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An efficient protocol for shoot organogenesis and plant regeneration in jackfruit

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ABSTRACT

The present investigation was carried out for the rapid clonal mass multiplication of disease free plants of jackfruit cv. Pant Garima. The nodal explants were collected in winter season proved to be the best with regard to maximum survival of explants. The effect of sterilants ($HgCl_2$ and ethanol) in various combinations was also assessed on survival percentage of explants. The MS medium was supplemented with five concentrations of BAP (1.0, 2.0, 3.0, 4.0 and 5.0 mgL^{-1}) along with control (0.0mgL^{-1}) and their effects on shootlets regeneration, and length of shootlets were evaluated. Maximum shoot proliferation was obtained in the medium supplemented with 4.0 mgL^{-1} BAP. Further, GA_3@ 1.0 mgL^{-1} added to MS medium supplemented with 4.0 mgL^{-1} BAP gave the best results for enhancing the length of the shootlets. For rooting, half strength of MS liquid medium fortified with 2.0 mgL^{1} IBA gave maximum rooting.

Key words: *Artocarpus heterophyllus*, micropropagation, BAP, GA_3, plant growth regulators.

INTRODUCTION

India is the world's largest producer of jackfruit, and has been part of country's staple diet, contributing a lot in ensuring food security at local level since ages (Sreeni,18). International demand for this 'super healthy food' has increased manifold in the last few years due to its high food value, health benefits and scope of its use as a meat alternative. Jackfruit tree, being quite hardy has enormous prospective to be grown successfully in the poor and marginal lands It is regular bearer and requires least care. The fruit is rich in several nutrients and is a great source of pectin. The fruits and seeds are consumed as vegetable, leaves as fodder, roots and leaves have medicinal properties and its wood also has high timber value (Swami and Kalse, 20).

'Pant Garima', used in the present investigation is a high yielding clonal selection with an average yield of 5 quintals/ tree/year. There is high demand for this variety amongst the orchardists of *tarai* region which cannot be fulfilled through vegetative methods of propagation. Mass multiplication of jackfruit is normally done through seeds which are recalcitrant in nature resulting in poor germination, if not sown immediately (Singh, 17). Besides, seedling plants lack in uniformity. Another method which is being commercially adopted is inarching, which although gives good result in terms of graft union, but is very cumbersome and produces very less number of plants from the source material. The other vegetative methods like air layering and softwood grafting are again uneconomical in terms of production of plants. These methods are also dependent on availability of quality rootstocks and favourable environmental conditions. There are not many orchards of jackfruit with true to type varieties in India, probably due to lack of clonal planting material of elite and productive varieties. Still, seedlings raised from locally available jackfruit trees are used as planting material by a number of nurserymen.

Many investigators have been attempted earlier the micropropagation in jackfruit using different combinations of auxin and cytokinins (Ali and Feyissa, 1; Amany *et al.*,2; Harb *et al.*,9; Miro and Acedo, 14). Faisal *et al.*(7) mentioned that incorporation of BAP @ 4.0mgL^{-1} in MS medium resulted in highest percentage of explant regeneration as well as maximum shoot multiplication. From the previous studies, it was found that production of multiple shoots with higher shoot length is a slow process in jackfruit micropropagation. Therefore, in the present investigation, synergistic effect of GA_3 and BAP on increasing shoot length was studied. The explants cultured in the MS medium supplemented with BAP and GA_3 produced significantly taller shoots in 4-5 weeks. The present study also focuses on standardization of season of explant collection along with exploring the best possibilities for sterilization of explants.

MATERIALS AND METHODS

The present study was carried out during 2019 in the tissue culture laboratory of the department

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of Horticulture, college of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar. The explants were collected from fifteen years old healthy mother plants of jackfruit cv. Pant Garima. Nodal segments from healthy shoots used as explants were collected during different seasons*viz*.,Spring (February - March), Summer (April -June), Rainy (July-August), Autumn (September - October) and Winter (November -January).

The explants were collected in a solution containing 1 % bavistin and 0.5 % Tween-20. The flask containing the explants and the solution was continuously agitated for 30 min followed by washing them under running tap water for 2 h. Further sterilization of explants was performed under the laminar air flow. The explants were surface sterilized with different concentrations of mercuric chloride ($HgCl_2$) and ethanol (either alone or in combination), for different durations (Table 2). Two drops of Tween-20 was added to $HgCl_2$ solution for enhancing the efficacy of the treatment as well as for easy removal of the residual Hg^{++} after treatment. The explants were properly rinsed four times with autoclaved distilled water after each treatment application. The sterilized explants were trimmed with a pre-sterilized scalpel blade to 1.0 cm length.

Murashige and Skoog's medium supplemented with 3 % sucrose and 0.7 % agar was used as the basal medium for both the establishment as well as proliferation stages. The MS medium was fortified with 0.0, 1.0, 2.0, 3.0, 4.0, and 5.0 mgL^{-1} BAP in the experiment for shoot proliferation. At the end of the experiments on shootlets proliferation, the media which gave best results was selected for next set of experiments with addition of GA_3@ 0.0,1.0, 1.5, 2.0 and 2.5 mgL^{-1} in order to study its effect on elongation of shootlets. Half strength liquid MS medium (without agar), containing 3% sucrose was used as the basal medium for rooting. IBA was added to the basal medium at various concentrations (0.0, 1.0, 2.0 and 3.0 mgL^{-1}). The pH of the medium in all the experiments was adjusted to 5.7±1, prior to autoclaving. In the rooting experiment, filter paper bridges were used upto 3rd sub-culturing inside the test tubes for providing support to the rooting shootlets. Sub-culturing was done at an interval of 8 days in shoot proliferation experiments and at 10 days in the rooting experiments. All the culture vessels were incubated at 26±2°C temperature, and a photoperiod 16/8 hours was maintained. Illumination was adjusted at 25µmol $m^{-2}s^{-1}$ photosynthetic photon flux intensity with the help of four cool white fluorescent tubes fixed at the top of every culture rack. Relative humidity was maintained at 80 - 85 per cent with the help of a humidifier.

All the experiments were performed in completely randomized design (CRD). Three replicates with 10 explants per replication were used in all the treatments. The data recorded was analysed with IBM SPSS software (Version 22). Significant differences among various treatments were compared using Duncan's Multiple Range Test at 0.05 % level of significance.

RESULTS AND DISCUSSION

Explant collection was done throughout the year to ascertain the most suitable season for achieving higher percentage of aseptic culture. The browning and survival percentage of explants was significantly influenced by the seasons of collection (Table 1). The highest survival of explants (93.33 %) along with lowest browning (13.33%), was observed during winter season. These explants were also observed to take minimal time for sprouting as well as shoot proliferation. The explants collected during rainy and summer seasons exhibited extreme leaching of phenols which resulted in browning of explants and the medium (Fig.1) due to which there was poor survival of explants (6.67 % and 26.67 %, respectively).Seasonal variations affecting browning and establishment of aseptic cultures have also been reported in peach (Bisht *et al*.,5) and karonda (Rai and Misra, 15). The explants collected in different seasons differ in the composition of phenolic compounds and the polyphenol oxidaseactivity which catalyses the oxidation of phenolic compounds into highly reactive quinines in the presence of oxygen, as has also been observed in the present study. Phenol exudation

Fig. 1. Browning of explants and medium due to leaching of phenols.

Table 1. Effect of different seasons on browning and survival percentage of explants in jackfruit.

Seasons	Browning (%)	Survival (%)
Spring (February-April)	53.33[b]	53.33[b]
Summer (May-June)	100.00[a]	26.67[c]
Rainy (July-August)	100.00[a]	6.67[d]
Autumn (September- October)	40.00[c]	46.67[b]
Winter(November-January)	13.33[d]	93.33[a]

Values with same character are at par

(browning) gradually decreased as the explanting season advanced from summer towards winter. There was active growth in the mother plants during summer and rainy seasons. The young tissues of explants collected during these seasons might have produced more secondary metabolites leading to greater production and exudation of phenols from the cut surfaces. Probably, as the season advanced towards winter, the growth rate slowed down, and tissues got matured resulting in less phenol exudation (Martini *et al.*, 12; Harb *et al.*,9).

The sterilization treatments significantly influenced the survival of explants (Table 2 and Fig. 2) registering highest survival (91.67%), with 25% ethanol (1 min)+ $HgCl_2$ @ 0.2 % (5 min).Treatment with $HgCl_2$ at lower concentration(0.1 %, 4 min) either alone or in combination with 25% ethanol (1 min) reduced the survivability of explants (67.67 - 68.67 %). The exposure of $HgCl_2$ at lower concentration for shorter

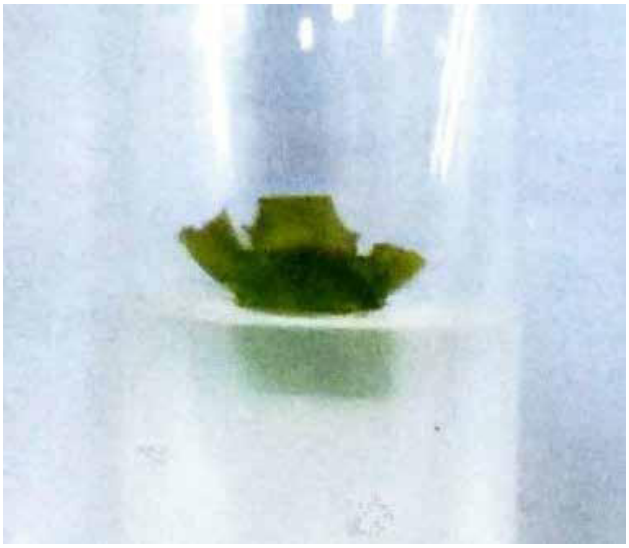

Fig. 2. Aseptic establishment of explants after treatment with 0.2 % $HgCl_2$ (5 min.) followed by 25 % ethanol (1 min.).

Table 2. Effect of sterilization treatments on survival percentage of explantsin jackfruit.

Treatments	Survival (%)
Distilled water (control)	0.00[i]
$HgCl_2$ @ 0.1% for 4 min	67.67[h]
25% ethanol for 1 min+$HgCl_2$ @ 0.1% for 4 min	68.67[h]
$HgCl_2$ @ 0.1% for 5 min	70.00[gh]
25% ethanol for 1 min+$HgCl_2$ @ 0.1% for 5 min	71.10[g]
$HgCl_2$ @ 0.2% for 4 min	82.33[c]
25% ethanol for 1 min+$HgCl_2$ @ 0.2% for 4 min	86.67[b]
$HgCl_2$ @ 0.2% for 5min	86.67[b]
25% ethanol for 1 min+$HgCl_2$ @ 0.2% for 5 min	91.67[a]
$HgCl_2$ @ 0.3% for 3 min	80.00[d]
25% ethanol for 1 min+$HgCl_2$ @ 0.3% for 3 min	75.67[e]
$HgCl_2$ @ 0.3% for 4 min	73.33[f]
Distilled water (control)	70.00[gh]

Values with same character are at par

time reduced the tissue injury and phenol exudation, but failed to effectively control the contamination, while higher concentration of $HgCl_2$ (0.3%) caused tissue injury, eventually reducing the survival rate of explants. Khan *et al.* (11) also concluded that at lower concentration of sterilants, the efficiency of treatments was drastically reduced, while enhanced intensity of treatments proved fatal for the survival of explants.

The shoot proliferation was assessed both by counting the number of shootlets formed per explant as well as the length of the shootlets (Table 3). After 32 days of inoculation, the average number of shootlets per explant in MS medium supplemented with 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 mgL^{-1} BAP was 0, 0.2, 0.30, 6.03, 10.70 and 0.93, respectively. Maximum number of shootlets (10.70 % on 32nd day) was formed in the MS medium supplemented with 4 mgL^{-1} BAP (Fig. 3). The results of present study differed with the findings of Amany *et al*. (2) who found that the medium enriched with 3.0mgL^{-1} BAP produced maximum number of multiple shoots in jackfruit, while Ashraffuzzaman *et al*.(3) found that 2 mgL^{-1} BAP was optimum for shoot induction in the jackfruit explants. There was sharp reduction in shoot formation at higher BAP concentration in the medium. Declining effects of higher concentrations of BAP have also been

Table 3. Effect of BAP on number and length of shootlets per explant in jackfruit.

Treatments	14 Days		20 Days		26 Days		32 Days	
	Number of shootlets	Length of shootlets (cm)	Number of shootlets	Length of shootlets (cm)	Number of shootlets	Length of shootlets (cm)	Number of shootlets	Length of shootlets (cm)
MS media (Control)	0.00^{e}	0.00^{e}	0.00^{f}	0.00^{e}	0.00^{f}	0.00^{f}	0.00^{f}	0.00^{f}
MS media + 1 mg L^{-1} BAP	0.00^{e}	0.00^{e}	0.20^{e}	0.00^{e}	0.20^{e}	0.22^{e}	0.20^{e}	0.38^{e}
MS media + 2 mg L^{-1} BAP	1.67^{c}	0.27^{d}	0.30^{d}	0.27^{d}	0.30^{d}	0.55^{d}	0.30^{d}	0.93^{d}
MS media + 3 mg L^{-1} BAP	3.07^{b}	1.69^{b}	5.30^{b}	1.85^{b}	5.70^{b}	2.53^{b}	6.03^{b}	3.63^{b}
MS media + 4 mg L^{-1} BAP	5.20^{a}	1.90^{a}	6.40^{a}	2.10^{a}	7.60^{a}	2.99^{a}	10.70^{a}	4.07^{a}
MS media + 5 mg L^{-1} BAP	0.30^{d}	0.32^{c}	0.40^{c}	0.32^{c}	0.40^{c}	0.77^{c}	0.93^{c}	1.07^{c}

Values with same character are at par

Fig. 3. Multiple shoot formation on MS medium supplemented with 4.0 mgL^{-1} BAP and 1.0 mgL^{-1} GA_{3s}.

observed in karonda (Rai and Misra, 15) and guava (Meghwal *et al.,*13). In the BAP free medium (control), the explants did not sprout at all even after 32 days of inoculation strongly suggesting the necessity of BAP for induction of such morphogenic development. The role of growth regulators during plant regeneration is well known and cytokinins especially play an important role in reinforcing regenerative responses like axillary shoot proliferation, probably due to its role in overcoming apical dominance. However, exogenous application of plant growth regulators are not the only factors affecting *in vitro* plant regeneration, other stresses like osmotic, mechanical or stresses due to pH changes in the medium are also responsible for the various developmental and morphogenic responses (Desjardins *et al.*,6).

The average length of shootlets per explant after 32 days of incubation in the medium supplemented with 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 mgL^{-1} BAP was recorded to be 0.0, 0.38, 0.93, 3.63, 4.07 and 1.07 cm, respectively (Table 3). Maximum shootlet length of 4.07 cm after 32 days was achieved in the MS medium containing BAP @ 4 mgL^{-1}, while the medium containing BAP @ 1 mgL^{-1} produced shootlets with an average length of 0.38 cm only. It was also observed that the shoot elongation was adversely affected by higher concentration of BAP @ 5.0 mgL^{-1} (1.07 cm) in the media. BAP inhibits shoot elongation by promoting ethylene production in the tissue culture vessel (Saha *et al*., 16).

Optimization of type and amount of plant growth regulators in the media, plays a very important role in the success of micropropagation. The use of plant growth regulators either beyond or below the optimum concentrations may induce deformities or encourage callusing in the explants, eventually reducing the micropropagation efficiency (Khan *et al*., 11). Sulusoglu and Cavusoglu (19) also reported that on increasing BAP concentration in the medium,callusing was induced at the base of the explants of *Prunus laurocerasus* L., decreasing the proliferation rate. The results obtained by Miro and Acedo (14) in jackfruit also confirm the findings of the present study. The physiological state as well as the cellular sensitivity of the source tissue towards the exposed PGR concentrations could be assigned to this variability (Ashrafuzzaman *et al.,* 3).

For greater elongation of shootlets, another experiment was conducted, where gibberellic acid (GA_3) at different concentrations (0.0, 1.0, 1.5, 2.0 and 2.5 mg L^{-1}) was added to the MS medium supplemented with 4.0 mg L^{-1} BAP (medium which showed best results in the previous experiments on shoot proliferation). The average length of shootlets recorded in MS medium + 4 mgL^{-1} BAP supplemented with 0.0, 1.0, 1.5, 2.0 and 2.5 $mgL^{-1}GA_3$ was found to be 3.98, 5.82, 5.38, 4.73 and 4.64 cm, respectively, after 32 days (Table 4). Longest shoot (5.82 cm) was obtained in the MS medium where 4 mgL^{-1} BAP was combined with 1.0 mgL^{-1} GA_3, however, it proved to be statistically similar with rest of the GA_3 concentrations.

Table 4. Effect of GA_3 on shootlets elongation in jackfruit.

Treatments	Length of shootlets (cm)			
	14 Days	20 Days	26 Days	32 Days
MS media + 4 mgL^{-1} BAP (Control)	1.84^{a}	1.95^{e}	2.95^{c}	3.98^{b}
MS media + 4 mgL^{-1} BAP + 1 mg L^{-1} GA_3	1.81^{ab}	2.54^{d}	4.37^{a}	5.82^{a}
MS media + 4 mgL^{-1} BAP + 1.5 mg L^{-1} GA_3	1.63^{ab}	3.62^{a}	4.30^{a}	5.38^{ab}
MS media + 4 mgL^{-1} BAP + 2 mg L^{-1} GA_3	1.55^{b}	3.45^{b}	3.87^{b}	4.73^{ab}
MS media + 4 mgL^{-1} BAP + 2.5 mg L^{-1} GA_3	1.47^{b}	3.24^{c}	3.83^{b}	4.64^{ab}

Values with same character are at par

Addition of GA_3 in the proliferation media pronounced the effects of BAP.The results apparentlysuggested synergistic effect of $GA_3$with BAP on increasing the length of shootlets. According to Gonbad *et al*. (8), addition of GA_3 effectively elongatedthe shoots without any noticeable effect on shoot multiplication in tea. There was comparable elongation of the proliferated shoots at all concentrations of GA_3, however the morphology and quality of shoots was poor when GA_3 concentration extended beyond 1.0 mgL^{-1}. The shoots developed in media supplemented with higher GA_3 concentration were relatively more tender, less green, thin with less and smaller leaves. The media where GA_3 was incorporated at higher concentration (2- 2.5 mgL^{-1}), signs of vitrification were also visible which further deteriorated the shoot quality. The observations with regard to poor morphological growth and development of shoots with higher concentration of GA_3 are in agreement with the findings of Bandaralage *et al*. (4) who witnessed vitrification along with slenderness of shoots and production of thin elongated narrow leaves, when the medium was aided with greater GA_3 concentrations during micropropagation of avocado. Application of IBA to half strength liquid MS medium was crucial for initiating the rooting process in the regenerated shootlets (Table 5). Root initiation was visible within 7-10 days. Application of 2 mgL^{-1} IBA resulted in greater percentage of rooting. No rooting was observed in the ½ MS liquid medium without IBA supplementation. The average number of roots formed per shootlet was significantly influenced by the IBA concentrations. After 40 days, maximum rooting (60.00 %), highest root number (1.48) and average length of roots per shootlet (3.36 cm) was obtained in the rooting medium supplemented with 2.0 mgL^{-1} IBA (Fig. 4). Incorporation of 1.0 and 3.0 mgL^{-1} IBA in the rooting medium significantly reduced

Fig. 4. Rooting of shootlets in half strength MS liquid media supplemented with 2.0 mgL^{-1} IBA.

Table 5. Effect of IBA on rooting percentage, number and length of roots per shootlet in jackfruit.

Treatments	10 Days			20 Days			30 Days			40 Days		
	Rooting (%)	Number of roots	Length of roots (cm)	Rooting (%)	Number of roots	Length of roots (cm)	Rooting (%)	Number of roots	Length of roots (cm)	Rooting (%)	Number of roots	Length of roots (cm)
½ MS media (Control)	0.00^{d}	0.00^{d}	0.00^{c}	0.00^{d}	0.00^{c}	0.00^{b}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}
½ MS media + 1 mg L^{-1} IBA	3.33^{c}	0.06^{c}	0.11^{c}	3.33^{c}	0.17^{b}	0.53^{b}	13.33^{c}	0.17^{c}	0.60^{c}	16.67^{c}	0.17^{c}	1.02^{c}
½ MS media + 2 mg L^{-1} IBA	23.33^{a}	0.74^{a}	0.90^{a}	23.33^{a}	1.23^{a}	2.04^{a}	56.67^{a}	1.29^{a}	2.72^{a}	60.00^{a}	1.48^{a}	3.36^{a}
½ MS media + 3 mg L^{-1} IBA	10.00^{b}	0.15^{b}	0.32^{b}	16.67^{b}	0.26^{b}	0.67^{b}	26.67^{b}	0.41^{b}	1.27^{b}	26.67^{b}	0.41^{b}	1.46^{b}

Values with same character are at par

rooting percentage of shootlets and produced less number of very small size roots.

A successful micropropagation depends on obtaining high rooting frequency of shootlets. Incorporation of specific auxin at a particular concentration is necessary for achieving optimum results. Auxins promote adventitious root development, and stimulate cell expansion by cell wall loosening. IBA has time and again proved to be the most efficient rooting hormone, and has significant effect on rooting as compared to NAA and IAA. It is not destroyed by IAA oxidase or other enzymes thus persist for longer time in the media (Harb *et al*., 9; Itoo *et al*., 10).

In the present study, micropropagation protocol for 'Pant Garima' cultivar of jackfruit was successfully developed. The results suggested that nodal segments collected during winter season can be successfully used as explant. Treatment with 25 % ethanol (1 min) followed by 0.2 % $HgCl_2$ (5 min.) resulted in maximum survival percentage of explants. MS medium supplemented with 4.0 mgL^{-1} BAP produced highest number of shootlets. The combination of 4.0 mgL^{-1} BAP with 1.0 mgL^{-1} GA_3 improved shoot elongation. Incorporation of 2.0 mgL^{-1} IBA in half strength liquid MS media resulted in maximum rooting of shootlets.

AUTHORS' CONTRIBUTION

Conceptualization of research and designing of experiment (RR, RS, RK, VPS), execution of experiment, data collection, data analysis and interpretation of results (D, RR), contribution of experimental material (P), preparation of manuscript (RR).

DECLARATION

The authors declare that there is no conflict of interest regarding the publication of this paper.

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