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In vitro regeneration of selected Kenyan papaya (*Carica papaya* L.) lines through shoot tip culture

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Papaya, an important fruit crop in Kenya is commercially propagated through seeds which lead to production of non-true-to-types plants. Conventional vegetative propagation methods are not carried out, hence, the need for micropropagation for mass multiplication of selected lines. An assessment for the effect of 6-benzylaminopurine (BAP) at 0.1, 0.5, 1.0 and 2.0 mg/l combined with α-naphthalene acetic acid (NAA) at 0.05, 0.1, 0.5 and 1.0 mg/l and a control on shoot multiplication and elongations, and indole-3-butyric acid (IBA) at 0, 0.1, 0.5, 1.0, 2.0, 2.5 and 3.0 mg/l on root induction were evaluated. Number of shoots and their length were recorded every three weeks for 12 weeks. Number of roots, root length and percentage rooting induction were recorded after eight weeks. The highest number of shoots was recorded in 0.5 mg/l BAP combined with 0.1 mg/l NAA and the longest shoots were recorded in 0.1 mg/l BAP combined with 0.05 mg/l NAA across the three lines. IBA at 2.5 mg/l produced the highest number of roots, root length and highest percentage of rooting induction. An *in vitro* regeneration of selected papaya lines through shoot tip culture was established.

Key words: *Carica papaya*, *in vitro* regeneration, shoots multiplication, rooting.

INTRODUCTION

Papaya (*Carica papaya* L.) is a fruit commonly eaten fresh all over the world. The ripe fruit is low in calories and rich in vitamins A and vitamin C (Farzana et al., 2008). Papain, a proteolytic enzyme present in the latex of green fruits, has many uses in beverages, food and pharmaceutical industries. These uses include chill-proofing beer, tenderizing meat and in drug preparation for alleviating digestive ailments (Nakasone and Paull, 1998). In Kenya, papaya is a widespread fruit crop especially where enough water is available for its cultivation (Imungi and Wabule, 1990). The fruit crop is believed to have been introduced into Kenya more than 50 years ago. The main varieties grown are hawaii, solo, honeydew, sunrise, and local types. These varieties were introduced from Hawaii, Philippines, India and Indonesia (Kamau et al., 1993).

Sexual propagation is the commercial method of propagation for papaya. Being heterozygous and a cross pollinated crop, sexual propagation has resulted in immense variation among populations for yield, size, shape, quality of fruit and disease susceptibility leading to production of non-true-to-type plants (Panjaitan et al., 2007). The signi-

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Abbreviations: BAP, 6-benzylaminopurine; **NAA,** naphthalene acetic acid; **IBA,** indole-3-butyric acid.

ficance of vegetative propagation in the maintenance of genetic uniformity and preservation of identity of an elite clone or cultivar is well recognized in horticultural crops. To develop true-to-type plants, an efficient vegetative propagation technique is required.

Conventional vegetative propagation methods in papaya such as grafting (Allan et al*.,* 2010) and rooted cuttings (Rajan and Markose, 2007) exist, but they are often not carried out on a large scale. Hence, the alternative method is micropropagation techniques for mass multiplication of elite materials. Development of an efficient *in vitro* regeneration system would be a remarkable progress for mass propagation and uniform plants for both commercial and research purposes.

Papaya is most commonly propagated *in vitro* by shoot tip or axillary bud (culture (Teixeira da Silva et al*.,* 2007). As such several protocols for *in vitro* plantlet regeneration from shoot tips of papaya have been developed in other regions of the world (Kabir et al., 2007; Panjaitan et al., 2007).

However, most of the protocols are genotype dependent and therefore cannot be reproduced (Mishra et al*.,* 2007). There are no documented studies on micropro-pagation of papaya lines in Kenya. The objective of this study was to develop an efficient *in vitro* regeneration system for micropropagation of Kenyan papaya lines through shoot tip culture.

MATERIALS AND METHODS

Plant material and sterilization procedure

Three local papaya lines namely line 1; line 2 and line 3 were selected from an existing papaya breeding project based at Jomo Kenyatta University of Agriculture and Technology (JKUAT) in Juja. The selection criteria were based on plant height, fruit yield and sex of mother plant. Seeds were extracted and established in greenhouse as stock plants. 1 cm shoot tips (explants) were harvested from three month seedlings and placed in a glass beaker and kept in running tap water for 30 min to remove physical impurities. Under a clean lamina flow hood, the explants were subjected to 70% (v/v) ethanol for 30 s and rinsed with double distilled water thrice to remove ethanol and then subjected to 20% (v/v) household bleach (jik®) containing 3.85% sodium hypochlorite and 100 µl/l Tween 20® for 20 min then rinsed three times with double distilled water.

Shoot multiplication and elongation

After sterilization, the peripheral surfaces of the tissues were trimmed leaving about 0.7 cm long explant which were cultured on Murashige and Skoog (MS) (1962) basal medium, supplemented with 30 g/l sucrose, and 6-benzylaminopurine (BAP) at concentrations 0.1, 0.5, 1.0 and 2.0 mg/l combined with naphthalene acetic acid (NAA) at concentrations 0.05, 0.1, 0.5 and 1.0 mg/l and a control where no plant growth regulator was applied for shoot multiplication and elongation, solidified with 2.5 g/l gerlite. Each concentration was replicated four times. The number of shoots produced per explant and their length were recorded after every 3 weeks for a period of 12 weeks. The explants were subcultured in a fresh media after every three weeks.

Root induction

Proliferated shoots, about 3 cm long were separated into individual explant and method described by Tsong et al. (2000) with modifications was used. The shoots were first subcultured in full strength MS containing 3% (w/v) sucrose, 0.28% gerlite supplemented with indole-3-butyric acid (IBA) at concentrations 0, 0.1, 0.5, 1.0, 2.0, 2.5, 3.0mg/l IBA for one week in darkness for root induction and thereafter transferred into vermiculite supplemented with half strength MS supplemented with 3% (w/v) sucrose for further root development. The experiment was replicated three times. Number of roots per explants, their root length and the rate (%) of shoots that produced roots were determined after 8 weeks.

Experimental design and statistical analysis

The experiments were carried out in factorial design with two factors (three papaya lines and varied hormonal treatments). Data collected was analyzed using analysis of variance (ANOVA) and means differing significantly were compared using student Newman Keuls (SNK) test at $p \leq 0.05$.

Acclimatization

Rooted plants were taken out from the culture bottles and washed carefully under running tap water for complete removal of remains of the medium. Pots (9x6 cm) were kept ready filled with forest soil, manure, sand and vermiculite in the proportion of 2:1:1:1, respectively. All the pots were covered with two layers of shade net, which was kept moist for providing high humidity to the plants. After one week, one layer of shade net was removed while the other layer was removed after another one week. Within three weeks in acclimatization chamber, *in vitro* regenerated plantlets had hardened and were taken to greenhouse for further growth.

RESULTS

Within seven days after culture initiation, new leaves started forming from the shoot tip (Figure 1A). After 28 days of culture, a number of axillary and adventitious buds emerged out of shoot tip (Figure 1B). Later, many of these buds developed as young shoots. The multiple shoots developed further after 12 weeks of culture (Figure 1C). In this study, different concentrations of BAP combined with NAA tested affected the mean number of shoot produced and mean shoot length per shoot tip. Increase in BAP up to 0.5 mg/l and NAA up to 0.1 mg/l increased the mean number of shoots produced per shoot tip (Table 1). However, the mean number of shoots produced per explant decreased on increasing the BAP concentration up to 1.0 mg/l and NAA up to 0.5 mg/l across all lines (Table 1). Explants cultured in absence of BAP and NAA, most of them senesced without producing shoots while other produced an average of 1 shoots per explants across the three lines. The mean number of shoots produced per shoot tip was influenced by interaction between papaya lines and BAP and NAA concentrations used ($P<0.05$). In papaya line 1, MS media supplemented with 0.5 mg/l BAP combined with 0.1 mg/l NAA produced the highest mean number of

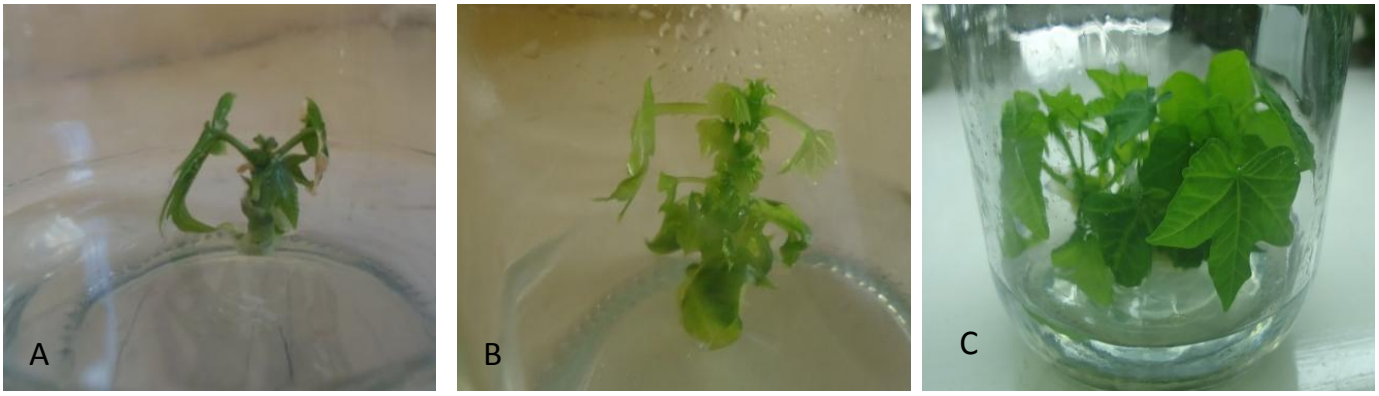

Figure 1. *In* vitro shoot tip development. A, Formation of leaves on shoot tips seven days after culture; B, shoot proliferation from shoot tips 28 days after culture; C, development of multiple shoots12 weeks of culture.

Table 1. Effect of different concentrations of BAP combined with NAA on mean number of shoot per shoot tip of three papaya lines(cm) after 12 weeks of culture, n=153.

BAP + NAA (mg/l)	Mean number of shoot		
	Line 1	Line 2	Line 3
0+0	0.8 ± 0.16^{h}	1.0 ± 0.44^{g}	0.6 ± 0.21^{h}
0.1+0.05	11.8 ± 0.91^{c}	8.8 ± 0.30^{c}	16.5 ± 1.54^{b}
0.5+0.05	13.8 ± 0.70^{b}	13.6 ± 0.95^{b}	16.8 ± 0.94^{b}
1.0+0.05	8.5 ± 0.22^{ef}	8.8 ± 0.47^{c}	6.8 ± 0.60^{efg}
2.0+0.05	6.5 ± 0.50^{fg}	7.8 ± 0.37^{cd}	6.5 ± 0.88^{efg}
0.1+0.1	11.0 ± 0.51^{cd}	12.3 ± 0.84^{b}	14.3 ± 0.91^{c}
0.5+0.1	24.3 ± 0.95^{a}	25.8 ± 2.08^{a}	19.3 ± 0.98^{a}
1.0+0.1	8.1 ± 0.47^{ef}	9.0 ± 0.25^{c}	10.6 ± 0.33^{d}
2.0+0.1	5.0 ± 0.36^{g}	7.6 ± 0.33^{cd}	5.8 ± 0.60^{efg}
0.1+0.5	4.1 ± 0.40^{g}	4.2 ± 0.30^{ef}	4.1 ± 0.40^{fg}
0.5+0.5	8.3 ± 0.66^{ef}	8.6 ± 0.21^{c}	8.5 ± 0.71^{de}
1.0+0.5	9.6 ± 0.49^{de}	8.1 ± 0.60^{cd}	8.8 ± 0.30^{de}
2.0+0.5	5.5 ± 0.42^{g}	5.8 ± 0.47^{de}	7.1 ± 0.65^{ef}
0.1+1.0	4.5 ± 0.34^{g}	2.3 ± 0.21^{fg}	4.6 ± 0.21^{fg}
0.5+1.0	4.1± 0.30^{g}	2.8 ± 0.40^{fg}	3.8 ± 0.30^{g}
1.0+1.0	4.6 ± 0.6^{g}	4.8 ± 0.54^{ef}	6.6 ± 0.49^{efg}
2.0+1.0	5.5 ± 0.42^{g}	4.0 ± 0.36^{ef}	5.6 ± 0.33^{efg}

Mean values within a column followed by the same letter are not significantly different by SNK (P≤ 0.05).

shoots of 24.5 within 12 weeks of sub culturing, followed by MS media supplemented with 0.5 mg/l BAP combined with 0.05 mg/l NAA with a mean of 13.8 shoots (Table 1). Control had the least mean number of shoots produced per explant with a mean of 0.8 shoots (Table 1).

In papaya line 2, the highest mean number of shoots produced per shoot tip was recorded in MS media supplemented with 0.5 mg/l BAP combined with 0.1mg/l NAA with a mean of 25.8 shoots per explant. This was followed by MS media supplemented with 0.5 mg/l BAP combined with 0.05 mg/l NAA with a mean of 13.6 shoots per explant and MS media supplemented with 0.1 mg/l BAP combined with 0.1 mg/l NAA with a mean of 12.3 shoots per explant. Control recorded the least with a mean of 1 shoot per explant (Table 1).

In papaya line 3, MS media supplemented with 0.5 mg/l BAP combined with 0.1 mg/l NAA recorded the highest number of shoots produced per explant with a mean 19.3 shoots. This was followed by MS media supplemented with 0.1 mg/l BAP combined with 0.05 and 0.5 mg/l BAP

Table 2. Effect of different concentrations of BAP combined with NAA on mean shoot length (cm) of three papaya lines after 12 weeks of culture, n=153.

BAP + NAA (mg/l)	Mean shoot length (cm)		
	Line 1	Line 2	Line 3
0+0	1.31 ± 0.017^{g}	1.38 ± 0.031^{g}	1.58 ± 0.033^{f}
0.1+0.05	3.25 ± 0.085^{a}	3.30 ± 0.082^{a}	3.28 ± 0.070^{a}
0.5+0.05	2.63 ± 0.042^{b}	3.03 ± 0.105^{b}	3.01 ± 0.087^{b}
1.0+0.05	1.80 ± 0.037^{de}	2.05 ± 0.043^{d}	1.73 ± 0.042^{e}
2.0+0.05	1.76 ± 0.049^{e}	1.68 ± 0.031^{ef}	1.60 ± 0.037^{ef}
0.1+0.1	2.71 ± 0.048^{b}	2.78 ± 0.079^{c}	2.16 ± 0.033^{c}
0.5+0.1	2.26 ± 0.042^{c}	2.11 ± 0.031^{d}	2.13 ± 0.056^{c}
1.0+0.1	1.70 ± 0.026^{ef}	2.10 ± 0.058^{d}	2.13 ± 0.056^{c}
2.0+0.1	1.43 ± 0.033^{g}	1.83 ± 0.033^{e}	1.96 ± 0.071^{d}
0.1+0.5	1.30 ± 0.026^{g}	1.51 ± 0.031^{fg}	1.73 ± 0.021^{e}
0.5+0.5	1.80 ± 0.026^{de}	1.80 ± 0.086^{e}	1.68 ± 0.031^{ef}
1.0+0.5	1.81 ± 0.031^{de}	1.80 ± 0.037^{e}	1.75 ± 0.022^{e}
2.0+0.5	1.85 ± 0.022^{de}	1.70 ± 0.037^{ef}	1.80 ± 0.037^{e}
0.1+1.0	1.56 ± 0.021^{f}	1.50 ± 0.026^{f}	1.58 ± 0.040^{ef}
0.5+1.0	1.71 ± 0.031^{ef}	1.63 ± 0.021^{ef}	1.68 ± 0.037^{ef}
1.0+1.0	1.95 ± 0.043^{d}	1.88 ± 0.040^{e}	1.68 ± 0.0373^{f}
2.0+1.0	1.70 ± 0.068^{ef}	1.70 ± 0.082^{ef}	1.73 ± 0.049^{e}

Mean values within a column followed by the same letter are not significantly different by SNK ($P \leq 0.05$).

combined with 0.05 mg/l NAA with a mean of 16.5 and 16.8 shoots, respectively. Control exhibited the least number of shoots with a mean of 0.6 shoots (Table 1). The mean shoot length (cm) per shoot tip was influenced by interaction between papaya lines and BAP and NAA concentrations used (P<0.05).

In papaya line 1, the longest shoot was recorded in MS media supplemented with 0.1 mg/l BAP and 0.05 mg/l NAA with a mean of 3.25 cm. This was followed by MS media supplemented with 0.1 mg/l BAP, 0.1 mg/l NAA, 0.5 mg/l BAP and 0.05 mg/l NAA with a mean of 2.71 and 2.63 cm, respectively.

The least shoot length was recorded in control with a mean of 1.31cm (Table 2). In papaya line 2, the longest shoot was recorded in MS media supplemented with 0.1 mg/l BAP and 0.05 mg/l NAA with a mean of 3.3 cm. This was followed by MS media supplemented with 0.5 mg/l BAP and 0.05 mg/l NAA with a mean of 3.0 cm. The least shoot length was recorded in control with 1.38 cm (Table 2).

In papaya line 3, the longest shoot was recorded in MS media supplemented with 0.1 mg/l BAP and 0.05 mg/l NAA with a mean of 3.28 cm. This was followed by MS media supplemented with 0.5 mg/l BAP and 0.05 mg/l NAA with a mean of 3.01 cm. The least shoot length was recorded in control with a mean of 1.5 cm (Table 2).

Root development was induced by pre-treating shoots in IBA for one week in darkness, followed by culture on vermiculite supplemented with half strength MS medium within 8 weeks (Figure 2). Meanwhile, shoots without IBA pre-treatment placed on medium with vermiculite supplement did not produce roots at all.

The rate of root induction was influenced by the interaction between papaya lines and IBA concentrations (P<0.05) used. IBA at 2.5 mg/l recorded the highest rate of root induction within 8 weeks across the three papaya lines with 75, 83 and 55% in papaya lines 1, 2 and 3, respectively (Table 3).

Significant effect of IBA concen-trations on the mean number of roots produced per shoot and the average root length (p<0.05) was recorded. IBA at 2.5 mg/l produced the highest number of roots and the longest root length within eight weeks with an average of 5.55 roots per shoot and mean root length of 2.46 cm (Table 4). After three weeks in acclimatization greenhouse, *in vitro* regenerated plantlets were ready for transplanting (Figure 3).

DISCUSSION

The production of shoots from axillary buds and shoot tip explants is the most reliable method of *in vitro* propagation of papaya (Teixeira da Silva et al., 2007). In this study, an *in vitro* regeneration system of three papaya lines through shoot tip culture was attempted. Different concentrations of BAP combined with NAA tested

Figure 2. Root formation in vermiculite supplemented with half strength MS 8 weeks after culture.

Table 3. The effects of different concentrations of IBA on the proportion of shoots (%) that rooted within 8 weeks, n=63.

Concentration of IBA (mg/l)	Proportion of shoots producing roots (%)		
	Papaya line 1	Papaya line 2	Papaya line3
0	0^{c}	0^{d}	0^{c}
0.1	5.66 ± 1.6^{c}	17.0 ± 1.5^{c}	5.667 ± 1.56^{c}
0.5	33.0 ± 4.08^{b}	33.0 ± 4.08^{bc}	33.0 ± 4.08^{b}
1.0	35.0 ± 5.2^{b}	33.0 ± 5.2^{bc}	33.0 ± 4.06^{b}
2.0	38.6 ± 5.6^{b}	38.6 ± 5.6^{b}	38.6 ± 5.6^{b}
2.5	72.3 ± 5.3^{a}	83.3 ± 9.5^{a}	55.6 ± 5.6^{a}
3.0	37.3 ± 4.88^{b}	33.0 ± 5.3^{bc}	33.0 ± 5.3^{b}

Mean values within a column followed by the same letter are not significantly different by SNK ($P \leq 0.05$).

Table 4. The effects of different concentrations of IBA on mean number of roots and the mean root length per shoot within 8 weeks of culture n=63.

Concentration of IBA (mg/l)	Mean number of roots	Mean root length (cm)
0	0 ± 0^{e}	0.00 ± 0^{e}
0.1	1.55 ± 0.557^{d}	0.50 ± 0.067^{d}
0.5	2.22 ± 0.333^{cd}	0.93 ± 0.120^{c}
1.0	3.11 ± 0.333^{c}	1.21 ± 0.058^{bc}
2.0	5.0 ± 0.577^{b}	1.16 ± 0.088^{bc}
2.5	5.5 ± 0.667^{a}	2.46 ± 0.120^{a}
3.0	4.88 ± 0.577^{b}	1.37 ± 0.115^{b}

Mean values within a column followed by the same letter are not significantly different by SNK ($P \leq 0.05$).

Figure 3. In *vitro* regenerated papaya plantlets.

affected number of shoot per shoot tip as well as shoot length. Increase in BAP up to 0.5 mg/l and NAA up to 0.1 increased the mean number of shoots produced per shoot tip. However, the mean number of shoots produced per explant decreased on increasing the BAP concentration from 0.5 to 1.0 mg/l and NAA from 0.1 to 0.5 mg/l among three lines.

The probable reason for this could be BAP at 0.5 mg/l and NAA at 0.1 mg/l were the optimum concentrations for shoot multiplication. Panjaitan et al. (2007) reported that BAP concentration more than 1.0 mg/l supported poor rate of shoot multiplication in field growth shoot tips of hermaphrodite papaya (*C. papaya* L. cv. Eksotika). Besides, excessive BAP has been shown to inhibit shoot growth and reduce proliferation rates in papaya (Drew, 1988).

There were statistically significant interaction between papaya lines and BAP and NAA concentrations used on the mean number of shoots produced per explant and mean shoot length (cm). Significant interaction between papaya lines and IBA concentration on rate of shoots (%) that produced roots was also noted. This shows papaya lines differences. Such interactions between papaya lines and hormonal treatment influences have been reported in tissue culture of papaya (Litz and Conover, 1977).

BAP at 0.5 mg/l combined with NAA at 0.1 mg/l produced higher number of shoots per shoot tip but were shorter than those produced in 0.1 mg/l BAP and 0.05 mg/l NAA shoots on the same explant share nutrients absorbed by the explant and the more the shoots, the higher the competition of the nutrients. There could have been less competition among the shoots in 0.1 mg/l BAP and 0.05 mg/l NAA leading to tall shoots.Pre-treatment of shoots in 2.5 mg/l IBA followed by culture on medium with vermiculite, was the most suitable for rooting of shoots derived from shoot tip explants of the three papaya lines. Panjaitan et al. (2007) reported that exposure of shoots to 1.0 mg/l IBA for 1 week followed by a transfer to a medium supplemented with vermiculite stimulated 90% of the shoots to produce roots.

The difference between the present results and those of Panjaitan et al. (2007) may be related to the genotype of the plant used. Good aeration in vermiculite must have contributed greatly to root development. Lai et al. (1998) observed that when papaya shoots were rooted in vermiculite under aerated conditions, the vessel ventilation was good, and no ethylene was detected and this may be the reason that individual shoots cultured in the aerated vermiculite medium for root development had vigorous growth and developed healthy roots.

Thus, a commercially viable protocol for micropropagation of selected papaya lines was developed. The shoot multiplication was optimal in MS media supplemented with 0.5 mg/l BAP and 0.1 mg/l NAA, shoot elongation in 0.1 mg/l BAP and 0.05 mg/l NAA and rooting in 2.5 mg/l IBA.

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