

Axillary Shoot Proliferation in Shoot Bud of *Zingiber officinales* (Ginger): Experience Based Review

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Abstract: *Zingiber officinale* Roscoe is a perennial herb belonging to the Zingiberaceae family. It is the most common ginger species in India and it is grown all over the country. The rhizome is horizontal, branched, fleshy, aromatic, white or yellowish to brown, which are generally effective in stomach disorders such as colic, spasms, vomiting, dyspepsia, flatulence, and other painful disorders. It accumulates high levels of important pharmacologically active metabolites, viz., [6]-Gingerols, which are some of the products of the phenylpropanoid pathway. The other constituents in *Z. officinale* are the pungent vanilloids, and [6]-paradol. The study on surface sterilization revealed that explants treated with 0.1 per cent mercuric chloride for 12 minutes, showed the highest aseptic culture establishment. Among the two different explants viz., shoot tips axillary buds treated, shoot tips gave maximum survival percentage and healthy culture establishment. We used amplified fragment length polymorphism to produce DNA fingerprints for the ginger species. The present investigation was undertaken to standardize the protocol culture establishment multiple shoot production in vitro rooting and also suitable hardening media for micro propagated plantlets of ginger. In the present study, we were tried to identify a suitable explant for in vitro propagation of ginger different explants. Among the various explants, shoot tips gave the quickest response for initial growth and the highest number of multiple shoots. On the other hand, Axillaries bud took more time for the regeneration of shoots. This difference in response among the different explants might be due to difference in physiology. This may also be due to the fact that, the shoot tip has meristematic region where cell division and differentiation occurs.

I. Introduction

Plant Tissue Culture is a practice used to propagate plants under sterile conditions, often to produce clones of a plant. Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation, including the production of exact copies of plants that produce particularly good flowers, fruits, or have other desirable traits. Ginger always has the same purpose, to be consumed; it's just the preparation that differs. Younger roots are cleaned and scraped to create white ginger which raw and tends to be more potent and hot. Older roots are usually cooked and dried for teas and spices; this is a form of black ginger. Each kind will add spicy seasoning and herbal benefits¹.

Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant (Totipotency). Single cells, plant cells without cell walls (protoplasts), pieces of leaves, or (less commonly) roots can often be used to generate a new plant on culture media given the required nutrients and Ginger was known in China as early as 400 BC. The Greeks and the Romans thought it was of Arabian origin because it was sent from India through the Red Sea. They used it as a spice. It was introduced into Jamaica and other islands of the West Indies by the Spaniards and ginger was exported from the West Indies to Spain in considerable quantities around the year 1547 AD.

Ginger is considered an herbal remedy in many cultures. Over the years, it has been used to reduce inflammation, help with digestion, improve cardiovascular health and even historically to ward off diseases like the plague. The rhizome can be sliced, chopped, minced or served fresh but will always carry the trademark "heat" that has made it a medicinal mystery. Historically, people used ginger to fight disease and cure a fever since people would sweat when they would eat the root. Although that helps, since it gets the metabolism racing and warms the body, we now realize that ginger offers additional medicinal benefits.^{2,3} The **Gingiberous** plants have been recognized as medicinally valuable reference of these plants are found in all system of Indian traditional and folk medicine about so genera and 1300 species of ginger are known to exist worldwide. The plant occurs mainly in Australia, Bangladesh, Haiti, Jamaica, Japan, Nigeria, Sri Lanka and most regions mainly around Chennai, Cochin, Assam, Arunachal Pradesh. *Zingiber officinales* is a perennial herb and most common ginger species; it is grown over all country.⁴ The rhizome is horizontal, branched, aromatic, white

or yellowish to brown which are generally effective in stoma-tic disorders such as colic, spasm, vomiting, dyspepsia and other painful disorders.



Figure showing the Young seedling



Figure showing the healthy Rhizome

It is very important in relieving severe nausea. In case of nausea, pregnant women can use this medicinal plant as a medicinal alternative. It can reduce arthritis' pain because it has anti-inflammatory substances. It can also benefit people who are suffering from gastric complications because it has a good effect in the digestion process. Gastric juice or liquids are stimulated by the chemical properties of ginger. *Zingiber officinales* can also relieve bowel problems and prevents from suffering Diarrhea. If someone having a bad breath or any smell problem, this medicinal plant can also act as freshener of mouth. It has also a strong anti-fungal substance which could be very effective in curing health problems caused by the bacteria fungi. Dizziness or vomiting is usually caused by travelling a long trip. This herbal plant is very effective in getting rid of this problem. Ginger medicinal plant is also effective in preventing colon, gastric or lung cancer because of its anti-cancer agents. It is also effective in lowering the cholesterol level of a person with heart problems. It is effective in relieving morning sickness, menstrual irregularity and headaches. Diabetic nephropathy disease can also be reduced by this miracle plant according to science studies. Ginger is a natural medicinal plant in curing colds and coughs. This prevents any cases and causes of flus. For those patients suffering from migraine, daily intake of ginger helps reduce migraine problems in curing colds, coughs and congestions; it is effective when a patient will take the Ginger drink with Tulsi in the morning⁵⁻⁸.

II. Materials And Methods

The main objectives of this project are to organize of plant tissue culture and its technology. The present project named as "Axillary Shoot Proliferation in Shoot Bud of *Zingiber officinales*(Ginger): Experience Based Review" was performed in our Tissue Culture Laboratory and the materials & methods used as given below.

2.1 Source of Materials:-

The experimental material such as shoots of ginger were obtained from the Pallishree Research Farm, Arambagh(WB) and Grow Tips Biotech, Bhopal(MP) to see the different variation of weather and climate condition of the different States. Murashige and Skoog's Medium (Murashige and Skoog, 1962) with 2.0 % sucrose and 0.7% Difco-bacto agar was used as the basal medium (MS). Only analytical reagent grade chemicals and the Scott Duran with Borosil glassware's were used.



Figure showing the buds of Rhizome



Figure showing the sterilized explants

2.2 Experimental Set Up:-

For raising cultures, the shoots of *Gingiber officinalae* were surface sterilized with 0.1% Mercuric chloride for 5 minutes. After repeated washing in sterile distilled water, the shoots were aseptically reared on MS Media alone and also MS Media supplemented with various growth adjuvant individually and in a few combinations. About three week old shoots were selected for experimentation and the various explants like shoot tips, nodal segment, epicotyls segments, hypocotyls segments, cotyledon segments and entire leaflets were aseptically excised and implanted on the suitable nutrient media. The present investigation was undertaken to standardize the protocol culture establishment multiple shoot production in vitro rooting and also suitable hardening media for micro propagated plantlets of *Gingiber officinalae*.

III. Results & Observation

3.1 Culture Establishment:-

3.1.1 Standardization of the Source of Explants:

In the present study, we identify suitable explants for in vitro propagation of different ginger explants was tried. Among the various explants, shoot tips gave the quickest response for initial growth and the highest number of multiple shoots. On the other hand, Axillaries bud took more time for the regeneration of shoots. This difference in response among the different explants might be due to difference in physiology.⁹ This may also be due to the fact that, the shoot tip has meristematic region where cell division and differentiation occurs.

3.1.2 Standardization of the Sterilization procedure for Different Explants:

The current investigation on effect of surface sterilants on reducing contamination rate and per cent of healthy cultured plants. It showed that $HgCl_2$ is better sterilant than $NaOCl$ in reducing contamination rate. This is because the most useful radical in $HgCl_2$ is probably the chlorite, commonly present as bichloride of mercury. Mercuric chloride is extremely poisonous due to high bleaching action of two chloride atoms and also mercuric ions which combine strongly with protein causing death of organism.¹⁰ Though $NaOCl$ consists of chlorine atom, its bleaching and disinfectant action is due to the slow decomposition of the salt to produce oxygen. The higher concentration of $HgCl_2$ at 0.1 per cent for 15 minutes observed more contamination and also death of the explants. This is due to the high bleaching activity of chlorine which killed the cells.



Figure showing the inoculation of explants



Figure showing the shoot formation

3.1.3 Standardization of the Growth Regulators for Shoot Growth:

In the present study, the less number of days for initiation and the highest number of multiple shoots were observed in 2 mg/l BAP supplemented media. This was in confirmation with the results of Dipti et al. (2005), who reported that the highest number of multiple shoots in media supplemented with 2 mg/l BAP in shoot tip. Similarly **Winnar and Winnar (1981)** reported that BAP 1 mg/l was most useful for development of multiple shoots.^{11-16,18}

Table No 1: Responses of shoot tip

S. No.	Nutrient Media (Mg/l)	Days to Shoot/ Root Induction	No. of Shoot	No. of Root
1.	Ms(h)	23	1.0	3.0
2.	Ms(f)	22	1.8	3.5
3.	Ms+ tdz(1mg/ml)h	22	2.3	5.0
4.	Ms+ tdz(1mg/ml)f	15	3.0	6.5
5.	Ms+ bap(1mg/ml)h	23	4.0	7.4
6.	Ms+ bap(1mg/ml)	20	2.1	7.5
7.	Ms+ iaa(0.5mg/ml+bap1mg/ml)h	16	1.9	6.5
8.	Ms+ iaa(0.5mg/ml+bap1mg/ml)f	16	1.3	5.8

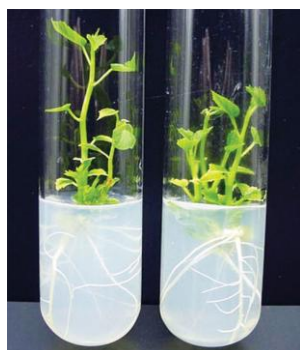


Figure showing the complete plantlet



Figure showing the hardening in Green House

IV. Discussion & Conclusion

Early responses for sprouting and better culture establishment of shoot tip were observed in ms medium among the cytokines BAP at different concentration 2.0 mg/ml BAP produced more number of multiple shoots and lowest length of shoots on Cytokinin free medium single shoots with maximum length were produced.

The present study of axillary shoot proliferation of Ginger(*Ginger officinales*) was conducted in the Tissue Culture Laboratory of School of Biological & Chemical Sciences, MATS University, Raipur(CG). Ginger is an important spice crop grown in India. It is herbaceous rhizomatous perennial plant. The method of propagation is through section of underground rhizomes however it has a dormancy period and sprouts only during the monsoon that to only 5 to 6 plants can be obtained from one single rhizome per year. The plant from proliferation is true type and free from disease.^{17, 19} The present investigation of Ginger was carried out to standardize surface sterilization of explants. Suitable explants type for culture establishment growth regulators for shoot multiplication and rooting and to evaluate suitable hardening media.

Among the various concentrations of $HgCl_2$ and $MnSO_4$ tried for surface sterilization among that the different concentration of $HgCl_2$ tried at 0.1% emerged as the best treatments. Different type of explants via shoot tip axillary bud, root tip was tried. Finally it concluded that shoot tip gave the best result and emerged as suitable explants for Ginger culture establishment among the different concentration of cytokines viz BAP and Kinetin, 2.0 mg/BAP gave the highest number of multiple shoots and among different auxin tried at different concentration for rooting of micro-shoots MS Medium with 1Mg/IBA gave the highest number of shoots.

Acknowledgement

We would like to thanks Dr. Shagufta Khan, Director of Grow Tips Biotech, Bhopal(MP) for giving us permission & providing us Research Materials to carry out research in the MATS School of Biological & Chemical Sciences, Raipur. Our deep gratitude towards the Director's of Pallishree Research Farm, Arambagh(WB) for their all time support and the positive response during the tenure of Research. We are also gratefully acknowledging the supportive nature of Mr. Vishwaprakash Roy & Dr. Ashish Saraf and all the Staff Members of MATS School of Biological & Chemical Sciences, Raipur (CG).

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