



Effect of Plant Growth Regulators (BA, KIN and NAA) on *In vitro* Propagation of Papaya (*Carica papaya*)

Nahida Hasan¹, Humayra Huq¹, Fahima Khatun^{1*} and Shamim Ara Sumi¹

¹Department of Biotechnology, Sher-e-Bangla Agricultural University, Bangladesh.

Authors' contributions

This work was carried out in collaboration among all authors. Author NH performed the research work and statistical analysis of the study. Author HH designed the experiment. Author FK wrote the protocol and prepared the manuscript. Author SAS managed the literature searches and assisted in designing the study. All authors read and approved the final manuscript.

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ABSTRACT

The present research was carried out in Biotechnology Laboratory of the Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207 from the period of September 2017 to June 2018. This research aims to study the effect of Benzyladenine (BA), Kinetin (KIN) and Naphthalene acetic acid (NAA) either in combination or alone on *In vitro* propagation of papaya (*Carica papaya*). The shoot tips of young shoots were used as explant, which was sterilized using freshly prepared 0.1% HgCl₂ mixing with few drops of Tween-20, were inoculated in MS media supplemented with 0.1% activated charcoal. The minimum days to shoot induction (10.25) were recorded on MS medium containing 0.5 mg/L BA. The highest shoots (4.5) and length of shoot (5.75 cm) observed in 1.0 mg/L BA. The combined treatment 1.0 mg/L BA+0.75 mg/LKIN gave the highest number of shoots (5.25) and length of shoot (5.78 cm).The minimum days (8.5) to root induction was reported in 2.0 mg/L NAA along with maximum 8.25 roots per plantlet. The highest length of root (6.92 cm) was observed in 2.0 mg/L NAA. In regenerated plantlets, 80% survival rates were observed in growth chamber conditions and 75% in the open

*Corresponding author: E-mail: saufahima@gmail.com;

atmosphere were achieved. Finally, the *in vitro* regeneration protocol described herein can potentially be used as a tool in molecular breeding programs for the improvement of different cultivars and genotypes of papaya.

Keywords: BA; KIN; NAA; explants; *Carica papaya*.

1. INTRODUCTION

Papaya, (*Carica papaya* L.) is an important tropical fruit crop in the family of Caricaceae which consists of herbaceous plants [1,2]. Ripened fruits are consumed fresh while unripened fruits can be used in salads or as a vegetable and as a source of papain. Papain, a proteolytic enzyme obtained from immature fruits is used in the pharmaceuticals, leather, wool and rayon industries [3]. The crop is believed to have originated in Central America in regions ranging from Mexico to Panama. The Caricaceae was originally comprised of 31 species in the *Carica*, *Jacartia* and *Jarilla* genera from Central America and the African *Cylicomorpha* genus [4].

Papaya is an important fruit plant of Bangladesh, having commercial importance for its high nutritive and medicinal value [5]. It is also a good source of vitamin A, C and ascorbic acid and the proteolytic enzyme papain and chymopapain [6,7,8]. *C. papaya* cv. Shahi is a popular local variety which is widely grown and contributes to major sources of income for the farmers in Bangladesh. Papaya is cultivated in about 71 countries of the world over an area of about 440,629 ha, producing 13,016,281 tonnes of papaya with an average productivity of 2.95 lakh hg/ha [9].

Papaya cultivation is encountered with the problem of its dioecious nature. The majority of plantations are established from seeds using dioecious cultivars. Hence seed propagation results in seedlings which are either male or female. However, the setback of propagating by seed is the production of non-true-to-type planting materials [10] due to the segregation of offsprings at the second filial generation that leads to the inherent heterozygosity [11]. It has been experimentally found that inbred hermaphroditic papaya cultivars are more susceptible to disease and environmental stress [12]. Moreover, as sex cannot be determined until the mid-development stage, three seedlings are established in each planting position, till flowering. Then they are thinned, retaining only the most vigorous female plant with one male to every 10 to 20 female plants. This results in

wastage of inputs. With a requirement to renew plantations every three years to ensure quality fruit production propagation by seed represents a significant cost to the producer. The multiplication rates are low in the vegetative propagation method like mound layering. Similarly, asexual reproduction is also often tedious and impractical when carried out on large scale [13].

Therefore to minimize these problems, efficient micropropagation of papaya has become critical for the multiplication of specific cultivars of papaya. This field level problem necessitated the substitution of seedling progeny with tissue culture propagules developed from female or bisexual plants. Micropropagation represents the only economic way of continuously producing uniform planting materials of known sex [14]. Micropropagation can produce large numbers of elite homogeneous clones, allowing for the planting of a single hermaphrodite in each hole and eliminating negative aspects of seed propagation. The regeneration of plants from tissue culture is imperative and essential technique of biotechnological research and sometimes genetic manipulation of plants are achieved through this technique [15]. Genetic improvement programs and genetic research will largely benefit from efficient protocols for papaya plant transformation. We focused to explore the variability of *in vitro* responses among different plant growth regulator. So, the present study was undertaken with following objectives:

- a. Assessment the role of phytohormone on *in vitro* propagation in papaya.
- b. Identification of appropriate hormonal dose for *in vitro* propagation in papaya.
- c. Establishment of *in vitro* propagation protocol of Papaya.

2. MATERIALS AND METHODS

2.1 Explants Preparation

The healthy, disease free shoot tips of 1-2 cm length were used as explants for the study for *in vitro* regeneration. The trimmed shoot tips were washed thoroughly under running tap water and then with autoclaved distilled water for several

times. Subsequently the explants were transferred to laminar airflow cabinet. Then they were treated with 70% ethanol for 1-2 minute and rinsed with autoclave distilled water for 3-4 times. After treating with 70% ethanol, the explants were immersed in 0.1% HgCl₂ within a beaker and added 3-4 drops of Tween-20 for about 4-5 minutes with constant shaking in clockwise and anticlockwise direction. Then explants were washed 3-4 times with autoclaved distilled water to make the material free from chemical and ready for inoculation in culture media.

2.2 Culture Media

MS [16] medium supplemented with different phytohormones as per treatments were used as a culture medium for shoot induction, shoot multiplication, maintenance, and regeneration of roots. Hormones (BA, KIN and NAA) were added either in combination or separately to the media according to the treatment. Four levels of BA (0.5, 1.0, 1.5 and 2.0 mg/L) with four levels of KIN (0.25, 0.5, 0.75 and 1.0 mg/L) and five levels of NAA (0.5, 1.0, 1.5, 2.0 and 2.5 mg/L) separately were used as treatment. The pH was adjusted to 5.8 before placing in microwave oven which was used for melting agar (semi solidifying agent).

2.3 Shoot and Root Proliferation

The explants were cultured on MS nutrient medium supplemented with different concentration of BA alone or in a combination of KIN. After successful shoot proliferation, the subculture was done with newly formed shoots. Newly formed shoots with adequate length were excised individually from the culture vial and transferred to rooting media containing different concentration of NAA (0, 0.5, 1.0, 1.5, and 2.5 mg/L) along with MS media. The observations on the development pattern of shoot and roots were made throughout the entire culture period.

2.4 Establishment of Plantlets

Regenerated plantlets were transplanted to pots (10×15 cm) containing sandy soil and cowdung in 1:1 ratio. An occasional spray of water was done to prevent sudden desiccations and maintain high humidity (98%) around the plantlets. Initially the plantlets were hardened in a controlled environment. Then after 2 weeks, exposed to lower humidity and higher light intensity (3000 lux). Finally, after 20 days plantlets were transferred to natural environment.

The experiment was one factorial set up in a completely randomized design (CRD) with five replications per treatment. Data were statistically analyzed by analysis of variance (ANOVA) technique and differences among treatment means were compared by using Duncan's multiple range test (DMRT) at 5% probability level using MSTAT-C program.

2.5 Data Collection and Statistical Analysis of Data

Data were recorded after 3, 6 and 9 weeks of culture, starting from day of inoculation on culture media in case of shoot proliferation. The experiment was one factorial set up in a completely randomized design (CRD) with five replications per treatment. Data were statistically analyzed by analysis of variance (ANOVA) technique and differences among treatment means were compared by using Duncan's multiple range test (DMRT) at 5% probability level using MSTAT-C program.

3. RESULTS AND DISCUSSION

The effect of BA, KIN and NAA was investigated with different concentrations for *in vitro* propagation of papaya using shoot tips as explants.

3.1 Effect of BA on Shoot Induction Potentiality

Significant variation was observed among a different concentration of BA on days to shoot induction, a number of shoots per explants and shoot length. The maximum days to shoot induction were recorded in control treatment (25.75 days) and 0.5 mg/ L of BA required minimum 10.25 days (Fig. 1). The treatment 1.0 mg/LBA gave the highest number of shoots (4.50) whereas the lowest number of shoots (1.2) was found with hormone-free media (Fig. 2). BA variations affecting shoot proliferation were also reported by Bhandari et al. [17] and Gantait et al. [18]. Baksha et al. [19] noticed 3.2 shoots per explant in media supplemented with 2.0 mg/ L BA in papaya.

The maximum length of shoot 5.75 cm was noticed in the 1.0 mg L⁻¹ BA which was statistically similar with 1.5 mg L⁻¹ BA (4.40 cm) and statistically different from rest of others whereas the minimum length 2.00 cm was in control (Fig. 3). Baksha et al. [19] noticed 2.5 cm length of shoot in 4.0 mg L⁻¹ BA. This decline in

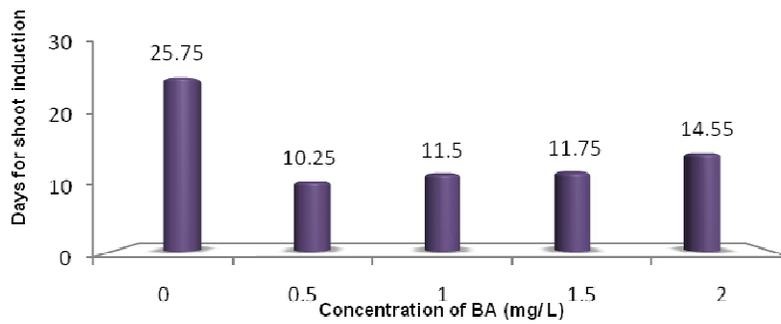


Fig. 1. Effect of BA on days to shoot induction in papaya

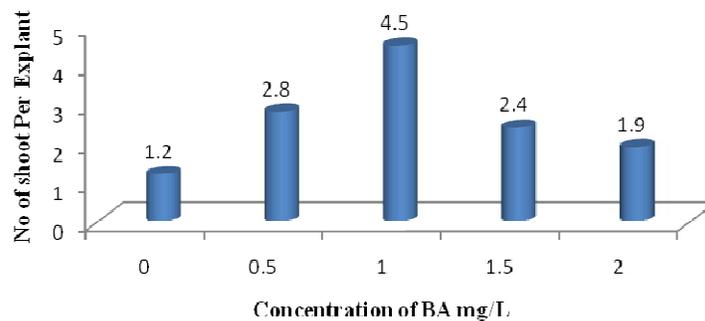


Fig. 2. Effect of BA on the number of shoots per explants in papaya

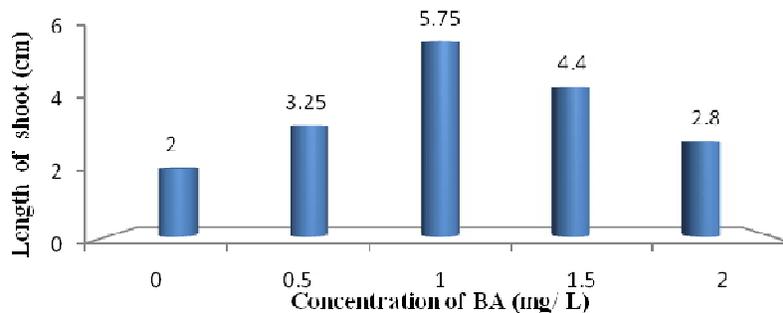


Fig. 3. Effect of BA on the length of shoot (cm) in papaya

the shoot length of papaya might be due to the inhibitory effect of BA, which provokes a little suppression of plant growth and activity of some proteolytic enzymes [20].

3.2 The Combined Effect of BA and KIN on Shoot Proliferation

There was a significant influence of different concentrations of BA and KIN on days to shoot induction, number of shoots per explants and length of shoot. The maximum days to shoot induction were recorded in control (28.00 days) and 1.0 mg/ L BA+0.75 mg/ L KIN required

minimum 16.80 days (Table 1). 1.0 mg/L BA+0.75 mg/L KIN gave the highest number of shoots (5.25) whereas the lowest number of shoots (1.25) was found with hormone-free media (Table 1). The average (5.78 cm) length of shoot was noticed from the 1.0 mg/L BA+0.75 mg/L KIN followed by 1.5 mg/L BA+0.75 mg/L KIN, whereas the minimum 0.7 cm was in control treatment. Islam et al. (1993) observed that BA at 0.5 mg L⁻¹ and NAA at 0.1 mg L⁻¹ gave best result in shoot proliferation in papaya. In papaya, Islam [21], Islam et al. [22] and Chowdhury [23] observed that KIN and BA at various concentrations were inducing

Table 1. The combined effect of BA and KIN on shoot induction potentiality in papaya

Name of the phytohormones	Phytohormones concentration (mg /L)	Shoot induction potentiality		
		Days for shoot induction	No. of shoots per explants	Length of shoot
BA+KIN	0.5+0.25	22.00 de	1.25 ef	0.7 f
	0.5+0.50	22.80 cd	1.35 d-f	1.28 ef
	0.5+0.75	23.80 cd	1.80 b-f	2.18 b-e
	0.5+1.00	21.60 def	2.10 b-f	1.93 d-f
	1+0.25	24.60 bc	1.70 c-f	1.68 d-f
	1+0.50	20.60 efg	1.85 b-f	2.15 b-e
	1+0.75	16.80 h	5.25 a	5.78 a
	1.0+1.00	19.20 g	3.17 b	2.63 b-d
	1.5+0.25	26.20 ab	2.33 b-f	0.85 ef
	1.5+0.50	27.00 a	2.65 b-d	2.9 bc
	1.5+0.75	23.80 cd	2.53 b-e	4.08 b
	1.5+1.00	22.20 de	2.75 bc	1.18 de
	2+0.25	23.40 cd	1.89 b-f	2.15 b-e
	2+0.50	22.00 de	2.80 bc	1.2 bc
	2+0.75	19.80 fg	1.95 b-f	3.72 bc
2+1.00	24.40 bc	2.35 b-f	1.53 d-f	
LSD (0.05)		1.93	1.16	1.20
CV (%)		5.75	5.25	5.45

*WAI=Weeks after Inoculation. Values in the column are the means of five replicates. In a column, mean values with the same letters are not statistically different from each other at 5% probability by DMRT

differentiations. However, Islam [21] observed that BAP with IAA in MS medium produced highest number and length of shoots and also days to shoot initiation occurred within 7-9 days.

3.3 Effect of NAA on Root Development

Hormonal concentration has a significant level of variation on days for root induction, number of roots and root length. The maximum 22 days to root induction was required in media lack of growth regulator. Minimum 8.5 days in case of 2.0 NAA mg/L (Fig. 4). Baksha, et al. [19] noticed that roots began to emerge from the tenth day in the medium with 0.5 mg/L of NAA.

The highest number of roots (8.25) was found with 2.0 mg/L NAA which was statistically different from other treatment. The minimum numbers of roots (3.1) were obtained in the control treatment (Fig. 5). Baksha et al. [19] noticed the highest number of roots per root was 4.8 with 0.5 mg/L NAA.

The maximum average root length (6.92 cm) in case of 2.0 mg/L NAA (Fig. 6). The minimum 1.00 cm average length of roots per explants (cm) was in the control treatment. Baksha et al. [19] noticed the highest average length of 3.5 cm with 0.5 mg/L NAA and 2.2 cm in IBA 1.5 mg/L.

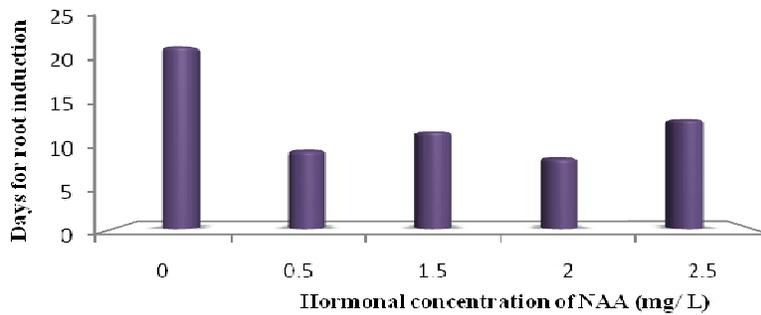


Fig. 4. Effects of NAA on days for root induction

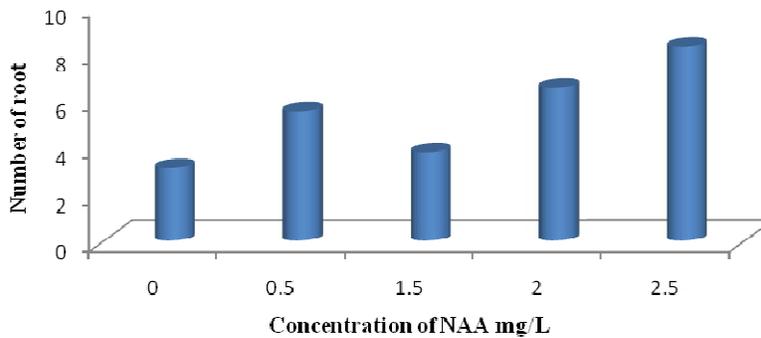


Fig. 5. Effects of NAA on number of root in papaya

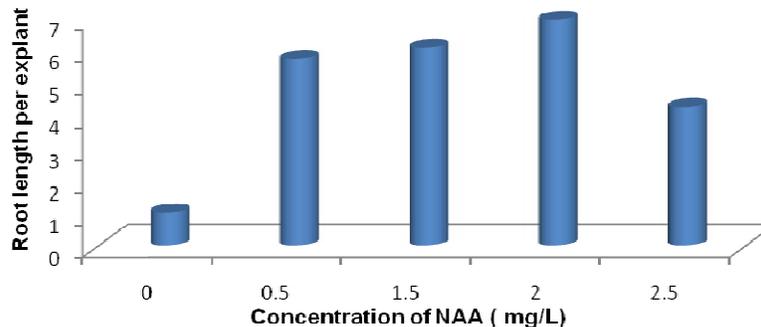


Fig. 6. Effects of NAA on length of roots (cm) in papaya

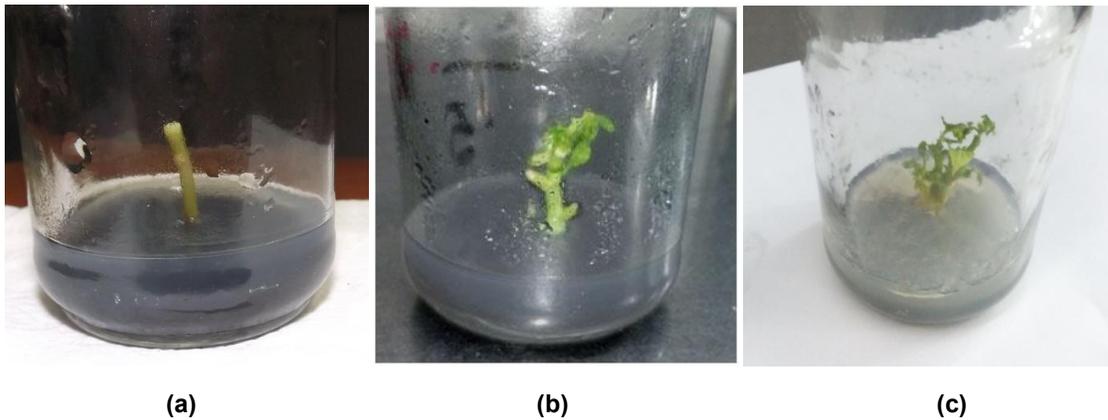


Plate 1. *In vitro* shoot regeneration of papaya in MS media with 1.0 mg/L BA treatments and 0.1% activated charcoal. (a) Inoculated Shoot tip, (b) & (c) Shoot multiplication



Plate 2. Effect of 1.0 mg/L BA + 0.75 mg/L KIN on shoot regeneration of papaya



Plate 3. Effect of 2.0 mg/L NAA on root regeneration in papaya

3.4 Acclimatization of Plantlets

The results of acclimatization or 'hardening' have been presented in Table 2 and Plate 3. After 35 days of culture on rooting media, the plantlets were taken for acclimatization.

Then the plantlets were shifted to shade house with less humidity (70% RH) and indirect sunlight. In the shade house, the top of the pots was covered with a transparent plastic sheet and grew at room temperature for 14 days with periodic irrigation (2 days interval). In these

Table 2. Survival rate of *In vitro* regenerated plants of papaya

Acclimatization	No. of plants transplanted	Duration of observation	No. of plants survived	Survival rate (%)
In controlled environment	20	15 days	16	80
In field condition	20	30 days	15	75

conditions, 80% of the plantlets showed survival (Table 2). After 3 weeks, the plantlets were transferred to the soil following de-potting and potting into different pots of bigger size. The plants were watered periodically whenever necessary. In open atmosphere, the survival rate was 75% (Table 2). It was also revealed that regenerated plants were morphologically similar to the mother plant. Bhandari et al. [17] observed plantlets that were transferred to the plastic pots in poly house showed 90% survival and under shade house (50%) it was found 80%. Baksha et al. [19] noticed well-developed rooted plantlets successfully transferred to the soil with 70% survival.



Plate 4. Acclimatization of regenerated plantlets of papaya

4. CONCLUSION

Findings of the present study showed that *in vitro* regeneration is an effective method in the proliferation of papaya. Shoot formation was best performed in 1.0 mg/L BA whereas in combine treatments 1.0 mg/L BA+0.75 mg/L KIN showed their best response. Root formation was significantly better in 2.0 mg/L NAA. The protocol thus optimized for the plant regeneration of local papaya variety and it provides a reliable propagation technique and will be of great use in genetic engineering or in a molecular breeding program for improvement of papaya genotypes.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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