academicJournals

Vol. 16(2), pp. 87-90, 11 January 2017
DOI: 10.5897/AJB2015.15191
Article Number: 8958BAD62378
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Short Communication

Protocol optimization for *in vitro* shoot multiplication of Jackfruit (*Artocarpus heterophyllus* L.)

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Received 23 December, 2015; Accepted 9 May, 2016

Jackfruit (*Artocarpus heterophyllus* L.) is a cross pollinated fruit tree valued mainly for its fruit and timber wood. Although the crop has been introduced to Ethiopia and adapted well to the Jimma area, its potential for production has not been exploited due to the absence of an efficient method for large scale propagation. Micropropagation is a method for production of large number of genetically uniform planting materials with desired characteristics. The present study was initiated to determine the optimum concentration and combination of benzylaminopurine (BAP) and naphthaleneacetic acid (NAA) for *in vitro* shoot multiplication. For this study, shoot tips were used as an explant source. The shoot tips from mature trees were sterilized with 70% ethanol and $HgCl_2$ before culturing on Murashige and Skoog (MS) medium supplemented with different combinations of BAP and NAA. The results show that, the combination of BAP and NAA resulted in significant ($P<0.01$) differences for shoot number, shoot length and leaf number, whereby 2 mg/L BAP alone was found to be the best with a mean shoot number of 5.12 and length of 0.89 cm. The optimized protocol could be used as a baseline for further studies on *in vitro* propagation of jackfruit.

Key words: *Artocarpus heterophyllus* L., mercuric chloride, auxiliary shoot.

INTRODUCTION

Jackfruit (*Artocarpus heterophyllus* L.) is a monoecious cross pollinated fruit tree of the tropical and sub-tropical region which belongs to the genus *Artocarpus* in the family Moraceae (Haq, 2006). It is an evergreen, medium-sized, latex-producing tree, with a somatic chromosome number of 2n=4x=56, valued mainly for its fruit and timber wood (Shyamalamma et al., 2008). The jackfruit tree produces higher yields than any other tree species, bearing the world's largest known edible fruit with an individual fruit weight of 0.5 to 50 kg. Jackfruit tree yields 20 to 250 fruits per tree per annum, sometimes reaching 500 fruits on a fully mature tree (Haq, 2006; Shyamalamma et al., 2008).

Jackfruit is mainly produced in South East Asian countries such as Bangladesh, India, Pakistan, Indonesia, Malaysia and Thailand (Haq, 2006). The center of origin

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of jackfruit is believed to be the Western Ghats region of India (Krishnan et al., 2015) and it has been spread in ancient times throughout tropics specially Southeast Asia (Haq, 2006). It was believed to be taken by Arab traders to the East African coast (Haq, 2006; Love and Paull, 2011) and to Ethiopia by Jimma Agricultural Research Center (JARC). Though it has been adapted in Ethiopia (Jimma area), it has a very low distribution because of various reasons but mainly due to the absence of an efficient method for large scale planting material propagation. Hence, it is still under maintenance at JARC.

The most common propagation method of jackfruit is by seed, but the crop being cross pollinated and highly heterozygous, the plants raised from seed never bear fruits that are true to the type of the mother plant. Furthermore, due to the recalcitrant nature of the seeds, its storage even for a short time results in loss of viability and poor germination. The vegetative propagation methods are generally tiresome, time consuming and seasonal with low multiplication rate making it difficult for effective and commercial level propagation. Hence, *in vitro* propagation is an alternative. So far, a lot has been reported on the effects of different plant growth regulators on the *in vitro* propagation of jackfruit (Rahman and Blake, 1988; Roy et al., 1991; Amin, 1992; Amin and Jaiswal, 1993; Lee and Keng, 2005; Mannan et al., 2006; Khan et al., 2010). However, there is no report on micropropagation of jackfruit genotypes grown in Ethiopia and the protocol developed in other countries for other genotypes cannot always be used as protocols are genotype specific. Hence, the present study was done with the objective of determining the optimum concentration and combination of BAP and NAA for *in vitro* shoot multiplication of jackfruit.

MATERIALS AND METHODS

The study was conducted in the tissue culture laboratory of Jimma University College of Agriculture and Veterinary Medicine, Jimma, Ethiopia. It was conducted from November 2011 to April 2013.

Explant preparation and surface sterilization

The explants used for this study were obtained from a healthy, mature, fruit bearing jackfruit tree grown in JUCAVM experimental field and shoot tips were used as explants. Actively growing new shoots of jackfruit of about 5.0 cm in length along with intact shoot cover (stipule) were excised from the tip of the branches of the selected mother tree and were placed in water till they were brought into the laboratory. The shoots were washed thoroughly under running tap water and detergent soap followed by rinsing in tap water prior to sterilization treatments in the laminar airflow cabinet. The explants were then treated with 0.3% Kocide (antifungal) for 45 min and immersed in 70% ethanol for 2 min. Then they were rinsed with distilled water and further sterilized by 0.5% mercuric chloride ($HgCl_2$) for 5 min. Finally, the shoots were rinsed three times with distilled water to remove traces of sterilants from the explant surface. After sterilization, the stipules were opened and the shoot tips were further trimmed from the basal ends to remove dead and chlorine affected tissues. Two centimeter sized shoots were then cultured on MS medium.

Media preparation

Murashige and Skoog (1962) medium supplemented with different plant growth regulators (PGRs) was used throughout the experiments.

Separate stock solutions of macronutrients, micronutrients, vitamins, $CaCl_2$ and Fe-EDTA were prepared by weighing and dissolving the powders of each component in distilled water. It was then stored in refrigerator at 4°C until it is used. Plant growth regulator stocks of benzylaminopurine (BAP), naphthaleneacetic acid (NAA) and indolebutyric acid (IBA) were also prepared separately by weighing and dissolving the appropriate amounts of the ready-made powders in the same fashion as MS components except the use of NaOH (for auxins) and HCl (for cytokinins) to facilitate the dissolving process.

Once the stock solutions were made, the culture media were prepared by taking the appropriate volumes of the stock solutions. Sucrose (3% w/v) was added in the media as a carbon source. The volume was then adjusted and the appropriate type and concentrations of growth regulators were added on the basis of the requirement to the treatments. Then, the pH was adjusted to 5.8 ± 2 using either 1 N NaOH or 1 N HCl solution before 0.8% w/v agar was added. It was then melted and 30 ml media was dispensed into the culture vessels and finally, it was autoclaved at a temperature of 121°C with a pressure of 15 Psi for 20 min.

The effect of BAP and NAA on shoot multiplication

In this experiment, MS medium containing different concentrations of BAP (1.0, 1.5, 2.0, and 2.5 mg/L) in combination with NAA (0, 0.1, 0.5, and 1.0 mg/L) was used. It was a two factor experiment factorially arranged (4 × 4) in Complete Randomized Design (CRD) with 15 explants per treatment where by, three explants were cultured per jar and randomly placed on shelves under uniform light. A growth regulator free medium was used as a control.

Culturing and culture conditions

The shoot tip explants cultured on MS medium supplemented with different plant growth regulators were maintained for 15 days on shoot multiplication medium. The cultures were maintained in a growth room at a temperature of 26 ± 2°C with a 16/8 h light/dark period under an illumination of 25 µmol $m^{-2}s^{-1}$ photosynthetic photon flux intensity provided by cool-white fluorescent light. Sub-culturing was done every 15 days. Data was collected on response variables of *in vitro* cultured shoot explants after two successive sub-culturing and the data include number of shoots, shoot length and leaf number

Data analysis was done using SAS statistical package, version 9.2 (SAS, 2008). Analysis of variance (ANOVA) was done and the means were separated using Duncan's New Multiple Range Test (DNMRT) at 5% level of significance.

RESULTS AND DISCUSSION

Effect of BAP and NAA on shoot multiplication

Analysis of variance showed that the interaction between BAP and NAA is highly significant ($p< 0.01$) for all the

Table 1. The effects of BAP and NAA on shoot multiplication after 45 days.

Treatments		Mean shoot number per explant ± SD	Mean shoot length (cm) ± SD	Mean leaf number per explant ± SD
BAP	NAA			
0.0	0.0	1.73 ± 0.28^{g}	0.74 ± 0.18^{c}	1.06 ± 0.05^{h}
1.0	0.0	2.00 ± 0.53^{fg}	1.08 ± 0.25^{bc}	1.67 ± 0.47^{gh}
1.0	0.1	2.80 ± 0.56^{cdefg}	1.69 ± 0.10^{a}	2.00 ± 0.94^{gh}
1.0	0.5	2.20 ± 0.50^{efg}	0.73 ± 0.21^{c}	2.73 ± 0.86^{defg}
1.0	1.0	1.87 ± 0.73^{g}	0.86 ± 0.50^{bc}	1.20 ± 0.12^{h}
1.5	0.0	2.64 ± 0.07^{defg}	1.15 ± 0.02^{bc}	2.27 ± 0.72^{fgh}
1.5	0.1	2.67 ± 0.41^{defg}	1.31 ± 0.06^{ab}	2.80 ± 1.57^{defg}
1.5	0.5	3.17 ± 1.01^{cdef}	1.14 ± 0.21^{bc}	4.13 ± 0.18^{bcd}
1.5	1.0	3.93 ± 0.27^{bc}	0.75 ± 0.23^{c}	4. ± 1.4400^{bcde}
2.0	0.0	5.12 ± 0.65^{a}	0.89 ± 0.38^{bc}	3.67 ± 1.1^{cdef}
2.0	0.1	3.13 ± 0.90^{cdef}	0.79 ± 0.22^{bc}	4.93 ± 0.28^{bc}
2.0	0.5	3.86 ± 0.38^{bcd}	0.88 ± 0.26^{bc}	4.06 ± 0.09^{bcde}
2.0	1.0	2.73 ± 0.76^{cdefg}	0.88 ± 0.21^{bc}	2.57 ± 0.06^{efgh}
2.5	0.0	3.26 ± 0.15cde	0.88 ± 0.27^{bc}	1.93 ± 0.68^{gh}
2.5	0.1	3.80 ± 0.65^{bcd}	0.79 ± 0.20^{bc}	5.46 ± 0.06^{b}
2.5	0.5	2.87 ± 0.45^{cdefg}	1.17 ± 0.27^{bc}	3.73 ± 0.04^{cdef}
2.5	1.0	4.54 ± 0.30^{ab}	0.88 ± 0.26^{bc}	8.00 ± 0.23^{a}
CV (%)		8.15	11.37	8.89

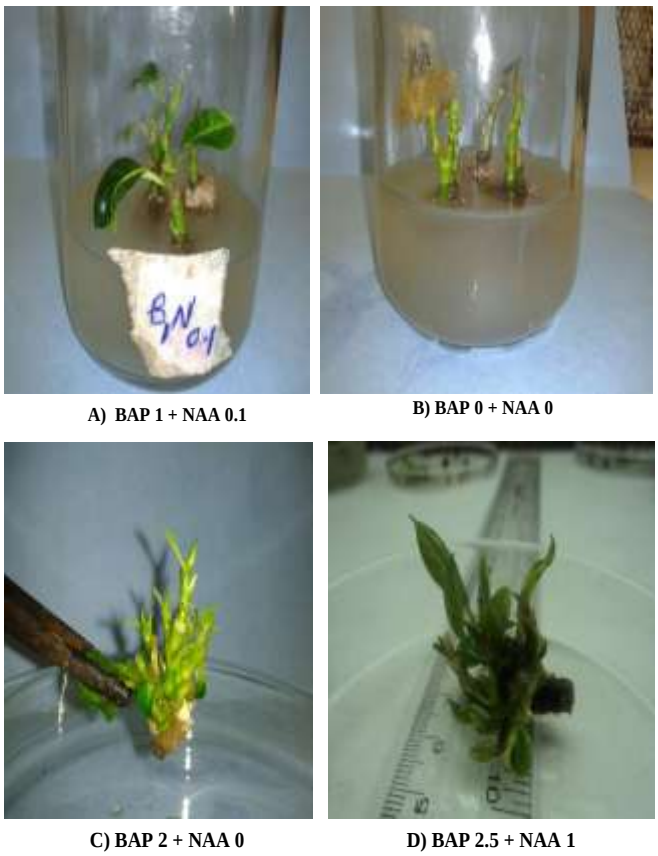
*****Means with different letters in a column are significantly different.

response variables; mean shoot number per explant, mean shoot length and mean leaf number per explant. It is apparent from the results of this experiment (Table 1) that all the growth regulator treatments including the control (Figure 1B) have induced shoot multiplication.

Results showed that medium supplemented with 2 mg/L BAP in the absence of NAA produced the highest number of shoots (5.12) per explant with a mean shoot length of 0.89 cm (Figure 1C), though the result from this treatment is not statistically different from that of a medium supplemented with 2.5 mg/L BAP + 1 mg/L NAA, which produced 4.54 shoots per explant with-a mean shoot length of 0.88 cm (Figure 1D).

From the result obtained, the best shooting was recorded for the shoots cultured on MS medium supplemented with 2 mg/L of BAP which is in agreement with the result of Amin and Jaiswal (1993) who found 3.3 shoots per explant. However, the shooting was significantly improved in this study (5.12). Ashrafuzzaman et al. (2012) also reported the highest shooting for cultures on medium supplemented with 2 mg/L BAP.

The axillary shoot proliferation enhancement observed on the shoots that were cultured on MS medium supplemented with BAP may be due to the role of cytokinins in overcoming apical dominance (Cline et al., 1997).



A) BAP 1 + NAA 0.1 B) BAP 0 + NAA 0

C) BAP 2 + NAA 0 D) BAP 2.5 + NAA 1

Figure 1. *In vitro* shoot multiplication on MS medium supplemented with A) BAP 1 + NAA, B) BAP 0 + NAA 0, C) BAP 2 + NAA 0, and D) BAP 2.5 + NAA 1.

Conflict of interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors would like to express their deepest thanks to Bayu Dume for his material and chemical support.

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