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IN VITRO CULTURE OF THREE ELITE CLONES OF JACKFRUIT

R. GAYATHRI* AND B. N. SATHYANARAYANA
University of Horticultural Sciences (Bagalkot),
Gandhi Krishi Vignana Kendra Campus, Bengaluru - 560 065
e-mail: gayathriacharya711@gmail.com

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***Corresponding author**

ABSTRACT
Studies on *in vitro* culture in three elite clones of jackfruit were carried out during 2012-13 at PG Centre, UHS (Bagalkot), GKVK, Bengaluru. Three elite clones of jackfruit (Clone no. 1, 2 and 3) were identified from Doddaballapur, Bengaluru Rural District, Karnataka and used for the study. In the *in vitro* study, survival of shoot tips and nodal segments was highest (62.5%) in January with least fungal (14%) and bacterial contamination (23.5%) whereas least survival was recorded during September (20%). Shoot tips were suitable explants with highest shoot length (3.04 cm) over nodal segments (1.84 cm) with highest leaves (3.18). Chloramphenicol (60 mg/L) recorded least bacterial contamination (6.33%) and highest survival (80.60%) of the culture against basal medium (86.32% bacterial contamination with culture survival of 7.16%). BAP (2 mg/l) gave highest leaves (2.86) and longest shoots (3.08 cm) *in vitro*. Number of shoots was more in clone no. 2 (1.93) and highest shoot length (2.57 cm) was recorded in clone no. 3. From the study, it can be concluded that explants survival was highest when collected during January, chloramphenicol as antibiotic was effective in reducing bacterial contamination and BAP (2 mg/l) was effective for shoot proliferation of *in vitro* culture.

INTRODUCTION

Jackfruit (*Artocarpus heterophyllus* L.) belonging to the family Moraceae. It has been reported that it is tetraploid with a somatic chromosome number of 56 (2n = 4x = 56) (Darlington and Habib, 1965). Commercial cultivation of jackfruit is still at a primitive stage in India, primarily because of the difficulty in procuring elite planting materials. Generally jackfruit is grown from seeds but the seeds are difficult to germinate even just after a short period of storage (Singh, 1986; Samaddar, 1990). The conventional seed-propagated plants have high variation in plant progenies and rarely identical to the parent plants. Breeding the desirable genotypes using conventional vegetative propagation methods such as grafting and air layering are time consuming and difficult (Amin and Jaiswal, 1993). Hence the alternative method is micropropagation having tremendous potential if exploited for mass multiplication of elite material. 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). 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. 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. Many investigators studied *in vitro* propagation (tissue culture) of the jackfruit, such as Adiga (1996) decided that sucrose was a good C source, as cultures supplemented with sucrose or sugar at 3 or 4% produced more shoots and GA3 (6 mg/l) promoted shoot length. Singh *et al.* (1996) mentioned that jackfruit was successfully micro propagated by culturing nodal segments on modified MS containing BA (18 mg/l) and IBA (0.2 mg/l). *In vitro* cultured nodal explants of *Artocarpus heterophyllus* on MS medium are induced to form multiple shoots when supplemented with BAP (1.0 mg/l) and kinetin (0.5 mg/l) (Roy *et al.*, 1990). Most of researchers have been successful with seedling explants in case of micropropagation of jack. However, some workers carried out micropropagation with mature explants also in recent years, but the protocols developed for one clone do not satisfy for another clone.

In view of the above factors, an investigation was undertaken to standardize methods of sterilization in jackfruit tissue culture, to standardize the source of explant for micropropagation and to study the effect of source of growth regulators on shoots proliferation of explants.

MATERIALS AND METHODS

The present study entitled "*in vitro* culture of three elite clones of jackfruit" was carried out during 2012-13. Three elite clones (Clone no. 1, 2 and 3) selected were identified from and around Doddaballapur taluk, Bengaluru Rural District, Karnataka State, and used for the study. The *in vitro* propagation of jackfruit was conducted in Plant Tissue Culture Laboratory, Division of Horticulture, UAS, GKVK, Bengaluru.

Culture media

Murashige and Skoog (1962) basal medium was used for all the experiments. While preparing culture media, the macronutrients, micronutrients, vitamins and sugar were first dissolved accordingly in double distilled water. The growth hormones were added as necessary. The final volume of the medium was made up by adding distilled water. The pH was adjusted to 5.8 by addition of 0.1 N HCl or 1 N NaOH as required. While boiling the solution, the gelling agents were added. Agar was used as a sole gelling agent at the rate of 6 g/l. For preparation of the medium with chloramphenicol, it is added to the culture medium before autoclaving as it is heat tolerant (added to the medium during preparation before checking the pH). The cultures were incubated in an air conditioned room at temperature of 25 $\pm$ 2^0 C, relative humidity (RH) of 60 per cent, under a photoperiodic regime of 16 hour light and 8 hour dark cycles.

Surface sterilization of explants

Explants (shoot tips and nodal segments) were washed with running tap water for 20 minutes and brushed with soft brush to remove adhering dirt followed by washing with double distilled water by giving 3 to 4 changes. They were surface sterilized with the combination of bavistin (1000 mg) + cetrimide (500 mg) + streptomycin sulphate (100 mg) per liter of water for 90 minutes by continuous stirring followed by surface sterilization inside the laminar air flow cabinet with mercuric chloride (0.1%) for 10 minutes (Adiga, 1996). Explants were thoroughly washed with sterile distilled water for 3 to 4 times and inoculated in half strength MS (Murashige and Skoog) medium at various concentrations of BAP.

Source of plant material

Shoot tips and nodal segments of young sprouts from mature tree were used as explants for initial establishment of cultures. Age of the tree was 42 years in clone no. 1, 30 years in clone no. 2 and 40 years in clone no. 3. The shoot tips and nodes as explant were collected from September to May and cultured on half strength MS medium containing BAP (Benzyl amino purine) 2 mg/l (Adiga, 1996). Observations on fungal contamination, bacterial contamination and survival of explants were recorded every 2 months from September to May with ten replications. Mean number of shoots produced per explants, mean length of shoots, mean number of leaves per shoot and per cent survival of explants were recorded. Type of response in terms of callus formation, bud break and shoot induction was recorded by visual observation (explants were inoculated on ½ MS media containing BAP 2 mg/l + chloramphenicol 60 mg/l, sucrose 3% and agar 0.6%). Completely Randomised Block Design (CRD) was used for analysis with ten replications.

To study the effect of chloramphenicol (antibiotic) on *in vitro* culture response, two treatments of chloramphenicol with 30 mg/L media and 60 mg/L media were used by using shoot tips and nodal segments as explants from mature tree with 10 replications (All explants were inoculated on ½ MS media containing 2 mg/L BAP, 3 per cent sucrose and 0.6 per cent agar). Per cent necrosis, per cent bacterial contamination and percentage survival of explants were recorded. Completely Randomised Block Design (CRD) was for analysis.

Effect of BAP on shoot proliferation on three different clones of jackfruit were studied by using three different concentration of BAP at 1.0 mg/L, 2.0 mg/l and 3.0 mg/L. Shoot tips from mature plants were used as explants (All explants were inoculated on ½ MS media containing chloramphenicol 60 mg/L, sucrose 3% and agar 0.6%). Mean length of the shoots (Length of shoots produced from explant was measured during subculturing {30 days after initiation} and expressed in centimeters), Mean number of shoots produced per explant (Shoots produced were counted and mean was calculated) and Mean number of leaves produced per shoot (Number of leaves produced from single explant was counted and mean of them was calculated) were recorded by using Factorial Completely Randomized Block Design (FCRD) with ten replications.

RESULTS AND DISCUSSION

Effect of season on *in vitro* survival of explants

The effect of different season on *in vitro* survival of shoot tips and nodal explants are presented in Fig. 1. Shoot tip explants collected in January resulted in highest survival (75%) with 8% fungal contamination and 17% bacterial contamination. Least survival of explants (30%) was resulted when shoot tips were collected in September with 40% fungal contamination and 30% bacterial contamination. Nodal segments collected in January resulted in highest survival (50%) with fungal contamination of 20% and 30% of bacterial contamination. Least survival of explants (10%) was resulted when shoot tips were collected in September with 50% fungal contamination and 40% bacterial contamination.

Mean survival of explants was highest in January (62.5%) followed by November (50%) and March (45%). Least survival was noticed during September (20%) proceeded by May (35%).

The seasonal influence on survival percentage of explants varied over different months. This may be attributable to the growth cycle, season, microbial load and physiological state of the mother plant at the time of collection of explants. The increase in survival percentage during January appears to have coincided with the growth cycle of the jackfruit in which new flushes are seen during January. Lower bacterial and fungal contamination may be due to less microbial load on the mother plant at the time of explant collection (Fig. 1). The effect of time of the year on *in vitro* propagation of jackfruit was studied by Mannan *et al*. (2006). Shoot tips as explant from fresh sprouts on the trunk of mature jackfruit were collected at three different times of the year from the same jackfruit plants. The explants collected and inoculated on January 5, 2005 maintaining the same media and cultural condition, survived and responded to proliferation.

Use of antibiotics against bacteria

Fungal contamination was reduced by following proper surface sterilization protocols. Bacterial contamination was serious problem which inhibited growth of jackfruit cultures and reduced survival percentage. Surface sterilization of explants with antibiotics did not inhibited bacterial growth on cultures. Rifampicin (antibiotic) was incorporated in culture medium in order to know the effect as an antibiotic (results were not

Table 1: Effect of chloramphenicol (antibiotic) on *in vitro* response of culture

Treatment	Necrosis (%)	Bacterial contamination (%)	Survival (%)
Control	6.46 (14.72) *	86.34 (68.31)	7.16 (15.51)
Chloramphenicol(30 mg/l)	7.54 (15.93)	45.60 (42.47)	46.86 (43.19)
Chloramphenicol(60 mg/l)	13.06 (21.19)	6.33 (14.56)	80.60 (63.87)
Grand mean	9.02 (17.28)	46.09 (41.78)	44.87 (40.86)
CD @ 5%	0.232	0.923	1.175

* Values in parentheses indicate arc sin transformed values

Table 2: Nature of response of different type of explants of jack fruit to culture condition

Explants	Callusing	Bud break	Shoot induction
Shoot tips from mature plants	-	√	√
Nodal segments from mature plants	√	-	√
(-) No response (√) Response			

Table 3: Effect of source and type of explants on culture establishment

Type of explant	Mean length of shoots/ explants (cm)	Mean number of shoots/ explant	Mean number of leaves/explant	Per cent survival of explant
Nodal segments	1.84	1.65	1.22	82.66 (65.43)
Shoot tips	3.04	2.57	3.18	83.66 (66.19)
Grand mean	2.443	2.11	2.20	83.16 (65.81)
CD @ 5%	0.369	0.646	0.967	NS

NS – Non significant

Table 4: Effect of different of BAP on shoot proliferation of three different clones of jackfruit

Clones	Mean shoot length (cm)				Mean shoots				Mean leaves			
	BAP 1mg/l	BAP 2 1mg/l	BAP 3 1mg/l	Mean	BAP 1mg/l	BAP 2 1mg/l	BAP 3 1mg/l	Mean	BAP 1 mg/l	BAP 2 mg/l	BAP 3 mg/l	Mean
Clone 1	2.37	2.96	2.17	2.50	1.60	2.30	1.30	1.73	2.00	3.00	1.70	2.23
Clone 2	2.02	3.04	1.71	2.26	1.80	2.80	1.20	1.93	2.00	2.90	1.60	2.16
Clone 3	2.25	3.24	2.22	2.57	1.50	2.20	1.50	1.73	1.90	2.70	1.50	2.03
Mean	2.21	3.08	2.03		1.63	2.43	1.33		1.96	2.86	1.60	
	S.Em ±	CD at 5%			S.Em ±	CD at 5%			S.Em ±	CD at 5%		
Clone	0.067	0.187			0.118	NS			0.135	NS		
BAP	0.066	0.187			0.118	0.332			0.135	0.382		
Clone X BAP	0.115	0.324			0.204	NS			0.235	NS		

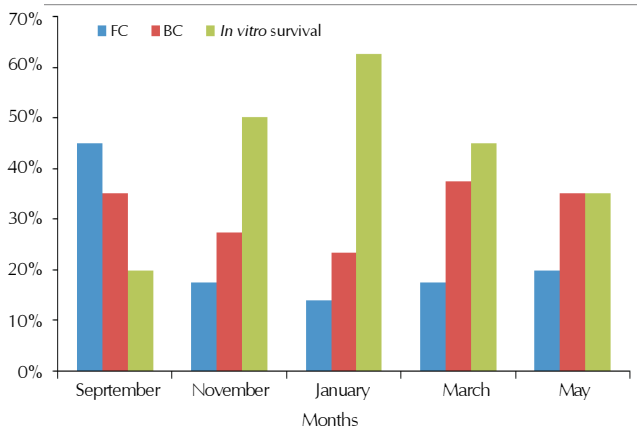
NS – Non significant

shown). Rifampicin was not effective in inhibiting bacterial growth on cultures. Further screening of the cultures was carried out to identify the type of bacteria. Bacteria were identified as gram positive type after gram staining. On the basis of this, chloramphenicol was chosen as an antibiotic which was found to be effective on gram positive bacteria. Various concentrations of chloramphenicol were used to know the effectiveness of the antibiotic.

Effect of chloramphenicol (antibiotic) on *in vitro* culture response

Experimental results on effect of chloramphenicol on explant response are presented in Table 1. Significant differences were recorded on per cent necrosis, per cent bacterial contamination and survival of explants. Necrosis was highest (13.06%) with 60 mg/l of chloramphenicol followed by 30 mg/L of chloramphenicol (7.54%). Least necrosis (6.46%) was observed under control (Basal medium). Lowest bacterial contamination (6.33%) was recorded in 60 mg/l of chloramphenicol in the culture medium followed by 30 mg/l chlramphenicol (45.60%). Highest bacterial contamination (86.32 %) was recorded with control (Basal medium). Highest survival percentage (80.60%) of cultured explants was recorded with 60 mg/L chloramphenicol followed by 30 mg/l chloramphenicol (46.86%). Survival was least (7.16%) in case of control (basal medium).

Endogenous microbial contamination is known to be one of the more serious problems in plant tissue culture, especially in tropical species. It is important for producers of tissue culture to use reliable procedures for detecting and differentiating bacterial contaminations. Furthermore, the use of bacteriostatic additives to the substrates has proven to be convenient. Over last few years, detailed identification of microbial infections in tissue culture has been carried out by different researchers (Attafuah and Badbury, 1989 and Boxus and Terzi, 1987). It has been reported that bacteria obviously can be found endogeneously in the *in vitro* plants rather than on the surroundings of MS medium (Podwyszynska and Hempel, 1987). In order to inhibit bacterial growth, antibiotics can be incorporated into the plant culture medium. In this context, it has to be taken into consideration that the repeated use of single antibiotics may cause resistance development by the bacteria. Thus antibiotics should be used to a very limited



FC: Fungal contamination BC: Bacterial contamination

Figure 1: Effect of season on *in vitro* survival of explants

extent either to clean contaminated cultures or during stage one. Okkels and Pederson (1983) demonstrated that a bacteriostatic effect could be obtained with chloramphenicol at 128 mg/l but some toxicity of the antibiotic against the plant tissue was observed at levels as low as 10 mg/l. Therefore, only those antibiotics should be applied which enable sufficient bacterial inhibition at a concentration level that does not harm the plant

Effect of source of explants on culture establishment

In an attempt to standardize explants for tissue culture of jack fruit, different types of explants from mature tree were used for *in vitro* shoot regeneration and multiplication. It was observed that explants exhibited differential response to the *in vitro* conditions. Kind of response, mean length of shoots/ explants, mean number of shoots/explant, mean number of leaves per explant and survival percentages of explants are presented in Table 2 and 3. Callus was formed when nodal segments were used as explants. Shoot tips has resulted in bud break and shoot formation. Mean length of shoots per explant was recorded highest (3.04 cm) in shoot from mature plants. The mean length of shoots was less (1.84 cm) when nodal segments from mature tree were used as explants. The number of leaves was highest (3.18) with shoot tips as explant. Mean number of shoots per explants recorded less when nodal segments were used as explants. No significant difference was observed on percentage survival of explants with shoot tips and nodal segments as explants. Percentage survival was 83. 66% and 82.66% with shoot tips and nodal segments respectively.

An explant constitute a living unit, independent of mother plant containing all its genetic information and presenting all the functions that the explant tissues or cells performed at their original location. Murashige (1974) recognized several factors that could be considered in explant selection including organ that is to serve as tissue source, the physiological and ontogenic age of the organ and size of explant. In the present study, it was revealed that shoot tips from mature tree exhibited maximum survival percentage than nodal segments and also the regeneration capacity was better in case of shoot tips derived from mature trees as explants. This indicates that the aptitude of cells belonging to *in vitro* cultured explants to differentiate is very unevenly distributed within various species, organs and tissues. The regenerative aptitude differs from cell to cell, tissue to tissue, organ to organ, species to species and even within same species, cultivar to cultivar. Similar results were obtained in Hortensia, citrus sp., *Annona cherimola* (Lazar and Cochita, 1984; Duran *et al.*, 1989 and Jordan *et al.*, 1991).

The above results have shown that shoot tips showed positive response on culture establishment. Hence shoot tips were used as explants to know the effect of BAP on shoot proliferation.

Effect of BAP on proliferation of three different clones of jackfruit

The results of the experiments conducted to study the effect of BAP (cytokinin) on shoot proliferation of jackfruit are presented in Table 4. Significant differences were observed among the treatments for mean number of shoots, length of shoots, mean number of adventitious buds and number of leaves.

Highest number of shoots (2.43) was observed on medium containing BAP 1 mg/l (1.63). The medium with BAP 3 mg/l (1.33) produced lowest number of shoots. There were no significant differences among three clones on number of shoots. Number of shoots was more in clone no. 2 (1.93). Mean number of shoots (1.73) were on par with clone no. 1 and clone no. 2. The maximum length of shoot (3.08 cm) was recorded with the medium containing BAP 2 mg/l. The shortest shoot (2.03 cm) was recorded with the medium having BAP 3 mg/l. highest shoot length (2.57 cm) was recorded in clone no. 3 followed by clone no. 1 (2.50 cm). Mean length of shoots (2.26) was less in clone no. 2. The mean number of leaves per explant was highest (2.86) with basal medium containing BAP 2.0 mg/l. The lowest number of leaves (1.60) was recorded with basal medium containing 3 mg/l BAP. No significant differences were observed among clones with respect to number of leaves.

The growth regulators are used to support basic level of growth. It is also equally important to direct the developmental response of the propagule (Hartmann *et al.*, 1997). *In vitro* culture of higher plants with growth regulators auxins and cytokinins are very significant to get the large propagules. Discovery of cytokinins has helped *in vitro* propagation of plants by shoot multiplication through regulating shoot growth (Skoog and Miller, 1957). In the present study, BAP (2 mg/L) has given better response with respect to shoot multiplication, length of shoot, mean number of adventitious buds produced and number of leaves per explant, followed by BAP at 1 mg/l (Table 4). Similar results were obtained by Adiga (1996) in jackfruit. However, Singh and Tiwari (1998) obtained maximum establishment and number of shoots per explants in jackfruit culture, when BAP 1.8 mg/l + IBA 0.2 mg/l combination was tried. But Roy *et al.* (1990) obtained higher establishment of explants and more number of shoots per explant in jackfruit when medium was supplemented with BAP 1 mg/l + kinetin 0.5 mg/l. Higher number of shoots (4.5) per culture and maximum length (3.38 cm) of shoot were recorded on MS medium containing 1.0 mg/l B A P + 1.0 mg/l kinetin with 3 per cent sucrose added in medium in pomegranate (Pushparaj Singh, 2014). Nazra Paiker and Kunul Kandir (2011), reported that most suitable medium found for shoot formation from shoot tips and leaf was a combination BAP (2.5 mgl) + IBA

(1.5 mg/l). 95% cultures exhibited shoot differentiation in above combination

It was confirmed from present investigation that BAP is the best source of cytokinin for induction of multiple shoots. This result is in conformity with the findings of previous workers in various species. The optimal level of BAP for shoot bud development from seedling explants of mangosteen (*Garcinia mangostana*) was 5.0 mg/l. However, higher concentrations were effective but shoot buds were clustered and stunted (Goh *et al.*, 1995). Among cytokinins tested, BAP was more effective than kinetin with maximum shoot proliferation from nodal explants in jack fruit when used at 0.5 mg/l (Rahman and Blake, 1988). Similarly, Upadhyaya (1995) opined BAP as a best source of cytokinin for shoot proliferation in neem.

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