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# A Review on *In vitro* Regeneration of Ginger: Tips and Highlights

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors TK, GR, NDS and GS wrote the first draft of the manuscript and managed the literature searches. Author SHM reviewed and edited the manuscript. Author FAA supervised the study and edited the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

Ginger (*Zingiber officinale*) is one of the oriental spices, widely used worldwide for multiple purposes. It is applied as an important ingredient in Ayurvedic preparations from time immemorial. Tissue culture is a practice that is utilized to propagate plants from cells or tissue under sterile conditions. This study is directed to create a review of the successful and reproducible convention for in vitro recovery of ginger with emphasis on effective initial culture establishment. Furthermore, it has dealt with the appropriate explant size and effectiveness of media quality on micropropagation of ginger. The current study recommends that the medium containing Benzyl Amino Purine (BAP) can be used for inducing shoot development of ginger. Among the diverse explants, shoot tips give the quickest response for starting development and the highest number of multiple shoots are produced. As well, it is demonstrated that a survival rate and proper shoot/root expansion can be obtained through the tissue culture methods. Emphasizing the tips and recommendations, this study would be a route-map towards time and cost saving for producing a better quality of ginger.

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## 1. INTRODUCTION

Ginger (*Zingiber officinale*) is herbaceous perpetual, monocot plant from the family *Zingiberaceae*. It is found to be everywhere throughout the world as a zest and medication. Ginger is planted in the tropics for its palatable rhizomes filling culinary and therapeutic needs [1]. The ginger rhizomes are small while the fibrous flesh is ash white in color. The ginger's taste and aroma are comparatively higher than other species and it is largely used for beverages [2,3]. Rhizome part of the ginger plant is usually utilized as toppings in sustenance readiness to add to the taste and flavor [4]. Apart from the flavor, ginger is stacked with bioactive photochemical, such as gingerols, shagols as well as unstable oils like sesquiterpenes (β-bisabolene and (−) zingiberene and monoterpenes (geranial and neral) [5].

Biotechnological approaches for crop improvement require efficient regeneration of crops from tissue culture. In vitro techniques considered as one of the best options that might be supplied an extensive number of planting materials for business planting and further investigations [6]. Ginger is mostly confined to propagate from shoot-tip culture [7-9]. A huge number of plantlets can be created within 1-2 years from a single explant. The tissue of ginger is cultured in vitro and therefore liberates of pathogens. High recurrence recovery of plants from in vitro culture tissues is a pre-requisite for effective use of tissue culture procedure to edit [10]. Development of in-vitro regeneration is very important for the rapid micropropagation of ginger which is by direct organogenesis or physical embryogenesis [6]. Different explants, for example, vegetative buds and shoot tips have been utilized as explants to set up in vitro of ginger [11].

A high diverse germplasm collection is the basis of the most successful plant breeding programmes [12,13]. Among options open to plant breeders to widen the genetic base, are exploitation of different variation and somaclonal variation [14]. Plantlets attained from in vitro culture may show a somaclonal variation which has been frequently heritable. It has been reported that valuable morphological, cytological, and sub-atomic varieties might be produced in vitro. Somaclonal variation caused by the tissue culture process more specifically characterise the prompting condition [3,6-10]. Somaclonal variety can be shown as either somatically or meiotically [11] stable occasion. Combining somaclonal variety strategies with strategic and proficient in vitro can prompt the generation of variations [8].

Moreover, ginger plants are inclined to parasitic, bacterial, and viral and mycoplasma infections such as *Pythium aphanidematum* causing delicate decay, *Fusarium oxysporum* causing yellowing of leaf, *Pseudomonas solanancearum* causing bacterial shrivel, *Phyllosticra zingiberi* causing leaf spot notwithstanding shoot borer *Conogethes punctiferalis* and root-tie nematode *Meloidogyne incognita* prompting crop misfortunes [15]. In addition, support of germplasm by yearly ranch is costly and laborious [16]. Tissue culture is the main strategy that can deliver a huge amount of clonal plants in a brief span with the high phytosanitary quality, thusly, it is imperative to produce malady free clones in extensive numbers in brief time and space by means of in vitro plant tissue culture method to guarantee a persistent supply of ginger to the ranchers. Different explants, for example, vegetative buds [11] and shoot tips [17] have been utilized as explants to build up in vitro culture of ginger. The present examination depicts a proficient convention for the micropropagation of ginger and the study has been attempted to exhibit the proficient proliferation of rhizome actuated by tissue culture in creating ordinary rhizome in ginger. This review is aimed to compile and recommend the proper steps of a ginger tissue culture protocol.

## 2. LITERATURE REVIEW

Ginger is a homestead and field economic harvest plant [18]. Generally, in vitro propagation of ginger is carried out to acquire infect free plants [19]. It relies on different factors such as the composition of media, culture temperature, explant and environmental conditions (photoperiod humidity) [20]. Throughout the years, ginger has been utilized to diminish irritation, help with the processing, enhance cardiovascular wellbeing and even generally to avert illnesses [21]. Since bacteria, viruses, and fungi are promptly transmitted through conventional practices, it is important to build up a proper micropropagation method. At the same time, it should be accessible for commercial use for producing disease-free ginger germplasm. In vitro regeneration of auxiliary and adventitious

shoots from the shoot, tips have already been attempted by callus culture [22]. Clonal multiplication methods through meristem tip culture have also been reported [23]. Plant tissue culture is a technique used to proliferate plants under sterile conditions to obtain pure clones [24] of a plant. Different procedures in plant tissue culture may offer certain favourable circumstances over conventional techniques for engendering, including the generation of particular plants [8].

Plant tissue culture mainly depends on the way that many plant cells can recover an entire plant (Totipotency). Single cells, plant cells without cell dividers (protoplasts), bits of leaves, or (less generally) roots can frequently be utilized to create another plant in culture media given the required supplements and in this regard Ginger is viewed as a homegrown cure in many societies [24-25]. Every kind will include zesty flavour and natural advantages [25]. The optimum multiple shoot regeneration is obtained from shoot tip explants of ginger on MS medium supplemented with 1.0 mg/L NAA + 3.0 mg/L BAP [21]. It has been reported that high contamination of cultures occurred when rhizomes or vegetative buds are used for micropropagation of ginger [26]. A technique called in vitro proliferation was built up to protect ginger germplasm resources [27]. In this technique plantlets with the complete rooftop system are recovered specifically from shoot tips (0 2-0.9 mm long) of ginger on MS medium containing 2.0 mg/L BAP and 0.6 mg/L NAA. Adventitious shoots and roots are also regenerated from leaf sheaths on MS medium containing 1.0 mg/L BAP and 0.6 mg/L NAA [28]. Auxiliary shoot expansion is the principal strategy for shoot multiplication on MS media, with the rate being around 6.0 increases for each month [21]. Shoot and bud production was the best in a high concentration of BAP with low levels of NAA. The shoots rooted best when transferred to a medium supplemented with 10% activated charcoal, with or without NAA at 0.5 mg/L [29].

The proliferation rate was not shown high to sufficiently acquire disease free quality. In vitro propagation has long been identified as an effective means for rapid clonal multiplication and conservation of important taxa [23]. However, in vitro culture is the best method as a persistent source of supply of disease-free planting material for commercial use [24]. The most important role of in vitro propagation is to conserve the genetic variation and evolutionary process in viable populations of ecologically and commercially viable varieties genotypes in order to prevent their potential extinction [25].

Micropropagation is the most widely recognized method which is basically important these days and could overcome the hereditary segregation of the plants developing from seeds [26]. The aim of culture creation by micropropagation technique is to put the explants into aseptic culture conditions for a better and consistent shoot production [27]. Micropropagation is the rapid vegetative proliferation of plants under in vitro condition of high light intensity, controlled temperature and specifies nutrient media [30]. The development of shoots is affected by the physical stability of nutrient media. Agar is the most common gelling agent for culture because of its inert nature that keeps its mediation with plant digestion [31].

## 3. MATERIALS AND METHODS

### 3.1 Plant Material

The gingers are covered with a wet cloth and left it for 2 weeks for a shoot growth. The gingers are kept on examine every two days to avoid any contamination.

### 3.2 Sterilization of Equipment and Utensils

#### 3.2.1 Air flow cabinet

The Laminar air flow cabinet is swabbed with 70% ethanol before all the autoclave utensils and the chemical is placed inside it. The UV light (250-270 nm) of the laminar air flow cabinet is switched on for 15 minutes to sufficiently eliminate microorganism present [32].

### 3.3 Glassware and Utensils

Glassware such as beakers, petri dish, culture tube, scoot bottles, conical flasks and forceps, scalpels are needed to use are washed with detergent thoroughly [22]. The glass wares are soaked with tap water containing detergent for 20 minutes [33]. Then, after 20 minutes, all the glassware and utensils are washed with tap water and rinsed thoroughly with distilled water and have placed in a clean container for 30 minutes to dry at room temperature [10]. Then, all the glassware and utensils wrapped with aluminium foil and autoclaved at the temperature 121ºC for 15 minutes [32].

## 3.4 Media Preparation

### 3.4.1 Preparation of stock solution

The stock solution for MS medium is prepared early. MS medium consists of macronutrients (10x), micronutrient (100x), vitamins (1000x), iron (100x), sucrose, plant growth regulator and distilled water. These prepared stock solution placed in the chiller at 4°C [12].

## 3.5 Preparation of Plant Agar

Plant agar is added into 300 ml of beaker at the ratio of 2.4 g/ml [34]. Then agar is heated and stirred by a magnetic stirrer to allow the agar to completely dissolve before being added to MS medium. After that, the medium is poured into tube culture at a volume of approximately 15 ml per tube [12]. The tube culture then is cooled and covered with the caps. All the tube culture is labelled and autoclaved at 121°C for 15 minutes. As soon as agar is solidified, the tube culture is tightened before storing in air-conditioned incubation room [10].

## 3.6 Preparation of Plant Material

Young shoots at different sizes ranging from 0.5 to 5.0 cm are collected and used as the source of explants [35]. The young shoot is washed under running tap water for an hour. The explants are washed thoroughly using with 20% of Clorox (5.25% NaOCL) of Tween-20 for 30-40 minutes before bringing inside the laminar air flow chamber to surface sterilize [21]. The explants are then rinsed several times with sterile distilled water. The outer layers of leaf sheaths of sterilized explants are removed until 5 to 8 mm size [11]. The explants are then cultured onto solidified Murashige and Skoog's (MS) (1962) basal medium supplemented with 3% sucrose with three different concentration of cytokinin 6-benzylaminopurine (BAP) (0, 1.0 and 5.0 mg/L). The pH of the medium is adjusted to 5.8 prior to autoclaving (15 min, 121°C) [19]. The cultures are incubated in the culture room under white fluorescent light with a light intensity of 50 µM m-2s -1 at a photoperiod of 16h at 25±2°C [36].

## 3.7 Observation of the Plant

Cultures should be examined regularly for contaminations and those presented with apparent infection symptoms are immediately discarded [21]. At the end of 8th week of the culture, the percentage of the callus formation and the explants which do not respond should be assessed [10].

## 3.8 Rooting

The extrinsic shoots that created on MS media are disconnected [22] from the callus material when they have framed little rosettes of 1 to 1.5 cm crosswise over comprising of three to five clears out. These rosettes are exchanged separately to 100 mL sterilin jars [37].

## 3.9 Shooting

Following a month and a half of culture foundation, the survived plant explant are transferred to new MS medium supplemented 5.0 mgL-1 BAP and 0.5 mgL-1 NAA for the arrangement of the micro-shoot augmentation [38]. The culture is maintained by subculture on the new medium [39].

## 3.10 Transfer the Ex-plant to Soil

The explants are recovered from the MS medium, washed with refined water to expel the agar and exchanged to the disinfected soil. The plant is kept in plastic pots and covered with plastic bags to maintain a high relative humidity. Finally, the explants are transferred to the greenhouse after the two weeks in the normal growth incubator and kept the plant in a greenhouse [40].

# 4. DISCUSSION

The supplemented MS medium with various concentrations and the combination of auxin and cytokinin is used to assess the growth of shoot and root induction [13]. The presence of cytokinin (kinetin or BAP) alone or with auxin (NAA or IBA) in MS media are usually used to give numerous shoots and their subsequent plantlet development in ginger [12]. Few researchers have detailed the utilization of different cytokinin is being helpful to improve multiplication rates in ginger [41]. It is found that the best various shoot regeneration is attained from the shoot tips of ginger on MS medium supplemented with 1.0 mg/L NAA + 3.0 mg/L BAP [21]. The observation of the effectiveness of cytokinin on the development of microshoots on the explants has been proven that cytokinin induces the growth of micro-shoot [42]. During tissue culture of plants adding cytokinin to the medium are very

important because they can induce the division and organogenesis and it can effect on other physiological and development processes [43].

The shoot and bud generation is shown to be the best in low levels of NAA with a high concentration of BAP [36]. The impact of NAA and BAP on shoot length in both solid and fluid medium has been demonstrated [10]. It is also shown that the NAA can expand shoot length in both solid and liquid medium [20]. Treatment with BAP induces better shoot multiplication to respond especially when it is treated along with NAA that can be led to the production of numerous shoots. The use of BAP in the culture medium influences on the rapid proliferation of meristem of many plants including ginger. BAP alone or in a blend with NAA is more successful for the shoot and root separation and generally utilized on micropropagation [36]. However, a higher concentration of BAP can cause a reduction in the shoot length and results in abnormalities [41]. It also proves that the addition of either NAA or IBA or IAA in the culture medium improves the response in terms of shoot growth [42]. Moreover, sucrose is used to supply carbon source [43] and energy and to keep up a stable or to maintain osmotic weight condition to support the development of in vitro cultures of plants [44]. Further, it could be seen that when sucrose concentration is low in the rhizome enlistment medium, the quantity of rhizomes is decreased under light condition [44-45]. The high amount of sucrose helps to arrange a high number of rhizome development. In this regard, the most elevated number of rhizomes generation is recorded with MS medium treated with 90 and 30 g/l of sucrose under light and dark conditions, separately [46]. Increasing the concentrations of benzyl aminopurine from 0.0 to a maximum level of 3.0 mg/L responsible for shoot multiplication. Effective plant regeneration is accomplished when embryogenic cultures are exchanged to MS medium containing 8.9 ppm of BAP [47]. Other than that, shoot tips give the fastest reaction to initial growth. The maximal number of multiple shoots is produced [48]. Then, axillary bud sets aside more opportunity for the regeneration of shoots [49].

## 5. CONCLUSION

In the presence of MS medium, the perfect hormone concentration for the formation of multiple shoots is expressed, whereas MS + NAA are the standard growth regulators for abundant rooting. Besides that, BAP in combination with the lower concentration of NAA synergistically improves the number of shoots. However, it unfairly decreases the shoot length, yet expands the quantity of root in combination with the higher concentration of NAA. Plantlet and root developments also parallel to the addition of sucrose that is recommending a synergistic impact. Similarly, the number of shoots increases and the mean length of shoots decreases since the concentration of cytokinin increases. Furthermore, shoot tips are well regenerative. The highest number of multiple shoots is produced by shoot tip explants. Among different concentrations of cytokinin, BAP, and kinetin, BAP gives the highest number of numerous shoots. Among the different types of explants the shoot tips possess the quickest reaction to initial growth and in turn, the highest number of multiple shoots are produced. This study suggests that the use of BAP, cytokinin, NAA and phytochrome concentration can avoid plant abnormalities. As well, media preparation should be conducted in a proper condition to avoid contamination that can inhibit the plant growth. Careful determination of factors including the source, type, formative stage and size of explants, cleansing of explants, growth regulator and medium and culture conditions are the important elements for the successfulness of in vitro propagation of ginger. The optimization of these factors is a guide for effective tissues in many plant species.

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## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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