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TISSUE CULTURE STUDY FOR MICROPROPAGATION OF ANY MEDICINAL PLANT

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ABSTRACT

For a substantial length of time, a large majority of plant species have been used for the production of therapeutic products. Nearly every country and culture make use of the therapeutic capabilities that plants possess as a primary or supplementary source of medical care. Denbath et al. (2006) state that medicinal plants play an important part in many health care systems. [Further citation is required] The estimations supplied by the World Health Organization (WHO) indicate that herbal medicines are capable of satisfying the needs of around 80 percentage of the world's population. This is especially true for the millions of individuals who make their homes in rural areas of emerging nations. Traditional treatments, many of which were derived from plants, formed the basis for the development of a significant portion of today's medications. According to some statistics, one-fourth of all drugs that can be purchased without a prescription either include plant extracts or are based on the chemical structures of plants. The most widely used pain medication, aspirin, was originally derived from several Salix and Spiraea species by chemical synthesis. Additionally, some of the most powerful anticancer medications, such as paclitaxel and vinblastine, are exclusively derived from plant sources. This is the case for both of these medications (Pezzuto, 1996).

Keywords: Culture, Study, Micropropagation, Medicinal, Plant

INTRODUCTION

The majority of plant species have been utilized for medicinal purposes for a significant amount of time. Plants have medicinal properties that are utilized as a source of medicine in almost every nation and culture. According to Denbath et al. (2006), medicinal plants have a significant role in health care systems. [Citation needed] According to estimates provided by the World Health Organization (WHO), herbal medicines meet the requirements of around 80% of the world's population. This is particularly true for the millions of people living in rural regions of developing countries. Many of today's pharmaceuticals originated from traditional remedies that were derived from plants. One quarter of legally available medications are said to include plant extracts or to be patterned on plant chemicals, according to some sources. Aspirin, the most common pain reliever, was initially synthesized from species of Salix and Spiraea. Additionally, some of the most effective anticancer medicines, such as paclitaxel and vinblastine, are entirely derived from plant sources (Pezzuto, 1996).

In wealthy countries, a significant number of medicinal herbs are used. In most circumstances, around 25% of the population in the UK uses herbal medicines as a kind of illness therapy. Almost two thirds of the 50,000 distinct kinds of medicinal plants are harvested from their natural environments. Only 10% of medicinal plant species that are produced for commercial purposes are found in Europe. At the moment, there is concern over the diminishing populations of medicinal plants, the loss of genetic diversity, the local extinctions, and the deterioration of their habitats. According to a number of estimates, the survival of more than 4,000 species of medicinal plants may now be in jeopardy (Goel and Singh, 2009).

MARKET FOR PLANT-DERIVED COMPOUNDS

medications, perfumes, tastes, and color additives alone is worth more than several billion dollars annually. For instance, chemicals derived from plants such as taxol, vincristine, vinblastine, colchicine, artemisinin, forkolin, and Saponin have widespread use in the fields of biology and medicine.

The cultivation and shipment of medicinal plants into international markets is gaining momentum. It is anticipated that the herbal industry, which is presently valued at $60 billion and expanding at a pace of seven percent, would be worth more than $5 trillion by the year 2050. (Denbath et al., 2006).

Significant increases are being seen in production, consumption, and worldwide commerce of medicinal plants and phytomedicines. Phytomedicines are plants that are used for medical purposes. In addition, the use of herbal medications is becoming more prevalent around the world. However, the collection of medicinal plants from their natural environments in order to use them as a source of raw materials results in the destruction of habitats and a loss of genetic variety. Even though herbal remedies have been used for the treatment of a wide variety of conditions since ancient times, vast quantities of medicinal plants continue to be collected from the natural environments in which they grow today (Kumari and Priya, 2020). Because there is no supervision over their harvesting, thus the agents of merchants and Vaidaya do harvest them brutally, and as a result, a number of species have either vanished completely or are on the brink of vanishing completely as a result of this (Kumari and Priya, 2020). It has been suggested that the methods of plant tissue culture might be a more acceptable option to cope with this concerning situation.

PROCESS OF VEGETATIVE DEVELOPMENT

In the field of plant biotechnology, the establishment of cell, tissue, and organ culture, as well as the regeneration of plantlets under in vitro conditions, has opened up new paths of research and development (Dagla, 2012). The process of vegetative development and multiplication from viable and regenerative cells in an aseptic and favorable state on adequate culture medium utilizing different plant tissue culture methods is known as micropropagation. Micropropagation is also known as cloning (Zhou and Wu, 2006). Micropropagation of various medicinal plants makes use of the technique of in vitro propagation because it makes it possible to produce large numbers of plants that are genetically identical in a short amount of time and within a confined space. These identical plants can then be used as planting materials.

The use of both conventional and biotechnological methods of plant breeding, with the goal of enhancing the genetic level in order to achieve improvements in yield and uniformity. The cultivation of plant tissue in vitro, also known as in vitro propagation, is an essential step in the manufacturing of high-quality medicines derived from plants. Micropropagation is one of the many techniques that may be used to accomplish this goal (Yushkova, 1998). Recent years have seen a rise in the commercial significance of secondary metabolites, which has led to an increased interest in secondary metabolism. This interest is particularly focused on the possibility of modifying the production of bioactive plant metabolites through the application

of tissue culture technology. According to Tripathi and Tripathi (2003), the end of the 1960s saw the introduction of a number of cell culture technologies that may be used as a tool for the research as well as the production of plant secondary metabolites. The use of a variety of in vitro systems has helped to optimize the generation of plant compounds.

In vitro plant culture is an essential technology that may be used for a variety of purposes, including the mass multiplication of plants, the eradication of plant diseases via the use of meristematic tissue culture, plant conservation, and crop enhancement by gene transfer (Sarasan et al., 2011). Because of this, preventing contamination from a variety of sources, including bacteria and fungus, is essential for the effective cultivation of medicinal plants via the use of in vitro propagation.

There is a lot of variation in the features of the plants since the majority of them are not farmed or micropropagated under the same settings.

The secondary metabolites of a plant change with the different seasons and stages of the plant's growth. The ability of the pharmaceutical business to produce pharmaceuticals in a sustainable manner is contingent on its ability to maintain a continual supply of healthy material, to which plants contribute significantly (Sahoo et al., 1997).

It is of the utmost importance to develop dependable replication procedures of these commercially significant medicinal plants via the use of micropropagation in order to facilitate both quick regeneration and the development of high-quality planting materials.

CULTURE PROPAGATION

Explants are any materials that are used in the process of tissue culture propagation. Because different kinds of cells do not have the same level of totipotency, the success of tissue culture is mostly dependent on the age, types, and positions of the explants (Gamborg et al., 1976). The most frequent sources of explants are the tips of shoots and nodes, as well as the tips of roots. According to the research conducted by Murashige and Skoog in 1962, large explants might raise the risk of contamination, but tiny explants such as meristems could indicate slower development.

Explants need to be sterilized before they can be used in in vitro micropropagation, which is one of the most important processes. Sterilization may be accomplished by the use of

chemicals such as calcium hypochlorite, sodium hypochlorite, ethanol, mercuric chloride, hydrogen peroxide, or silver nitrate (Mihaljevic et al., 2013). The use of sterilization is necessary in order to lessen the risk of contamination and get explants that are free of illness.

OBJECTIVE

1. To Study On The Tissue Culture Study For Micropropagation Of Any Medicinal Plant

2. To Study On The Develop Dependable Replication Procedures

REVIEW LITERATURE

Ghriosa superba A summer-growing bulbous plant known for its vital medicinal effects and well as a fascinating decorative, L is a member of the family Lilliaceae and is indigenous to southern Africa. It is rarely accessible in several regions of India, especially the North Eastern Region (Narain, 1988).

Ghriosa superba is a kind of herbaceous plant that grows upright and is often used in gardens for its decorative value (Prain, 1963). It is a branching herbaceous climber that has a perennial fleshy tuberous rhizome in the form of a 'V' and spectacular, big blooms that bloom singly (Kanjilal, 1939). It has been described as a shrubby perennial species with bright red, showy flowers and cylindrical, sometimes irregularly bent 60-90 cm long; 1.3-2 0 cm thick light yellowish brown, smooth often with short transverse shallow fissures at the regions of bends with acrid roots in some of the published works that have been done on the subject.

According to Prain (1963), the purgative characteristics of the roots of Ghriosa superba were effective in the treatment of leprosy, piles, colic discomfort, boils, and to expel roundworms. Additionally, these roots were useful in the treatment of snake bites and scorpion stings. It has been stated that the tubers of the Ghriosa superba plant have both medicinal and poisonous effects (CSIR). The tubers are used as a tonic, for stomachaches, and as an anthelmintic; they are very dangerous in high amounts; they are also used to enhance labor pain and as an abortifacient; and they are used topically for neuralgic pains and skin problems (Gupta, 1982).

Sivkumar & Krishnamurthy, 2000,Colchicine is the predominant alkaloid that may be found in the seeds. Some derivatives of colchicine, including cornigerine, 3-demein-yl-N-formyl-N-

deacetyl P- lumicalchicine, 3- demethyl-y lumicolchicine, and 3-demethyl colchicines, have been isolated from the plant. Other derivatives of colchicine, including p-sitosterol, its glucoside, a long chain fatty acid, P and y-lumiccolchicines from fresh tuber There is evidence that conns contain yet another significant alkaloid called eoichicoside.

RESEARCH METHODOLOGY

In the month of April, corms that had already sprouted from the Gloriosa superba plant were gathered from their natural environment and then planted in the department's experimental garden. The plants were cared for according to standard agricultural techniques and received watering whenever it was necessary to do so. After being cared for for a year, these plants were eventually employed as source material in future tests (Platel).

METHODS

In order to determine which of the four types of tissue culture basal media, namely those developed by Murashige and Skoog (1962), Gamborg (1968), Nitsch's (1969), and White medium (1934), is the most effective at fostering the growth and multiplication, in vitro, of various kinds of explants for the purposes of micropropagation as well as for callus culture experiments, these media were tested.

DATA ANALYSIS

As a result, the current inquiry was undertaken with the intention of discovering an effective protocol for the preservation of Gloriosa superba by the use of tissue culture technology. A comparative isozyme study of a few enzymes between in vitro grown plants and in vivo grown plants was another feature of the current inquiry. The goal of this part of the investigation was to determine whether or not the in vivo plants differed in any way from the wild grown ones. A comparison of the levels of colchicine, the most important and valuable alkaloid that can be found in different portions of the Gloriosa superba plant, was carried out between the various plant components as well as between in vitro and in vivo produced plants. Under their respective subheadings, the following table presents the findings of each and every experiment that was conducted.

TISSUE CULTURE

Micropropagation

It was determined by doing experiments with micropropagation of Gloriosa superba using four distinct culture media: MS, Gamborg, Nitsch's, and White. The MS culture medium proved to be the most successful. Corm bud explants were inoculated in each of the four different media, and their performance was assessed based on factors such as the number of days needed for bud break and the number of shoots that grew per corm bud after 30 days. After one week of culture in MS media, shoot initiation was seen. This was followed by 10 days of culture in Gamborg medium, and then 15 days of culture in both Nitsch's and White medium. Only a single shot developed in Nitsch's media, and only single shoots developed in White medium. After 30 days, the MS media generated an average of 4.5 shoots, whereas the Gamborg medium only resulted in 2.3 shoots (Table 4.1, Figure 4.1). On the basis of these findings, MS basal medium was determined to be the superior option, and as a consequence, it was chosen for the purpose of conducting all of the tissue culture tests.

Table 1 Effect Of Different Tissue Culture Basal Growth Medium On In Vitro Response Of Gloriosa Superba Corms

Medium		Response		Mean shoot
				no.
	Gestation period	Kind	Percent	
	(days)			
MS	Multiple shoot	7	90	4.5
B 5	Multiple shoot	10	90	2.3 •'
Nitsch	Multiple shoot	15	70	1.0
White	Single shoot	15	80	1.0
'F' test		*		*

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SEd		1.74		1.08
CD (5%)		2.9		1.8

Both Nitsch's and White medium were unable to provide any evidence of multiple shoot production on the corm explants. In the case of the shoot tip nodal explant, however, none of the tested media resulted in the development of numerous shoots. Nodal explants perished after being kept for an extended period of time in the same media. This was preceded by an initial browning that began at the tip. There was evidence of apical development of the shoot tip explants in all of the media; however, the MS medium required the shortest amount of time for bud break (Fig. 2). On the basis of this finding on corm explants in the four different media that were tested, MS was discovered to be the best, and it was used for additional tissue culture studies utilizing various growth regulators as a supplement.

In the month of April, fully developed corms in the form of a 'V' were harvested once the dormant phase of the corms had passed. There was at least one inactive eye present on each of the corm segments.

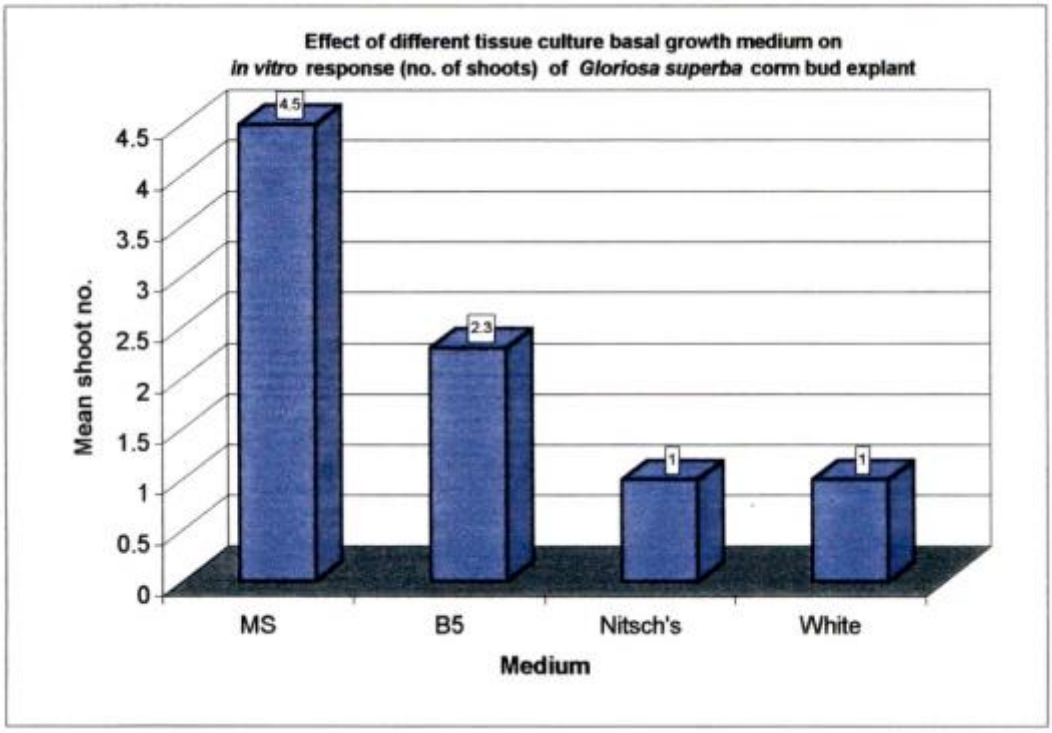

Fig. 1 Effect Of Different Tissue Culture Basal Growth Medium On In Vitro Response (No. Of Shoots) Of Gloriosa Superba Corm Bud Explant

cultivated in MS basal medium and allowed to sprout. At first, substantial fungal contamination was the cause of death for eighty percent of the cultures. In order to get rid of the contamination, the period of the fungicide treatment was extended, and the concentration of the fungicide was raised. As a consequence of this, there was a 10% reduction in the level of fungal contamination in the cultures. Conns began to sprout in vitro seven days after being inoculated, but only when contamination was removed first. Corms were used to prepare two distinct varieties of explanans: in one, the entire conn was inoculated as such on MS medium with various growth regulator treatments; in the other, the sprouts were dissected out by making a split across, with a small portion of basal corm attached to it aseptically, and inoculated into MS medium with a supplement of BAP/Kn/BAP+IAA/BAP+Kn. Both of these explanans were then The number of shoots generated by the two different types of explants that were formed from the corms was noticeably different from one another.

Table 2 Effect Of B Ap/Kn In Ms Media On In Vitro Response Of Corm Explants (Whole Corm & Splitted Sprout)

GR Conc. (mg/l)	Mean shoot number			
	Whole corm		Splitted sprout	
	BAP	Kn	BAP	Kn
0.1	1.5	1.0	2.9	2.2
0.5	2.1	1.5	4.5	3.2
1	3.5	2.5	6.5	6.3
2	2.9	3.2	8.5	4.2
3	2.0	2.3	6.3	3.9
F Test	NS	NS	*	*
SEd			1.1	1.1
CD (5%)			1.9	1.9

shows that the concentration of BAP in supplemented medium was much lower than in supplemented medium without BAP. The largest average number of shoots (6.3) was produced by the MS medium that was supplemented with kinetin at a concentration of 1 mg/1, which was higher than any of the other Kn concentrations that were examined. The

The number of shoots ranged from 2.9 to 8.5 in different BAP concentrations (0.1-3 mg/1) that were examined, and it ranged from 2.5 to 6.3 in different Kn concentrations (0.1-3 mg/1) that were tested. The best result was observed at a concentration of lmg/1 yielding 6.3 number of shoots per corm. This concentration was chosen from among the several kinetin concentrations.

The addition of auxin to a medium that had already been treated with cytokinin did not result in an increase in the number of multiple shoots produced from corm bud explants (data not shown).

Table 4. 3 Effect Of Type And Combination Of Pgr On Shoot Tip And Nodal Explain Culture Of Gioriosa Super bct In Ms Medium.

Explant	PGR concentration (mg/l)	Kind of response	Remark
	BAP	Apical shoot growth	Fast growing
	Kinetin	Apical shoot growth	Slow growing
Shoot tip	NAA	Nodular structure at base	Non regenerative
	BAP+IAA	Nodular structure at base	Regenerative
	BAP+NAA	Nodular structure at base	Regenerative
	BAP+Kinetin	Direct multiple shoot regeneration	Multiple shoot along with corm
	BAP+AS	Nodular structure	Regenerative
	Kn + IAA	Multiple shoot from base	Shoot formation preceded by little nodular structure
Nodal explant	BAP/Kn/BAP+IAA/Kn+IAA	No response	Browning followed by death of explant
	BAP+Kn	Multiple shoot formation	Weak, Fragile shoots

combined with IAA or NAA was put through a series of tests to see how effective each one is at triggering numerous shoots from a single shoot tip. The kind of response that was produced in the shoot tips that were grown in MS medium that contained either cytokinin (BAP/Kn) alone or in combination with an auxin (IAA and NAA) or two cytokinins (BAP and Kn, BAP and AS) together has been presented in Table 4, and the specific details of those responses have been presented under various subheadings.

REPERCUSSIONS OF BAP AND KINETIN

The impact of cytokinin on the in vitro response of the shoot tip was examined by supplementing MS medium with BAP/Kinetin at concentrations ranging from 0.1 to 4 mg/1.

There was no indication of multiple shoot induction at any of the amounts that were investigated for both hormones. After one week after inoculation, the apical shoot bud

underwent differentiation into a shoot, giving birth to a single shoot per explant in all of the treatments for both BAP and kinetin.

Even though the number of shoots generated from each explant was just one, regardless of the concentration and kind of growth regulator that was utilized, the length and vigor of the shoots, however, remained consistent.

Table 4 Effect Of Bap/Kn On Shoot Tip Culture Of Gloriosa Superba Cultured In Ms Media After 45 Days

GR Conc. (mg/l)	Days to bud break		Mean shoot length (cm)	
	BAP	Kn	BAP	Kn
0 1	7	7	2.5	2.9
0.2	7	7	2.8	3.3
0.5	6	7	4.3	4 0
1.0	6	6	5.2	4.5
2.0	5	7	6.0	5.5
3.0	5	6	7.5	4.0
4.0	6	6	4.8	2.9
'F test	NS	NS	*	*
SEd				0.79
CD (5%)				1.3

shifted depending on the treatments (Table5, Fig. 5). After 45 days, the shoot length was greatest (7.5 centimeters) in the medium that included 3 mg/1 of BAP. This was the case regardless of the BAP concentration that was used.

In a second series of experiments, auxin and cytokinin were mixed together at various concentrations, and the impact of this combination on the development of many shoots from a single shoot tip was investigated. BAP and Kn, BAP and IAA, BAP and NAA, BAP and AS, and Kn and IAA were the many combinations of growth regulators that were used.

Effector BAP+Kn

The effect of BAP and kinetin combined in MS medium on in vitro shoot multiplication of shoot tip explants was examined by adding BAP at concentrations ranging from l to 4 mg/l

together with Kn at concentrations ranging from 2-4 mg/l. BAP and Kn alone in MS media did not lead to the introduction of numerous shoots, despite the fact that they did cause.

Table 5 Effect Of Bap+Kn In Ms Media On In Vitro Shoot Multiplication Of Gloriosa Superha.

PGR concentration mg/l		Average no. of shoots	
BAP	Kn	Shoot tip	Nodal explant
1.0	2.0	-	-
1.0	3.0	-	-
1.0	4.0	-	-
2.0	2.0	3.9	1.5
2.0	3.0	4.2	2.0
2.0	4.0	5.9	3.5
3.0	2.0	7.5	3.9
3.0	3.0	10.3	5.7
3.0	4.0	8.3	4.2
3.0	1.0	4.2	2.9
4.0	2.0	5.0	2.0
4.0	3.0	2.3	1.5
4.0	4.0	-	-
F test		*	NS
SEd		1.09	
CD (5%)		1.8	

BAP and Kn, when coupled together, resulted to direct multiple shoot production on the shoot tip explants. these results were obtained by using shoot tip explants (Table 6). BAP at a concentration of lmg/1, on the other hand, did not generate any numerous shoots on the explants even though there was no Kn present. The highest possible average number of shoots was generated in a medium that included BAP and Kn at concentrations of 3 mg/l each, with corm development occurring simultaneously at the base of the shoots. This resulted in a total of 10.3 shoots (Table6, Fig.6, Plate 4c). When shoot tips were cultivated in

MS media that was supplemented with BAP (3 mg/l) and Kn (4 mg/l), the number of multiple shoots generated was 8.3, which was the second best number (Plate 4b). On average, 7.5 numbers of shoots were generated when BAP was applied at 3 mg/l and Kn was applied at 2 mg/l (Plate 4a). At a concentration of BAP (4 mg/l) and Kn (3 mg/l), the minimal number of shoots that could regenerate was 2.3. Complete inhibition of multiple shoot regeneration was seen after the addition of BAP and kinetin at a dose greater than 3 mg/1 in both cases.

THE IMPACT OF BAP AND IAA

Cytokinin and auxin were both present in the medium, but when combined, they elicited a distinct sort of response in the shoot tip explants (Table4). At the time when BAP was introduced to the media with IAA,

Table 6 Effect Of Pgr Combinations (Bap+1aa) On Shoot Tip Culture Of Ghriosa Superba In Ms Medium.

GR Conc.(mg/l)		% response	Days to respond	Type of Response	Mean shoot length (cm) ± S.E
BAP	IAA				
2	0.05	75	7	Apical shoot growth	3.1±0.12
2	0.1	80	7	Apical shoot growth	2.8±0.05
2	0.5	80	8	Apical shoot growth	5.3±0.23
3	0.05	75	7	Apical shoot growth, slight nodular structure	3 5±0.96
3	0.1	95	15	Nodular structure at the base	Inhibition of apical shoot growth
3	0.5	80	8	Apical shoot growth	4.3±0.19

certain quantities, as well as some specific combinations of concentrations, caused a basal nodular structure on the tips of the shoots. The kind of reaction that was shown by the Gloriosa superha shoot tips changed depending on the concentration combinations of BAP and 1AA. The concentrations of BAP and IAA that were utilized as supplements in MS medium varied between 0.1 mg/l and 3 mg/1 for BAP and between 0.05 mg/l and 0.3 mg/1 for IAA, respectively. Every combination with a smaller proportion of BAP to IAA failed to

elicit any multiple shoots from the explants and instead produced only one shoot from each shoot tip explant. After two weeks of culture, the development of a basal nodular structure was induced in the shoot tips by BAP at a concentration of 3 mg/1 in combination with 0.1 mg/1 of IAA (Table 7). This was preceded by the apical bud's termination of its upward expansion (Plate 6a). They needed to be subcultured into new media since the nodular structure continued to expand despite the fact that the media continued to be used up gradually. After the third subculture, or after two months of inoculation, there was abundant branching occurring downward, approximately equal in diameter, with thick roots, the color of which was light green (Fig. 14, Plate 6c, 6d & 6e). The subsequent growth of one or two adventitious buds per culture, which led to the regeneration of shoots, took place after this (Plate 6f). When they were sectioned longitudinally in another set of experiments, and each section was transferred to fresh medium both liquid (Plate 7b) and solid medium separately, the number of multiple shoots that regenerated increased, with significant differences in their extent between liquid and solid medium.

Sivkumar & Krishnamurthy, 2000After producing longitudinal sections, there was an increase of up to 12-15 shoots per explant in the number of shoots that were formed in solid medium (Plate 7a).

REGENERATION IN A MEDIA CONSISTING OF LIQUID

Some longitudinal parts of the nodular structures were transplanted into liquid MS medium with the same growth regular combinations, which resulted in an increase in the number of shoots generated by a factor of 25-30. (Table8, Plate7c, Fig. 7). After increasing the BAP concentration up to 4 mg/I while maintaining the same level of IAA concentration, it was not possible to increase the number of shoots generated any more. There was no discernible difference in the amount of multiple 58 shoots produced whether the BAP concentration was raised beyond 4 mg/I or decreased to 2 mg/I while maintaining the same level of IAA concentration. This was also the case when the BAP concentration was dropped below 2 mg/I. An increase in the amount of IAA in the water had a detrimental effect on the

CONCLUSION

The performance of several tissue culture media, including Gamborg's, Nitsch, White, and MS media, was analyzed based on the kind of in vitro response generated by these media on corm bud explants after 30 days of culture. The media in question were Gamborg's, Nitsch,

White, and MS media. The results of this experiment were used to choose the medium for the subsequent tests that were to be conducted.When corms were inoculated in MS basal medium, it took one week to regenerate a single shoot from the sprout. This was followed by 10 days and 15 days for Nitsch's, Gamborg's, and White mediums, respectively. The results showed that MS medium had the best performance overall, requiring the fewest number of days to initiate a response on the explant and producing the greatest number of shoots per explant after the observation period.

REFERENCES

1. Akerele, O., Heyword, V. and Singe, H. (1992)(Eds.) Conservation of medicinal plants Cambridge University Press, Cambridge, and U.K.pp. 145-189

2. Amin, M.N., Jaiswal, V.S. (1993) In vitro response of apical bud explants from mature trees of jackfruit (Artocarpus heterophyllus). Plant Cell Tiss. Org. Cult. **33 :** 59-65

3. Andrade, M. W., Luz, J, M. Q., Lacerda, A. S, and Melo, P. R. A. DE. (2000) Micropropagation of Urandary (Myracrodonon ueundeuva Er. All) Cienciae. Agrotechnologia. **24 (1):** 174-180

4. Animuddin, J.K., Anus, M. and Balasubramanium, V.R. (1996) Regeneration of Piper kettle from callus tissue. Curr. Sci. 65: 795-796

5. Animuddin, M., Johri, J. K., Anus, M. and Balasubramanium, V. R. (1993) Regeneration of Piper betel from callus tissue. Curr. Sci. 65: 795-796

6. Anzidei, M., Vivona, L., Schiff, S., Bennici, and A. (1996) In vitro culture of Foenicttlum vulgare: callus characteristics in relation to morphogenesis. Plant Cell Ttss.Org.CultAS: 263-268

7. Aradhya, M.K., Zee, F. and Manshardt, R.M. (1994) Isozyme variation in cultivated and wild pineapple. Euphytica 79: 87-94

8. Arockiasamy, S. and Ignacimuthu, S. (1998) Plant regeneration from mature leaves and roots of Eryngittm Foetidum L., a food flavouring agent. Curr. Sci. 75(7): 664-666

9. Arora R. and Bhojwani S.S. (1989) Plant Cell Rep. 8: 44-47*

10. Arrebola, M. L., Socorro, 0 . and Verpoorte, R. (1997) Micropropagation of Isoplexis canariensis (L.) G. Don. Plant Cell Tiss. Org. Cull. 49: 117-119

11. Arya, S., Liu, J.R., Eriksson, T. (1991) Plant regeneration from protoplast o f Panax ginseng (C.A.Meyer) through somatic embryogenesis. Plant Cell Rep. Id: 277-281

12. Asahira, T. and Yazawa, S. (1970) Study on bulb formation o f Dioscorea batatas by in vitro stem culture. In: S. Baba (ed.) Second symposium for plant tissue culture. Kyoto Univ. Coop. Press, Kyoto, pp.2