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DEVELOPING A TISSUE CULTURE PROTOCOL FOR PRODUCTION OF PLANTING MATERIALS OF PAPAYA (*Carica papaya* L.) HYBRID

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ABSTRACT

Papaya (*Carica papaya* L.) is a very important fruit crop grown in the world including sri Lanka. This study was conducted to develop a procedure for the rapid tissue culture propagation of papaya hybrid plantings at commercial scale. Shoot tips were surface sterilized with 20% NaOCl for 20 minutes before establishing on hormone free Murashige & Skoog (MS) medium. MS medium supplemented with 3 mg/l Benzyl Amino Purine (BAP), 0.3 mg/l Naphthalene Acetic Acid (NAA) and 500 mg/l Casein was the most suitable medium for formation of shoots. Well grown roots were obtained in MS medium containing 2 mg/l IBA and 500 mg/l Casein. Successful hardening (100% survival rate) was achieved when the plantlets were transplanted in pots containing moist mixture of sand: top soil: compost to a 1:1:1: ratio at sterile condition together with a weekly application of Albert solution. Flowering was observed at 7 weeks after the field planting. Micro propagated plants produced fruits with average fruit weight of 1.405 kg and average Brix value of 14.5, which were similar to fruits obtained from plants grown from seedlings.

Key words: Tissue culture, Papaya hybrid, Micro propagation.

INTRODUCTION

Papaya is considered as one of the important fruit crops growing in tropical and subtropical countries including Sri Lanka. It is popular in sub-tropics for easy cultivation, rapid growth, quick economic returns and adaptation to diverse soil and climates. Papaya has a good demand in both local and export markets. Non-availability of high quality fruits throughout the year is the main problem in exporting papaya. Therefore, production of high quality planting material is very important to papaya cultivation in Sri Lanka. Conventional method of propagation through seeds with open pollinated flowers always result a mixture of genotypes. Therefore, hybrid seeds are

used for commercial cultivation. Hybrid papaya seeds are currently imported to Sri Lanka and they are very expensive. Local hybrid papaya seeds are producing in Sri Lanka however; it is not easy method for mass seed production. Therefore, micro propagation is successful and economical way of producing uniform, true type planting materials continuously.

Axillary buds, small laterals and shoot tips proliferation is technique widely used in commercial micro-propagation (Drew and Smith, 1986; Mondal *et al*, 1990; Abeyratne and Abeysekera 2000; Roy and Hakim, 2012). Therefore, this study was conducted with the aim of developing a suitable protocol for micro propagation of papaya hybrid variety using shoot tip as explants.

MATERIALS & METHODS

This study was carried out to optimize surface sterilization, multiplication, rooting, acclimatization and field planting of papaya. The hybrid seeds were transplanted in poly bags containing sand and soil (1:1) in a greenhouse at plant virus indexing centre, Homagama. Seedlings of about 30 cm height were selected and about 5 cm from the top was cut to enhance the development of lateral shoots.

Surface sterilization of papaya shoots

Murashige and Skoog (MS) (1962) medium supplemented with 30 g/l of sugar, Casein 500 g/l and 0.1 g/l of myo- insitole was used. Media containing (35 ml/jar) jars were sterilized at 120 ^{0}C and 1.06 kg/cm^{3} steam pressure for 20 minutes using auto clave. Shoot tips (2 cm long) from newly developed lateral shoot sprouts of papaya plants were collected to a jar containing water to prevent from dehydration and to minimize the flow of latex. Then shoot tips were washed with running tap water for 15 minutes followed by washing 3 times with liquid Vim solution. Thereafter, shoot tips were treated with 0%, 5%, 10%, 20% and 30% of Clorox for three different time durations (10min, 20min, and 30min). Each treatment was replicated 5 times. The sterilized shoot tips were cut in to1cm long pieces that, established

on hormone free MS medium and incubated at 23±1°C under 16 hours' photo period. The numbers of survived explants were recorded weekly interval.

Optimization of shoot proliferation of papaya

Survived shoots were transferred to MS basal medium with 5 different combinations of BAP and NAA (Table 1). For all treatments 500 mg/l of casein was added. Sub culturing commenced after eight weeks. Extent of the shoot proliferation was estimated with the number of shoots formed.

Optimization of condition for root induction of *in vitro* raised papaya shoots

The *in vitro* proliferated shoots were transferred to four different combinations of IBA and BAP (Table 2) in the MS medium. For all treatments 500 mg/l of casein were added. The experiments were arranged as Completely Randomized Design (CRD) and SAS (Statistical Analysis System) software package (Windows 9.0-English) was used to analyze experimental data.

Acclimatization and field planting of tissue cultured papaya plantlets

The *in-vitro*-rooted plantlets were removed from the agar using sterilized water and the plantlets were transferred to plastic pots containing sterilized sand, top soil and compost 1:1:1 ratio. They were placed in a propagator for a week. Albert solution was applied weekly. Eight weeks after acclimatization they were planted in the field pits (60 cm x 60 cm x 60 cm) containing top soil, compost and cow dong 1:1:1 ratio and applied with DOA recommended fertilizer. Fruits weight and brix values were measured and compared with Hybrid papaya seedlings planted at the same condition.

RESULTS AND DISCUSSION

Optimization of surface sterilization

Well grown lateral shoots were observed after removal of shoot tips. Four weeks after incubation 100% contamination was observed in Clorox free condition. The mean comparison test of survived explants with the concentrations of Clorox and time durations showed a significant effect of Clorox concentration on surface sterilization ($p= 0.0001 < \alpha= 0.05$). The mean

number of survived explants in 20% NaOCl concentration and 20 min time duration are significantly higher than other treatments (Figure 1). Therefore 20 % NaOCl and 20 min time duration are suitable for surface sterilization of hybrid papaya shoot tips.

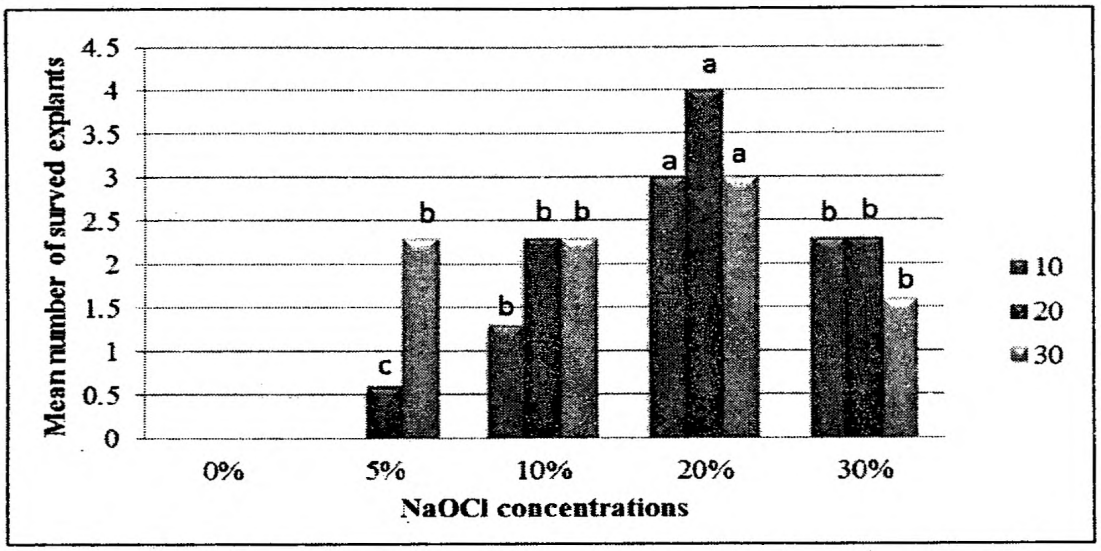

Figure 1. Effect of NaOCl concentrations (0%, 5%, 10%, 20%, and 30%) and time durations (10, 20, 30min) for surface sterilization of papaya shoot tips

Note: Same letters are not significant different according to the Duncan multiple mean comparison test)

Culture media for shoot proliferation of papaya

Shoot formation was started seven weeks after inoculation in multiplication media Three weeks after incubation the base of explants were enlarged in treatment 4 (3mg/l BAP+0.3mg/l NAA). Well grown papaya shoots were shown, 2 months after inoculation in the multiplication media. According to the one-way ANOVA results, a significantly higher treatment effect was observed in the media containing 3mg/l BAP + 0.3mg/l NAA (Treatment 4) of papaya *in vitro* raised shoot tips for shoot formation ($p= 0.0001 < \alpha = 0.05$) (Table 1; Figure 2). Callus formation was observed in treatment 4 in 6^{th} level of sub culture. Similar results were observed by Gunathilake and Jayathilake (1988), where shoot multiplication on sub culturing of adventitious buds was necessary after four weeks and at each subsequent four-week interval.

Table 1. Effect of BAP and NAA combination for shoot proliferation of papaya

Treatments(BAP+ NAA mg/l)	Mean number of shoot proliferated ±SE
T_1(1.0+0.0)	2.43 ± 0.083
T_2 (2.0+0.1)	2.50 ± 0.085
T_3 (2.5+0.2)	2.45 ± 0.083
T_4 (3.0+0.3)	4.20 ± 0.088
T_5 (2.5+0.3)	3.25 ± 0.087
T_6 (0.0+0.0)	0.00

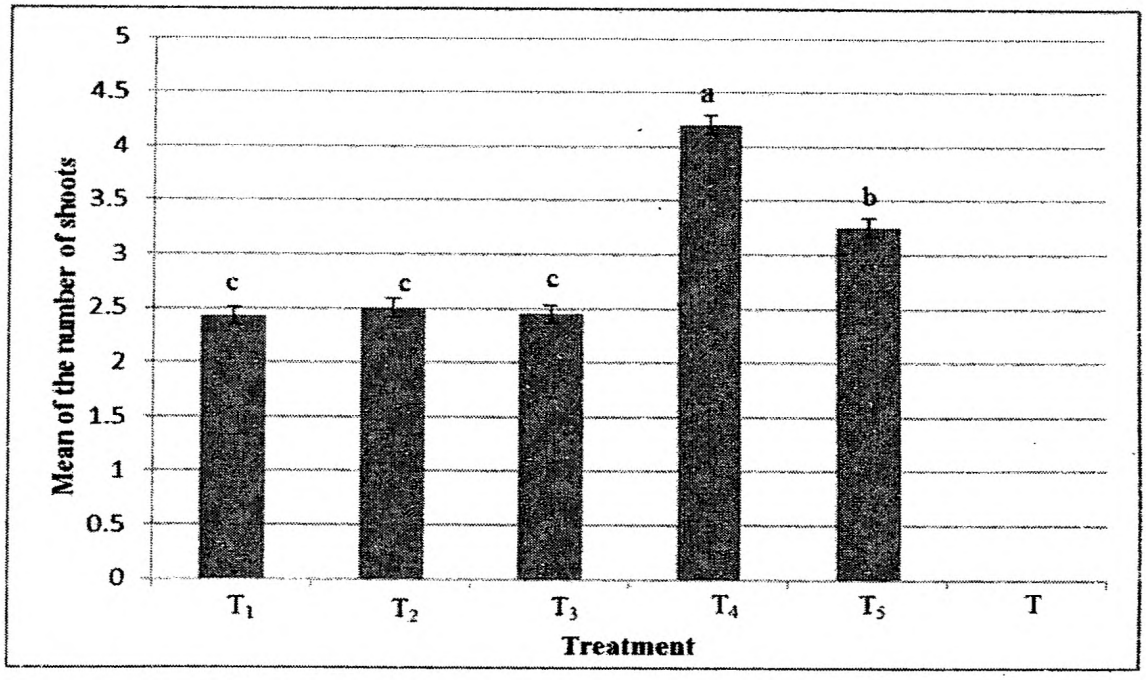

Figure2. Effect of different combination of plant growth regulators in culture media on shoot proliferation of papaya (DMRT mean comparison).

Optimization protocol for root formation of *in vitro* raised shoots papaya.

Data presented in Table 2 indicated that addition of IBA 2 mg/l+ BAP 0.2 mg/l showed the optimum number of root formation. These results are close to those of Rejeevan and Pandey (1986) and Mondal *et al*, (1990). They used medium containing 2 mg/l IBA for *in-vitro* rooting of papaya. However, the highest number of roots was observed with 2.5mg/l IBA+ 0.3mg/l BAP and it was impossible to separate plantlets of these treatments and shoot to root ratio also very low. Low numbers of survived shoots were observed after inoculation in rooting media (Table 2).

Table 2. Effect of IBA & BAP combinations for *in vitro* root formation of papaya (After three months)

Treatments IBA mg/l +BAP mg/l	Mean number of roots ± SE
T_1 (1.0+0.0)	1.3 ± 0.68
T_2 (1.5+0.1)	2.2 ± 0.79
T_3 (2.0+0.2)	8.2 ± 0.81
T_4 (2.5+0.3)	33.5 ± 0.91
Control (0.0+0.0)	0.0

Acclimatization

All acclimatized plantlets were survived and they were transplanted in the field. Under single propagator system 100% plants were survived after acclimatization.

Field Planting

Flower initiation was observed 7weeks after Field planting. First Ripened fruit was observed after 5 months. The average fruit weight 1.405 kg was observed. Orange colour skin of the ripened fruit was observed. Dark orange colour and 14.5 Brix values in the flesh was observed. Same results were observed in hybrid papaya seedling plants. Our findings are similar to the reports of Bindu *et al.* (2015); Naomi *et al.* (2013) and Mondal *et al.* (1990).

CONCLUSION

In vitro regeneration of papaya shoot tip culture was attempted using shoot tips from lateral buds of papaya hybrid variety. Surface sterilization by 20% Chlorox for 20 minutes of papaya shoot tip explants, MS medium supplemented with 3 mg/l BAP and 0.3 mg/l NAA and 500 mg/l casein for shoot proliferation, and 2 mg/l IBA with 0.2 mg/l BAP for root formation in MS medium were identified as optimum media compositions for micro propagation of hybrid papaya. The results indicate the possibility of mass production of hybrid papaya planting material using tissue culture technology.

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