In vitro shoot initiation of *Artocarpus heterophyllus* Lam. (Jak Fruit) Effect of the explant type and the season of explant collection

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Abstract

A method for rapid propagation of mature Jack fruit was developed. Four types of explants (mature embryos, apical meristems of young seedlings, apices from mature plants and nodal segments) were used. It has been found 88% of young apical meristems produced shoots in Campbell and Durzan (CD) medium compared to 60% in Murashige and Skoog (MS) medium. Only 1/3 of them produced multiple shoots. Shoot initiation from nodal segments was very rare. Mature apices produced callus. Although removal of the sheathing cover around mature buds enhanced the shoot initiation but success rate was low in growth regulator free medium. Embryos respond to the CD medium but not to the MS medium. Embryos from seeds soaked in water for 24 hours produced shoots after 8 weeks of incubation and the success rate was 70% while embryos from dry seeds only produced roots. There was no significant effect of cold storage (refrigeration) for 7 days on shoot initiation from mature embryos (65%) but the ability for shoot induction declines with storage time (55% after 21 days of cold storage).

Mature axillary buds were established in Modified Campbell and Durzan (CD) medium supplemented with 0.5mg/l and IBA. There was a significant difference in the growth performance of shoots according to the period of the year in which explants were collected. Highest (60%) was observed in November-January period. It was only 30% when the explants were collected in February-April or May-July and decreased to 20% in August-October. The shoots produced in November-January showed a higher vigor than those produced in other months. Since Jak fruit show seasonal changes in fruit bearing and shedding of leaves, it can be

suggested that the difference in growth performances of tissues cultured in artificial culture media would have been affected by endogenous rhythms.

Keywords: CD medium, cold storage, multiple shoots, seasonal changes, sheathing cover,

Introduction

Artocarpus heterophyllus is recognized as a versatile multipurpose tree species. There are variety of types which differ from each other from the shape and size of the fruit and the sweetness and colour of the pericarp which is edible. Seed propagation is the cheapest, easiest and most convenient method, but has disadvantages in that the progeny may differ from parent plants due to the high level of cross-pollination. Grafting and vegetative propagation methods also studied for rapid propagation of high quality Jak. Vegetative propagation is useful but has a relatively poor success rate (Gunasena *et al*, 1996). Nagy *et al* (1990) reported that, there was an effect on the month on epicoyl grafting with mature scion shoots. And air layers taken in August were more successful than those in July (Sing *et al*, 1995).

There are records on *in vitro* propagation of *Artocarpus* from embryo culture. Hossain *et al*(1991) induced shoots through callus culture from embryos. Higher concentration of sucrose (35g/l) and 0.1mg/l IAA or IBA induced roots from the callus induced shoots (Roy *et al*, 1992).Physiological age of the explant significantly effect the shoot growth (Rajmohan and Mohankumaran, 1988). Developing tissue culture technique for clonal propagation from mature apices will be very useful for quantitative improvement of the plant. Since period of the explant collection seems to be having an effect on vegetative propagation, explants were colleted from mature plants in different months and studied the growth *in* vitro. Pink, sweet pericarp type (Pink waraka) which is widely used in productionof jam, jelly and syrup and also rare in nature was used in all experiments.

Materials and methods

As the starting material, four types of explants were used (mature embryos, apical meristems from young seedlings, apices from mature plants and nodal segments). Except for embryo culture all other explant types were surface sterilized and 2 - 5mm sized explants were taken. Prepared explants were blotted dried on a sterile filter paper before being transferred into the culture media. To excise mature embryos, seeds were washed thoroughly with running tap

water and surface sterilized with 10% Clorox. Then the seed coat was removed under sterile conditions, and the embryos were excised.

Culture media were prepared with the addition of 3% sucrose and 0.7% w/v agar. The pH of the medium was adjusted to 5.6. All the cultures were incubated at 251C in a 16 hour day. A completely randomize design was used in all experiments. Analysis of variance and Tukey's pairwise comparison was used to analyse the data obtained.

Effect of different treatments to the seed on mature embryo culture:

Seeds were subjected to four different treatments (T1 - T4) in order to induce shoots from embryos. They were cultured in growth regulator free MS or CD medium and response to the culture medium was assessed. Twenty replicates were used in each treatment. Counts were made on the number of explants producing shoots or roots in different time intervals

Different treatments used are as follows:

T1 - Seeds soaked in water for 24 hours

T2 - Dry seeds (air dried)

T3 - Seeds stored in a refrigerator for 7 days

T4 - Seeds stored in a refrigerator for 21 days

Induction of shoots from different explant types:

Apices from young (21-day-old) seedlings, nodal segments of young seedlings, matureapices (axillary buds) from older plants with and without sheathing cover, were cultured in growth regulator free CD and MS media in order to determine the shoot initiation. After 6 weeks of incubation, number of explants producing shoots and callus was counted.

Effect of different growth regulators on shoot induction from mature apices:

As shoot induction from mature apices was very low in growth regulator free media, either NAA or IBA was added to the medium to enhance shoot induction. Apical meristems were established in CD medium with either NAA or IBA (Table 1) in different concentrations (0.5 - 2.0mg/l). Sub cultures were made in every 4 weeks interval in order to reduce the accumulation of phenolic compounds. Elongated shoots were decapitated after 16 weeks to induce elongation of axillary buds.

Effect of the period of the year which explants were collected:

Axillary buds were collected at different months of the year and were cultured in CD medium supplemented with 0.5mg/l IBA after removal of the sheathing cover. Mean number of leaves/shoot, mean leaf width and mean shoot length (mm) were measured. This was continued over a period of three years and the percentage of explants producing shoots were observed.

Results and discussion

Effect of different treatments to the seed on mature embryo culture:

Embryos from seeds soaked in water for 24 hours (T1) produced shoots after 8 weeks of incubation and the success rate was 70%. There was a significant effect on air drying of seeds on shoot initiation *in vitro*. Embryos from dry seeds (T2) only produced roots. Cold storage (refrigeration) of seeds does not show any significant effect on shoot initiation from mature embryos (Table 2) after 16 weeks of incubation. Shoot induction was very low up to the 8th week in medium when the seeds were subjected to cold storage for longer period (T4). Embryos did not respond to MS medium. Increasing ability of shoot induction by seed imbibition was reported in Corsican pine. As seeds soaked in water, the shoot induction from embryo enhanced and the cold storage of seed always in favour of the induction of callus or roots than shoots (Senarath, 1996). Embryos activate as the seeds imbibe water and the actively growing meristems produce growth regulators, which favoured the shoot elongation in culture medium. Long term storage of seeds in moist conditions were determined by Tang and Fu (1994) and found that when seeds were placed at 5-10oC, chilling injury occurred after 30-40 days. Refrigeration of seeds for 7 days mighthave retarded the activity of growth regulators in embryos which slow or cause inhibition of shoot growth *in vitro*. Cold storage for 21 days may have cause chilling injuries in embryo.

Induction of shoots from different explant types:

It has been found that only 88% of young apices produced shoots in CD medium and it was only 60% in MS medium. Only 1/3 of them produced multiple shoots. When shoots from young plants were used, MS medium produced shoots in the presence of 0.05mg/l NAA and 1mg/l IBA (Roy and Hadiuzzaman, 1991).

Although Roy *et al*(1990) reported that, nodal explants produced multiple shoots when MS

medium was supplemented with 1mg/l BAP and 0.5mg/l K, shoot initiation from nodal segments in both CD and MS medium was a rare event (16% and 12% respectively) in this study. Mature buds with sheathing cover produced callus. Mature buds cultured in CD medium favoured the induction of white mucilaginous embryogenic callus. Removal of the sheathing cover around the bud enhanced the shoot initiation from mature apices in both CD and MS media reducing the callus formation (32% and 8% respectively) (Table 3).

a. b. c.

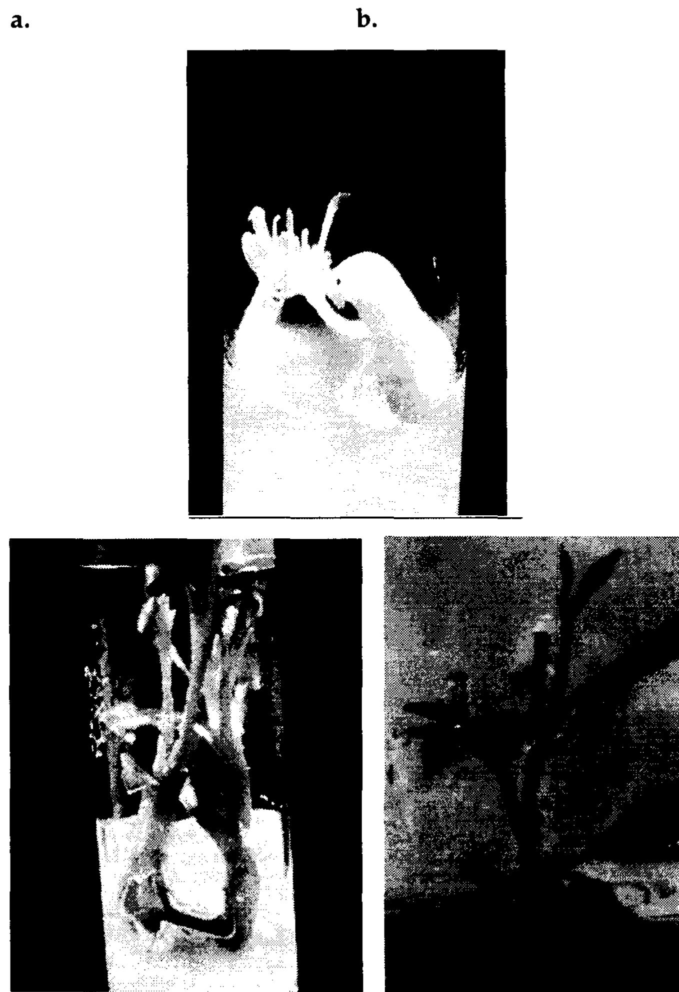

Fig 1a: Shoot initiation from mature embryos (T1) after 8 weeks of incubation. **1b:**Multiple shoot induction from mature apices after removal of sheathing cover. **1c:** Elongation of axillary buds after decapitation of the shoot

Effect of different growth regulators on shoot induction from mature apices:

It has been found that the 0.5mg/IBA with no added NAA (T1) was the best treatment to produce shoots from mature apices. Decapitation of actively growing shoot enhanced the elongation of axillary buds *in vitro* (Figure 1c). Auxin regulates the apical dominance and the auxin level in the stem inhibits the growth of the axillary buds. Decapitation of shoot apex increases the level of auxin in auxiliary bud promoting the elongation. When shoot tip and axillary buds were used as explants, *A. heterophyllus* responded to MS medium with 0.05 mg/l NAA and 1. 0 mg /l BA (Roy and Hadiuzzaman, 1991). Roy *et al* (1993) reported that BAP and NAA at 8.88mol and 2.68mol concentrations in MS medium induced multiple shoots from stem explants after 7 subcultures. Elongation of solitary shoots from the buds obtained from actively growing apices in MS medium in the presence of a range of 4.5 - 9.0mol BAP or Kinetin was reported and higher concentrations of cytokinin inhibited the bud breaking and favoured the callus formation (Amin and Jaiswal, 1993).

Effect of the period of the year which explants were collected:

In November-January period, 60% of the apical meristems produced shoots. It was only 30 35% when the apices were cultured in February-April and in May-July. It was decreased (to 20%) in August-October (Table 4). Similar pattern was observed in all three tested years.

The shoots produced in November-January period showed a higher vigour in number of leaves per shoot (2.90.15), mean leaf width (7.51.1) and mean shoot length (26.00.96) than those produced in other months (Table 5). Reducing the accumulated phenolics at the base by repeated subcultures of the explant enhanced the growth rate. There was a significant difference in the growth performance of shoots according to the period of the year in which explants were collected.

Plants are richly rhythmic and biological clocks regulate number of key metabolic pathways and are controlled by genes. Amin and Jaiswal (1993) reported November to January as the best season for initiation of cultures from field grown Jack trees. Senarath (1996) reported that there is an effect of the period of the year which explants were collected on shoot initiation in Corsican pine. The results obtained from these experiments revealed that, there is an effect of the period of the year on *in vitro* shoot induction in *Artocarpus heterophyllus*. Since Jackfruit show seasonal changes in fruit baring and shedding of leaves it can be suggested that difference in growth performances of tissues cultured in artificial media would have been affected by endogenous rhythms.

*Artocarpus heterophyllus*easily multiply in growth regulator free culture media from mature embryos. Long term storage of seeds is not recommended as it decreases the ability of embryos

to produce multiple shoots *in vitro.* Since *Artocarpus* is an out breeding species, use of embryos in clonal propagation has limitations. Therefore, another alternative type of explant such as mature apices should be considered. This study revealed that apices from mature plants which are readily available throughout the year can be used after removal of sheathing cover as explants for clonal propagation. To obtain higher vigour in plantlets it is recommended to collect explants during the November January months.

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Table 1: Different growth regulator combinations used

Treatment code	Concentration of IBA	Concentration NAA
T1	0.5	-
T2	1.0	-
T3	1.5	-
T4	2.0	-
T5	-	0.5
T6	-	1.0
T7	-	1.5
T8	-	2.0

Table 2: Number of apices responding in MS and CD medium after 6 weeks of incubation period. (Twenty-five replicates per treatment)

Explant type	CD medium	**MS mediu**
Young apic	22	15
Nodal segments	4	3
Mature buds (+ sheath)	*callu	callus
Mature buds (- Sheath)	8	2

* = mucilaginous callus

Table 3: Number of embryos responding in CD medium. Twenty replicates per treatment.

Incubati	**Number of embryos responding in culture**			
Period (Week	T1	T2	T3	T4
2	0	0	0	0
4	4	3*	3	2
8	14	8*	13	9
16	14	9*	13	12

* = only produced roots

Table 4: Percentage of explants which produced shoots after 8 weeks of incubation. Twenty replicates per treatment

Year	February-Apri	lMay-July	August-September	November-January
1	30%	30%	20%	60%
2	35%	35%	20%	60%
3	30%	35%	20%	60%

Table 5: The difference in vigour of the *in vitro* produced shoots in first year. Twenty replicates per treatment.

Month	Mean no. of leaves per shoot	Mean leaf width (mm)	Mean shoot length (mm)
February-April	2.40.51	6.50.18	16.00.16
May-July	1.20.16	4.01.02	15.00.98
August-October	1.50.19	4.50.54	16.00.53
November-January	2.90.15	7.51.10	26.00.96
LSD (5%)	0.09	0.00	0.01