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In vitro Rapid Propagation of Jackfruit (*Artcarpus heterophyllus* Lam.)

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Abstract: Propagation of jackfruit plant (*Artcarpus heterophyllus* Lam.) from seeds is not widely accepted, because of high heterozygosis. To maintain the true type quality fruit; tissue culture technique could be used for the vegetative propagation of jackfruit throughout the year. Healthy and juvenile shoot tips and nodal segments were collected during the four different seasons (winter, spring, summer and autumn) and were cultured on Murashige and Skoog (MS) medium. Explants, which were collected in winter gave the highest survival percentage (100%) without browning of explants. Also, the explants collected in winter gave the highest growth to survival percentage (90 and 50% for shoot tips and nodal segments, respectively). Shoots could be multiplied on MS medium supplemented with 6-bezylaminopurine (BA) (0.0, 1.0, 2.0 and 5.0 mg/l) and kinetin (Kn) (0.0, 0.5, 1.0, 1.5 and 2.0 mg/l). The maximum number of proliferated shoots (6.6 shoots/explant) was obtained on MS medium supplemented with 2.0 mg/l BA + 0.5 mg/l Kn. The highest increase in shoot length (5.14 cm) was recorded on medium containing 3.0 mg/l gibberellic acid (GA_3). These shoots were then cultured on half-strength MS medium supplemented with indole-3 butyric acid (IBA) and β-naphthalene acetic acid (NAA) at concentrations of 0.5 and 1.0 mg/l, both auxins were supplemented to the medium either individually or in combination. It was observed that the medium containing 1.0 mg/l of both IBA and NAA gave the highest rooting percentage (80%), average number of roots/explant (4.3) and average root length (4.0 cm).

Key words: Moraceae · Jackfruit · *Artocarpus heterophyllus* · *In vitro* culture · Vegetative Propagation · Shoot Tip · Nodal Segment

INTRODUCTION

Jackfruit (*Artcarpus heterophyllus*) belongs to the family Moraceae along with other fruit bearing plants like fig (*Ficus carica*) and mulberry (*Morusalba*) (Fig. 1a). It is often confused with the closely related species *Artcarpus integer*. Evergreen leaves are oblong, oval or liptic in shape, 10-15 cm in length, alternate, glossy and dark green in color. The juvenile leaves are lobed. The tree is monoecious, producing male and female flowers. The male flowers are produced amongst the leaves above the female flowers and when mature, become covered in pollen that falls rapidly after flowering. The female flowers are borne on short twigs that develop from the trunk, branches and sometimes from below the soil level at the base of older trees. Jackfruit is the largest tree-borne fruit in the world, reaching up to 50 kg in weight and 60-90 cm in length [1]. A mature tree produces up to 700 fruits per year, each weight 0.5-50 kg. The rind of the compound fruit is greenish yellow when fully ripe. Inside, the fruit is made up of large, yellow bulbs enclosing oval light-brown seeds. There are 100-500 seeds in a single fruit. Jackfruit pulp is rich in calories (84%), carbohydrates (18.9%), proteins (1.9%), vitamin A (540 IU) as well as being a good source of iron (500.1 mg) and potassium (35 mg) [2]. The juicy pulp or ripe fruit is eaten fresh as a desert or preserved in syrup. The seeds are cooked, roasted or fried. Unripe fruit is consumed as a vegetable. The Chinese consider jackfruit tonic prepared from pulp and seeds are useful in overcoming the influence of alcohol on the digestive system [2]. Jackfruit is native to India and is the most common and popular fruit tree of

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Bangladesh. It was declared as a national fruit of Bangladesh and cultivated in the coastal areas of Pakistan. Jackfruit is widely cultivated in many tropical and subtropical countries of the world [3]. Jackfruit plants flourish best in humid and warm climate [4, 5], such as hills-tops up to an elevation of 1500 m. It is also grows well in arid and warm plains [6]. It requires plenty of soil moisture, open textured deep alluvial soil and good drainage tree roots are very sensitive to water-logging. Well known cultivars of jackfruit are Rudrakshi, Singapore, Ceylon, Khaia Allah Abad and Varikka. Jackfruit is a cross pollinated plant and seed is not suitable for true to type plant multiplication. However, the plant is generally grown from seeds, which are difficult to germinate even after a short period of storage. Seeds are viviparous in nature [7]; however high variation is found among the trees of seedling origin. Propagation of jackfruit plant from seeds is not widely accepted because of high heterozygosis [8, 9]. Clonal propagation through grafting of selected genotype is highly desirable but the number of plants produced by these conventional methods is relatively low [9, 10]. The application of tissue culture methods for improvement and large-scale propagation of fruit trees has been well demonstrated. The successful *in vitro* propagation of jackfruit seedlings has also been demonstrated [11, 12]. Regeneration of plantlets from bud and nodal explants of mature jackfruit trees has also been reported [13-15].

The present investigation was therefore undertaken to establish a micropropagation protocol for large-scale production of true to type jackfruit plants as a rare and economically important fruit tree.

MATERIALS AND METHODS

Plant Material and Explant Preparation: The plant material used in this study was obtained from 40-50 year-old tree of jackfruit grown in the Botanical Island, Aswan, Egypt. Explants (shoot tips and segments) were excised from healthy and juvenile shoots and were wrapped with wet paper and transferred in ice box to the lab of Tissue Culture Unit, Plant Genetic Resources Department, Desert Research Center, Cairo, Egypt. Nodal segments and shoot tip explants (2-3 cm long) were washed under running tap water for 60 min to remove the traces of dust and dirt. They were then, swabbed with an alcohol (70% v/ v) followed by two times of rinsing with sterile distilled water in a laminar air flow cabinet. Then were immersed in an aseptic solution of 2.5% sodium hypochlorite (NaOCl) with two drops of tween 20 for 25 min. All traces of the used disinfectants were removed by soaking and rinsing the explants seven times with sterile distilled water.

Nutrient Medium: The basal nutrient Murashige and Skoog (MS) medium containing macro and micro elements was applied throughout the study according to Murashige and Skooge [16], plus 100 mg/l myo-insoitol 0.30 g/ l sucrose and 0.4 mg/l thiamine HCl. The pH value of the nutrient media was adjusted at 5.7 to 5.8 with adding few drops of 0.1 N of NaOH or HCl, prior the addition of 7 g/l DifcoBacto agar. The media were dispensed into 25x150 mm tissue culture tubes, each contained 20 ml of culture medium. Sterilization of the medium was achieved by autoclaving the tubes containing media under pressure of 1.1 kg/ cm^2 and at 120ºC for 20 min. The tubes were transferred to the culture cabinet and left to cool in slanted position till they were used. Cultures were incubated under fluorescent light (2500-3000 lux) at 25°C ± 2 with a 16-hour photoperiod.

Effect of Seasonal Variation on Bud Break: In order to obtain the best regeneration capacity, the shoot tips and nodal segments were collected several times in the four different seasons: spring, summer, autumn and winter. The explants were cultured onto MS medium supplemented with 2 g/l activated charcoal (AC) without any plant growth regulators. The percentage of survived explants, percentage of browning and growth were measured after eight weeks of culture.

Multiplication Stage: Multiplication experiment was carried out using the most vigorous shoots from the establishment stage. Nodal segments (2-3 cm long) containing two axillary buds were cut and cultured on MS medium supplemented with 6-bezylaminopurine (BA) at concentrations of 0.0, 1.0, 2.0 and 5.0 mg/l in combination with kinetin (Kn) at concentrations of 0.0, 0.5, 1.0, 1.5 and 2.0 mg/l. Explants were cultured on these media for six weeks, thereafter; the average shoots number/explant and average shoot length (cm) were recorded. The multiplication cycle consisted of the regular subculture of explants onto fresh medium.

Elongation and Rooting: Individual proliferated shoots were excised and cultured on elongation media of MS basal medium supplemented with 2 g/l AC and different concentrations of gibberellic acid (GA_3) (1, 2 and 3 mg/l).

The increase in length of shoots was determined after 8 weeks of culture. For rooting, individual shoots of 2-3 cm long excised from the elongated shoots were cultured on half-strength MS medium supplemented with 0.5 and 1.0 mg/l indole butyric acid (IBA) and/ or β-naphthalene acetic acid (NAA). Percentage of rooted shoots, number of roots/ shoot and average root length (cm) were recorded after six weeks of culture.

Acclimatization: The rooted plantlets were taken out from tubes, washed to remove agar gel adhered to the roots and transplanted to plastic pots with sand and peat (v/v) for hardening. The plantlets were covered with plastic bags and kept in a chamber at 80% relative humidity, 32±2°C under a 12 hour photoperiod for acclimatization and the survival percentage was recorded.

Statistical Analysis: At least ten cultures were raised for each treatment. The experiments were subjected to completely randomized design. Analysis of variance (ANOVA) and Duncan's multiple range test [17], as modified by Snedecor and Cochran [18], were performed to analyze the obtained data. The differences among averages of the recorded parameters for all treatments were tested for significance at 5% level.

RESULTS AND DISCUSSION

Effect of Different Seasons on *in Vitro* Culture Establishment: The season of explant collection is a critical factor in the establishment and growth of *in vitro* culture [19, 20]. In certain cases, the season of explants collection is more important than the selection of right kind of media. From data presented in Table 1, it is clear that season affects the survival, browning and growth to survival percentages of the sterilized explants of Jackfruit.

Generally, it was noticed that nodal segments and shoot tips of Jackfruit, which were collected in winter surpassed the other seasons and gave the highest survival percentage (100%) and the lowest browning percentage (0.0%) for both shoot tips and nodal segments (Fig. 1b). While, shoot tips and nodal segments taken in summer gave the lowest survival percentage (20 and 10%, respectively and the highest browning percentage (80 and 90%, respectively). Also, the explants collected in winter gave the highest growth to survival percentages of 90 and 50% for shoot tips and nodal segments, respectively. While, the explants collected in summer gave the lowest growth to survival percentage (40 and 10% for shoot tips and nodal segments, respectively). Amin and Jaiswal [21] 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. Micropropagation of mature trees using vegetative parts as explants has been a difficult task due to various factors, like juvenility vs. maturity, inherent slow growing habit, exogenous and endogenous infection, presence of phenolic compounds, long complex life cycles and great genetic variations, etc. [22-25]. *In vitro* propagation of plant species is influenced by factors such as genotype, age and source of initial tissue/organ, which in turn are related to their endogenous hormonal status [26]. Most of the hardwood species form phenolic compounds after wounding, which is also encountered in *Artcarpus lakoocha*. The phenolic substances on oxidation get converted into quinines, which cause tissue blackening and inhibit new *in vitro* morphogenetic

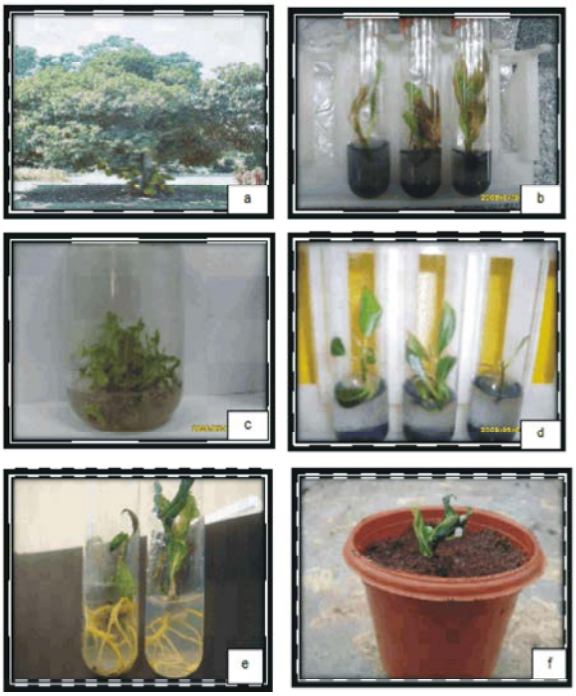

Fig. 1: Vegetative propagation of jackfruit by tissue culture technique

a. Jackfruit tree.
b. Establishment of Jackfruit after 8 weeks.
c. Multiple shoot proliferation after 6 weeks.
d. Elongation of shoots after 8 weeks.
e. *In vitro* rooted plantlets after 6 weeks.
f. A plantlet acclimatized in sand and peat (v/v).

Table 1: Effect of seasonal variations and explant type on survival, browning and growth percentages of *Artocarpus heterophyllus* cultured on MS medium supplemented with AC

	Survival %		Browning %		Growth to survival %	
Season	Shoot tips	Nodal segments	Shoot tips	Nodal segments	Shoot tips	Nodal segments
Winter	100a	100a	0c	0c	90a	50a
Spring	70b	60b	30b	40b	60ab	20b
Summer	20c	10c	80a	90a	40b	10b
Autumn	70b	50b	30b	50b	50b	20b

Table 2: Effect of BA and Kn combinations in MS medium on shoots multiplication of *Artocarpus heterophyllus*

Cytokinins concentration (mg/l)			
BA	Kn	Average number of shoots/ explant	Average shoot length (cm)
0.0	0.0	2.12f	3.7a
1.0	0.5	2.60ef	3.2a
1.0	1.0	3.20def	3.4a
1.0	1.5	3.72cdef	3.8a
1.0	2.0	4.00bdef	3.4a
2.0	0.5	6.60a	2.8a
2.0	1.0	5.60abcd	2.9a
2.0	1.5	6.00abc	3.1a
2.0	2.0	6.26ab	2.8a
5.0	0.5	4.40abcdef	3.4a
5.0	1.0	4.50abcdef	3.4a
5.0	1.5	4.60abcde	1.8a
5.0	2.0	4.20abcdef	1.8a

responses in plants. Phenolics could be minimized by keeping explants right from the time of collection to inoculation in antioxidant solution comprising of ascorbic acid and citric acid [24, 27].

Shoot Multiplication: The shoot initials (2-2.5 cm) obtained from the *in vitro* derived culture were subcultured in MS medium supplemented with combinations of BA (1.0, 2.0 and 5.0 mg/l) and Kn (0.5, 1.0, 1.5 and 2.0 mg/l) for multiplication. Average number of shoots/explant and shoot length are presented in Table 2. The cytokinin free medium gave the least average number of shoots, indicating strong apical dominance. BA and Kn stimulated the production of axillary shoots regardless of their concentrations. On the other hand, BA affected multiple shoots induction more than Kn. A similar observation was reported for *Artocarpus heterophllys* [21], *Ficus religiosa* [28], *Azadirachta indica* [29] and *Ficus benghalensis* [30]. The highest shoot multiplication occurred in the medium supplemented with 2.0 mg/l BA and 0.5 mg/l Kn (6.6 shoots/explant) (Fig. 1c). Higher levels of BA (5.0 mg/l) reduced multiplication of shoots. Increasing Kn concentration to the medium containing 1 mg/l BA enhanced the elongation of shoots (reached 3.8 cm using 1.5 mg/l Kn). Similar observation was found in *Gymneme saylvester* [31] and *Holarrhena antidysenterica* [32].

Shoot Elongation: Plant tissue culture can generally be induced to grow and differentiate without giberellins, although GA_3 may become an essential ingredient of media for culturing cells at low densities [33]. When GA_3 is added to culture media, it often produces effects, which are of a similar nature to those of auxins. Separated individual shoots from multiple shoots were transferred to MS medium augmented with different concentrations of GA_3 (1.0, 2.0 and 3.0 mg/l) for shoot elongation. The highest increase in shoot length (5.14 cm) was recorded on medium containing 3.0 mg/l GA_3(Fig. 1d), followed by 2.0 mg/l GA_3 (5.05 cm). The lowest concentration of GA_3 (1.0 mg/l) caused slight elongation of shoots (Table 3).

Root Initiation: About 5-6 cm long shoots, harvested from *in vitro* elongated shoots, were used for rooting experiment (Table 4). The individual shoots were transferred to rooting media containing half-strength of the basal MS medium supplemented with two auxins, IBA and NAA at concentrations of 0.5 and 1.0 mg/l. Both auxins were supplemented either individually or in combination. The highest rooting percentage (80%), average number of roots/shoot (4.3) and average root length (4.0 cm) were obtained when shoots were cultured on half-strength MS medium supplemented with a combination between IBA and NAA, both at 1.0 mg/l

Table 3: Effect of GA_3 in MS medium on shoot elongation of *Artocarpus heterophyllus*.

	Shoot length (cm)		
GA_3 concentration (mg/l)	Before treatment	After 8 weeks	Increase in length (cm)
0.0	1.45 a	3.56 b	2.11 b
1.0	1.40 a	4.74 b	3.34 b
2.0	1.35 a	6.40 a	5.05 a
3.0	1.40 a	6.54 a	5.14 a

Table 4: Effect of IBA and NAA in ½ MS medium on rooting of *in vitro* derived shoots of *Artocarpus heterophyllus*.

Auxins conc. (mg/l)				
IBA	NAA	Rooting %	No. of roots/ shoot	Average root length (cm)
0.5	0.0	30bc	1.60b	2.20a
1.0	0.0	40bc	1.75b	2.80a
0.0	0.5	10c	1.00b	2.50a
0.0	1.0	30bc	1.00b	2.20a
0.5	0.5	60ab	3.30a	3.60a
1.0	1.0	80a	4.30a	4.00a

(Fig. 1e), while the lowest root induction (10%) and average number of roots/ shoot (1.0) were obtained on half-strength MS medium supplemented with 0.5 mg/l NAA. IBA was more significant and efficient for rooting than NAA. This result is in agreement with that obtained by Abd Alhady [34], who mentioned that IBA gave better response for rooting of *Stevia rebaudina* than NAA. The use of half-strength MS medium for root induction of jackfruit was supported by Beena *et al*. [35] who reported that half-strength MS medium free from growth regulators induced more roots compared to full-strength MS medium in *Ceropagiacan delabrum*. In the root meristem, auxin is implicated in regulating the pattern of cell division and differentiation [36]. According to Puente and Martin [37], if the shoots are competent to root, rooting rate could be increased easily. It has been reported that shoot characteristics, such as size and shoot culture origin fail to attain a stabilized growth phase or apparent rejuvenation and also lead to variable rooting response [38].

Acclimatization: Plantlets regenerated *in vitro* with well-developed root system were transferred to plastic pots containing sand and peat moss mixture (v/v) and were covered with translucent plastic bags to ensure high humidity around the plants. The use of this procedure during the acclimatization phase ensured that most of the plantlets transplanted to *ex vitro* conditions continued to grow vigorously. After two months, when the plastic bags were completely removed, 60% of the plantlets survived in the greenhouse and showed no sign of water stress (Fig. 1f). Thereafter, the regenerated plants showed normal growth. In conclusion, this study describes a protocol for direct shoot multiplication of jackfruit from shoot tips and nodal segment explants. This protocol provides a successful and rapid technique that can be used for large-scale vegetative propagation of jackfruit to produce true to type plants to the mother plant.

- Jackfruit tree.
- Establishment of Jackfruit after 8 weeks.
- Multiple shoot proliferation after 6 weeks.
- Elongation of shoots after 8 weeks.
- *In vitro* rooted plantlets after 6 weeks.
- A plantlet acclimatized in sand and peat (v/v).

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