



EFFECT OF SEASON AND GROWTH REGULATORS ON *IN VITRO* PROPAGATION OF JACKFRUIT (*ARTOCARPUS HETEROPHYLLUS* LAM)

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Abstract: The present study was conducted at the Laboratory of Plant Tissue Culture of Agrotechnology Discipline, Khulna University, Khulna, during the period from September, 2004 to May, 2005 with a view to study the effect of time of the year and growth regulators on *in vitro* propagation of jackfruit. Shoot tips from fresh sprouts on the trunk of mature jackfruit trees (*Artocarpus heterophyllus* Lam.) were used as explants. The explants were collected at three different times of the year from the same jackfruit plants viz. 5/09/04, 18/10/04, 5/01/05. The collected explants were cultured on MS medium supplemented with NAA-0.5 mg^l⁻¹ and fortified with different levels (1.0, 1.5 and 2.0 mg^l⁻¹) of BA to study the proliferation and multiplication of shoots from the cultured shoot tips and their subsequent growth and development. The *in vitro* shoot bud proliferation, multiplication and survivability were found better when the explants were cultured in January. BA at a concentration of 1.5 mg^l⁻¹ was found most suitable for shoot proliferation and multiplication. And all the three concentrations of BA varied significantly. The survivability of the proliferated shoot buds was found better in concentration of BA at 1.0 and 1.5 mg^l⁻¹.

Key words: Growth regulators, *In Vitro* propagation, Jackfruit

Introduction

Jackfruit (*Artocarpus heterophyllus* Lam.) is the national fruit of Bangladesh and is one of the most common, important and delicious fruits in the country. It ranks third in area next to mango and banana and second in production (Anon, 2000). Jackfruit is a highly cross pollinated crop due to its monoecious habit, and plants raised from seed never bear fruits true to the type of the mother plant. The seeds are recalcitrant and difficult to germinate even after a short period of storage (Singh, 1986; Samadder, 1990).

Propagation of jackfruit by air layering, approach grafting, and budding on seedling root stock have been reported successful. But these clonal propagation methods are slow, laborious and very expensive with many limitations and could not be recommended for effective and commercial multiplication (Dhar, 1998). On the other hand *in vitro* propagation of fruit and forest trees offer the advantage of fast multiplication rates (Mott, 1981). The application of micropropagation techniques to the fruit crops is likely to continue in future in the production of new cultivars, difficult to propagate elite genotypes, and large quantities of root stocks (Hammerschlag, 1986).

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The plants produced through this advanced technique also provide a method for storing clonal materials in a tissue bank for future use (Prokash, 1993).

Tissue culture methods for the propagation of jackfruit were introduced by several workers using various plant parts and a number of media. Roy *et al.* (1990) cultured nodal explants of *Artocarpus heterophyllus* on MS medium and induced to form multiple shoots when supplemented with 1.0 mg l⁻¹ BAP and 0.5 mg l⁻¹ kinetin. Amin and Jaiswal (1993) reported that a tissue culture technique of rapid vegetative propagation of mature jackfruit trees using apical bud culture had been developed.

According to reports concerning tissue culture, success of *in vitro* propagation of jackfruit depends on the season, when explants are collected and on the source of explants, physiological state of plant and nutrient environment. In this circumstance an attempt has been made in the present study to investigate the effect of time of the year and growth regulators on shoot proliferation and subsequent development of jackfruit shoot from matured tree.

Materials and Methods

The experiment was carried out in the Tissue culture Laboratory of Agrotechnology Discipline, Khulna University, Khulna, Bangladesh, during the period from September 2004 to May 2005. The plant materials used in this study were the shoot apices and axillary buds (4-6 cm), which were collected from the selected mature trees of Jackfruit (*Artocarpus heterophyllus* Lam.) from Khulna University campus. The shoot tips were collected at the same day of explantation during morning hours. Explants were cultured on agarified MS (Murashige and Skoog, 1962) medium, supplemented with 0.5 mg l⁻¹ NAA (α -Naphthalene Acetic Acid) fortified with different levels of BA (6-Benzyladenine) *viz.* 1.0, 1.5, and 2.0 mg l⁻¹. Seven g l⁻¹ agar-agar, 30 g l⁻¹ sucrose, different ratio of auxin and cytokinin for shoot proliferation and multiplication of proliferated shoots.

Preparation of explants: In jackfruit, the apical buds remain covered with a cap like structure called stipule. Unopened buds with one or two leaves along with shoot tip were collected from the selected plant and placed in water and then brought into the laboratory and used as plant materials. After removing the expanded leaves, the materials were washed thoroughly under running tap water. The materials were then suspended in 0.7% PVP solution, an antiseptic plus detergent containing 2% sucrose and were agitated by a magnetic stirrer for 15 minutes. They were then washed thoroughly to remove PVP. After that the plant materials were transferred to the laminar air-flow cabinet. Then the plant materials were dipped in 70% ethanol for 30 seconds and immediately washed with autoclaved sterilized distilled water. This was followed by surface sterilization with 0.2% mercuric chloride (HgCl₂) for 10 minutes followed by washing with sterile double distilled water giving 3-5 changes. The surface water of treated material were then dried with the autoclaved tissue paper and kept for few minutes in laminar air-flow on an open Petridish for final drying (Dhar, 1998).

Inoculation of explants for shoot proliferation The explants were prepared by removing the outer covering of the green stipules and excised the inner buds enclosed within the creamy white stipules. The excised explants were then placed vertically on the surface of the medium. The excised explants were cultured with the help of sterilized forceps into the conical flasks (100 ml) containing 30 ml of nutrient medium per conical flask. In each conical flask a single explant was inoculated during first and subsequent sub-cultures. For each treatment 15 explants were cultured for 1st culture, and for subsequent sub-cultures.

Incubation of explants for shoot proliferation: After inoculation of the explants, culture vessels were kept in a growth chamber with 16 hours photoperiod at about 2000 lux and at a temperature of 25±1 °C. Proliferation of the shoot started within 7-10 days after inoculation. The explants were sub cultured twice on the same medium at 30 days interval. Then finally nodal segments were isolated aseptically from the proliferated shoots and cultured on the same medium.

Experimental design, data collection and statistical analysis: The study was conducted following Completely Randomized Design (CRD). Data on number of shoot proliferated, average number of explants

forming callus, average number of nodes per plant, average shoot length, explant proliferation percentage, callus production percentage, relative amount of callus, average number of shoot buds per explants and survivability of cultured shoots were recorded. The means of all the treatments were calculated and the analysis of variances (ANOVA) for all the characters were performed by F-test. The means of treatments were compared by Duncans Multiple Range Test (Gomez and Gomez, 1984).

Results

Effect of time of the year on survivability of shoot bud and their proliferation: It is apparent from the result of the present study that the explants collected and inoculated on September 5, 2004 and October, 2004 did not show any response in culture and ultimately all the explants were perished (Table 1). On the other hand, 95.56% of the explants, collected and inoculated on January 5, 2005 maintaining the same media and cultural condition, survived and responded to proliferation. Amin and Jaiswal (1993) reported that November to January is the best time for initiation of cultures from the field grown jackfruit trees, which is in agreement with the present study.

Effect of different levels of BA on shoot proliferation and callus induction: The effect of the different concentration of BA on shoot proliferation in inoculated shoot buds was significant (Table 2). It was evident from the results that 100% shoot buds were proliferated on the medium supplemented with BA of 1 mg l⁻¹ and 1.5 mg l⁻¹ whereas 87.5% inoculated shoots were proliferated on the medium containing BA of 2 mg l⁻¹.

Table 1. Effect of time of the year on survivability of shoot buds and their subsequent proliferation.

Date	Explants inoculated	Explants proliferated	% proliferated
5/09/04	105	0b	0b
18/10/04	60	0b	0b
5/01/05	45	43a	95.56a
Level of significance		**	**

** p = <0.01

Average number of shoot buds per plant: There was significant differences among the treatments in respect to average number of shoot buds per explant (Table 3). The highest number of shoot bud per explant (2.33) was obtained from treatment no. 2 (NAA 0.5 mg l⁻¹ + BA 1.5 mg l⁻¹) followed by (1.47) in the treatment no. 3 (NAA 0.5 mg l⁻¹ + BA 2 mg l⁻¹) and that was the lowest (0.87) in treatment no. 1 (NAA 0.5 mg l⁻¹ + BA 1 mg l⁻¹). This is in partial agreement with Dhar (1998) who reported that 3-4 shoots proliferated per explant in the supplemented medium of NAA and BA.

Table 2. Effect of different levels of BA on shoot proliferation and callus induction in shoot bud culture of Jackfruit.

Concentration of BA (mg l ⁻¹)	Explant proliferated (%)	Explant producing callus (%)	Relative amount of callus
NAA-0.5 + BA-1.0	100a	23.33b	Slight
NAA-0.5 + BA-1.5	100a	46.98a	High
NAA-0.5 + BA-2.0	87.5b	40.00a	Moderate
Level of significance	*	*	

* p = <0.05

Average length of shoot: The concentration of BA showed significant effect on average length of shoot (Table 3). The longest shoot (1.45 cm) was found in treatment no. 2 (NAA 0.5 mg l⁻¹ + BA 1.5 mg l⁻¹) and the shortest shoot (1.17 cm) was found in treatment no. 1 (NAA 0.5 mg l⁻¹ + BA 1.0 mg l⁻¹). The present experiment revealed that the highest length per explant was recorded from the medium which was supplemented with 1.5 mg l⁻¹ BA. This is in agreement with the findings of Dhar (1998) who reported that longer shoots were recorded in 1.0-1.5 mgL⁻¹ BA supplemented medium with or without auxin.

Average number of nodes per explant: The average number of nodes per explants was significantly varied with the concentration of growth regulators (Table 3). The maximum number of nodes per explant (3.0) was observed in treatment no. 2 (NAA 0.5 mg l⁻¹ + BA 1.5 mg l⁻¹)

followed by (2.8) treatment no. 3 (NAA 0.5 mg l⁻¹ + BA 2 mg l⁻¹) and the minimum number of nodes (1.86) was found in treatment no. 1 (NAA 0.5 mg l⁻¹ + BA 1 mg l⁻¹).

Survivability of proliferated shoot buds:

The concentration of growth regulators had significant effects on survivability of proliferated shoot buds both before and after sub-culture (Table 4). The survivability of shoot buds decreased with the increase of concentration of cytokinin. The highest survivability was recorded from T₁ (NAA - 0.5 mg l⁻¹ + BA - 1.0 mg l⁻¹) followed by T₂ (NAA 0.5 mg l⁻¹ + BA 1.5 mg l⁻¹) and the lowest in T₃ (NAA 0.5 mg l⁻¹ + BA 2 mg l⁻¹). Similar results were also observed by Amin and Jaiswal (1993) in their experiment.

Discussion

The present experiment revealed that season when the shoot bud of jackfruit collected and inoculated *in vitro*, played a vital role in the survivability of shoot buds and their proliferation. It was found that explants inoculated on September 5, 2004 and October 18, 2004 did not proliferate at all, while explants collected on January 5, 2005 from the same plants responded to proliferation. Amin and Jaiswal (1993) reported that November to January is the best time for initiation cultures from the field grown jackfruit trees, which is in agreement with the present study.

Three levels of BA were used in the nutrient medium to verify their effect on shoot proliferation and caulogenesis. It was found that concentration of BA @ 1.0 and 1.5 mg l⁻¹ performed better in shoot proliferation. On these media 100% inoculated shoot buds responded to proliferation. When the concentration of BA increased to 2.0 mg l⁻¹, proliferation was declined. Similar situation was also observed by Amin and Jaiswal (1993) in their experiment.

Average number of shoots per explant, average number of nodes per explant and average length of shoots per explant were observed almost similar in the shoot inoculated on three different media. However, maximum number and length of shoots were found from the shoot buds cultured on the medium containing 1.5 mg l⁻¹ of BA. It was evident from the result of the present study that, the highest number of shoot buds per explant (2.33) were in the medium, supplemented with BA 1.5 mg l⁻¹. This is in agreement with Dhar (1998) who reported that 3-4 shoots proliferated per explant. The present experiment revealed that the highest length per explant was recorded from the medium which was supplemented with 1.5 mg l⁻¹ BA. This is in agreement with the findings of Dhar (1998) who reported that longer shoots were recorded in 1.0-1.5 mg l⁻¹ of BA supplemented medium with or without auxin.

Conclusion

The results revealed from the present study that micropropagation of mature jackfruit trees for shoot proliferation as well as shoot multiplication through tissue culture is possible and the use of BA is the most effective cytokinin with NAA concentration for the successful shoot tip culture. In the light of the observed results, 1.5 mgL⁻¹ of BA was found to be the suitable concentration for

Table 3. Effect of different concentration of BA on caulogenesis in the apical buds.

Concentration of BA (mg l ⁻¹)	Mean number of shoot buds per explant	Mean length of shoot (cm)	Mean number of node per explant
NAA-0.5 + BA-1.0	0.87b	1.17b	1.86b
NAA-0.5 + BA-1.5	2.33a	1.45a	3.0a
NAA-0.5 + BA-2.0	1.47ab	1.23b	2.80a
Level of significance	**	**	**

** p = <0.01

Table 4. Survivability (%) of the proliferated shoot buds before and after the treatments.

Treatment	Before	After
T ₁	100a	95a
T ₂	97b	85b
T ₃	95b	79c
p =	<0.01	<0.01

T₁ = NAA 0.5 mg l⁻¹ + BA 1.0 mg l⁻¹;
T₂ = NAA 0.5 mg l⁻¹ + BA 1.5 mg l⁻¹;
T₃ = NAA 0.5 mg l⁻¹ + BA 2.0 mg l⁻¹

high frequency of multiple shoot induction and shoot proliferation. The present study also proved that season has marked influence on shoot proliferation. It was evident from the present study that the month January was significantly influences the shoot proliferation and multiplication which results 95.56% shoot proliferation.

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