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Proliferation Studies in Garcinia mangostana L. and Acclimatization Accomplishment

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Abstract: ***In continuation with the Initiation response, the mass propagation was achieved by multiplication process using the set of trials performed with 6BAP resulted with the emergence of shoots. Being a medicinal plant, the availability and their easy multiplication efficiency should be the key tools for the success rate of the propagation. In addition to 6BAP, Gibberellic acid also added the excess proliferation of shoots. Mangosteen was raised with the elongated shoots and roots when the media was supplemented with NAA, 6BAP along with the coconut water which contains cytokinins hormones that signal the plant to divide cells in the roots and growing shoots. The healthy plants after the root induction were transplanted in the soil for acclimatization and noted the survival of plants after hardening with fertilizers. This study enabled the ease of the mangosteen micropropagation which can be easily propagated and benefitted in several ways for their medicinal values.***
Keywords: Mangosteen, Proliferation, 6BAP, Gibberellic acid, NAA, Coconut water, Acclimatization, Medicinal values.

I. INTRODUCTION

A. Botanical Review

Mangosteen is the most valued tropical fruit. Mangosteen is a slow-growing and shallow-rooted evergreen tree reaching up to 25 m in height. The leaves are thick and leathery, while the flowers are fleshy and 4 to 5 cm in diameter. The flowers are often green on the outside and yellow to red on the inside, with 4 sepals and 4 petals.

The fruit is round, 2.5 to 7.5 cm in diameter, and weighs about 75 to 150 g. The rind is smooth and 0.6 to 1 cm thick. The exterior is pale green when immature and dark purple when fully ripe. The inner pulp contains 4 to 8 juicy white segments that are sweet and faintly aromatic. The fruits may or may not contain seeds.

B. Status Of Mangosteen Propsgation

Propagation of mangosteen is challenging though it possess various medicinal values due to limited seed set, slow seed growth rate, and difficulty with root formations. The poor root system found in mangosteen seedlings represents a significant problem in mangosteen propagation. In order to overcome this propagation challenge in mangosteen, *In vitro* propagation through tissue culture techniques could be an alternative solution. This technique may also help production of more uniform and high quality plants. Slow growth rate of seedling is due to the lack of root system in which root hair was a few. In addition, the seedling planted via conventional propagation gives fruit after 10-15 years of planting. This takes an advantage for the tissue culture.

C. Significance

Numerous studies have found high concentrations of xanthones, a class of polyphenolic compounds, in mangosteen. Xanthones have antioxidant, antibacterial, antifungal, anti-inflammatory, antitumor, antiplatelet aggregation, antithrombotic, and vasorelaxant activities, prevent oxidative damage of low-density lipoprotein, histamine, and serotonin receptor blocker activity, and inhibit HIV. The xanthones and tannins of the mangosteen pericarp protect against insects, fungi, plant viruses, bacteria, and animals while the fruit is still immature. Of the 200 known xanthones, nearly 50 are found in mangosteen. The major xanthones are alpha-mangostin, beta-mangostin, gamma-mangostin, and methoxy-beta-mangostin, and the most abundant is alpha-mangostin. Calcium, phosphorus, iron, thiamine, riboflavin, niacin, and ascorbic acid are found in mangosteen.

II. MATERIALS AND METHODS

The study was continued at Genewin Biotech, Research Laboratory, Hosur for the next stages. After the initiation response, the culture was preceded to the next stage for the mass proliferation.

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A. Proliferation Trials

Proliferation stage results in the development of new shoots in a large number from a single culture. After the response of bud break, the responded shoots were trialed with 6BAP and Gibberellic acid (GA). The growth was frequently monitored every week and recorded. The cultures were inoculated in following MS basal media treatments + Sucrose 3% with various growth regulator concentrations.

PM 1: 6BAP – 1 mg/l
PM 2: 6BAP – 3 mg/l
PM 3: 6BAP – 5 mg/l
PM 4: 6BAP – 1 mg/l + GA – 1 mg/l
PM 5: 6BAP – 3 mg/l + GA – 1 mg/l
PM 6: 6BAP – 5 mg/l + GA – 1 mg/l

The explants were placed in the prepared media and the mean parameters were calculated. The inoculated jars were incubated (Ika Rostika et al., 2008).

B. Culture Conditions

The explants are subjected under light intensity for 10-12 hours in the growth room. Photoperiod provided by cool white fluorescent lamps of 1500-3000 lux, temperature of about 25 ± 2 °C and humidity of 35 - 40%. The observation will be recorded after 4-5 weeks (Sompong Te-Chato, 2004).

C. Root Induction Trials

Development of healthy roots was attained in the Rooting stage from the regeneration of new shoots in 15 – 25 days. The growth was frequently monitored every week and recorded. The cultures were inoculated in following MS basal media treatments + Sucrose 3% with various growth regulator concentrations.

RM 1: IBA – 1 mg/l
RM 2: IBA – 2 mg/l l
RM 3: NAA – 1 mg/l
RM 4: NAA – 2 mg/l
RM 5: NAA – 1 mg/l + IBA – 1 mg/l
RM 6: NAA – 2 mg/l + IBA – 2 mg/l

The cultures were placed in the prepared media and the mean parameters were calculated. The inoculated jars were incubated (Ika Rostika et al., 2008).

D. Culture Conditions

The explants are subjected under light intensity for 10-12 hours in the growth room. Photoperiod provided by cool white fluorescent lamps of 1500-3000 lux, temperature of about 25 ± 2 °C and humidity of 35 - 40%. The observation will be recorded after 4-5 weeks (Sompong Te-Chato, 2004).

E. Acclimatization

Acclimatization is done to harden the plants in contact with the soil as media in the greenhouse environment for the plants to withstand the temperature and humidity in the small stage itself so as to sustain themselves when they are planted in the fields. It is of 2 satges:

1) Stage 1 - Primary Hardening

The rooted plants were sent for hardening in the Green house at Genewin Biotech for acclimatization of plants to the environment. In primary hardening, the plants were kept and maintained in the tunnel for a period of 45 days in order to avoid over exposure to sunlight which leads to mortality.

2) Stage 2 - Secondary Hardening

After primary hardening, the plants were transferred to the secondary hardening stage wherein the plants were removed out of tunnels and given 50% sun light inside the green house for its acclimatization to the environment. This stage includes another 45 days.

III. RESULTS AND DISCUSSION

A. Proliferation Outcome

The below table summarizes the proliferation efficiency of mangosteen using various growth regulators and observed the formation of shoots.

Table 1 Proliferation Of Mangosteen

TRIAL MEDIA	MEAN SHOOT LENGTH (cm)	MEAN NUMBER OF SHOOTS (cm)	MULTIPLICATION RATIO				OUTCOME
6BAP (mg/l)			1st SUB-CULTURE	2nd SUB-CULTURE	3rd SUB-CULTURE	4thSUB-CULTURE	
1	0.51	1	0.41	0.79	0.93	1.1	Small and dull single shoots
3	0.87	1	0.57	0.82	1.12	1.85	Better multiplication rate
5	2.21	3	1.34	2.41	2.84	3.01	Faster multiplication with healthy shoots
6BAP + GA							
1 + 1	1.22	1	0.95	1.19	1.33	1.67	Fresh and slow multiplication rate
3 + 1	1.13	1	0.91	1.15	1.34	1.71	High multiplication in the beginning and rate slowed down gradually
5 + 1	1.28	2	1.04	1.27	1.51	1.83	Faster multiplication gradually but could not gain the freshness

According to the observation, the multiplication efficiency was found be higher with the number of shoots and the height of the shoots in the media trialed with 6BAP – 5 mg/l. When GA was added in combination with 6BAP, the growth is slightly dull and freshness is lost during the sub-culturing and rate is slow.

Fig.1 Proliferation trials

B. Root Induction

Followed by the multiplication, the cultures were moved on to the rooting stage for the development of roots using various root inducers.

Table 2 Root Induction

TRIAL MEDIA	MEAN ROOT LENGTH (cm)	MEAN ROOT NUMBERS (cm)	RESPONSE %
RM1	5.6 ± 0.11	7	96 ± 0.15
RM2	5.72 ± 0.15	5	68 ± 0.25
RM3	3.6 ± 0.11	5	54 ± 0.32
RM4	2.4 ±0.07	6	56 ± 0.32
RM5	2.1 ±0.04	4	42 ±0.39
RM6	2.1 ±0.04	3	30 ± 0.41
RM7	2.22 ±0.043	3	24 ± 0.41
RM8	2.24 ±0.043	4	16 ± 0.43

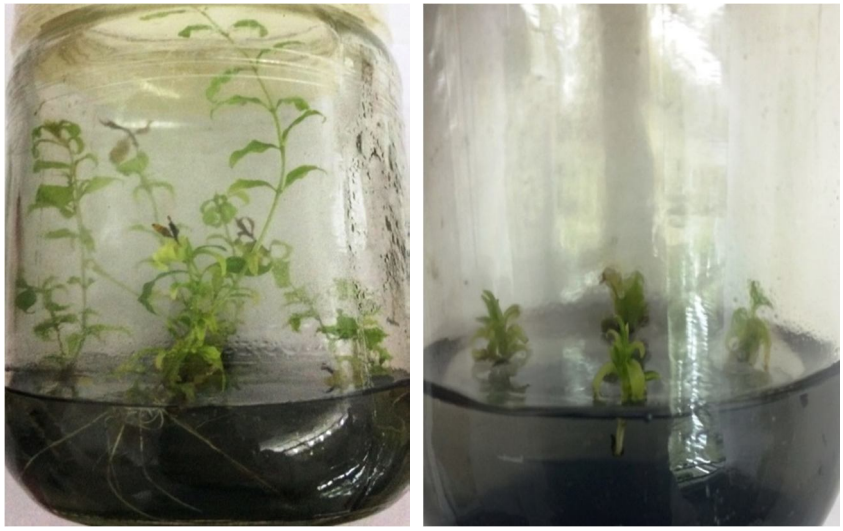

Fig.2 Rooting trials

C. Acclimatization

The rooted plants were ready for hardening which were kept in the green house for acclimatization of light, temperature and humidity. The rooted plants were trialed with various combinations of soil for the survival efficiency of the plants and the results were tabulated.

TABLE 3 Hardening

MEDIA TRIALS	MEAN HEIGHT OF PLANT (cm)	MORTALITY (%)	SURVIVAL RATE (%)
PRIMARY HARDENING			
Compost	4 ± 0.8	3.5	96.5
Vermicompost	2.6 ± 0.5	14.4	85.6
SECONDARY HARDENING			
Red Soil	5.3	10	90
Compost	6.1	3	97
Red soil + compost + sand	8.2	2.1	97.9
Vermicompost	6	6	94

The above table represents the ability of the media used for hardening that enables the plants to attain the maximum survival rate and height of the plants. Primary hardening used compost and vermicompost which showed 96.5% survival rate in compost media and 85.6% survival rate in vermicompost media. In continuation with primary hardening, secondary hardening responded well with the combination of red soil, sand and compost in the ratio 1:1:1.

Fig.3 Hardened plants

IV. CONCLUSION

Mangosteen responded to tissue culture positively that led to the production of plantlets in large numbers in a short duration of time. The fruits are processed for the medicinal uses for the treatment of several diseases. The various parts of this plant are commercially being used for various applications. In order to meet the demand of mangosteen, tissue culture option can be chosen to speed up the production process and produce the healthy plantlets which can be encouraged for the natural medicine.

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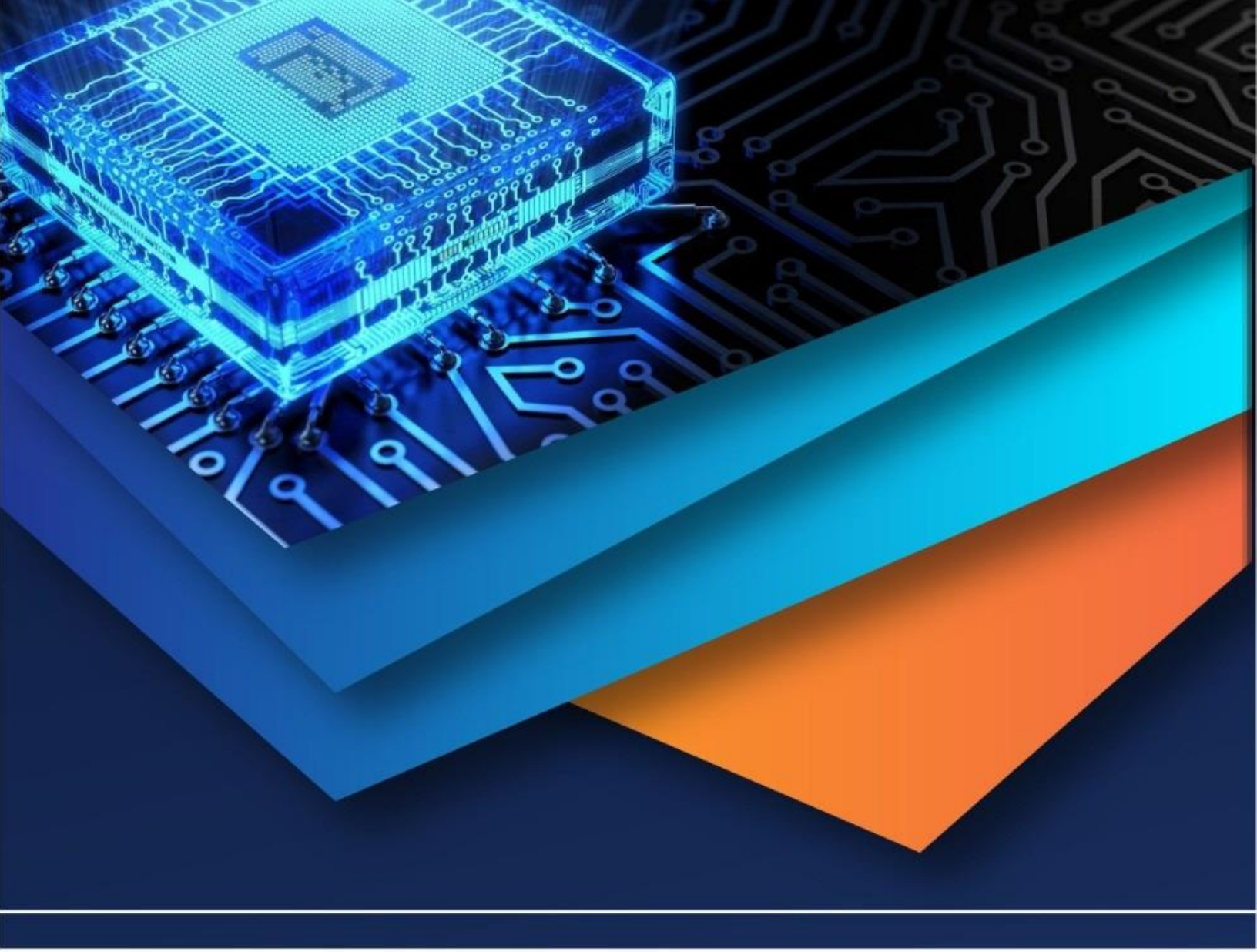

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