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Review Article

INVITRO PROPAGATIONS AND MICRO RHIZOME INDUCTION OF *ZINGIBERACEAE OFFICINALE*

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Abstract *Ginger (Zingiber officinale Rosc.), is a rhizomatous crop that belongs to the Zingiberaceae family. Its bioactive ingredient has gained much attention for its many uses in medicine, food, and industry. Traditional rhizome propagation fails because of disease susceptibility, limited multiplication rates, and seasonal limitations. In vitro micropropagation and micro-rhizome induction may provide disease-free, genetically uniform plants throughout the year. Ginger micropropagation encounters contamination, soma clonal variance, hyperhydricity, Subculturing, and rigorous sterilizations. To mitigate these issues this review evaluates ginger micropropagation and in vitro micro-rhizome formation techniques. Ginger tissue culture requires explant selection and sterilization. Shoot tips, lateral buds, and rhizome segments exhibit variable responses to media compositions and sterilization methods. The fundamental medium is the Murashige and Skoog (MS) medium, which comprises organic compounds and carbon sources. The cytokine 6-benzylaminopurine influences shoot induction and proliferation. Reduced levels of NAA or IAA enhance shoot elongation and quality. To stop hyperhydricity and callus formation, which slow down shoot growth and regeneration, the right amounts of cytokinin and auxin must be present. The multiplication and storage of ginger depend on the production of micro-rhizomes, which requires certain concentrations of growth regulators and suitable environmental conditions. Rhizome development needs 6–8% sugar as an osmotic agent and energy source. Cytokinins and auxins, such as NAA and IBA, enhance micro-rhizome biomass. Photoperiod and temperature influence micro-rhizome development, with 16 hours of light and temperatures of 25 ± 2°C promoting growth. Plantlets must acclimate to soilless or substrate medium after in vitro cultivation. The micropropagation of ginger and the induction of micro-rhizomes may enhance industrial output. This review aims to address the knowledge gap on the micropropagation of ginger by synthesizing and critically evaluating previous studies. It functions as a comprehensive resource for researchers and those aiming to improve ginger farming using sophisticated biotechnology methods.*

Keywords*:* *Ginger; Micropropagation; Tissue culture; Acclimatization; Shoot formation; Micro rhizome*

Introduction

Ginger (*Zingier officinale Roscoe)* is a valuable income crop that can be used as a spice, medicinal, and condiment (Cao et al., 2020). In almost all countries that are tropical or subtropical plant ginger is commercially grown. It's thought to have come from Southeast Asia (Junaid and Gokce, 2024; Prasath et al., 2018). Majorly it has grown in Asian countries 78.3%, African countries 19.8%, North and South America 1.7%, and only 0.3 % in Oceania (Thakur et al., 2024). Every variety will have a zesty flavor and natural benefits (Kasilingam et al., 2018). Additional expected uses for ginger are still somewhat contested; it has consistently been found to help with nausea and vomiting in pregnancy, inflammation, metabolic problems, digestive function, and signs of colorectal cancer (Anh et al., 2020). Additionally, it is a low-maintenance annual crop with a great economic return. The poor flowering and seed formation significantly negatively impact ginger breeding (Jaffar Shaik, 2018). Rhizomes of ginger are bulky and expensive to manage as planting material. So, in vitro propagation is a good choice for producing ginger effectively (Arsahd et al., 2024; Chatta et al., 2024; Nisar et al., 2021). It is a good substitute for effective ginger production so it enables large-scale seedling production, excellent profitability, high phytosanitary quality, and genetic stability with rapid turnover (Zhou et al., 2022). Vegetative propagation carries the risk of disease and is a slow process, As a result, the in vitro process is an effective way to mass produce disease-free plants in a significant number in a short time (Sathyagowri & Seran, 2011). While ginger is a species that is not fertilized, it does not generate seeds. It typically reproduces vegetatively through its rhizomes (Abbas et al.,

204ab; Alqadasi et al., 2022). It is propagated through in vitro to produce disease-free plants in a short time (Manisha et al., 2018). Its rhizomes that grow underground are usually used as planting materials and that's their conventional approach, although, has a low rate of multiplication (Zuraida, 2016). Ginger is sensitive to many diseases at all stages of its growth. The presence of various diseases causes considerable yield reductions in ginger. In vitro propagation is the best way to preserve their germplasm and mass production. As an explant, a rhizome sprouting bud is used (Jaffar Shaik, 2018). Therefore, a suitable substitute for generating healthy clones of ginger plants and a solution for these challenges is plant tissue culture technology (Irfan et al., 2024; Lincy & Sasikumar, 2010). From a little space; food crops must be raised in higher quantity and quality to satisfy the rising demand for it (Baral et al., 2021). A rhizome sprouting bud is used (Jaffar Shaik, 2018; Sami et al., 2023) as an explant. Tissue culturing is a method of propagating crops aseptically to obtain true copies (Rehamn et al. 2024; Haroon et al., 2024; Kasilingam et al., 2018). Plant tissue culture is primarily dependent on how many plant cells are required to replenish a plantlet (Totipotency). When given the necessary supplements, single cells, plant cells without cell dividers (protoplasts), parts of leaves, or (less commonly) roots can commonly be used to develop another plant in culture media, and ginger is generally viewed as a homegrown remedy in several cultures (Kasilingam et al., 2018). The best shoot proliferation regeneration is created by Explants of ginger shoot tips produced on MS basal medium with 1.0 mg/L NAA and 3.0 mg/L BAP (Shakya, 2016). However, the best way to guarantee a steady supply of disease-free planting material for commercial usage is through in vitro culture (Kasilingam et al., 2018). Hyperhydricity, somaclonal variation, contamination, subculturing, and stringent sterilizations are all encountered during ginger micropropagation. This study aims to address the information gap regarding ginger micropropagation by combining and critically assessing earlier research. It serves as a thorough resource for researchers and individuals looking to use advanced biotechnology techniques to enhance ginger growing.

Selection of Explant

Tissue culture techniques provide for the possibility of rapid multiplication that is free of disease; nevertheless, the quality and quantity of the explants that are used are essential factors in determining the effectiveness of these procedures. Explants of ginger grown in tissue culture should have a low risk of contamination and a high capacity for regeneration (Mol et al., 2016). A key factor impacting the success of culture start and advancement depends on the size and source of explant in ginger tissue culture. If the transplant size is too small, it could not have enough meristematic cells to regenerate. There is a higher chance of culture compromise with larger explants, as they are more susceptible to containing contaminants (CZ et al., 2016; Estouka et al., 2021; Inden et al., 1988; Jaffar Shaik, 2018; Sathyagowri & Seran, 2011). The ideal size for ginger explants is between half a centimeter to 2cm (Amgai et al., 2017). The most common explants used for ginger tissue culture are rhizome buds. Their rapid regeneration rate is a direct result of their high levels of meristematic activity. Rhizome buds are usually used with a length ranging from half a centimeter to one centimeter (Ali et al., 2016; Amgai et al., 2017). Rhizome buds outperform other types of explants when it comes to shoot formation and regeneration capability. Buds are easily contaminated thus it's important to sterilize their surfaces thoroughly (Mol et al., 2016; Ayenew et al., 2012; David, Ji, & Gansau, 2016). Moreover, shoot tips, often obtained from invitro-produced plants or sterilized field-grown plants, are commonly used because of the vigorous growth of their apical meristem. Shoot tips often measure between one and two centimeters in length tips (Tewelde et al., 2020; CZ et al., 2016; Malamug et al., 1991).

In comparison to rhizome buds, shoot tips exhibit a lower level of contamination and may generate many shoots during cultivation. The growing of shoot tips may be technically challenging since it requires meticulous care to avoid damaging the meristem (Bhagyalakshmi & Singh, 1988). Axillary buds serve as a prevalent option for the establishment of ginger cultures. The buds are derived from the nodes of the rhizomes located at the plant's base. The typical length of an explant ranges from 0.5 to 1.5 cm buds (Sharma & Singh, 1997). Axillary buds can produce many branch types and are often less prone to contamination compared to rhizome buds. Despite the slower development of axillary buds compared to shoot tips, they may nevertheless generate shoots under suitable cultural conditions (Sharma & Singh, 1997; Kavyashree, 2009) and Axillary bud (Ayenew et al., 2012). Although less often utilized, the leaf sheaths of juvenile ginger plants may serve as explants in tissue culture. This is especially beneficial for callus induction and the advancement of somatic embryogenesis. The length of leaf sheath segments generally varies from one centimeter to one and a half centimeters. Callus may be generated from leaf sheaths, which may then be used for study on regeneration. The regeneration effectiveness of leaf sheath explants is inferior to that of buds and shoot tips (CZ et al., 2016; Malamug et al., 1991).

Sterilizations of Explant

Several issues and irregularities inhibit the use of in vitro micropropagation, particularly in PTC labs.

When it comes to the development and maintenance of plants inside in vitro cultures, the efficacy of the in vitro sterilization procedure is a very important factor. The proper sterilization of biological material, such as the selection of explant, is the essential component of the successful commencement of in vitro culture. Nystatin, Flugal, Bavistin, Ridomil gold, Sodium hypochlorite, Alcohol, Ethanol, Tween 20, and Mercuric chloride are some of the sterilants that are used in this procedure to sterilize explants. Enhancing the survival and regeneration capacities of a large number of selected explants is vital for enhancing the efficiency of plant tissue culture systems. The standardization of these procedures can accomplish this role (Babu et al., 2022). All types of ginger explants were thoroughly washed with tap water and detergents to eliminate dirt particles. Subsequently, they were rinsed with sterile distilled water until all residues were removed. The explants were then placed in a laminar airflow chamber for surface sterilization using 20% Clorox® (NaOCl) and 50 µL of Tween-20 for 30-40 minutes on a rotary shaker. Following this, the explants were sterilized with 75% (w/v) ethanol for 1 minute (CZ et al., 2016)**.** Several researchers have reported using for sterilization, 70% alcohol solution, 5% sodium hypochlorite, povidone-iodine, fungicides, bactericides, and antibiotics. Tween-20 and 4 drops of 25% HCl were applied for 20 minutes. Additionally, Clorox (0.1% w/v Sodium Hypochlorite) and mercuric chloride were used to remove traces of explants (Mol et al., 2016).

Paul & Shylaja (2023) reported that after removing the scale leaves, they washed the light-yellow sprouts from the rhizomes, submerged them in temporal for 10 minutes, and rinsed them again with distilled water. After 30 minutes with 0.1% Indofil M45, the sprouts were surface sterilized with 0.1% HgCl2 for 10 minutes before inoculation. After washing and drying remove the sterilant solution. Gezahegn et al. (2023) suggest that the explants were periodically soaked in 70% ethanol for 10 minutes and shaking. After removing the ethanol, then cleaned it three times with sterile distilled water and exposed it to a 2% chlorinated detergent solution for 15 minutes, then rinsed with distilled water four more times. (Perera et al., 2023) recommended that the first sterilize ginger buds with 75% ethanol, then 20% bleaching solution. In ginger micro-propagation, sterilization with 70% ethanol with 10% (w/v) bleaching powder and sterilized distilled water (control) did not reduce contamination (Table 1). Salim et al. (2024) using the process of sterilizing the shoot bud with a solution consisting of 20% (v/v) Clorox and Tween20 for thirty minutes and 95% (v/v) ethanol for one minute, it was possible to produce an explant that was free of contamination by 58%. Tewelde et al. (2020) reported that after three weeks of incubation of explant for break dormancy, 0.50% v/v NaOCl was added for 20 minutes, followed by 0.25% HgCl2. Explants exhibited an 80% absence of contaminants and a 70% viability rate. After three weeks of culture, cefotaxime at concentrations of 50, 100, and 200 mg/L, together with streptomycin at 25, 50, and 100 mg/L, yielded contamination-free micro shoots ranging from 87% to 93% efficacy. Roik et al. (2019) reported that the Ginger buds should be three centimeters or less to ease infection and found that sterilizing ginger buds with 0.1% mercuric chloride for 45–50 minutes works best. After this treatment, the sterility index varied from 50 to 63.9%, and the yield of viable buds from 61 to 81.9% across varieties. The rhizomes were subsequently removed and washed under running water for 30 minutes before reinstallation. These rhizomes were immersed in 70% ethanol and 2.5% sodium hypochlorite for 45 seconds to disinfect their surfaces. Subsequently, they were administered 0.05 percent mercuric chloride for 10 minutes. The fragments, cut to a size of four millimeters, contained developing rhizome buds. Following three rounds of washing with clean distilled water, the shoots were excised from the rhizomes and sectioned to a length of one centimeter (Miri. 2020). Sumon et al. (2019) recommended that rinse the rhizome buds and shoot tips under running tap water after collecting them from the garden then twenty minutes in liquid detergent (Surf Excel), they were washed four times with distilled water and three distilled water washes, the explants were treated with fungicide (Babystin) for three minutes. After transferring the explant into an autoclaved flask, the laminar flow cabinet sterilized the surface with 70% ethanol for 30 seconds and 0.1% mercury chloride for 9–10 minutes, the flask was constantly swirled. To remove particles, the explants were five times washed in sterile distilled water as recommended by Sumon et al. (2019).

Table 1 Summary of previous reported work on the Sterilization of explant of ginger

Cultivar	Explant source and size	Sterilization	Result	Days	References
Deribo	Used Shoot tips that are two to three centimeters in length	A solution of NaOCl (0.50% v/v) for twenty minutes and ethanol (70 percent v/v) for five minutes.	73% contamination free	3 weeks	(Tewelde et al., 2020)

Bentong	Buds	70% Clorox for 30 min	75% contamination-free	4 weeks	(Nisar Ahmad Zahid et al., 2021)
Red ginger	Buds (1.5 to 3.0 cm)	For ten minutes, first with 70% (v/v) ethanol then with 0.1% (w/v) mercuric chloride (HgCl2).	80%	4 weeks	(George et al., 2022)
Local	Buds 0.5-0.8cm	Carbendazime (0.1%) for 45 minutes	70.99% contamination-free	4 weeks	(Manisha et al., 2018)
Etlingera coccinea	Buds 5.0±0.5cm	Used 5.25%Clorox and 2 drops Tween-20 in water for 20 min.	100% contamination-free	4 weeks	(Hore & Tanti, 2018)
Rangun	Buds 0.5-1.0 cm	Twenty-five percent sodium hypochlorite was used for a period of thirty minutes.	60%-70% contamination-free	4 weeks	(Swarnathilaka et al., 2017)
Rubrum (ZOR)	Buds 0.5-1 cm	20% of Clorox® (5.25% NaOCL) and 50 uL (micro liter) of Tween-20 for 30-40 mins on a rotary shaker	65% contamination-free	45 days	(Zuraida A.R et al.,2016)
Red ginger Var. (Rubrum)		Sterilized by exposing it to a solution of 70% alcohol for one minute and a solution of 20% sodium hypochlorite for ten minutes	60%	4 weeks	(Sukarnih et al., 2021)
African ginger (Mondia whitei)	Leaves	Fungicide—0.3% (w/v) GOLDazim 500 for twenty-five minutes. One nine percent sodium hypochlorite for fifteen minutes.	80% contamination free	4 weeks	(Patricia et al., 2021)
Local	Rihzome buds	3% of Sodium hypochlorite (NaOCl) was used for 20 minutes	90%, contamination free	4 weeks	(Estouka et al., 2021)

Culture Medium

Miri, (2020) showed the MS medium with 10 mg/l BA produced the greatest shoots, 20.6 per explant from sprouting bud explants. The best medium for shoot initiation was MS supplemented with 10 µM zeatin, yielding 4.28 shoots per explant (Zahid et al., 2021). Lincy & Sasikumar (2010) recommended that cultures were kept alive in a climate-controlled environment at a temperature of 22°C with a photoperiod regime of 16 hours of light 8 hours of darkness and 3000 lux of light intensity from Philips cool white, fluorescent bulbs. Shaaban et al. (2023) recommended that the optimal number of ginger plants were obtained in MS medium supplemented with different concentrations of (BA and NAA) to achieve optimum vegetative growths and also found that ginger plants' vegetative growth is enhanced when 0.1 mg NAA is added to 1 or 2 mg/l BA. Estouka et al. (2021) used various concentrations of BAP administered to plantlets grown on Murashige and Skoog's (MS) medium. MS medium supplemented with 3 mg/l BAP yielded the highest shoot and root count, averaging 5.4 shoots per explant. Each shoot had an average of 10 leaves and 17.7 roots. CZ et al. (2016) reported the highest survival rate (55–65%) and average number of micro shoots per explant (3.2±0.06) were achieved from surface-sterilized early buds measuring 0.5–1 cm and 2–4 cm, cultivated on Murashige and Skoog (MS) medium supplemented with 4 mg BAP. Sterilized sprouting buds were grown on MS media supplemented with various growth regulators. Shootlet multiplication was greatest in MS-medium augmented with 4.5 mg/l BAP (Abbas et al., 2011). The rhizome bud explants cultured on Murashige and Skoog's (MS) medium with BAP (4.0 mg/l) and IAA (1.0 mg/l) produced the most shoots (13.1 ± 0.9) and roots (43.1 ± 2.8) (Chavan et al., 2018). Syahid et al. (2023) recommended that the 1.0 mg/l of BA and 0.1 mg/l of kinetin, the ideal medium for the first stage yielded the highest rate of shoot induction and lasted two and four weeks respectively Ayenew et al. (2012) found that shoot tip explants on 2 mgl^{-1} BA and 1 mgl^{-1} kin achieved an average of

seven shots per explant, which was better than other media combinations (Swarnathilaka et al., 2016) reported that for bud development 0.5 mg L-1 NAA and 3.0 mg L-1 BAP are used to choose the best culture for the establishment.

Shoot proliferation

In vitro shoot multiplication is one proposed approach for overcoming these limits. This method of production enables rapid and widespread development of disease-free plants that are genetically identical. Ginger tissue culture growers must have growth regulators to help in shoot induction and multiplication. Kinetin, Zeatin and 6-benzylaminopurine are the primary cytokinin's used for shoot induction and proliferation. In tissue cultures, where they are used to encourage the formation of axillary buds. Cytokinin's have a particularly significant impact. Cytokinins are essential growth regulators that play a role in the construction of lateral shoots. They do this by influencing the expression of genes that are responsible for cell division in the cells that make up the shoot meristem. It is possible that the different degrees of gene expression that cytokinins exhibit are the cause of the different impacts that BAP has on the proliferation of shoots. The majority of research cantered on using cytokinin's with shoot cultures (Mohamad et al., 2022). Cardenas-Aquino et al., (2023) found that lemongrass exhibited improved upregulation of genes involved in cytokinin signaling (AHK3 and B-type RRARR1), stem cell maintenance (STM, CLV3, and REV), and cell cycle (CYCD3). This, in turn, led to improved shoot initiation and proliferation, resulting in a greater number of shoots. For ginger (*Zingiber officinale* Rosc) micropropagation, the ideal concentration of sucrose in MS medium is 30 g/L. This allowed the plantlets to grow and develop to their full potential, particularly in terms of shoot quantity, root length, and shoot length. This was shown by the outcomes. In contrast to the ideal concentration of 30 g/L of sucrose in the medium (Estouka et al., 2022). Alqadasi et al. (2022) found Increases in shoot length, root count, and shoot multiplication had the greatest impact in explants cultured in MS basal medium supplemented with 2.0 mg L^{-1} Kin and 1.0 mg L^{-1} IBA. The medium's optimum pH is 5.8. The explants were placed for three to four weeks after being cultivated, surface sterilized, and maintained at a temperature of 25 degrees Celsius, relative humidity of 90-95%, and a light: dark photoperiod of 14:10 hours. (Gnasekaran et al. (2021) recommended that the overall effect of red (660 nm) LED-guided shade avoidance syndrome development was a rise in shoot length, taller plants with longer and thinner leaves, and an overall improvement in plant health. The micro shoot culture was established by first multiplying and then growing them in MS media with 1.0 mg/l BA and 0.1 mg/l NAA added for shoot differentiation. (Manisha et al., 2018). To induce shoot multiplication in micropropagation of ginger, KNO3 considerably increased ginger's in vitro proliferation rate in both full-strength and half-strength media (Villamor, 2010). The maximum shoot regeneration rate was obtained when 0.05 mgl^{-1} NAA and 4.0 mgl^{-1} BAP were combined (Nkere & Mbanaso, 2010), 4.5 mg/L BAP, 0.009 mg/L IBA increased number of shoots according to (Minas, 2009). The maximum shoot multiplication was observed in Thidiazuron (TDZ) at 0.5 mg/L (Hamirah et al., 2007).

Researchers showed that BAP concentrations between 1.0 and 3.0 mg/L considerably promote shoot proliferation. Auxins like IAA and NAA work synergistically to promote shoot elongation and root induction at low dosages. However, excessive cytokinin concentrations may promote hyperhidrosis, deformed shoots, and slower regeneration. Adenine sulphate (100 mg/l), 1 mg/l of IAA, and MS basal media supplemented with BA were shown to be the most beneficial for the in vitro multiplication of shoots. From a single explant, 8.2 shoots were generated by this medium within 30 days of the initial culture. Successive subcultures were found to have a constant rate of multiplication (Mohanty et al., 2008). MS media supplemented with 3.0 mg/L BAP and 0.4 mg/L NAA constituted the ideal environment for shoot growth. (Jagadev et al., 2006). The most promising explants for ginger culture establishment were the shoot tips, which produced the greatest results, with MS basal medium supplemented with 2 mg/L BAP, the greatest response for shoot multiplication was seen in the axillary buds (4.0 shoots/explant) and shoot tips (5.1 shoots/explant) (Rajani et al., 2008). (David et al., 2016) showed After seven days of culture, treatment with 3.0 mg/L BAP + 1.0 mg/L NAA produced the first observation of shoot development. KIN at 3.0 mg/L exhibited the highest shoot induction rate (90%) and the shortest duration (13.00 days). The maximum shoot induction occurred at 3.0 mg/L KIN combined with 1.5 mg/L GA3. The combination of 3.0 mg/L KIN and 1.5 mg/L GA3 yielded the highest number of shoots (4.6), leaves per explant (10.00), and a shoot length of 4.56 cm (Abbas et al., 2011). Salathia et al. (2022) reported that the MS medium with BAP (4 mg/L) and NAA (0.5 mg/L) showed the greatest mean number of shoots (12.3±33) and the longest shoot length. The Rangun and Chinese buds in the MS treated with 4.0 mg l^{-1} BAP and 0.25 mg l^{-1} NAA exhibited the highest rate of multiplication, with 7.33 and 7.20 shoots per explant, respectively (Swarnathilaka et al., 2016). Local ginger had the highest rate of multiplication in MS medium that was supplemented with 2 mg L^{-1} BAP and 0.25 mg L^{-1} NAA. On the other hand, Rangun and Chinese

cultivars required 4.0 mg L^{-1} BAP and 0.25 mg L^{-1} NAA. However, to improve shoot development, it is necessary to transfer shoots that are growing in liquid media to a solid medium. The outcomes suggested that the liquid culture was more effective than the solid culture in terms of multiplication (Swarnathilaka et al., 2017).

The most efficient medium for redifferentiation of somatic embryos among the various that were investigated was the one that included 0.2 mg/L of 2,4 dichlorophenoxyacetic acid and 6.0 mg/L of BAP (Thingbaijam et al., 2014). Kinabalu. (2015) investigated a shoot elongation medium containing 2.5 to 20 µM BAP that was added to the regenerated shoots before they were transferred to it. The explant was promoted by regenerated shoots in MS medium supplemented with 5.0 µM BAP. In Subcultures BAP concentrations showed significant variations. Under the first subculture, 4.06 sprouts per explant came from 2.22 µM BAP concentration. Although it was already present in the second and fourth subcultures, increasing cytokinin concentration produced sprouts at 4.05 and 4.96 per explant at maximum BAP concentration. BAP raised sprout emissions according to reported data. At doses of 2.22, 8.88, and 11.10 µM, this cytokinin induced the highest multiplication rates in the first, second, and fourth sub-cultivations (de-Oliveira et al., 2016). Shaik. (2018) reported that the explants that were grown on MS basal medium that was supplemented with 2.5 mg/l BAP and 1 gm/l NAA concentration exhibited the highest rate of shoot multiplication. In some cases, reported when 2 to 3 mg BAP are used with 0.5mg to 1mg NAA also gave better results and 4 to 7 shoot induced per explants (CZ et al., 2016; David, Ji, & Gansau, 2016; Jaffar Shaik, 2018; Jain et al., 2016; Manisha et al., 2018; Patricia et al., 2021; Sathyagowri & Seran, 2011; Swarnathilaka et al., 2016).

Root Formation and Hardening

A critical phase in the micropropagation of ginger is the in vitro growth of roots. This phase determines the plantlet's capacity to survive and thrive in conditions outside in vitro environments. The efficacy of rooting may be significantly improved by the manipulation of auxin type and concentration, basal media composition, and ambient conditions (Table 2). Auxins are important plant growth regulators that are involved in many morphogenetic process's implants. It has been suggested that the exogenous auxin was responsible for the upregulation of the endogenous auxin biosynthesis genes, such as the TAA1 and YUCCA genes, which were first discovered by Savina et al. (2020) in Arabidopsis thaliana. Auxins are crucial for promoting root formation in ginger crops. The auxins often used for promoting root growth include indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) (Ayenew et al., 2012; Nandagopal & Kumari, 2007; Swarnathilaka et al., 2016; Nisar Ahmad Zahid et al., 2021; Zhou et al., 2022). auxins that are added to the rooting media cause the expression of transcription factors such as WOX11 and WOX12, which in turn activate root-promoting genes such as LBD16, LBD29, and WOX5 (Liu et al., 2014). Concerning this approach, IBA has shown very favorable outcomes. According to Ayenew et al. (2012) when MS media is supplemented with 2 mgl-1 BA and 1 mgl-1 kinetin the rate of formation of roots is high. Abbas et al. (2011) using a medium containing 1.0 mg/l of NAA and half the strength of B5, shootlets were shown to have a strong growth of roots. During the greenhouse period, the greatest percentage of acclimatization, hardening, and rhizome formation for plants that were produced in vitro was between 80 and 100 percent. Similarly, the 2 to 3 mg BAP and 0.5 to 1mg IAA also increased the rate of root formation (Zhou et al., 2022). Sultana et al. (2009) reported that the root induction concentration was maximum (85.40%), achieved by combining Suruchi, leaf, MS, and 1 mg/l IBA. Jagadev et al. (2006) showed during rooting that MS treated with NAA at a 0.5 mg/L concentration performed better. This was due in part to its highest root length (2.0 cm), the largest number of roots per shoot (13.3), and the least amount of time needed to start root development (10.3 days). Growing in vitro, the plantlets were pre-hardened in a liquid solution with ½ MS. The most successful combination of hardening and acclimatization media for the survival of the plantlets present in ginger turned out to be one comprised of soil, sand, and farmyard manure in a 1:1 ratio. According to David et al. (2016) ginger seedlings germinated much more rapidly in media treated with 2.0 mg/L NAA. After 10 weeks of culture, the treatment caused an average root length of 4.52 ± 0.20 cm and the induction of up to 34.40 ± 1.81 roots per explant. Three weeks after transplanting, 64% of the plantlets survived after being acclimated in a container with a medium combination of sand and clay (1:4). Mehaboob et al. (2019) investigated the regeneration of the shoots; they were transferred to an in vitro rooting medium containing 1.0 mg/L of IBA. Then, fully developed plantlets with established root and shoot systems had a two-step hardening procedure. Seventy-one percent of plantlets successfully endured secondary hardening without displaying any abnormal morphology. Kango et al. (2024) recommended nutrient solutions that affected plantlet phylogenesis, rhizogenesis, and height development. Successful plantlet development requires optimal temperatures of 26.54˚C and humidity of 96.16%. One compost, two soils, and two coconut peat substrates boosted 1C2T2TC survival to 92.5%. Plantzym solution promoted plantlet height (0.6 cm), root (30 roots),

and leaf (03 leaves) growth during hardening. The plantlets that were developed in a lab are unable to compete with soil bacteria or adapt to their surroundings when they are transferred to the field. The plantlets' morphology, anatomy, and physiology have been transformed as a result of the in vitro cultivation settings. Controlling the physical and chemical environment as well as bio hardening of micro-propagated plantlets are priorities to boost growth and decrease mortality in plantlets at the acclimatization stage (Hazarika, 2003, 2006; Pospóšilová et al., 1999). After removing the culture jars, the plant was placed in a growth incubator with temperature control of 25 + 2 C and continuous lighting at an intensity of 9 mol m^{-2} s^{-1} for two weeks. then placed in a pot with (2:2:1) topsoil, sand, and organic soil (Keng & Hing, 2004). The resulting plants were exposed to ex-vitro conditions for two weeks in the greenhouse. Plantlets were then transplanted to peat moss-filled pots, taking extra care not to damage the roots, and uniformly hydrated at regular intervals (Estouka et al, 2021). Before transferring potting medium mixture of peat, soil, and vermiculite is 1:1:1. To keep the plants moist, plastic bags were placed on top of the plants (Hamirah et al., 2007). The optimum acclimation medium for ginger was a mixture of sand, soil, and peat (1:1:1) (Hamirah et al., 2007; Naz et al., 2009). Ginger plantlets were effectively acclimated in a 1:1:1 mixture of soil, sand, and farmyard manure with a 95% survival rate (Kambaska & Santilata, 2009; Manisha et al., 2018; Sharma & Singh, 1997).

Table 2 Previous studies reported an efficient combination of plant growth regulators and their effects on shoot multiplication and root formation of ginger

Cultivar	Auxin	Cytokine	No. of Shoot length and shoot length	No of Roots	References
Kaski Local	0.5mg/L NAA	5 mg/L BA	20.27cm	9.71	(Resham et al., 2017)
Elite Ethiopian ginger	1 mg l/L IAA	2 mg l/L BA	4 & 4.2cm length	8 to 11	(Ayenew et al., 2012)
Local cultivar	1 mg / L NAA	2.5mg / L BAP	4.9 & 5.1 shoot length		(Jaffar al., 2018)
Local cultivar	0.25 mg / L NAA	2mgl-1 BA	5	5.5 roots with 3.64 cm	(Sathyagowri et al., 2011)
Wynad local	o.5mg/L IBA	1mg/L BAP	6 & 3.5 cm length	2 & 2.5 cm length	(Bhagyalakshmi & Narendra 2015)
ZOR	0.5 mg/L NAA	3 mg/L BAP	19 & 4cm length	7 & 3.4 cm	(Zuraida et al., 2016)
Tambunan	1mg/ L NAA	1mg/L BAP	4 and 1.23 cm length	4 & 1.91cm	(David et al., 2016)
Chinese	1mg/L NAA	3mg/L BAP	5 & 4.5 cm length	17 & 3.9cm	(Zahid et al., 2021)
Rangun & Chinese	0.25 mg/L NAA	4.0 mg/L BAP	7	5 & 3.64 cm	(Swarnathilaka,2016)
Exotic		5mg/L BAP	3 & 3.18 cm Length	10 & 3.89 cm length	(George et al., 2022)

Micro rhizome induction

The vegetative growth of ginger is accomplished using the rhizome part as a seed source. Rhizome rot infections, primarily spread by damaged seed rhizomes, inhibit the growth of ginger. Micropropagation can assist produce planting material free of diseases. While in storage and cultivation, rhizomes intended for vegetative propagation may develop rhizome rot and bacterial wilt caused by *Pythium* and *Ralstonia solanacearum*, respectively. Bacterial wilt is a significant challenge in southern and southwestern areas, where ginger is extensively cultivated for commercial purposes. The global incidence of field disease ranges from 10% to 40%, although it may result in crop mortality rates of 80% to 100%. Gezahegn et al. (2024) reported that the regeneration of healthy planting materials is one of the most effective strategies to mitigate disease effects. Consequently, alternatives for generating disease-free planting materials are essential. Consequently, in vitro development and formation of disease-free micro-rhizomes in modified Murashige and Skoog medium. " Best fresh weight (3.72 g) and most micro rhizomes (9.6) were obtained with 16 hours of light and 4 hours of dark in solid medium. VIVEK (2024) reported that micro rhizome induction by carbon, plant hormones, and gelling agents. Following 12 weeks, sucrose (70 g/l) produced micro rhizomes best; table sugar (60 g/l) followed. 3 mg/l BA and 0.5 mg/l NAA were shown to be the most efficient medium for micro rhizome

induction in MS medium with 70 g/l sucrose (Mim12) and 60 g/l table sugar.

Table sugar, isabgol, tapioca powder replaced sucrose and agar helped to lower microrhizome costs for production. Micro rhizome induction was greatest in G5 (14% tapioca powder) and G3 (3% isabgol and 0.4% agar). Yu et al. (2024) examined ginger microrhizome induction, genetic homogeneity, and agricultural effectiveness. Plants grown with 3.0 mg·L^{-1} BAP, 100 g·L^{-1} sucrose, and a 12-hour photoperiod generated the most micro rhizomes but had a lower average fresh weight after 60 days. Larger micro rhizomes were more desiccation-resistant and germinated faster. Thus, extra efforts were taken to expand micro rhizomes. After 30 days of supplemental culture and in vitro growth of small micro rhizomes, the fresh weight increased to 280.3 mg and 403.4 mg, respectively, under optimal conditions. Gezahegn et al. (2024) reported that the medium containing 80.0 g/l sucrose, and 6.0 mg/l BAP yielded the greatest viable micro-rhizome count (5.67) and shoot count (10.33). Swarnathilaka et al. (2016) showed that 4.0 mgL^{-1} BAP with 0.1 mgL^{-1} NAA induced micro rhizomes best within 60 days, followed by 6.0 mgL-1 BAP. Increased NAA did not induce micro rhizomes. MS medium with 90 g L-1 sucrose had the highest fresh and dry weight of micro rhizomes, followed by 60 g L^{-1}. Plantlets treated with more than 90 g L^{-1} sucrose had decreased micro rhizome weight but increased root induction and root fresh weight, possibly due to water buildup. DAVID et al. (2016) reported that the explant responded better on MS medium with 60 g/L sucrose and 6 mg/L BAP after three months of culture for micro rhizomes. Meetei et al. (2015) suggest inducing micro-rhizomes, the plantlets were grown separately on MS semi-solid media with 0.6% agar, 8% sucrose, and 2 mg/L BAP in a 16L/8D photoperiod yielded the most sensitive micro-rhizomes, weighing 634.3 ± 20.53 mg and requiring 26.7 ± 1.15 days for induction (Table 3). Rhizome growth was evaluated using two different carbon sources, a stress hormone and a polyamine (Devi et al., 2017). findings showed an average of 2.92 cm for rhizome length, 1.36 g for rhizome weight, and 8.50 rhizomatous buds. The most effective medium was 7% sucrose in MS media.

Table 3 Summary of Efficient medium used for micro rhizomes induction of ginger reported in previous studies

Cultivar	Type of Media	Finding	References
Volvo	MS medium included 80.0 g/l sucrose and 6.0 mg/l BAP.	Produced micro rhizome (5.67), shoot (10.33). After one month in soil, 80% of micro-rhizomes sprouted.	(Gezahegn et al., 2024)
Tambunan	MS medium containing 60 grams of sucrose per liter and 6 milligrams of BAP per liter.	After 3 months, 7 2.90-g micro rhizomes produced 35 buds. After 21 days of acclimatization, these micro rhizomes produced new shoots and roots with 88% survival.	(DAVID et al., 2018)
Local	MS medium containing 9 mg/L BAP and 90 g/L sucrose was utilized under 16-h photoperiod.	Produced 15 micro rhizomes per jar after 10 weeks under darkness.	(Abbas et al., 2014)
Local	80 g/l sucrose, 2 × MS macro-elements, 1 × MS micro-elements, 24L:0D photoperiod.	Maximum micro rhizome weight of over 0.25 g under shaded conditions after 10 weeks. After transplanting in vitro ginger plantlets with small rhizomes to soil, 100% survival was obtained.	(Zheng et al., 2008)
Baishey & Nadia	MS medium contains 2 mg L^{-1} BAP, 1 mg L^{-1} NAA, 8 g L^{-1} sucrose, and 11 µM silver nitrate.	On average, both ginger types produced the most micro rhizomes (Baishey, 34.25±.09 & Nadia, 38.25±.09) during 35-45 days of incubation. Both varieties survived 75–90% ex vitro soil acclimatization	(Singh et al., 2014)
Local	In 1/2 strength MS medium, add 2.4 µM NAA, 32 µM BAP, and 9% sucrose.	The micro rhizomes developed in 9% sucrose exhibited the biggest dimensions and the highest average fresh weight. Following the four-month-old plantlets	(Punyarani et al., 2021)

Conclusion

In vitro micropropagation and micro-rhizome induction are regarded as reliable and efficient methods for the multiplication, preservation, and global dissemination of ginger germplasm. To enhance the agricultural and pharmaceutical industries, further refinement of existing technologies and the use of biotechnological approaches may unlock more possibilities for sustainable ginger production.

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Not applicable.

Consent for Publication

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Authors' Contribution

Conceptualization, Haider Ali and Kazim Ali; Data curation, Iqbal Hussain, Shoukat Ali, Ghulam Muhammad Ali; Funding acquisition, Kazim Ali, and Iqbal Hussain; Investigation, Haider Ali; Resources, Kazim Ali, and Iqbal Hussain; Supervision, Kazim Ali; Writing – review & editing, Haider Ali, Kazim Ali, Iqbal Hussain and Ghulam Muhammad Ali.

Conflict of interest

There is no conflict of interest among the authors of the manuscript.

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