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Evaluation of Growth Rate and Phenotypic Traits of Meristem-cultured Papaya Plants

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EKG and FKR designed the study, wrote the protocol and interpreted the data. Authors EKG and AWK anchored the field study, gathered the initial data and performed preliminary data analysis. While authors EKG, FKR and MMW managed the literature searches and produced the initial draft. All authors read and approved the final Manuscript.

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ABSTRACT

Papaya *in vitro*-based micropropagation has been attempted for a number of cultivars. This effort has mainly been geared towards production of multiple unisexual planting stocks in large-scale production of papaya. In this study, a comparison was made on growth performance between tissue-culture generated and direct-seeded papaya plants. Key morphological traits ranging from leaf length to days taken to flowering were employed in evaluating the effect of *in vitro* propagation on papaya compared to conventionally, seeds generated papaya plants. Three papaya lines for each experimental group were selected at 5-leaf stage and transplanted in a greenhouse. These were set in a completely randomized design with three replicates and the plants subjected to the same soil and water treatment. Data on growth characteristics was collected weekly from transplanting to plant flowering. One way ANOVA was used to generate means which were then separated using Student Newman's Kules test at p≤0.05. The results showed that direct-seeded and the *in vitro*-generated papaya plants varied slightly in respect to leaf length and width with both falling within

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the normal range (leaf width; 30 – 60 cm, leaf length; 45 – 90 cm). It was also noted that, the tissue cultured plants had shorter and narrower leaves compared to the seeds generated plants. On average tissue cultured plants took 95 days to flowering compared to 100 days taken by the direct seeded papaya plants. On average, the stem diameter variation (0.6 cm) was not significantly different at 95% confidence interval. In addition, the average internode length of seeds generated papaya was slightly longer than that of the tissue culture generated plants. From the seven criteria used in the comparison, this study did not record a consistent difference between seeds and tissue cultured papaya plants. In conclusion, *in vitro* regeneration process does not change the genetics of the papaya plant but provides a rapid production of true-to-type planting materials per unit time.

Keywords: Papaya; micropropagation; true-to-type; tissue culture.

1. INTRODUCTION

Papaya (*Carica papaya* L.) of the Caricaceae family has over the years become an important fruit crop in both tropical and sub-tropical regions. The major producing countries include India, Brazil, Indonesia, Dominic Republic and Nigeria [1]. The nutritive value of this fruit has been a major contribution to balanced human diet. Particularly, the fruit is highly rich in vitamins A, B and C, in addition to being a good source of iron and calcium [2]. Unripe papaya fruits and latex from stem have also been used as a source of two key proteolytic enzymes – papain and chymopapain [3].

In the past, seed propagation has been the major route of setting up papaya commercial plantations [4]. This method however is curbed with a number of challenges owing to the dioecious nature of this crop in addition to eminent cross-pollination imposing heterozygosity in the resultant cultivars [5,3].

In an attempt to circumvent these challenges, *in vitro* clonal propagation techniques are recently being employed in establishing papaya large-scale cultivation. Different protocols are currently available for *in vitro* propagation of a number of papaya cultivars and optimizations of the regeneration conditions are carried out to fit a specific cultivar [6]. Particularly, use of various combinations of phytohormones to initiate callogenesis, followed by embryogenesis and eventual organogenesis has been widely investigated in a number of papaya cultivars [7-9].

From these studies, auxins ranging from NAA, IBA, 2, 4-D and cytokinins BAP, kinetin, zeatin have in various combinations and proportions been used in papaya *in vitro* regeneration. This clonal propagation route of generating planting materials for commercial use has achieved a number of advantages compared to the conventional direct seedling method. The most favorable aspect of the former is the ability to generate massive clones over a short period of time. Additionally, clonal propagation has made it possible to generate disease-free and unisexual papaya planting stock [10-12].

Irrespective of the method used to generate papaya planting materials, optimal growth performance and high yield are the targets of any papaya commercial production set up. It is therefore necessary to evaluate the differences in performance between direct seeded and clonally propagated papaya plants. Of importance in this evaluation includes plant's leaf characteristics, height, internode length, stem girth and time taken to flowering.

Papaya leaves varies from 30 – 60 cm in width and each leaf blade is irregularly divided into 5 – 9 segments. The width and the length of the overall leaf determines the surface area, a feature integral in trapping sunlight for photosynthesis [13]. Whether clonal propagation improves the leaf characteristics of these plants, therefore, becomes an important aspect when selecting a system to adopt. Papaya plant under ideal field conditions grows at a rate of 1.8 – 3 m per year and reaches up to 6 – 9 m height at maturity [7]. The time taken to maturity is key in any commercial production set up in order to cut down on time spent for each harvesting cycle. This study took to evaluate the differences in growth performance between the two groups of papaya plants produced clonally and sexually.

2. MATERIALS AND METHODS

2.1 Plant Establishment

Three papaya lines were selected at 5-leaf stages and transplanted in a greenhouse at Jomo Kenyatta University of Agriculture and Technology. The spacing in each block was set

at 2 X 2 m with holes of 60 X 60cm in a completely randomized design with three replicates. During transplanting, the top soil was mixed with 5 kg farm yard manure and 100 g Di ammonium Phosphate (DAP) fertilizer. The mixture was returned into the planting holes mixed and watered to field capacity. The established plants in each block were watered uniformly and regularly to maturity. Pests and diseases were controlled during the plants growth. Insect pest were controlled by use of insecticide spray while fungal infections were controlled by fungicide sprays. Data collection was started after two weeks of crop establishment.

2.2 Data Collection and Analysis

Data on leaf length, stem girth, leaf width, plant height, flowering height and days to flowering was collected weekly and recorded. This data was subjected to Analysis of Variance (ANOVA) using SAS® version 9.1.3 and significant means separated by Student Newman's Kules (SNK). The means and standard error of the mean for each growth characteristic was used in plotting bar graphs showing the difference in papaya lines between the meristem culture and sexually-generated papaya using GraphPad Prism® version 6.0.2.

3. RESULTS

The three selected papaya lines compared rather closely relative to the evaluated morphological traits as shown on Fig. 1.

3.1 Plant Height

The flowering height for the direct seeded papayas was generally higher than the tissue cultured papaya for the three papaya lines evaluated as indicated in Table 1. The height ranged from a mean of 35.98 cm to 40.30 cm in direct seeded while meristems cultured papaya mean range was 29.03 to 33.85 cm in height (Fig. 1). This difference however was not significantly different at 95% Confidence Interval.

3.2 Stem Girth

There was a marginal significant difference in plant girth between different lines in direct seeded and tissue cultured papayas. In the direct seeded group, line 2 had the highest mean girth of 6.64 followed by 6.25 in tissue cultured papaya of line 3 (Fig. 2). The lowest mean girth was recorded in TC line 2 with a mean stem girth of 4.36 cm.

3.3 Plant Internode Length

The direct seeded papaya lines had a marginally longer internodes than the meristem cultured lines. Direct seeded lines had the highest mean length of 31.22 cm and the lowest mean length of 26.17. The tissue cultured papaya internodes length ranged from 22.45 to 28.61 (Fig. 3). In average, there was no significant morphological difference between the two groups of papaya in terms of internode length.

3.4 Leaf Width

There was an observed marginal difference between the leaf widths of direct seeded and meristem cultured papaya lines at 95% confidence interval with the direct seeded leaves recording in average wider leaves. The widest leaf width was recorded in direct seeded papaya line 2 with 49.67 cm while the lowest value in this group was recorded in line 1 with 44. 50 cm. Tissue cultured papaya lines in average recorded lower leaf widths. This meristem cultured group had the highest mean value of 41.50 in line 3 and the lowest mean value of 30.08 in papaya line 2 (Fig. 4).

3.5 Leaf Length

Direct seeded papayas had longer leaves as observed with a mean range of 22.58 shortest and longest at mean of 32.97 cm. The tissue culture papayas leaf length ranged from 21.16 cm to 29.33 cm (Fig. 5). At 95% confidence interval, there was a marginal significant difference between the average leaf lengths of direct seeded and tissue cultured papaya plants.

3.6 Days to Flowering

Tissue cultured papaya lines took a shorter time to flowering than their counterparts direct seeded papaya lines. These meristem cultured papaya took in average 95 days to flowering while direct seeded papaya took 100 days. The difference in time to flowering, however, is only statistically marginal at 95% confidence interval ($p \leq 0.05$).

4. DISCUSSION

In papaya, *in vitro*-based micropropagation has been attempted for a number of cultivars. This has so far provided a number of advantages including reduced time to produce new planting stocks, ease of maintaining genetic uniformity and production of unisexual plants. [14,15,11,16].

Micropropagated trees also produce fruits lower on the stem with benefit of earlier and greater yields. [17,15,16] In this study, a comparison was made on growth performance between tissue culture generated and direct seeded papaya. Key morphological traits were employed in evaluating the effect of *in vitro* propagation on papaya compared to conventionally, seedling generated papaya.

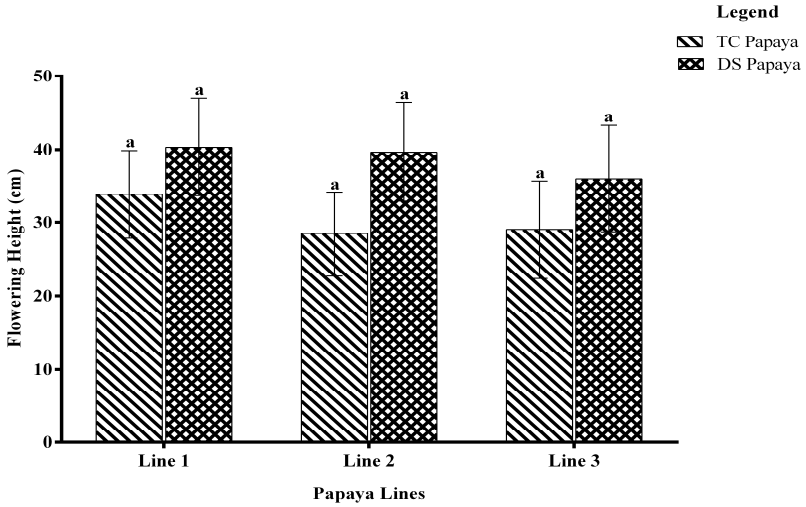

Fig. 1. Flowering height of direct seeded and meristem cultured papaya lines

Table 1. Means and SEM of six morphological traits compared between the direct-seeded (DS) and tissue culture-generated (TC) papaya plants

Line	Flowering height	Stem girth	Plant height	Internodes length	Leaf width	Leaf length
1 DS	40.31 ± 6.65[a]	6.11 ± 0.45[b]	56.28 ± 4.72[a]	31.19 ± 1.53[a]	44.50±1.28[b]	30.66±0.82[a]
2 DS	39.64 ± 6.75[a]	6.64 ± 0.53[a]	59.95 ± 5.45[a]	31.22 ± 1.80[a]	49.67±0.48[a]	32.97±2.75[a]
3 DS	35.98 ± 7.28[a]	5.47 ± 0.56[c]	48.31 ± 5.81[b]	26.17 ± 1.64[b]	45.58±0.77[b]	22.58±1.68[b]
1 TC	33.85 ± 6.02[a]	5.81 ± 0.44[b]	50.72± 4.67[a]	27.93 ± 1.73[a]	41.47±0.54[b]	29.33±0.82[a]
2 TC	28.45 ± 5.72[a]	4.36 ± 0.35[c]	40.06 ± 4.17[b]	22.45 ± 1.40[b]	30.08±1.54[c]	21.16±0.66[b]
3 TC	29.03 ± 6.64[a]	6.25 ± 0.51[a]	50.36 ± 5.39[a]	28.61 ± 1.64[a]	41.50±1.4[b]	24.17±0.45[b]
p-value	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05

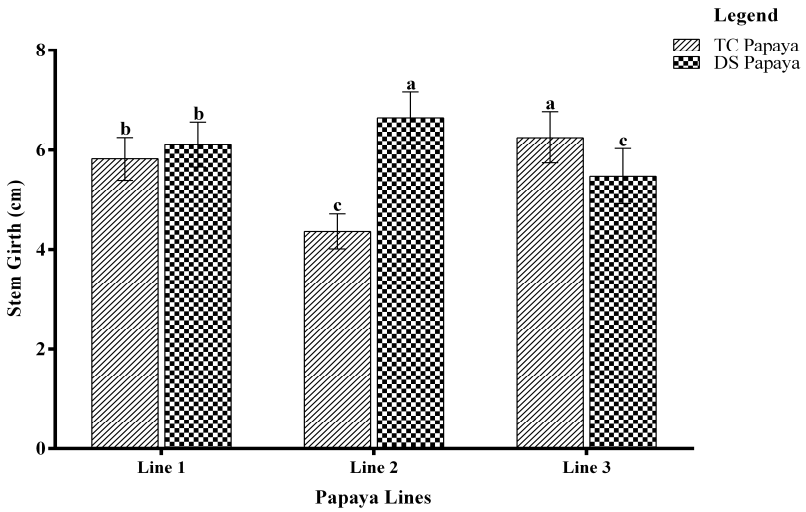

Fig. 2. Stem girth diameters of direct seeded and meristem cultured papaya lines

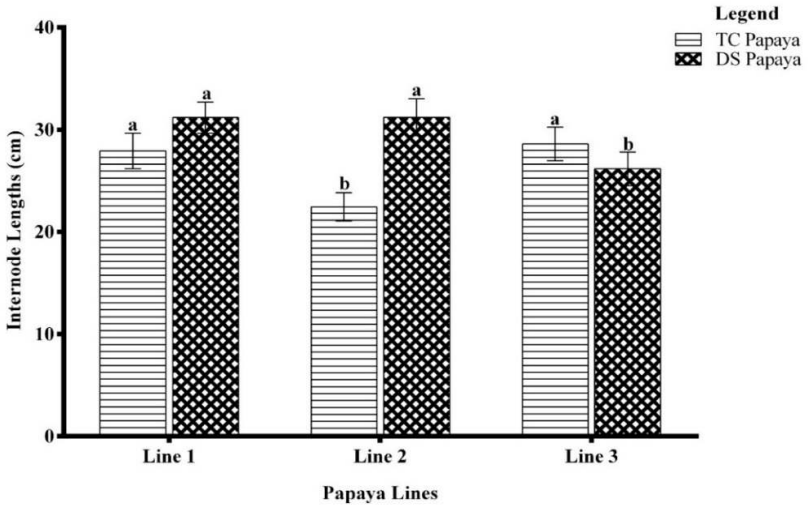

Fig. 3. Internode lengths of direct seeded and tissue culture generated papaya lines

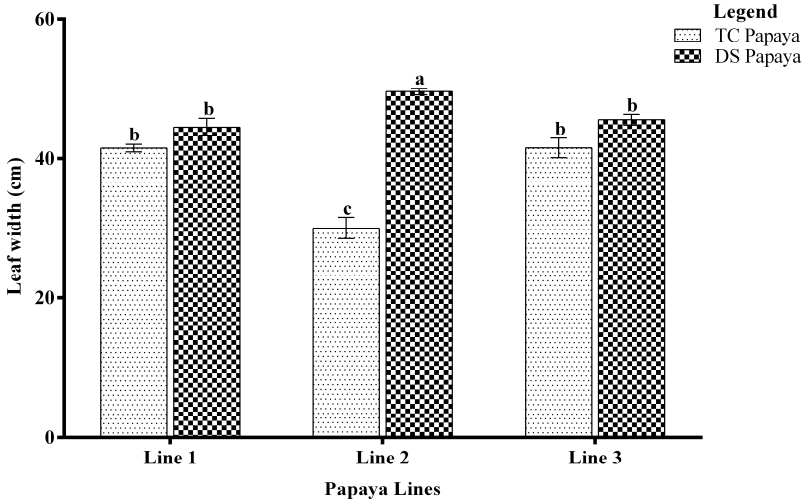

Fig. 4. Leaf width of direct seeded and tissue cultured papaya lines

From the seven criteria used in the comparison, this study did not record a consistent difference between seedlings and micropropagated papaya plants as observed from juvenile stage. This observation concurs with a report [18] who observed that tissue culture propagated papaya plants through organogenesis gave plantlets similar to their mother plants. They also observed that tissue culture propagated papaya plants and their fruits showed no morphological differences from their conventionally propagated mother plants. In comparison to the mother plants, the *in vitro* raised plants were all hermaphrodites showing no distinct phenotypic variation as was also reported [19].

Papaya leaves varies from 30 – 60 cm in width and each leaf blade is irregularly divided into 5 – 9 segments. The width and the length of the overall leaf determines the surface area, a feature integral in trapping sunlight for photosynthesis [13] In this study, the direct seeded and the *in vitro* generated papaya plants only varied slightly in respect to leaf length and width with both falling within the normal range (leaf width; 30 – 60 cm, leaf length; 45 – 90 cm). However, it is worth noting that in both cases, the tissue cultured plants had shorter and narrower leaves compared to the seedling generated plants. This is possibly due to tissue exposure to numerous stress factors including wounding and

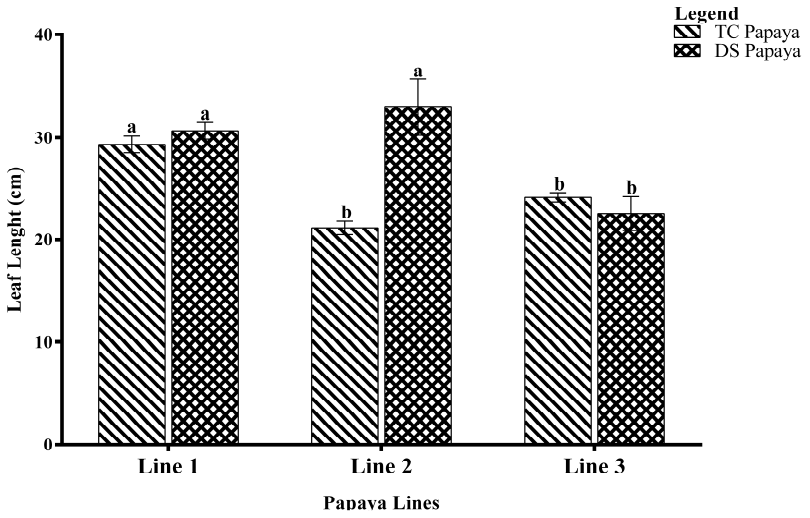

Fig. 5. Leaf lengths of direct seeded and tissue culture generated papaya lines

exposure to sterilants during sterilization and leading to oxidative stress damage that commonly arise from the process of *in vitro* regeneration in tissue culture environment [20] .

The duration of each papaya vegetative stage varies depending on cultivar and the existing climatic conditions [21]. Meristem culture in papaya reduces juvenile stage when compared with direct seeded plants. This was demonstrated by the shorter period of time taken by the tissue culture generated papaya to flowering. This reduction in days to flowering may be due to the optimized growth conditions provided in the regeneration process allowing the plants to access adequate nutrients required during the growth stages. Controlled relative humidity, temperature, and photoperiod has been shown to improve maturity time in pawpaw as reported by [22]. However, statistically, the difference in days to flowering between the seedling generated and meristem cultured papaya lines was only marginally significant with the former taking an average 100 days compared to 95 days taken by the latter.

Papaya plant under ideal field conditions grows at a rate of 1.8 – 3 m per year and reaches up to 6 – 9 m height at maturity [7]. Being a large herb, the height attained to flowering and the accompanying stem girth of the stem becomes important in carrying the fruit set and surviving environmental stress in strong winds and floods [23]. In this study, seedling generated papaya plants attained a higher height at flowering than their meristem cultured counterparts. Twenty one days after transplanting, the direct seeded papaya plants had a lager girth than the tissue culture generated plants. On average, this stem diameter variation (0.6 cm) was not significantly different at 95% confidence interval.

Plant internode length in papaya has been found to have more influence on plant height than the number of nodes [24]. The resultant height varies in different varieties as well as the growth conditions present. In this study, the average internode length of seedling generated papaya was slightly longer than that of the tissue culture generated plants. Consequently, the resultant tissue culture generated plants were marginally shorter in average than their direct seeded counterparts. The shorter internode lengths in the latter could be as a result of dwarfism resulting from the tissue culture process. This phenomenon of dwarfism in tissue culture papaya has been reported previously as well as in other plants [25-28]. However, the difference in internode lengths between the two groups obtained in this study (3.2 cm) was not statistically significant.

From the evaluated morphological characteristics compared between tissue culture generated and direct seeded papaya plants, this study did not record a consistent significant differences between the two groups. This is supported by the fact that micropropagation in plants is only a technique for rapid generation of many and clean planting stocks [29] Other than the mild morphological variations encountered, this process does not change the genetics of the

plant. Therefore the growth performance between *in vitro* generated and seedling generated plants is naturally regarded as tantamount.

5. CONCLUSION

Evaluation was done of the growth rate and morphological traits of both meristem cultured and directly seeded papaya plants. From this, only slight differences were recorded between the two treatment groups. Notably, the tissue culture generated plants took less time to flowering, implying reduction of the juvenile stages by the regeneration process. On the other hand, the direct seeded papaya plants were taller in average. The shortness on the meristem cultured plants was as a result of short internode lengths, possibly due to dwarfism arising from multiple stress factors encountered in the tissue culture process. From the results of this work, it was evident that micropropagation in papaya does not change the genetic fidelity of the resulting plants. A conclusion was hereby arrived at that there was no significant difference in growth performance between the meristem cultured and the direct seeded papaya plants. Regardless, the integration of *in vitro* micropropagation into the existing papaya propagation methods towards generation of many, clean planting stocks stands a viable addition into commercial papaya production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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