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In vitro Propagation of Papaya (*Carica papaya* L.Cv. *Tainung No.2*) from Nodal Segments in Response to Nutritional and Growth Hormonal Factors

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Abstract

Background:

Papaya (*Carica papaya* L.) is a significant commercial fruit crop in the tropics, and successful micropropagation is crucial for the mass production of superior true hermaphrodite genotypes such as '*Tainung No. 2*'.

Objective:

This study aimed to establish an effective protocol for rapid in vitro regeneration and clonal multiplication from nodal explants of hermaphrodite papaya '*Tainung No.2*'.

Methodology:

Nodes used for explants were cultured on MS medium containing hormone BAP (0-1.0 mg L⁻¹) along with Kinetin (10 mg L⁻¹) or NAA (0.1-0.5 mg L⁻¹) for shoot formation. Multiplication and elongation were determined by observing the response of BAP, NAA, and casein hydrolysate (CH) at varying concentrations (000–500 mg l⁻¹).

Results:

The maximum value of shoot induction was recorded as 66.67 %, using 0.5 or 1.0 mg l⁻¹ BAP and 0.1 mg l⁻¹ NAA. Maximum multiplication and elongation had been observed when 0.5 mg l⁻¹ IBA, 0.2 mg l⁻¹ NAA, and 200-300mg l⁻¹ CH were used. Rooting was successful in a half-strength MS basal medium containing 1.5 mg L⁻¹ IBA and 200 mg L⁻¹ CH.

Conclusion:

The developed protocol enables a high rate of clonal propagation of papaya '*Tainung No.2*' and successful acclimatization of the plantlets.

Keywords: *Carica papaya*, vitropagation, MS concentration, growth regulator, casein hydrolysate.

1. Introduction

Papaya is a commercially valuable fruit produced in many tropical and subtropical regions. Global production 2013 was estimated to be 12,420,585 tons from 441,042 hectares of land (Faostat, 2015). Papaya is widely known for its nutritional value, containing large quantities of vitamins A, B, and C, minerals, proteins, papain, and chymopapain enzymes (Barret, 1985; Manshardt, 1992). Furthermore, papaya contains a negligible amount of sodium, fat, and calories and has no starch content at all, thus making it suitable for heart-conscious people (Sampson, 1986). Papaya is a polygamous species, exhibiting three sex types: Male flowers, female flowers, and bisexual flowers (Paull, 2011). For commercial, planting only hermaphrodite is encouraged as it results in the production of quality fruits. However, propagation through seeds has led to variability because many plants are heterozygous, and there are unwanted male plants (Hsu, 2012; Usman, 2002), which make up about 30–50% of the field (Jordan, 1983). Also, using seeds in propagating the crop results in variability in disease resistance, fruit quality, and yield (Allan, 1995; Teixeira, 2007). To overcome these drawbacks, the production of true-to-type plants through asexual reproduction is very crucial.

Traditional techniques like cutting and budding have proven effective (Airi, 1986; Chong, 2008; Ramkhelawan, 1998), but these are time-consuming, unsuitable commercially, and can never eradicate the disease (Setargie, 2015). Micropropagation can effectively propagate uniform and high-quality planting materials that are genetically as good as the source or the mother plant (Mumo, 2013; Roy, 2012). This technique helps tackle the limitations always associated with the traditional approach to plant breeding for fast clonal multiplication.

Traditional reports of micropropagation of papaya using both sexual and asexual organs have been widely discussed. Still, the regeneration potential of papaya consequently, standard operating procedures that can be unique to specific cultivars or explant types are critical in the propagation process (Ambasta, 2013; Kabir, 2007; Panjaitan, 2007; Setargie, 2015; Teixeira, 2007; Wu, 2012; Yu, 2003). However, the stages of *in vitro* propagation of papaya respond differently to vary of explants, genotypes, and nutritional and hormonal media compositions (Chan, 1993; Falcone, 1986; Mishra, 2007; Mumo, 2013; Saha, 2004; Usman, 2002). Therefore, suitable protocols must be established for different papaya varieties and used explants.

Taiwanese '*Tainung No.2*' cultivated in Vietnam, is characterized by medium fruit size (1.1kg), attractive orange-red pulp, good taste, and high production rates. Today, it reproduces mainly through seeds but has limited success through grafting and cutting. However, only the micropropagation using nodal stem explants of '*Tainung No.2*' has not been documented. This research aims to determine the best procedure for micropropagation '*Tainung No.2*' papaya using nodal segments while addressing the role of growth regulators, casein hydrolysate, and media composition.

2. Methodology

All the experiments of this work were done in the Plant Cell Technology Laboratory, Institute of Life Sciences, Thai Nguyen University of Agriculture and Forestry, Vietnam.

2.1. *Plant Material and Explant Selection*

Hermaphrodite '*Tainung No.2*' papaya plants were employed in the present investigation and grown under the protected greenhouse structure. Plants one year old were cut to 50 cm from the top to stimulate new shoots' growth. Gunnera shoots of 5–10 cm in length were harvested, and the leaves of the collected material were identified and removed. Lateral buds were surface disinfested using 70% ethanol for 30 seconds and then Clorox containing 10 a few drops of Sigma Tween 20 USA for 15 minutes on the specimen. After this process, the buds were gently washed five times using sterile distilled water. For lateral buds, once necrotic tissue had been removed, they were cut into single-node explants measuring about 6 mm in length and grown on MS medium containing 30 g l⁻¹ sucrose and 0.65% (w/v) agar. Each explant was checked for contamination for 20 days, after which it was transferred to shoot initiation media.

2.2. *Shoot Induction*

Seeds of explants were surface sterilized and then cultured on MS medium enriched with 6.5 g l⁻¹ agar, 30 g l⁻¹ sucrose, and various concentrations of 6-benzylaminopurine (BAP: Culturing explants were treated with different concentrations of BA (0, 0.5, 1.0 mg l⁻¹) or kinetin (10 mg l⁻¹) or NAA (0.1 to 0.5 mg l⁻¹). The experiment was conducted in a completely random setup, and the treatments were replicated five times, each containing 12 explants from the parent plant. The

percentage regeneration of explants into shoots, the number of shoots developed per explant, and the mean shoot length were recorded on shoot-inducing medium after 40 days of culture.

2.3. Effect Photomultiplication and Photoelongation

Explants used for the study were derived from the 40-day cultures on MS basal medium supplemented with BAP (1.0 mg L^{-1}) and NAA (0.1 mg L^{-1}), where the shoots were cut to about 0.5 cm in length with three developed leaves on their apex. These shoots were cultivated on MS basal medium containing sucrose 30 g l^{-1} and agar 6.5 g l^{-1} with BAP ($0.0, 0.5,$ and 1.0 mg l^{-1}) alone or with NAA ($0.1 - 0.5 \text{ mg l}^{-1}$). In another experiment, trimmed shoots were cultured on modified MS medium (MSC) containing MS basal salts, 6.5 g l^{-1} agar, 30 g l^{-1} sucrose, BAP (0.5 mg l^{-1}), NAA (0.2 mg l^{-1}), and casein hydrolysate (CH: $0-500 \text{ mg l}^{-1}$). Record of shoot multiplication rate, the measure of shoot length, and the number of leaves per shoot were done after 30 days.

2.4. Rooting of Shoots

Shoots approximately 1.5 cm in length from the 30-day cultures on MSC medium with CH (200 mg l^{-1}) were excised and then transplanted into rooting media with full or half-strength MS salts, 6.5 g l^{-1} agar, 30 g l^{-1} sucrose, CH (200 mg l^{-1}), and various concentrations of indole-3-butyric acid (IBA: $0.0-4.0 \text{ mg l}^{-1}$). Hence, the performance variables, including the percentage of shoot developing roots, number of roots, growth rate in root length, shoot height, shoot diameter, and the number of leaves, were determined after 30 days.

2.5. Culture Conditions

The pH of the media was adjusted to 5.8 using 1.0 N NaOH or 1.0 N HCl before steaming at 1.1 kg cm^{-2} and a temperature of $121 \text{ }^{\circ}\text{C}$ for 20 minutes. Plants were cultured at $25 \pm 2^{\circ}\text{C}$ under 16/8-hour light-dark conditions with a light intensity of $45 \mu\text{mol m}^{-2} \text{ s}^{-1}$ using cool white fluorescent lamps (**Figure 1**).

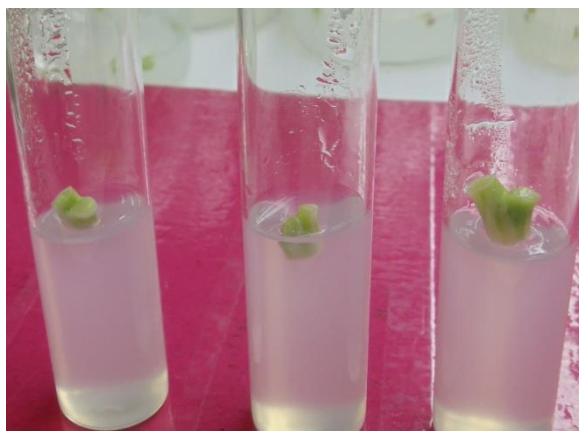


Figure 1. *Culturing nodal explants of papaya on MS medium.*

2.6. Statistical Analysis

All experiments were carried out employing the absolute random method. Data on shoot initiation and multiplication were subjected to one-way analysis of variance (ANOVA), while data on rooting were subjected to two-way ANOVA at $p \leq 0.05$. Comparisons of treatment means found to be significantly different are stated at $p < 0.01$ using Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

3.1. Shoot Induction

The nodal explants possessed a high regeneration capacity on the MS media supplemented with PGRs during in vitro culture. The differentiation of the shoot and the regeneration process was identified from the fourth week of culture through the budding of the nodal regions of explants. No shoot induction was observed on Media containing MS basal salts and no PGRs, suggesting that shoot regeneration depends on supplemented PGRs. The culminating outcomes of shoot induction after 40 days are presented in **Table 1** below.

Table 1. *Effect of BAP, Kinetin in combination with NAA in MS medium shoot induction from nodal explants of papaya (after 40 days of culture).*

Growth regulators (mg l ⁻¹)			Percentage of explants produced shoots (%) (mean±SE)	Mean number of shoots (mean±SE)	Shoot height (mm) (mean±SE)
BAP	Kinetine	NAA			

0.0	0.0	0.00±0.00	h	0.00±0.00	f	0.00±0.00	j
0.5	0.0	20.00±4.56	def	1.07±0.15	cd	1.15±0.08	cdef
1.0	0.0	23.33±3.73	cde	1.13±0.18	cd	1.10±0.07	gh
0.5	0.1	65.00±3.73	a	2.08±0.11	a	2.16±0.06	a
1.0	0.1	66.67±5.89	a	2.31±0.07	a	1.82±0.06	abc
0.5	0.2	40.00±3.73	b	1.08±0.11	cd	1.76±0.08	bcd
1.0	0.2	43.33±3.73	b	1.25±0.07	cd	1.80±0.02	abcd
0.5	0.3	16.67±5.89	ef	1.00±0.00	d	1.72±0.05	bcd
1.0	0.3	23.33±3.73	cde	1.27±0.15	cd	1.73±0.08	bcd
0.5	0.4	6.67±3.73	gh	1.00±0.00	d	1.34±0.11	efg
1.0	0.4	13.33±4.56	fg	1.10±0.22	cd	1.28±0.10	efg
0.5	0.5	3.33±4.56	h	0.40±0.55	e	0.44±0.61	i
1.0	0.5	5.00±4.56	h	0.60±0.55	e	0.74±0.68	ih
10	0.0	16.67±5.89	ef	1.00±0.00	d	1.46±0.06	def
10	0.1	28.33±4.56	c	1.60±0.09	b	1.96±0.06	ab
10	0.5	26.67±3.73	cd	1.37±0.07	bc	1.77±0.08	bcd
10	1.0	23.33±3.73	cde	1.37±0.07	bc	1.81±0.03	abcd
10	1.5	20.00±4.56	def	1.00±0.00	d	1.53±0.06	cde
10	2.0	20.00±4.56	def	1.00±0.00	d	1.50±0.04	fg

Means followed by the same alphabet are not significantly different based on DMRT (at $p < 0.01$)

MS medium fortified with BAP (0.5 or 1.0mg l⁻¹) and NAA (0.1mg l⁻¹) produced the highest percentage of shoot formation at 65% and 66.67%, respectively, as illustrated in **Figure 2**.

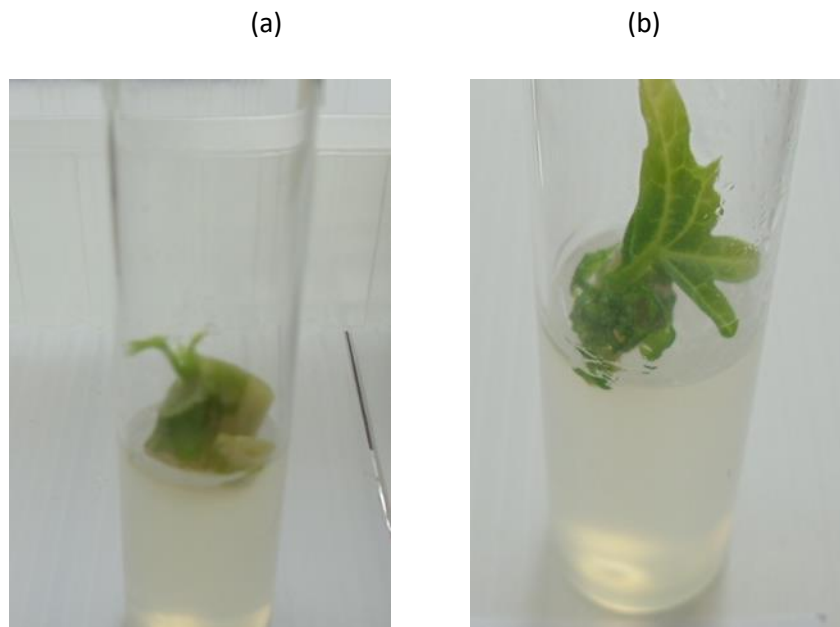


Figure 2. Shoot induction from nodal explants of papaya. (a), shoot regeneration from the node part of the explants in MS+ BAP (1.0 mg l^{-1}); (b) proliferation of shoot from the node part of the explants in MS+ BAP (1.0 mg l^{-1}) + NAA (0.1 mg l^{-1}).

In addition, these media produced the highest mean shoots per explants (2.08 ± 0.69 and 2.31 ± 0.74) and shoot length (2.16 ± 0.41 and 1.28 ± 0.33), respectively. The results presented here correlate with previous studies that documented the additive impact of cytokinins (BAP) and auxin (NAA) in shoot induction and formation (Suksa-Ard, 1997; Sultana, 2003; Tsay, 1989). For *in vitro* propagation of papaya, the same opinion was used in most of the previous research (Panjaitan, 2007; Roy, 2012; Setargie, 2015).

Thus, BAP appeared more effective than the two cytokinins tested when combined with NAA. Litz and Conover also worked on shoot regeneration and concluded that BAP with NAA is best suited for this purpose (Litz & Conover, 1978). Genetic factors are likely to be a reason for these result differences (Jabeen et al., 2005; Setargie et al., 2015). The outcomes validate that it is essential to fine-tune PGR combinations further to enhance papaya's micropropagation *in vitro*.

3.2. Shoot Multiplication and Elongation

3.2.1. Effect of PGR Combinations on Shoot Multiplication and Elongation

Shoot multiplication and elongation data are summarized in **Table 2**. The maximum micropropagation rate of shoots was obtained in MS medium containing BAP (0.5mg/L) + NAA (0.2mg/L) having a multiplication rate of 6.03, shoot length of 7.10 mm, and number of leaves per shoot of 4.24. This combination was significantly higher than MS medium without PGRs in which shoots were multiplied at the rate of 1.05 fold; shoot length was 5.55 mm, and three leaves per shoot were developed.

The observed results also confirm that cytokinins break apical dominance and shoot proliferation. At the same time, auxins such as NAA contribute to shoot elongation as proposed by (Skoog, 1957). Similarly, the efficacy of BAP and NAA for enhancing the shoot multiplication in papaya has been studied by (Ambasta, 2013; Anandan, 2011; Mumo, 2013).

Table 2: Effect of different concentrations of BAP, or combination of BAP and NAA, on multiplication and elongation of shoot (after 30 days of culture)

Growth regulators (mg l ⁻¹)		Multiplication of rate (folds) ± SE		Mean shoot length (mm) ± SE		Mean No. of leaves per shoot(leaves) ± SE	
BAP	NAA						
0.0	0.0	1.05±0.04	j	5.55±0.16	g	3.00±0.10	h
0.5	0.0	2.52±0.09	g	5.68±0.06	g	3.55±0.04	f
1.0	0.0	3.19±0.07	f	5.19±0.15	h	3.29±0.07	g
0.5	0.1	6.03±0.03	a	6.18±0.04	b	3.90±0.03	b
0.5	0.2	5.98±0.07	a	7.10±0.05	a	4.18±0.03	a
0.5	0.3	3.82±0.06	e	7.04±0.06	a	4.24±0.06	a
0.5	0.4	1.83±0.06	h	5.96±0.06	de	3.62±0.05	ef
0.5	0.5	1.35±0.03	i	5.81±0.03	f	3.59±0.03	ef
1.0	0.1	4.14±0.06	d	5.59±0.08	g	3.58±0.05	ef
1.0	0.2	4.70±0.06	c	6.03±0.11	d	3.73±0.07	de
1.0	0.3	5.04±0.04	b	6.26±0.05	b	3.85±0.03	bc
1.0	0.4	3.91±0.07	e	6.07±0.09	cd	3.78±0.06	cd
1.0	0.5	2.43±0.09	g	5.85±0.06	ef	3.67±0.04	ef

Means followed by the same alphabet are not significantly different based on DMRT (at p< 0.01)

3.2.2. Effect of the Casein Hydrolysate on Shoot Formation and Extension.

The data showed that cultivation on MSC medium supplemented with casein hydrolysate enhanced shoot elongation and multiplication ($F = 105.64$; $P < 0.01$). The treatment of MSC medium

containing 200 or 300 mg l⁻¹ CH produced the maximum shoot elongation and the highest number of shoots per plant with an average of 4.4 new leaves, as illustrated in **Figure 3**.



Figure 3. The shoot clusters developing in MSC media containing CH of 200 mg l⁻¹

At higher CH concentrations (400–500 mg l⁻¹), shoot length was significantly lowered, perhaps due to osmotic stress or hormonal disruption (**Figures 4 and 5**).

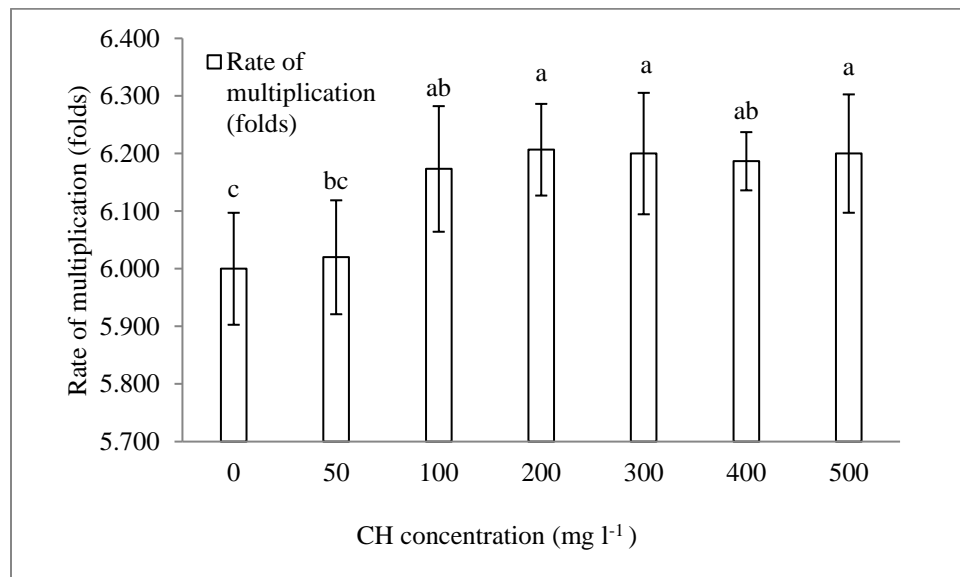


Figure 4. CH concentration affects shoot multiplication rate (30 days of subculture). Different letters above bars show significant differences (Duncan's multiple range test, $P < 0.01$). Error bars indicate SE.

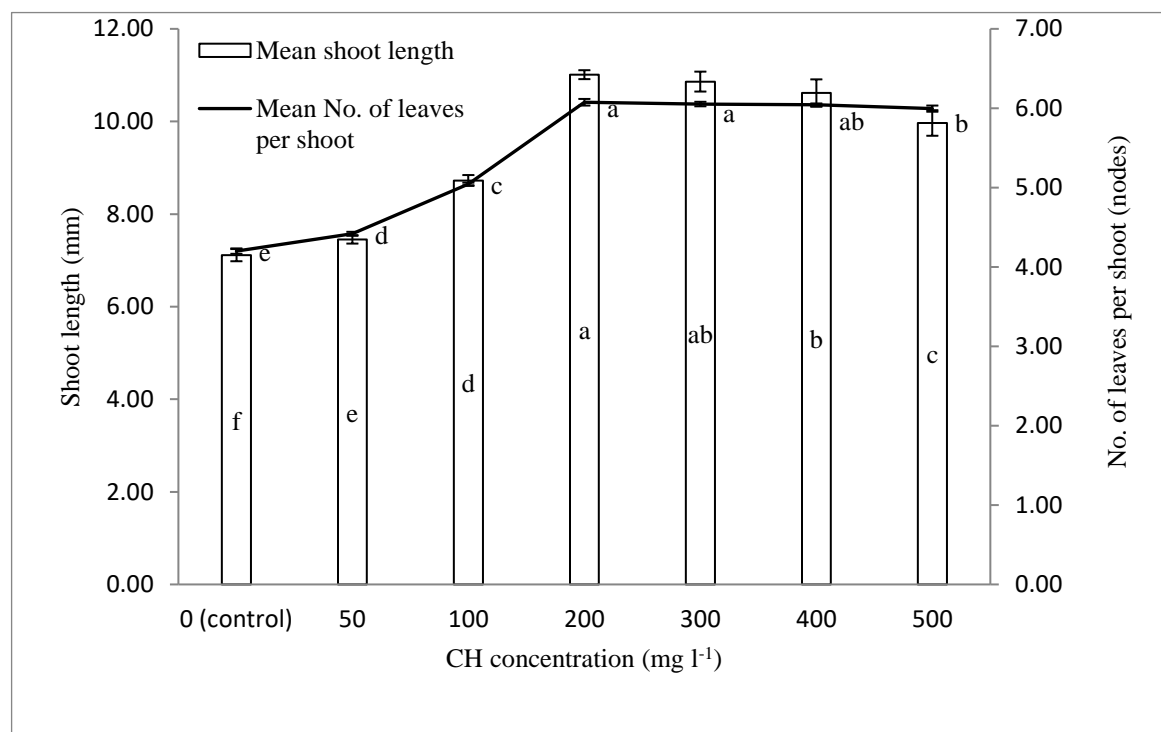


Figure 5. CH concentration affects the length and number of leaves per shoot (30 days of subculture). Different letters, central bars, or right lines show significant differences (Duncan's multiple range test, $P < 0.01$). Error bars or lines indicate SE.

The nutritive values of CH, such as amino acids, vitamins, and minerals, were established in a study by (George, 1984). The results regarding the positive impact of CH on improving the quality of papaya shoots correspond with the findings of the current investigation (Chan & Teo, 1993; Roy et al., 2012). From these results, it can be concluded that CH at a concentration of 200–300 mg l⁻¹ is suitable for shoot multiplication and elongation of 'Tainung No.2.'

3.3. Rooting of Shoots

Rooting responses varied significantly across the different media and about the IBA concentrations ($P < 0.01$). Overall, the best shoots were developed from shoots cultured on half-strength MS medium containing 1.5 mg l⁻¹ IBA; the minimum period required for root initiation was noted

(11.28 days), maximum rooting percentage (91.67%) and maximum root length (35.80 mm) (**Table 3**). Similarly, these results suggest that the lack of IBA caused the media not to promote rooting.

IBA in higher concentration ($\geq 3.0 \text{ mg l}^{-1}$) caused callus formation at the base of the shoot excision to prevent the further extension of roots (**Fig. 6 b, c**). Similar observations stating the adverse impacts of high amounts of auxin on roots were made by (B. Jarvis, 1986; Roy et al., 2012).

The result is consistent with the findings of Andrade et al. (1999) and Yu et al. (2000), which stated that the half-strength MS medium is better than the full-strength MS medium in producing root (**Fig. 6 a**) (Andrade et al., 1999; Yu et al., 2000).



Figure 6. Rooting of micro shoots from rooting media with 1.5 mg l^{-1} IBA (a) or high IBA concentration (b, c)

Plantlets of such a combination grew normally and vigorously after greenhouse acclimation and transplanted well (**Fig 7 a, b**).



Fig. 7. In vitro regenerated plant developed well in substrate (a) and in soil pot (b)
Thus, it is very challenging to get a high rooting percentage and quality of adventitious root systems from in vitro individual shoots of *C. papaya* on a medium (Drew, 1986; Winnaar, 1988). Adjusting the medium concentration to half-strength MS did not limit shoot growth and got better results for shoot and roots than full-strength MS (**Table 3**).

Table 3. Effect of different IBA concentrations on growth and rooting of papaya shoots cultured on half and full-strength MS medium (after 30 days of culture)

Media culture	IBA concentration	Time required for root formation (days)	Rooting of shoot					Growth of shoots						
			Percent of shoot produced root (%)± SE	No. of roots (roots) ± SE	Root length (mm) ± SE	Height shoots (mm) ± SE	Diameter of shoots (mm) ± SE	Mean of leaves ± SE						
Factor A(IBA concentration)														
	0.0	-	0.00 ± 0.00	f	-	-	15.95 ± 0.45	h	1.52 ± 0.02	f	2.77 ± 0.16	g		
	0.5	23.19 ± 0.81f	38.33 ± 4.56	e	1.80 ± 0.20	g	34.34 ± 0.24	b	19.26 ± 0.49	g	1.56 ± 0.02	f	3.77 ± 0.14	f
	1.0	11.52 ± 0.23 f	51.67 ± 6.97	d	6.76 ± 0.21	a	35.98 ± 0.35	a	24.60 ± 1.06	f	1.66 ± 0.02	de	5.55 ± 0.20	e
	1.5	11.28 ± 0.19 e	91.67 ± 5.98	a	6.72 ± 0.19	a	35.80 ± 0.13	a	36.72 ± 0.14	a	2.07 ± 0.05	a	8.40 ± 0.39	a
	2.0	13.38 ± 0.76 d	93.33 ± 3.73	a	6.10 ± 0.19	b	27.09 ± 0.17	c	33.60 ± 0.90	b	1.98 ± 0.06	b	8.34 ± 0.15	a
	2.5	15.61 ± 0.37 c	88.00 ± 4.31	ab	5.26 ± 0.12	c	24.77 ± 0.15	d	31.92 ± 0.39	c	1.89 ± 0.05	c	7.68 ± 0.30	b
	3.0	18.65 ± 0.39 b	81.67 ± 3.73	b	4.83 ± 0.18	d	23.36 ± 0.11	e	30.70 ± 0.74	d	1.85 ± 0.02	c	7.48 ± 0.21	b
	3.5	23.30 ± 0.30 b	66.67 ± 0.00	c	3.15 ± 0.10	e	23.27 ± 0.18	e	25.95 ± 0.77	e	1.69 ± 0.05	d	6.83 ± 0.24	c
	4.0	26.15 ± 0.22 a	55.00 ± 4.56	d	2.58 ± 0.36	f	20.37 ± 0.24	f	25.30 ± 0.73	ef	1.62 ± 0.02	e	6.25 ± 0.48	d
Factor B(MS concentration)														
	MS/2	14.30 ± 0.24 b	68.52 ± 2.62	a	4.88 ± 0.06	a	25.38 ± 0.14	a	31.48 ± 0.20	a	1.76 ± 0.03	a	7.00 ± 0.08	a
	MS	17.48 ± 0.15 a	57.33 ± 6.25	b	3.38 ± 0.07	b	24.63 ± 0.06	b	22.74 ± 0.32	b	1.76 ± 0.03	a	5.69 ± 0.30	b
Interaction of A and B factors														
	0.0	-	0.00 ± 0.00	f	-	-	16.20 ± 0.45	h	1.51 ± 0.02	i	2.73± 0.35	g		
	0.5	26.20 ± 1.64 b	20.00 ± 9.13	e	1.30 ± 0.45	g	34.70 ± 0.67	b	19.70 ± 0.25	g	1.53 ± 0.02	hi	3.73 ± 0.25	f
MS/2	1.0	8.67 ± 0.20 i	40.00 ± 0.00	d	6.77 ± 0.52	b	38.37 ± 0.25	a	26.00 ± 1.82	e	1.62 ± 0.03	fg	5.53 ± 0.45	d
	1.5	8.20 ± 0.22 i	100.00±0.00	a	8.03 ± 0.32	a	38.45 ± 0.21	a	45.00 ± 0.71	a	2.12 ± 0.04	a	8.80 ± 0.27	a
	2.0	10.53 ± 1.48 h	100.00±0.00	a	7.00 ± 0.20	b	27.27 ± 0.37	e	38.80 ± 0.84	b	2.00 ± 0.07	b	8.83 ± 0.24	a
	2.5	14.67 ± 0.26 g	100.00±0.00	a	6.70 ± 0.14	b	24.42 ± 0.18	g	38.40 ± 0.55	b	1.90 ± 0.07	cd	8.70 ± 0.18	a

	3.0	15.13 ± 0.36 g	100.00±0.00	a	6.77 ± 0.09	b	22.71 ± 0.11	i	38.20 ± 0.84	b	1.88 ± 0.04	cd	8.80 ± 0.22	a
	3.5	20.63 ± 0.25 e	80.00 ± 7.45	b	3.92 ± 0.11	d	22.88 ± 0.19	i	30.26 ± 1.00	c	1.64 ± 0.06	fg	8.00 ± 0.48	b
	4.0	24.73 ± 0.19 c	76.67 ± 9.12	b	3.43 ± 0.13	d	19.65 ± 0.25	k	30.81 ± 1.33	c	1.61 ± 0.05	fgh	7.83 ± 0.53	b
	0.0	-	0.00 ± 0.00	f	-		-		15.70 ± 0.67	h	1.52 ± 0.03	i	2.80 ± 0.27	g
	0.5	20.18 ± 0.34 e	56.67 ± 9.13	c	2.30 ± 0.24	f	33.98 ± 0.26	c	18.83 ± 1.01	g	1.59 ± 0.03	ghi	3.81 ± 0.22	f
	1.0	14.37 ± 0.31 g	63.33 ± 7.46	c	6.75 ± 0.31	b	33.59 ± 0.48	cd	23.20 ± 0.38	f	1.69 ± 0.02	ef	5.57 ± 0.15	d
	1.5	14.36 ± 0.38 g	83.33 ± 8.62	b	5.40 ± 0.13	c	33.16 ± 0.15	d	28.43 ± 0.64	d	2.02 ± 0.08	b	8.00 ± 0.91	b
MS	2.0	16.22 ± 0.14 f	86.67 ± 7.46	b	5.20 ± 0.20	c	27.01 ± 0.19	e	28.40 ± 0.99	d	1.96 ± 0.06	bc	7.85 ± 0.50	b
	2.5	16.55 ± 0.62 f	76.00 ± 8.62	b	3.81 ± 0.21	d	25.13 ± 0.17	f	25.43 ± 1.12	e	1.89 ± 0.07	cd	6.67 ± 0.46	c
	3.0	22.17 ± 0.47 d	63.33 ± 7.46	c	2.88 ± 0.39	e	24.01 ± 0.16	gh	23.20 ± 0.76	f	1.82 ± 0.06	d	6.16 ± 0.37	cd
	3.5	25.97 ± 0.34 b	53.33 ± 7.46	c	2.38 ± 0.29	ef	23.65 ± 0.18	h	21.63 ± 0.71	f	1.73 ± 0.06	e	5.67 ± 0.37	d
	4.0	27.57 ± 0.44 a	33.33 ± 1.78	d	1.73 ± 0.75	g	21.09 ± 0.41	j	19.80 ± 0.77	g	1.63 ± 0.02	fg	4.67 ± 0.59	e

In high IBA concentration compared with opinion concentration, roots were fewer, and many callus at the base of the shoot (**Fig 6 b, c**). This was due to the callus formation at the base of the roots and also might be the inhibitive effects of IBA at higher concentrations (B. C. Jarvis, 1986; Roy, 2012). IBA of 1.5 mg l⁻¹ was the optimum concentration to add a rooting medium, and the results were found by (Chommalee & Attathom, 1988; Setargie et al., 2015). Such observation was also made by (Hidaka et al., 2008; Kumar & Kumari, 2013). These outcomes clearly showed that the right concentration of IBA and strength of MS media are very important factors in the rooting process

4. Conclusion

This research aims to determine an effective protocol for the rapid micro clonal propagation of 'Tainung No.2' papaya *in vitro*. As per the present experiment, the outcomes showed that shoot induction was at its best in BAP supplemented of 0.5 or 1.0 mg L⁻¹ and NAA of 0.1 mg L⁻¹ for shoot multiplication and elongation, and with the supplementation of 200–300 mg L⁻¹ casein hydrolysate to the media. The best results were obtained using half-strength MS media with 1.5 mg l⁻¹ IBA; these had well-developed roots and healthy shoots.

These protocols offer an efficient and consistent way of generating uniform and true-to-type plantlets to yield large-scale commercial clonal propagation of papaya. It solves the problems associated with seed propagation and plays an essential role in producing healthy and quality cultivars.

5. References

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