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Study of in Vitro Shoot Production of Off-season Jackfruit (Artocarpus Heterophyllus Lam)

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ABSTRACT

In vitro shoot production efficiency of off-season jackfruit (var. BARI jackfruit -2) plant was observed. There were 2 factors in this experiment. Factor A consisted of four benzyl amino purine (BAP) concentrations and factor B consisted of two nepthalene acetic acid (NAA) concentrations. Four BAP concentrations were 0.0 (Control), 1.0, 2.0 and 4.0 mg-L of BAP and two NAA concentrations were 1.0 and 2.0 mg-1 of NAA. The highest (85.36) percentage of explants regenerated in MS medium supplemented with the highest (4.00 mg-1) BAP concentration. The medium when fortified with 2.00 mg-1 BAP took the shortest (8.25 days) time. The highest (4.90 and 5.05, respectively) number of multiple shoots at both subcultures were obtained from the medium when enriched with 4.0 mg-1 BAP. Higher NAA concentration (2.00 mg-1) showed better performance than lower one. Number of multiple shoots increased at third subculture than second subculture in respect to both NAA concentrations. The shortest (8.17 days) time to shoot initiation was taken when 2.00 mg-1 each of BAP and NAA interacted with each other in MS medium. The longest (4.8 cm) micro shoots were obtained from the medium fortified with 4.00 mg-1 BAP and 2.00 mg/l NAA, respectively.

Keywords: *Jackfruit; Multiple Shoot; In Vitro; Shoot Tip; Subculture.*

1.0 Introduction

Jackfruit (Artocarpus heterophyllus L.) is a monoecious cross pollinated fruit tree of the tropical and sub-tropical region (Ali et al., 2017). It is under the family moraceae is the largest among the edible fruits (Bose et al., 2002). It is an evergreen, medium – sized, latex producing tree with a somatic chromosome number of 2n=4X=56. The main jackfruit producing countries are Bangladesh, India, Pakistan, Indonesia, Malaysia and Thailand (Ali et al., 2017). Jackfruit considered as a good source of carbohydrate, proteins, vitamins and minerals (Ashrafuzzaman et al., 2012). It is the national fruit of bangladesh and is one of the most common, nutritious and delicious fruit of this country. Jackfruit ranks third in area next to mango and banana and second in production (Anon, 2000). In bangladesh it is extensively grown in Dhaka, Mymensingh, Sylhet and Chittagong Hill Tracts. It is a costly fruit compared to others and more costly during off-season. There is a off-season jackfruit variety in our country released from BARI which produces fruits during off-season i.e., January to April.

Jackfruit is a highly cross pollinated crop due to its monoecious habit and plants produced from seed never be true to the mother plant. Jackfruit can be propagated by air layering, approach grafting and budding. But these conventional propagation methods are slow, laborious and expensive with many limitations and may not be recommended for effective and commercial multiplication (Dhar, 1998). Without these, since there is original mother plant (OMT) of BARI jackfruit-2 in our country, so we will have to multiply it immediately for rapid extension. The advantages of in vitro propagation is that it offers fast multiplication rates (Mott, 1981). Chawla (2002) mentioned the significant advantages of micropropagation by which a large number of plants can be produced from a single individual in a relatively short span of time and space. There are advantages of tissue culture for rapid vegetative propagation of mature jackfruit trees using apical bud. Keeping in mind the above facts, the present research works was taken to develop and establish a rapid in vitro multiplication protocol for off-season jackfruit variety.

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2.0 Materials and Methods

2.1 Plant material

Shoot tips of BARI jackfruit – 2 were used as explants.

2.2 Methods

The experiment was carried out at the Biotechnology Laboratory under the Department of Genetic engineering, University of Chittagong, Chittagong-4331. There were 2 factors in this experiment. Factor A consisted of four benzyl amino purine (BAP) concentrations and factor B consisted of two concentrations of nepthalene acetic acid (NAA). Four BAP concentrations were 0.0 (Control), 1.0, 2.0 and 4.0 mg^{-1} of BAP and two NAA concentrations were 1.0 and 2.0 mg^{-1} of NAA. So total number of treatments were 8. Each treatment consisted of 6 test tubes/vials and replicated 3 times. The experiment was set under Completely Randomized Design (CRD). A nutrient medium for plant regeneration usually consists of organic and inorganic salts, irons, a carbon source, some vitamins and growth regulators. In this study, MS (Murashige and Skoog, 1962) medium was used as basal medium for plant regeneration.

Shoot tips were collected from OMT (Original Mother Tree) of BARI jackfruit–2 of HTARS, BARI (Bangladesh Agricultural Research Institute**)**, Ramgarh, Khagrachari Hill District. For establishing the plants in the media only the tender and actively growing shoot tips having 1.00-1.25 cm long were prepared to use as explants. To maintain aseptic condition, precautions were taken in every step of works. All inoculations and aseptic manipulations were carried out in a laminar airflow cabinet. It was usually started half an hour before use. The cabinet was wiped with 70% ethyl alcohol (C_2H_5OH) to reduce the chances of contamination. The inoculating instruments like scalpels, forceps etc. were sterilized. Other required materials like distilled water, hard papers etc. were sterilized by autoclave. Hands were properly washed with soap before starting work in laminar airflow cabinet. During operation, hands and cabinet base were rubbed with 70% ethyl alcohol frequently for maintaining clean condition. To obtain possible contamination free condition in clean bench proper care was taken during explant preparation. Shoot tips were prepared inside the laminar airflow cabinet using a fine sterile forcep and scalpel. The excised shoot tips were then inoculated on to the culture test tubes/vials containing various concentrations and combinations of BAP and NAA for in vitro multiple shoot regeneration. The physical conditions for growth and development of cultures were maintained at the temperature of 25 ± 1^0C and a light intensity of 2000-3000 lux provided by fluorescent tube. The photoperiod was maintained at 16 hours light and 8 hours dark (16L/8D) and the relative humidity was 60-70%.

Successful shoot formation became evident when small green fresh leaves began to emerge. It was the first sign of regeneration. These tiny leaves, when developed in their actual shape, were transferred into fresh media containing the same hormonal concentration and combinations for further proliferation and development of shoots. First subculture was carried out at 25 days after shoot initiation. Second and third subculture were carried out at 50 and 75 days after shoot initiation, respectively. Multiple shoots were counted at second and third subculture only. The regenerated multiple shoots were carefully removed from the culture tubes/vials and placed on a sterile hard paper. Each shoot was cut from the basal end and was transferred to new media containing different concentrations and combinations of BAP and NAA for further multiple shoot induction.

Data on number of explants regenerated, days to shoot initiation, percentage of explants regenerated, number of multiple shoots per explant at second and third subculture and shoot length (cm) were recorded. The data were analyzed using MSTAT-C statistical software. Differences among the means were compared following Duncan's Multiple Range Test (DMRT) at 5% level of significance.

3.0 Results and Discussion

3.1 Influence of cytokinin (BAP)

Significant differences were observed among the BAP concentrations in all the parameters studied (Table 1). The highest (85.36 %) percentage of explants regenerated in MS medium supplemented with the highest (4.00 mg^{-1}) BAP concentration. The lowest (36.78 %) percentage of explants regenerated from the BAP free MS media. The same media (0.00 mg^{-1}) took the highest (11.87 days) time to shoot initiation. Figure 1 was showed the shoot initiation in inoculated jackfruit explant. But the medium when

fortified with 2.00 mg^{-1} BAP took the shortest (8.25 days) time. In case of multiple shoot production, the highest BAP concentration was found superior to all other tested concentrations. Shoot production was little bit increased in third subculture. After three weeks of explant inoculation Ashrafuzzaman et al. (2012) carried out first subculture and they reported that MS medium fortified with 2 mg/l BAP produced the highest significant values of shoot inducing explants (8) which followed by 3 mg/l (6), 4 mg/l (4), 1 mg/l (5) and 0 mg/l (0) of BAP. Ali et al. (2017) was counted the highest number of shoots (5.12) when MS medium supplimented only with BAP @ 2.0 mg/l. Amany et al. (2007) mentioned that medium when enriched with 3.0 mg^{-1} BAP produced the highest (43.67) number of multiple shoots. They were counted the multiple shoots twelve weeks after shoot inoculation.

This finding differed with present finding as the highest number of multiple shoots produced in this investigation in the medium supplemented with 4.0 mg^{-1} BAP. Actually more success obtain from woody species like jackfruit is difficult due to different contaminations, phenolic compound formation, vitrification etc. The longest (4.83 cm) micro shoot was measured in the medium fortified with the highest BAP concentration followed by 2.00 mg^{-1} BAP (4.67 cm).

Figure 1: Shoot Initiation from Shoot Tip Explant of Jackfruit

The lowest BAP concentration produced the shortest micro shoot (Table 1). Ashrafuzzaman et al. (2012) recorded the highest shoot length (3.84 cm) from MS medium supplimented with BAP @ 2.0 mg/l. Amany et al. (2007) reported that using 3.0 mg^{-1} BA in the medium they obtained the highest (2.53 cm) shoot length. Present finding agreed with the finding of Ashrafuzzaman et al. (2012) but differed with Amany et al. (2007). The cytokinins are generally supplemented to a culture medium to stimulate cell division, to induce shoot formation and axillary shoot proliferation and to inhibit root formation (Torres, 1989).

Ara et al. (2000) was reported that the best response regarding shoot multiplication was obtained when medium was enriched with 1.5 mg/l BAP. They also reported that 1.5 mg/l BAP was the best for shoot growth and proliferation, followed by 1.0 mg/l BAP. The shoot multiplication rate increased with the number of subculture increased when the woody plant medium was fortified with 1.5 mg/l BAP after two and half months later. They obtained 4-5 shoots per node culture which supports the present findings from the point of view that in this investigation 4.91 shoots were recorded after same time.

Table 1: Shoot Regeneration and Multiple Shoot Production from Shoot Tips of Jackfruit as Influenced by Different Concentrations of BAP

Concentration of BAP (mg/l)	% of explants regenerated	Days to shoot initiation	Number of shoots/ explant at 2nd subculture	Number of shoots/ explant at 3rd subculture	Length (cm)
0.00	36.78 d	11.87 a	0.65 d	0.91 c	0.68 c
1.00	44.19 c	10.00 b	2.62 c	3.64 b	3.67 b
2.00	75.79 b	8.25 d	4.65 b	4.91 a	4.67 a
4.00	85.36 a	8.92 c	4.90 a	5.05 a	4.83 a

Khan et al. (2010) mentioned that BAP @ 1.5 mg/l gave the maximum number of shoots followed by 2.0 mg/l BAP. These findings almost near about present findings. In an investigation of in vitro jackfruit propagation Harb et al. (2015) reported that the highest (6.6) number of shoots were obtained when medium supplemented wiyh 2.0 mg/l BAP + 0.5 mg/l Kn. In present work, MS medium fortified with 4.0 mg/l BAP produced maximum number of shoots at both subcultures. But Harb et al. (2015) also mentioned that BA @ 5.0 mg/l reduced the number of shoots. In this investigation, we didn't use 5.0 mg/l BAP concentration, so the effect of this concentration was unknown. Cytokinin enriched medium reinforces regenerative responses like somatic embryogenesis, adventitious shoot formation

in callus (indirectly) or directly from explants and axillary shoot proliferation, depending on the source of explants and the genotypes (Narayanaswamy, 1994).

Means in a column followed by uncommon letter (s) varied significantly at 5% level of significance.

3.2 Influence of auxin (NAA)

From the table 2 it was revealed that higher NAA concentration (2.00 mg^{-1}) showed better performance than lower one. Both concentrations showed significant difference in all the parameters studied except percentage of explants regenerated and days to shoot initiation. Number of multiple shoots increased at third subculture than second subculture in respect to both NAA concentrations. Amany et al. (2007) reported that concerning the effect of different NAA concentrations, they obtained the highest (45.00) number of multiple shoots using 0.1 mg^{-1} NAA which is differed with present findings.

Ara et al. (2000) were observed no shoot proliferation in medium when supplimented with either BAP + NAA @ 1.0 mg/l or Kn + NAA @ 1.0 mg/l. These findings differed with present findings from the point of view that in present investigation shoots were produced when MS medium supplemented with 1.0 and 2.0 mg/l NAA, respectively. Actually NAA and other auxins generally added in a culture medium to stimulate callus production and cell growth, to initiate shoots and particularly roots and to induce somatic embryogenesis and stimulate growth from shoot apices and shoot tip cultures (Torres, 1989).

Table 2: Shoot Regeneration and Multiple Shoot Production from Shoot Tips of Jackfruit as Influenced by Different Concentrations of NAA

Concentration of NAA (mg^{-1})	% of explants regenerated	Days to shoot initiation	Number of shoots/explant at 2nd subculture	Number of shoots/explant at 3rd subculture	Length (cm)
1.00	59.37	9.86	2.96	3.33	3.25
2.00	61.69	9.66	3.45	3.93	3.64
Level of significance	ns	ns	**	**	**
CV (%)	4.91	4.59	3.06	3.44	6.20

Means in a column followed by uncommon letter (s) varied significantly at 5% level of significance.

Table 3: Interaction Among Different Concentrations of BAP and NAA on Shoot Regeneration and Multiple Shoot Production from Shoot Tips of Jackfruit

Concentration of BAP (mg^{-1})	Concentration of NAA (mg^{-1})	% of explants regenerated	Days to shoot initiation	Number of shoots/explant at 2nd subculture	Number of shoots/explant at 3rd subculture
0.00	1.00	35.86 e	11.96 a	0.00 f	0.00 e
	2.00	37.69 de	11.78 a	1.30 e	1.82 d
1.00	1.00	42.38 cd	10.06 b	2.47 d	3.56 c
	2.00	46.00 c	9.94 b	2.78 c	3.71 c
2.00	1.00	74.45 b	8.33 cd	4.51 b	4.76 b
	2.00	77.12 b	8.17 d	4.79 a	5.06 a
4.00	1.00	84.77 a	9.10 c	4.88 a	4.98 ab
	2.00	85.96 a	8.74 cd	4.92 a	5.13 a

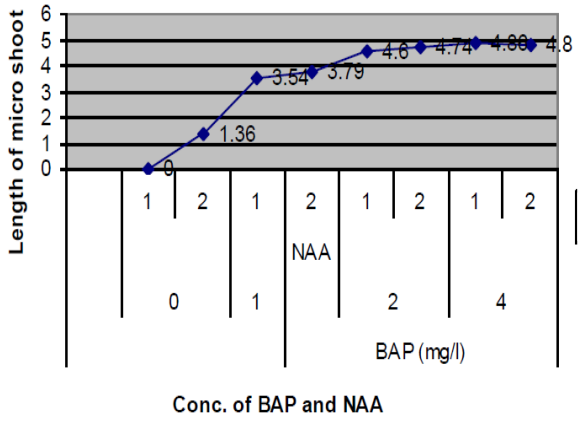
Figure 2: Shoot Production in MS Medium Fortified with 4.00 mg/l BAP and 2.00 mg/l NAA

Means in a column followed by uncommon letter (s) varied significantly at 5% level of significance.

3.3 Interaction between BAP and NAA

Interaction between BAP and NAA was found significant in all the parameters studied (Table 3). The highest (85.96 %) percentage of explants regenerated was observed in the medium, when fortified with the highest concentration of both BAP and NAA (4.00 and 2.00 mg^{-1}, respectively).

Figure 3: Length (cm) of Jackfruit Micro Shoots as Influenced by Different Concentrations of BAP and NAA



The lowest (35.86 %) percentage of explants regenerated was observed in the medium, when fortified only with lower NAA concentration (1.00 mg^{-1}). The shortest (8.17 days) time to shoot initiation was taken when 2.00 mg^{-1} each of BAP and NAA interacted with each other in MS medium. In both subcultures, the highest (4.92 and 5.13, respectively) number of multiple shoots were produced in the medium supplemented with the highest BAP and NAA concentration. Figure 2 showed the shoots of off season jackfruit. The longest (4.8 cm) micro shoots were obtained from the medium fortified with 4.00 mg^{-1} BAP and 2.00 mg^{-1} NAA, respectively (Fig. 3). Ali et al. (2017) was mentioned that MS medium supplemented with 2.5 mg/l BAP + 1 mg/l NAA produced 4.54 shoots per explant with a mean shoot length of 0.88 cm. Ali et al. (2017) was reported that the combination of BAP and NAA resulted in highly significant ($P<0.01$) for all the response variables; mean shoot number per explant, meean shoot length and mean leaf number per explant. They also reported that 2 mg/l BAP alone was found to be the best with a mean shoot number of 5.12 and length of 0.89 cm. They commented that axillary shoot proliferation enhancement observed on the shoots that were cultured on BAP fortified MS media may be due to the role of cytokinin in overcoming apical dominance.

4.0 Conclusions

From the above findings it was concluded that MS medium fortified with 4.00 mg/l BAP and 2.00 mg/l NAA was the best for in vitro shoot multiplication of off-season jackfruit plant. This protocol could be used for further micropropagation and genetic transformation studies. This study also would be helpful for cryopreservation program of jackfruit and other species of moraceae family.

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