vitro* culture and used *in vitro* grafting. When the stem axis of the shoots reached a length of about 2.0 cm the upper 1.0 cm of the tips were excised and used as scion, while remaining portion was used as rootstock. Wedge type of grafting was used. The scions were placed in the incisions made in the rootstock with both the cut surfaces in good physical contact. The stock and scion were held together at the point of graft with sterile cactus thorn. The scions and rootstocks were used interchangeably with the scion and rootstocks of the same line being the control. Grafted assemblies were cultured on MS medium solidified with 2.5 g/l gerlite and containing 30 gL-1 sucrose and 0.1 mg/l BAP combined with 0.05 mg/l NAA.

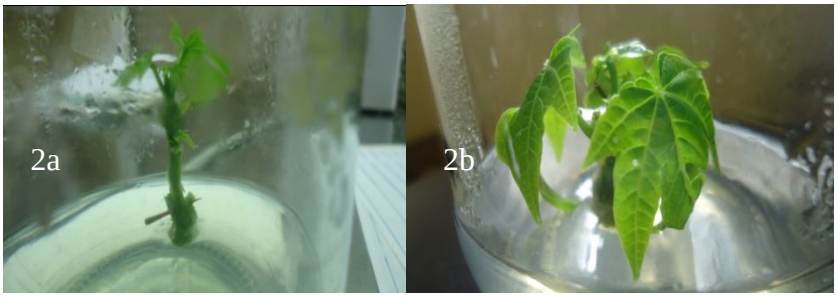
The experiment was laid in a completely randomized design with 3 replications. Number of leaves and scions length (cm) was also recorded every week for 4 weeks. After 4 weeks, grafting success was determined by recording the numbers of scions still alive out of all grafts. The data on shoot length and the number of leaves was subjected to analysis of variance and mean separated by least significant differences (lsd) at 95% level of confidence.

3 Results

Seven days after grafting, calluses were formed at the junction of a graft union, arising from the living cells of both, scion and rootstock (Plates 1 (a) (b) and (c). The intensity of callus formation differed among grafts tested with some grafts producing more callus (plate 1 (a), others less calluses (plates 1 (b) and (c). New leaves started forming seven days after grafting (Plate 2a) and after 28 days a fully formed graft union was observed (plate 2b). In vitro grafting success was determined by whether the graft union formed or not, and the subsequent growth of the bud on the scions. The ability of the graft unions to

form successfully varied from 45 to 80% between the grafts. The success rate of the controls 1 (papaya line 1 grafted on its own rootstock) and control 2 (papaya line 2 grafted on its own rootstock) was significantly higher than the other two treatments, with 75% and 80% of the graft unions forming within 4 weeks (Table 1). Contrary, papaya line 2 grafted on papaya line 3 rootstock and papaya line 3 grafted on papaya line 2 rootstock had the least success rate of 45%. Four weeks after grafting, number of leaves and shoot length for each graft were compared with the controls. There were significant differences in leaf induction and shoot length when control 1 was compared with the other grafts. When control 1 was compared with other grafts (except other controls) significant differences on leaf number and shoot length was observed. The control 1 had the highest number of leaves with an average of 8 leaves per graft and the longest shoot length with an average of 2.5 cm (Table 2). When control 2 was compared with other grafts, significant differences on leaf number was observed but there was no significant difference in shoot length seven leafs were recorded in control 2 within 4 weeks of growth (Table 3). At the same time, no significant differences in leaf induction and shoot length were recorded when control 3 was compared with other grafts (Table 4). Papaya line 1 grafted on papaya line 3 rootstock had the least number of leaves compared with other graft combinations.

Plates 1: Callus forming at the point of graft union (a) more intense callus (b) and (c) less intense callus



Plates 2: New leaves formation seven days after in vitro grafting (2a) and successful in vitro grafted shoot (2b)

Table 1: The effect of the rootstock and scion on success rate of in vitro grafting of selected papaya lines

Scion	Rootstock	No. of shoots grafted	Graft survival	% successful grafts
	Papaya line 1	40	30	75
	Papaya Line 2	40	24	60
Papaya line 1	Papaya Line 3	40	20	50
	Papaya Line 1	40	28	70
	Papaya Line 2	40	32	80
Papaya Line 2	Papaya Line 3	40	18	45
	Papaya Line 1	40	20	50
	Papaya Line 2	40	18	45
Papaya Line 3	Papaya Line 3	40	26	65

Table 2: The effect of rootstock and scion on shoot length and number of leaves (compared with control 1) n=18

Papaya Lines graft combinations	No. of leaves	Shoot length (cm)
Line 1/Line 1(control)	8±1.15b	2.5±0.037b
Line 1/Line2	6±1.41ab	2.3±0.043a
Line 2/Line 3	6±1.50ab	2.2±0.028a
Line 2/Line1	6±1.32ab	2.3± 0.032a
Line 3/Line 1	6±1.49ab	2.2± 0.025a
Line 3/Line 2	6± 1.35ab	2.1± 0.031a
Line 1/Line 3	4±0.98 a	2.2 ±0.028a
p value	0.05	0.01

Mean values within a column followed by the same letter are not significantly different by LSD (*P<0.05*).

Table 3: The effect of rootstock and scion on shoot characteristics (compared with control 2) n=18

Papaya Lines graft combinations	No. of leaves	Shoot length (cm)
Line 2/Line 2 (control)	7 ±1.62b	2.3± 0.045a
Line 1/Line 2	6 ±1.41ab	2.3 ±0.043a
Line 3/Line 2	6± 1.35ab	2.1±0.031 a
Line 3/Line1	6±1.49ab	2.2±0.025 a
Line 2/Line 1	6±1.32ab	2.3±0.032 a
Line 2/Line 3	6±1.50ab	2.2±0.028 a
Line1/Line 3	4±0.98a	2.2±0.028 a
p value	0.0409	0.308

Mean values within a column followed by the same letter are not significantly different by LSD (*P<0.05*).

Table 4: The effect of rootstock and scion on shoot characteristics (compared with control 3) n=18

Papaya Line graft combinations	No. of leaves	Shoot length (cm)
Line 3/Line 3 (control)	6±1.51a	2.2±0.023 a
Line 2/Line 3	6±1.50a	2.2±0.025 a
Line 3/Line 1	6±1.49a	2.2±0.025 a
Line 1/Line 2	6±1.41a	2.3±0.043 a
Line 2/Line 1	6±1.32a	2.3± 0.032a
Line 3/Line 2	6±1.35a	2.1±0.031 a
Line1/Line 3	4±0.98a	2.2± 0.028a
P value	0.157	0.442

Mean values within a column followed by the same letter are not significantly different by LSD (*P<0.05*).

4 Discussion

Grafting success was determined by callus formation. The firm placement of the scion onto the rootstock to ensure good contact was essential for the formation of the graft union. Calluses tended to be formed at the junction of a graft union, arising from the living cells of both, scion and rootstock. It has been reported that the formation of callus is a good indication of grafting success, since the callus provides the initial pathway for water until vascular connections are formed between the rootstock and scion (Hartmann *et al.,* 1997). Dislocation of the grafts resulted in drying out of the scion, in which no callus formation was observed. According to Tirtawinata (2003), success rates of micrograft are highly dependent upon the cambium union of rootstocks and scion. Partial contact of rootstock and scion cambium will contribute to unsuccesful joining. Estrada–Luna *et al*. (2002) described these cases as "translocated" graft incompatibilities and this might be the cause of the differences in success rate of in vitro grafting observed. A low or incorrect callus formation between the rootstock and scion could lead to defoliation, reduction of scion growth and low survival of grafted plants (Oda *et al.,* 2005). This could also be associated with differences in success rates of in vitro grafting we observed.

The formation of vascular bridges between rootstock and scion provides an important developmental index for compatible grafts. Controls 1 and 2 had high number of leaves and shoot length compared with other grafts. The number of leaves and shoot length are parameters which allow the assessment of growth and development in plants. Greater number of leaves indicate a greater productive capacity of the plants by increasing the capacity of photosynthesis. As such, shoot grafted on their own rootstock showed better growth and development than those grafted on different rootstock. The differences observed on shoots grafted on different rootstock on number of leaves and shoot length could be as a result of scion rootstock interaction which could influence the vigour of the plants.

5 Conclusion and Recommendations

In vitro grafting of Kenya's papaya was successful. By ensuring good contact between the scion and the rootstock was essential for the graft unions to form successfully. More work is geared towards rooting in order to study the root systems of the rootstocks. In addition, evaluation of agronomic traits in vitro grafted plants will be necessary to compare their performance with conventionally produced seedling plants in growth rate, agronomic performance and fruit quality.

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