IN VITRO SHOOT AND ROOT PROLIFERATION OF JACK FRUIT AS AFFECTED BY DIFFERENT CONCENTRATIONS OF GROWTH REGULATORS

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ABSTRACT

The research work relating to the micro-propagation of Jack fruit was carried out in the tissue culture lab of the Horticultural Research Institute, NARC, Islamabad during the year 2004 with the objective of finding a suitable protocol for its mass multiplication. Explants grown from seedlings in the greenhouse were surface sterilized with NaOCl. For this purpose, four levels of NaOCl i.e., 0.5, 0.75, 1.0, 1.25 % w/v were used. MS basal medium containing different concentrations of BAP (1.0, 1.5, 2.0 and 2.5 mg l⁻¹) was used for shoot proliferation. Different concentrations of IBA (1.0, 2.0, 3.0, 4.0, and 5.0 mg l⁻¹) were used for determining the in-vitro rooting potential. The best survival was observed with 0.75% NaOCl which gave maximum survival (46.66 %) while BAP @ 1.5 mg l⁻¹ exhibited the highest number of shoots. Higher rooting rate (%) and number of roots per plant were obtained with IBA @ 3.0 mg l⁻¹.

Key Words: Jack fruit, BAP, IBA, Sodium Oxy-Chloride, In-vitro shoot and root proliferation

INTRODUCTION

Jack fruit (Artocarpus heterophyllus lam) belongs to the family ‘moraceae’ along with other fruit bearing plants like fig (Ficus carica) and mulberry (Morus alba). It is native to India and is the most common and popular fruit tree of Bangladesh. Jack fruit is widely cultivated in many tropical and subtropical countries of the world (Amin, 1992) , was declared as a national fruit of Bangladesh, and is cultivated in the coastal areas of Pakistan. Jack fruit plants flourish best in a humid, warm climate such as hills-tops up to an elevation of 1500m. It also grows well in arid and warm plains (Ochse et al., 1981). 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 jack fruit are Rudrakshi, Singapore, Ceylon, khoa Allah abad and Varikka. Jack fruit is a monoecious, cross pollinated plant and seed is not suitable for true to type plant multiplication. However, the plant is generally grown from seed which are difficult to germinate even after a short period of storage. Seeds are viviparous in nature (Maurya, 1987); however high variation is found among the trees of seedling origin. Jack fruit 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 (350 mg) (SCUC, 2006). The juicy pulp of ripe fruit is eaten fresh as a desert or preserved in syrup. The seed are cooked, roasted or fried. Unripe fruit is consumed as a vegetable. The Chinese consider jack fruit tonic prepared from pulp and seeds is useful in overcoming the influence of alcohol on the digestive system (SCUC, 2006).

Recently, it has become popular due to its special nutritional profile, taste and flavor. But certified nursery plants are scarcely available in the country as it is generally grown from seed but due to the short storage life, seed shows poor germination as it looses its viability in a very short span of time. Through micropropagation, it is possible to multiply certified, disease free plants at a much faster rate than using conventional propagation techniques. Hence, an experiment was conducted to find a reliable, rapid and efficient protocol for in-vitro plant regeneration.

Plantlets from bud and nodal explants of mature jack fruit tree have also been reported (Rahman and Blake, 1988 and 1988b) but the multiplication rate was very low. The present investigation was therefore undertaken to establish protocol for large-scale propagation of jack fruit through tissue culture.
MATERIALS AND METHODS

The experiment on “in-vitro shoot and root proliferation of jack fruit as affected by different concentration of growth regulators” was conducted (in the tissue culture lab of Horticultural Research Institute, NARC, Islamabad) during the year 2004. The experiment was organized in a complete randomized block (CRD) design with 4 replications (having 5 glass bottles per replication). Plant material for this experiment was obtained from three sources: seedling grown in the laboratory through in-vitro means, one year old seedling grown in the greenhouse of the Horticultural Research Institute, NARC, Islamabad, mature trees of jack fruit (Jinnah Garden Lahore).

Shoot tips 3-5cm long and nodal segments collected from jack fruit seedling were surface sterilized in order to control contamination. Chlorox was used with following concentration i.e. 0.5, 0.75, 1.0, 1.25% for 15 minutes with two drops of Tween 80 (Sigma, USA) polyoxyethylene sorbitan monolaurate per 50 ml of solution. Explants were agitated in the sterilizing solution for 10 minutes, the solution was decanted and the tips were rinsed 5 times with sterile distilled water using flamed forceps. The longer outer leaves and most of the stem were cut off leaving about 2-3 cm long shoot tips. MS (Murashige and Skoog, 1962) basal medium was used throughout the studies for shoot proliferation and rooting of jack fruit. The disinfected cutting and nodal segments were placed on hormone free MS media for culture establishment. In vitro obtained shoots were transferred for application on MS basal medium. The concentrations of BAP mg l⁻¹ tested were 1.0, 1.5, 2.0, and 2.5. In-vitro formed shoots were separated and transferred to MS basal medium containing different levels of IBA, 1.0, 2.0, 3.0, 4.0, mg l⁻¹. All the cultures were incubated at 27±1°C under 16 hours light (2,000 lux) photoperiod with fluorescent lamp (Philips TL 40 W/54).

The data was recorded after 4 weeks of culturing on the basis of necrosis %, infestation %, and survival % of explants using the following formulas:

Necrosis % = number of plants showing necrosis / total number of explants cultured × 100
Infestation % = number of plants showing infection / total number of explants cultured × 100
Survival % = number of plants showing normal growth / total number of explants cultured × 100

Number of shoots and roots produced on respective media were counted. Root proliferation rate was calculated as follows:

Root Proliferation rate % = Number of plants producing roots / total number of plants × 100.

Before initiating analysis, data presented as percentages were arcsine-square-root transformed (into degree values) using MS-Excel worksheet by formula:

\(= \frac{\text{ASIN}(\%\text{value}/100)}{\pi/180}.\)

After transformation, the data were statistically analyzed through analysis of variance (ANOVA) following the procedure given by Steel & Torrie (1997) and ranking of genotypes was made using LSD test. All the analysis was performed in MstatC statistical software (MSU, 1982).

RESULTS AND DISCUSSIONS

Effect of different levels of NaOCl on the establishment and disinfestations of explants

Explants from mature trees were completely contaminated and hence future studies were carried out only on explant seedlings obtained from the greenhouse. Plants obtained from the open field always have high numbers of exogenous as well as endogenous microbes however plants grown under control condition perpetually carry very limited numbers of micro-organism which can be easily disinfected and thus culture establishment is more readily achieved (Ahmad, 2002). Results relating to the response in terms of of necrosis, infection and survival (%) in different concentrations of sodium hypochlorite (NaOCl) are presented in (Table I). From the results it is evident that (0.75 % NaOCl) gave the maximum survival (46.66 %), followed by (1.0 % NaOCl) with 26.66 % survival while the minimum survival (6.6 %) was observed in (1.25 % NaOCl). It is clear from results that infections were reduced (0 %) with an increase in concentration of NaOCl (1.25 %). The same trend is depicted by the graph in (Fig. 2). In contrast to infection %, necrosis % increased with the increase in NaOCl concentration (Fig.1). Results showed that both higher and lower concentrations greatly affected the cultured shoot tip explants. Lower concentrations enhance contamination whereas higher concentrations were toxic to the plant as it damaged the tissues (Fig.3).
Fig. 1 Effect of NaOCI (%) on the necrosis (%) of in vitro culture explant of Jack fruit.

Fig. 2 Effect of NaOCI (%) on the infection (%) of in vitro culture explant of Jack fruit.

Fig. 3 Effect of NaOCI (%) on the survival (%) of in vitro culture explant of Jack fruit
Table-1  Effect of different levels of NaOCl on necrosis, infection and survival percentage of cultured shoot tip explants.

<table>
<thead>
<tr>
<th>Treatment (NaOCl %)*</th>
<th>Necrosis (%)*</th>
<th>Infection (%)*</th>
<th>Survival (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>6.6 d (3.78)</td>
<td>80.00 a (53.43)</td>
<td>13.33 c (7.66)</td>
</tr>
<tr>
<td>0.75</td>
<td>20 c (11.53)</td>
<td>33.33 b (19.47)</td>
<td>46.66 a (27.82)</td>
</tr>
<tr>
<td>1.0</td>
<td>60 b (36.91)</td>
<td>13.33 c (7.66)</td>
<td>26.60 b (15.42)</td>
</tr>
<tr>
<td>1.25</td>
<td>93 a (68.89)</td>
<td>0.0 d (0.00)</td>
<td>6.60 d (3.78)</td>
</tr>
<tr>
<td>LSD at 0.05%</td>
<td>6.310</td>
<td>6.206</td>
<td>2.642</td>
</tr>
</tbody>
</table>

Means not sharing a common letter in a column differ significantly at P< 0.05.

*15 explants were used in each treatment.

Values in brackets are first converted from % to Arc-sine transformed value, and then LSD test was applied.

Effect of different BAP concentrations on the in-vitro shoot proliferation rate (%) and number of shoots per plant

Medium containing BAP initiated shoot proliferation in all treatments regardless of the concentration, whereas control explants did not generate any shoots (Table II). Significant differences were observed between the treatments for the number of shoots per proliferated explant. The control treatment did not respond, however 1.5 mg l-1 BAP gave the best result in terms of maximum number of shoots (4.66) per proliferated explant. It was followed by 2.0 mg l-1 BAP and 1.0 mg l-1 BAP, which produce 2.66 and 2.11 shoots per proliferated explant, respectively (Table II). Poor results were obtained at 2.5 mg l-1 BAP with 1.2 shoots per proliferated explant. Sajid et al., (2006) also observed poor shoot proliferations in grape germplasm when low levels of BAP and auxins were used. Fig. 4 clearly indicates that shoot proliferation was lower at low concentrations of BAP and gradually increased with the increase in BAP concentration. It became maximum at 1.5 mg l-1 BAP, but again decreased as BAP concentration further increased. It was generally recognized that concentration of cytokinin in the medium should be optimum for getting increased shoot proliferation of woody plants (Sharp et al., 1984).

Effect of different IBA concentrations on in-vitro rooting rate (%) and number of roots per plant

Significant differences were noted between the treatments for rooting rate. Results in (Table III) show that the control treatment did not respond at all whereas 3.0 mg l-1 IBA showed the highest percentage of rooted shoots (80 %) followed by 2.0 mg l-1 IBA and 4 mg l-1 IBA which produced 70 and 50 percent rooted shoots respectively (Table III).

It has been reported that IBA enhances rooting at low concentration but has an inhibitory effect at higher concentration (Zimmerman, 1984). These results are also supported by Amin (1992) who found highest rooting (90%) success at low level of IBA (2 mg l-1). Significant differences were recorded between the treatments for the number of roots per plant (Table 3). Among all the treatments, 3 mg l-1 IBA gives maximum number of roots per plant (6.9), followed by 2 mg l-1 IBA having 5.4 roots per plant. Non-significant differences were observed between 1 mg l-1 IBA and 4 mg l-1 IBA. Next to the control the least number of 2.8 roots per plant was initiated on IBA @ 5 mg l-1. Swamy & Sahijram (1998) obtained 47.6 % rooting in Bougainvillea when 5 mg/l IBA was added to MS medium. Root proliferation rate showed almost similar trend as in the case of shoot proliferation as it initially increased with the increase in IBA concentration, attained the maximum at IBA @ 3 mg l-1, but declined again at 5 mg l-1. (Fig.5 and Fig. 6).

Table-II  Effect of different levels of BAP on shoot proliferation rate (%) and number of shoot per proliferated explant.

<table>
<thead>
<tr>
<th>Treatment (BAP mg/l)</th>
<th>Shoot proliferation rate %</th>
<th>Number of shoots per proliferated explant</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.0 (0.00)</td>
<td>0.000 e</td>
</tr>
<tr>
<td>1.0</td>
<td>100 (89.96)</td>
<td>2.133 c</td>
</tr>
<tr>
<td>1.5</td>
<td>100 (89.96)</td>
<td>4.269 a</td>
</tr>
<tr>
<td>2.0</td>
<td>100 (89.96)</td>
<td>2.668 b</td>
</tr>
<tr>
<td>2.5</td>
<td>100 (89.96)</td>
<td>1.200 d</td>
</tr>
<tr>
<td>LSD at 0.05%</td>
<td>---</td>
<td>0.460</td>
</tr>
</tbody>
</table>

Means not sharing a common letter in a column differ significantly at P< 0.05.
Table-III. Effect of different concentration of IBA on in-vitro rooting % and no. of roots per plant of Jack fruit.

<table>
<thead>
<tr>
<th>Treatment (IBA mg L⁻¹)</th>
<th>Rooting rate %</th>
<th>Roots per plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.0 f (0.00)</td>
<td>0.00 e</td>
</tr>
<tr>
<td>1.0</td>
<td>40 d (23.57)</td>
<td>3.25 c</td>
</tr>
<tr>
<td>2.0</td>
<td>70 b (44.53)</td>
<td>5.40 b</td>
</tr>
<tr>
<td>3.0</td>
<td>80 a (53.43)</td>
<td>6.90 a</td>
</tr>
<tr>
<td>4.0</td>
<td>50 c (30.01)</td>
<td>3.55 c</td>
</tr>
<tr>
<td>5.0</td>
<td>20 e (11.53)</td>
<td>2.80 d</td>
</tr>
</tbody>
</table>

LSD at 0.05% 6.208 0.354

Means not sharing a common letter in a column differ significantly at P<0.05.

Values in brackets are first converted from % to Arc-sine transformed value, then LSD test was applied.

Fig.4 Effect of BAP (mg/L) on the number of shoots per proliferated explant of Jack fruit

Fig.5 Effect of IBA (mg/L) on the rooting rate (%) of proliferated explant of Jack fruit
CONCLUSIONS AND RECOMMENDATIONS

On the basis of findings of the above experiment, it may be concluded that for efficient and rapid micro-propagation of jack-fruit, NaOCl @ 0.75 %, BAP @ 1.5 and IBA @ 3.0 may be used for getting higher survival % of explants cultured, greater number of shoots proliferated, enhanced root initiation rate and number of roots obtained, respectively.

REFERENCES


