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Review Article

Critical Review on Plant Micropropagation of Ethiopian Plants Reported So Far: Existing Gaps, Required Standardization, and Future Research Direction

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Plant micropropagation research in Ethiopia requires concerted efforts to meet desired levels of application for sustainable utilization of the country's diverse plant genetic resources. The purpose of the present review is to provide an update on the results of plant micropropagation conducted so far in Ethiopia. It assessed their strengths and identified gaps in order to standardize research methods and indicate future research directions. Two cereals, three oil crops, three spices, five medicinal plants, two high-value crops, six fruit plants, nine root crops, and one endangered multipurpose shrub were reviewed. The assessment of previously published research was carried out in terms of methods used in the selection of *ex plants* and their disinfestations, culture vessels, and media used with a variety of combinations and concentrations of plant growth regulators, macro- and micronutrient requirements, culture environments, and genetic stability of regenerated *plantlets*. Further assessments include the utilization of plant growth-promoting microbes and applications of "omics" research in order to establish standardized, efficient, and cost-effective micropropagation techniques. The findings of the assessments are summarized and current advances are highlighted, along with recommendations for future plant micropropagation studies in the country.

1. Introduction

Plant micropropagation is one of the applied biotechnology tools that have been utilized in horticulture, forestry, plant breeding, conservation, and disease-free plant production. Its utility can be expanded to culture-based *in vitro* cloning, the production of bioactive compounds, secondary metabolites, and engineered molecules such as vaccines and multiple pharmaceuticals [1].

Micropropagation is essential in the understanding of somatic embryogenesis [2], haploid plant production [3], and provision of experimental systems for physiological, proteomics, transcriptomics, and epigenetic analysis to study plant cell "*dedifferentiation*" [4–7] or *trans-differentiation*. Standardization of plant micropropagation impacts current advances in biochemistry, genetics, and cell biology through which applications of micropropagation can be widened to improve quality, yield, disease resistance, and stress tolerance by editing and selecting genes and genomic regions [1, 2]. These expensive applications and their detailed processes require standardization, repeatability, and specifications for sustainable utilization of plant resources. Such detailed processes of standardization can be exemplified by various steps followed to solve problems of optimization in meristem culture for disease-free plant production and *cryopreservation* for the conservation of *recalcitrant* species like coffee.

Plant micropropagation for various applications has various challenges and is usually addressed through different approaches, including through appropriate selection of *ex*

plants and their proper disinfection, culture media optimization, as well as the utilization of different types of nitrogen and carbon sources, growth regulators, macronutrients, micronutrients, and culture environments. Other modern approaches that can help alleviate the challenges of plant micropropagation include methods to test for genetic stability, application of plant growth-promoting microbes (PGPMs), and current advances in genomics, transcriptomics, and proteomics. The critical evaluation of the methods used and the results derived from each of these methods would provide crucial information that would lead to the design of cost-effective and repeatable research directions and protocols for the effective utilization of plant genetic resources in the public and private sectors.

In Ethiopia, both the private and public sectors have had a limited number of plant micropropagation protocols for the delivery of disease-free plants [8]. Case studies have reported fifty *in vitro* protocols across all plant micropropagation laboratories in the country [9]. These protocols, however, should be further optimized to increase their efficiency and reduction of costs incurred (particularly in hardening *in vitro* derived plantlets). For the realization of this, it is crucial to have labs equipped with competent staff who can effectively analyze the physiological genetic processes of the micropropagated genotypes that transition from *in vitro* regeneration to in *vivo* conditions. Other limitations of plant micropropagation in the country include technical, administrative, financial, laboratory designs, facilities [8, 9], and consumables. These limitations have been exacerbated by poor maintenance of the facilities installed before the required manpower to run and maintain the facilities and also by recent unrest and war in the north and northwest parts of the country.

Many of the current scientific reports on plant micropropagation protocols, including those that are presently peer-reviewed, are not cost-effective, as they were presented in generalized forms without specifying the genotypes used. Avoiding using such inadequately developed protocols or replacing them with appropriate protocols developed for properly defined genotypes adds value to the economical utilization of plant bioresources. Limitations that arise from hasty generalizations due to poorly designed studies and usage of some of the conceptually unclarified terms should also be avoided. Terminologies such as *in vitro, in vivo*, and *plantlet* [2, 4, 6] have been widely accepted and used in plant micropropagation reports. However, terminologies such as *totipotent* are being challenged currently [6]. This terminology has been challenged by indicating that plant cells can regain *totipotency* although they are not necessarily *totipotent.* Hence, it should be clearly defined with regard to plant micropropagation so that the problems related to misconceptions that have an impact on plant regeneration, micropropagation, and other plant science research can be avoided [6]. Exogenous auxin and wounding trigger callus formation in different ways [4]. However, the use of the term *dedifferentiation* in relation to this has also been challenged. Ikeuchi et al. [5] stated that *dedifferentiation* is erroneously used and should be understood as *transdifferentiation* that may lead to increased developmental *potency* as callus formation. It appears that the controversy arises from the mixing of genetic and developmental biology viewpoints on cellular differentiation. Plant micropropagation reports in Ethiopia should thus pay attention to these presumed controversies and contribute their share to address some of the existing controversies in order to expand the application of micropropagation with the desired repeatability and standards. Diverse plant species including cereals, oil crops, spices, medicinal plants, high-value crops, fruit plants, root crops, and endangered multipurpose shrubs were utilized in various forms of plant micropropagation in Ethiopia [10–20], [21–31], [32–42], [43–55]. Critical assessment of these studies would facilitate the development of repeatable protocols that have wide applications.

Quite often we find issues associated with repeatability, reproducibility, and replicability that impact almost all areas of science [56–58]. So these issues are not restricted to plant micropropagation and the societal costs associated with these problems are high [59]. This calls for working out the details of basic design and background factors behind these issues. Some of the societal costs of plant micropropagation culture in Ethiopia can be minimized through the application of plant growth-promoting microbes (PGPMs). However, PGPMs are not currently in use in *in vitro* plant micropropagation studies on Ethiopian plants. Few studies elsewhere have reported that inoculation with PGPMs in micropropagation [60–62] reduces the cost of production of *in vitro* plants during the micropropagation process due to better survivability and increased resistance to water stress.

The present review provides updated literature with an assessment of strengths and gaps in Ethiopian plant micropropagation studies conducted in Ethiopia, in order to show the need for standardized methods for optimal and cost-effective utilization of plant diversity. These together with future research on the genetic and physiological bases of micropropagation can serve as reliable research directions for the sustainable utilization of existing biodiversity.

2. Materials and Methods

A survey of published articles and other literature on Ethiopian plant micropropagation studies was carried out to collect data on plant species studied so far. The keywords used in the survey were (1) plant tissue culture research in Ethiopia, (2) plant micropropagation in Ethiopia, and (3) *in vitro* plant regeneration in Ethiopia. The Google Scholar branch of the Google search engine was used for searching literature with these key words. The obtained publications were filtered and the most relevant and informative ones were used to generate the data sets presented in Tables 1–3.

The surveyed data include objectives of the various studies, reported results, *explants* used, surface sterilization procedure followed, nutritional and nonnutritional media components used, utilization of different sources of nitrogen, carbon, micronutrients, plant growth regulators (PGRs), type of culture vessel used, *in vitro* growth and regeneration conditions followed, methods of transfer of propagules from culture to the soil, and genetic stability tests applied (Tables 1–3). The status of the applications of plant

Table 1: List of plant species studied in Ethiopia, published and reported so far, culture vessels, *in vitro* growth and regeneration conditions, and nonnutritional components used.

List of species	Culture vessel used	Growing conditions	Nonnutritional components used	Ref.
Prunus salicina Lindl.	Magenta GA-7	$T = 25 \pm 2^{\circ}C$; LD = 16 h; LI = 37.8 μmol/m^2/s)	Agar (0.8%)	[37]
Vitis vinifera L.	Magenta GA-7	T = 27 ± 2°C; = 16 h; LI = 40 μmol/m^2/s);	Agar (0.7%)	[38]
Zingiber officinale Roscoe	Culture jar	T = 20–25°C	Agar (0.8%)	[39]
Guizotia abyssinica (L.f.) Cass.	Culture jar	T = 25 + 2°C; LI (40 μmol·m^{-2}·s^{-1}); For CI:- the dark at	Agar (0.7%)	[40]
Carica papaya L.	Magenta	T° = 25 ± 2°C; LD = 16 h; LI = 28–35 μmol/m^2/s; RH 70%	Agar (0.8%)	[27]
Saccharum officinarum L.	Glass culture jar	T = 25 ± 2°C LD = 16; LI = 35 μmol/m^2/s; RH = 75–80%	Agar (0.8%)	[42]
Ensete ventricosum (Welw.) Cheesman	CV = 250 ml jars	T = 27 ± 2°C; LD: 14 h LI: 40 μmol/m^2/s; RH: 60–70%	Agar (0.8%) & activated charcoal (0.3%)	[28]
Triticum aestivum L., *Var.; Yerer, Simba, Ude and Galama,*	Petri dish	T: 24 ± 4°C; LD = 16 h; LI = 19.09–28.80 μmol/m^2/s	Agar (0.8%)	[43]
Manihot esculenta Crantz.	Test tube	T = 22–23: LD:16 h; LI:14 μmol/m^2/s	Agar (0.8%)	[44]
Ananas comosus (L.) Merr.	Test tubes	T = 25°±2°C; LD = 16 h; LI = 28–42 μmol/m^2/s	Agar (0.8%); Enset starch (60 g/l) & agar (2 g/l) for rooting and phythogel 3 g/l	[45]
Sesamum indicum L.	Magenta GA7&Petri dish	Vials incubated under continuous light for rooting	Not mentioned	[46]
Coffea arabica L.	Magenta-GA7	T = 25°±2°C; LI = 40 μmol μmol/m^2/s; LD = 16 h	0.8% Agar	[11]
Cordeauxia edulis Hemsl.	Magenta	T: 25°±2°C, LD = 16 h	0.7% agar and Charcoal	[55]
Musa x paradisiaca L.	Test tube	T° = 25 ± 2°C: LD = 16 h cool white fluorescent	Agar (0.8%)	[47]
Echinops kebericho Mesfin,	Magenta jars	T = 25 ± 2 C; LD = 16 h; LI = 22 μmol/m^2/s	Agar (0.8%)	[13]
Capsicum annuum L.	Test tube	T = 27 ± 2°C, LD = 12 h; LI = 14–28 μmol/m^2/s	(0.7% agar-agar)	[23]
Coccinia abyssinica (Lam.) Cogn.,var. 29)	Tubes or jars	T = 25 ± 2°C	Agar (0.8% w/v)	[52]
Aframomum corrorima (Braun) Jansen,	100 ml baby food jar	T = 25 ± 2°C LD = 16 h; LI = 28 μmol/m^2/s	Agar-agar 0.7%	[49]
Solanum tuberosum L., var.Hunde and var. Ararsa	culture jar	T° = 24°C LD = 16 h; LI = 35 μmol/m^2/s	Agar 0.8%	[35]
Ipomoea batatas (L.) Lam.,var. Beletech, Awassa-83 &Belela	Magenta jars	Grown under florescent light at 25 ± 2°C	Agar 0.6%	[53]
Brassica carinata A. Br., Holeta -1 and yellow dodola	Magnet box	T = 25 ± 2°C; LD: 16/8 h; LI: 37.8 μmol/m^2/s)	Agar 0.6%	[29]

g = gram, h = hours, l = liter, LD = light duration per day in hours, LI = light intensity in μmol/m^2/s (= micromole per meter square per second), ml = milliliter, RH = relative humidity, s = seconds, *T* = temperature in degree Celsius,

growth-promoting microbes for micropropagation was also recorded. The data gathered are organized into distinct but interconnected themes for gap identification and assessments in the discussion session. The discussion is followed by conclusions, current advances, recommendations, and brief highlights about future research directions for plant micropropagation in Ethiopia.

2.1. Results Recorded following the Materials and Methods Described above. The overall stated objectives and results of some of the representative micropropagation studies made so far are provided in Table 3. The stated objectives and results of the various micropropagation studies have wide coverage and include virus cleaning and disease-free *plantlet* production, screening for salt-tolerant plants, *in vitro* micropropagation of medicinal and high-value crops, identification of better microtuber induction under various levels of sucrose, optimization of various concentrations of sterilants and plant growth regulators, and *in vitro* conservations [10–20], [21–31], [32–42], [43–55]. Other objectives include hormonal and media treatments to overcome *hyperhydricity* (vitrification) of micropropagated shoots [17].

The studies on plant micropropagation conducted so far in Ethiopia used *explants* that covered immature embryos, cotyledons, hypocotyls, seeds, lateral buds, nodes, shoot tips, shoot buds, and leaves. The types of surface sterilization agents applied are predominantly 70% v/v ethanol and 1 to 20% w/v sodium hypochlorite. The exposure time to ethanol

Table 2: List of species studied in Ethiopia, with background information on surface sterilization, plant growth regulators, and their applied rates.

S.N.	Species name	Explant used	Surface sterilization		Media ± plant growth regulator (PGR) Type	Ref.No
			Type (Concentration)	**Exposure time**		
1	*Prunus salicina* Lindl.	Nodes	Soap sol. + washing alcohol (70% (v/v))	5 m to 20 m 30 s	For Shooting: BAP or Kin ± IBA: For rooting: ½ Ms + IBA or IAA	[37]
			HgCl2 (0.1%(W/V))	7 m		
			NaOCl (2%) + Tween-20	20 m		
2	*Vitis vinifera* L.	Single-node shoots	Ethanol (70%) NaOCl 2%	7 m	For shooting: MS + BAP + IBA For rooting: MS + IBA	[38]
3	*Zingiber officinale* Roscoe.	Shoot tips and auxiliary bud	Ethanol (70%)	Brief immersion	for Shootin: i. MS + BA + Kin and ii. BA + NAA	[39]
			Active chlorine (5%) + Tween-20	15 m		
4	*Guizotia abyssinica* (L.f.) Cass.	Hypocotyl; Micropropagation,	Ethanol (70%)	2 m	Shoot initiation & proliferation: BAP + NAA; MS + NAA + IAA + BAP; MS + BAP + NAA	[40]
			CaOCl2 10 (%w/v) + Tween −80	15 m + 7 m		
			HgCl2 (0.1%)	5 m		
5	*Carica papaya L*	Shoot buds	Hot water, tween 20Kocide solution	3×15 m^2 drops 0.3% w/v	Shoot tip initiation: MS + BAP + Kin	[27]
6	*Saccharum officinarum* L.	Shoot tips	Hot water treatment (3×15) min	2 h (50oC)	Shoot tip initiation: MS + BAP + Kin	[42]
			Kocide sol. (0.3% (w/v))	30 m		
7	*Ensete ventricosum* (Welw.) Cheesman,	Shoot tips	Ethanol (70%) NaOCl (4%) + Tween 20	20 m	For shooting: MS + BAP + NAA For rooting: BA	[28]
			Ethanol NaOCl (2%)	5 m 10 & 20 m		
8	*Triticum turgidume& T. aestivum* L, *Var.; Yerer, Simba, Ude and Galama,*	Unfertilized ovary (Immature spikes)	Ethanol (70%)	1 m	MS or N6 or Chu or B5 + 2,4-D; EF: 2,4-D + (Kin) + 30 g/l maltose; Reg. of shoot: Cold pretreatment at + 60 g/l sucrose	[43]
			NaOCl (5.25%) + Tween 20	10 m		
9	*Manihot esculenta* Crantz.	Nodes	Kocide-103 sol. (0.3%)	30 m	Shooting: BAP and Kin;RI: NAA and BAP for root induction	[44]
			Ethanol (70%)	1 m		
			NaOCl (0.1%) + tween −20	10 m		
10	*Ananas comosuss* (L.) Merr.	Shoot tips	Running water		MS + 2 mg/l BA rooting: ½MS with 3 mg/l IBA	[45]
11	*Sesamum indicum* L.	Anther	Ethanol (70%)	45 m	callusing: MS + 2, 4-D + BAP shooting: MS + BAP + BAP + NAA Rooting: ½MS + IBA + NAA	[46]
			Kcl (5%)	10 m		
			Hgcl2 (0.1%)	1 m		
12	*Coffea arabica* L.	Leaf	ethanol (70%)	20–30 s	CIM:-BAP + IAA, and/or (2, 4-D); EC formation:- calli transferred 2,4-D + BAP; EGM: BAP + GA3	[11]
			3% NaOCl	15 m		
13	*C. edulis* Hemsl.	Seed & shoot tips	Kocide sol. (30 g l^{-1})	30/60 m	(MS and ½ MS) or (B5&1/2 B5) + (BAP, GA)	[55]
			ethanol (70%)	3 m		
			NaOCl (5%)	(5,8,10 &15 m)		
14	*Musa* x *paradisiaca* L.	Shoot meristems	detergent sol.	15–20 m	For shoot initiation: BAP + IAA for shoot proliferation: BAP and IAA; For rooting: NAA & IAA or IBA	[47]
			Ethanol (70%)			
			NaOCl (2%)	10 m		

Table 2: Continued.

S.N.	Species name	Explant used	Surface sterilization		Media ± plant growth regulator (PGR) Type	Ref.No
			Type (Concentration)	**Exposure time**		
15	*Echinops kebericho* Mesfin.	Seed and shoot tips	Running Water Ethanol (70%) NaOCl (5.25%) + Tween 20	10 m 9 m 5 m	Shoot induction: BAP and Kin Shoot multiplication: BAP + Kin + TDZ ± NAA rooting: Full,½ and 1/3 MS + IBA and NAA	[13]
16	*Capsicum annuum* L.	Node & shoot tip	Ethanol (70%) NaOCl (2%–4%)	1 m 10–25 m	Cytokinins: BAP, Zeatin Kin, Auxins; IAA and IBA	[23]
17	*Coccinia abyssinica* (Lam.) Cogn.,var. 29)	Shoot tips & nodal segments	Mancozeb sol. (0.3%) ethanol (70%) NaOCl (5.25%) + Tween -20	20 m 30 s 5,10 & 1 m	For culture establishment; MS + BAP and kinetin Shoot regeneration; BAP ± IAA For rooting: ½ MS + 15 g/l sucrose + IBA	[52]
18	*Aframomum corrorima* (Braun) Jansen.	Axillary buds	Ethanol (70%) NaOCl + Tween-80(6% + 2 ml)	1 m 5 and 10 m	Shooting:MS + 5% coconut water + 3 mg/l BA and 1 mg/l Kin	[49]
19	*Solanum tuberosum* L. Var.Hunde'& var.'Ararsa'	Lateral buds	Ethyl alcohol (70%) NaOCl (10%) + Tween-20	10 s 20 m	MS + GA3, NAA SC: (30 gl^{-1})	[35]
20	*Ipomoea batatas* (L.) Lam. var.,Beletech, Awassa-83 and Belela	Apical meristem	Ethanol (70%) NaOCl (0.1%)	1 m 4 to 7 m	Cytokinin (BAP and Kin) and auxin (IBA and NAA)	[53]
21	*Brassica carinata* A. Br., var. Holeta and yellow Dodola	Hypocotyl & cotyledon leaves	Ethanol (70%) NaOCl (1%)	1 m 20 m	callus induction:2.4-D, BAP, NAA SC = 2% for rooting	[29]

BAP = 6-benzylaminopurine, B5 = Gamborg medium, $CaOCl_2$ = calcium hypochlorite, CIM = callus induction medium, 2,4-D = 2,4-dichlorophenoxyacetic acid, EC = embryonic callus, EGM = embryo germination medium, GA3 = Gibberellic Acid, Hgcl2 = mercuric chloride, h = hours, IAA = indol acetic acid, IBA = indole-3-butyric acid, Kin = kinetin, KOcide = contact protect ant fungicide, MS = Murashige and Skoog, NAA = α-naphthalene acetic acid, m = minutes, NaOCl = sodium hypochlorite, s = seconds, PGRs = plant growth regulators, RI = root induction, Sol.= solution, SC = sucrose, TDZ = Thidiazuron, v/v = volume per volume, w/v = weight per volume.

was 1 m while it ranged from 5 to 25 m in the case of sodium hypochlorite depending on the species utilized. The exposure time used in the case of sodium hypochlorite did not correlate with the concentration applied. The hypochlorite used was partly an unstandardized local product called "Berekina." The vessels used for culturing were predominately Petri dishes followed by magenta and test tubes. Plant growth regulators (PGRs) and their concentration and tissue culture growing and regeneration conditions differed slightly between the species studied and the *explants* used. In most cases, the reported studies were conducted with no comparative experimental assessments.

In nearly all cases, Murashige and Skoog media was used, but without comparison with other media. The growing conditions and nonnutritional components applied were also similar. Molecular markers were not used for the identification of genotypes studied, and hence, the results reported so far may not be repeated on other genotypes of the same species. Local cultivar names were used as a marker, underestimating the genetic variation that exists within each local cultivar. In most cases, what we call protocol development lacks a comparative assessment. The growth temperature used was predominantly within the range of 25–27°C, and the relative humidity was mostly 70%. However, this information was not provided in some studies. The duration of light hours was predominantly 16 hs, while the light intensity used varied among the various studies. In some cases, the parental *ex plants* from ecologically different sources were treated as if they were the same [25], thus ignoring the impact of preceding environmental growing conditions on the micropropagation of the genotypes under study.

The types of *in vitro* tissue culture approaches for regeneration and mass propagation of the plant species studied are summarized in Table 3 as callus culture, meristem culture, somatic embryogenesis, and organogenesis. The culture types reported in the studies are mostly for micropropagation except in two cases of somatic embryogenesis [11, 24], a single case for unfertilized ovary [43], anther culture [46], callus induction for shoot regeneration [15], and two cases for *in vitro* germination of seeds and shoot induction [13, 23, 55]. These studies identified MS media with some growth regulators individually and/or in combinations for the shoot and root developments [27, 37–40, 42] and determined the minimum number of days for root induction [28], the highest percentage of callus production [29], effect of PGRs on the number of roots [45], and shoot length and shoot fresh weight [27, 37, 53, 55]. The studies also determined the survival rate of micropropagated plants in greenhouses [38, 45] and identified responsive explants and polyploidization, for example, to produce larger bulb size in garlic [26], by anticipating an increase in

Table 3: Objectives of the study, reported results, and culture types for some representative species studied with their published references.

Species studied	Objectives of the study	Results reported	Culture type	Ref.
1. *Prunus salicina* Lindl.	Micropropagation and virus cleaning	The best shooting was obtained on MS + 0.5 mg/l BAP in combination with 0.1 mg/L of IBA; similarly, the best rooting response was obtained on ½ MS media supplemented with 1.0 mg/L IBA	Micropropagation from nodal segment	[37]
2. *Vitis vinifera* L.	Salt tolerant cultivar identification	The degree of NaCl tolerance varied among the cultivars evaluated	Shoot culture from shoots with a single node	[38]
3. *Zinger officinale* Roscoe	Micropropagation & Virus cleaning	Better shooting was reported when MS was supplemented with (2 mg/l and 1 mg/l BAP; 7 shoots per ex plant was produced	Micropropagation from auxiliary bud and shoot tip	[39]
4. *Guizotia abyssinica* (L.f.) Cass.	Indirect in vitro regeneration Virus cleaning	NAA (5 mg/l) + BAP (1 mg/l) gave better CI from hypocotyl ex plant (98.3%); the highest percentage of shoot formation was obtained when cotyledons were cultured on a medium supplemented with 3.0 mg/l IAA + 1.0 mg/l BAP. Maximum number of shoots per explant (20.3) was obtained from a medium containing 0.1 mg/l NAA + 1 mg/l BAP. Best shooting was on 0.5 mg/l IBA. Shoot survival in soil was 65%	CI from hypocotyl and cotyledon for *in vitro* shooting and rooting	[40]
4. *Carica papaya* L.	Micropropagation	The highest mean, mean number of shoots, leaf, and shoot length were obtained on 1 mg/l BAP and 0.5 mg/l NAA. A minimum (9.5 days) and longer (13.5 days) for shoot initiation were recorded on BAP at 0.5 mg/l + 0.5 mg/l NAA and BAP at 2.0 mg/l + 0.5 mg/l NAA, respectively. Half-strength MS with 2 mg/L IBA is the most suitable treatment for root induction. The maximum number of roots (16.25) and root length (3.92 cm) were measured on shoots pretreated on MS media supplemented with 1.5 mg/l IBA.	Shoot bud culture for shoot initiation (micropropagation)	[27]

Table 3: Continued.

Species studied	Objectives of the study	Results reported	Culture type	Ref.
5. *Saccharum officinarum* L.	Micropropagation and Virus cleaning	Genotype N52 showed a maximum of 6.95 ± 0.19 shoots per explant with 4.75 ± 0.06 cm shoot length and 5.65 leaves per shoot on liquid MS medium fortified with 2 mg/l BAP + 0.5 mg/l kinetin while genotype N53 produced a maximum of 6.30 ± 0.26 shoots per explant with 3.94 ± 0.03 average shoot length and 5.83 leaves per shoots on liquid MS medium supplemented with 1.5 mg/l BAP + 0.5 mg/l kinetin	Shoot tip culture for micropropagation	[42]
6. *Ensete ventricosum* (Welw.) Cheesman	Micropropagation, virus cleansing	The minimum number of days for multiplication of shoots (11.6) and maximum shoot mass number of shoots (23.0) were obtained on 4.5 mg/l BAP and 1.5 mg/l for enset cultivar Mezia. The minimum number of days (10.5) for root induction was observed for the same cultivar on media with 1.5 mg/l IBA and the maximum root number (3.8) was recorded at 2 mg/l IBA	Shoot tip culture for micropropagation	[28]
7. *Triticum turgidum* and *Triticum aestivum* L., Var.; Yerer, Simba, Ude & Galama	Micropropagation, somatic embryogenesis, and anther culture	Genotypes, types of media, concentrations of 2,4-D and Kin and durations of cold pretreatment at 4°C affected direct formation of embryonic tissues independently. Stage II of wheat spikes, MS medium containing 1 mg/l of each of 2,4-D and Kin and 15 days of cold pretreatment were found to be the best conditions for direct formation of embryonic tissues; the highest frequency of shoots was regenerated from the cultured embryonic tissues of variety Yerer (41.6%) and Simba (41.3%) on medium containing 0.1 mg/l 2,4-D; From a total of 14,524 cultured unfertilized ovaries, 1,100 embryonic tissues (7.6%) and 75 regenerants were obtained. The average percentage of embryonic tissues and regenerants were 9.0 and 1.1% from 3,444; 9.8 and 0.55% from 4,732; 5.6 and 0.17% from 2,988; 4.7 and 0.12% from 3,360 cultured unfertilized ovaries for varieties Yerer, Simba, Ude, and Galama, respectively	unpollinated ovary cultures for direct formation of embryogenic tissue and subsequent shooting	[43]

Table 3: Continued.

Species studied	Objectives of the study	Results reported	Culture type	Ref.
8. *Musa esculenta* Crantz.	Micropropagation	BAP and Kin, For shooting, NAA and BAP for root induction augmented with 0.75 mg/l BAP and Kin gave the highest shooting (7.3/explant); NAA 0.5 mg/l gave the highest rooting (6.14 root/explant)	Auxilar nodal bud culture (micropropagion)	[44]
9. *Sesamum indicum* L.	Micropropagation	The highest mean shooting (87.15%) was obtained on 0.25 mg/l IBA + 0.5 mg/l NAA. A survival rate of 66.7% and the highest shooting (38.33%) were obtained. The highest callusing was obtained with (2,4-D 2 mg/l and BAP (1 mg/l) with 56% callusing and callus weight of 8.33g	Anther culture	[46]
10. *Coffea arabica* L.	Micropropagation	Leaf explants cultured on MS with 0.05 mg/l Kinetin in combination with 0.1 mg/l IBA resulted in embryonic callus. The highest number of embryos that germinated per ex plant (14.0 ± 1.7) was obtained on MS with 2.0 mg/l BAP in combination with 0.5 mg/l GA3. The maximum number of roots per plantlet (3.0 ± 1.0) was obtained on a 1/2 MS medium containing 0.5 mg/l IBA	Leaf explant culture for Embryogenic callus formation	[11]
11. *Cordeauxia edulis* Hemsl.	Micropropagation&Optimization of sterilization protocol	5% NaOCl for 10 m is the most effective in surface sterilization. The sterilized seed cultured on ½ B5 media was most suitable with a germination of 26.67%. The highest shoot initiation (89%) of explants produced shoots), the number of shoots per explant, and the number of leaves per shoot were obtained from cotyledonary node explants cultured on MS media supplemented with 2 mg/l BAP within nine weeks. But, the highest shoot length and shoot fresh weight were recorded from control (free BAP) and 6.00 after 42.57 ± 0.58 days of culture BAP, respectively. The highest shoot multiplication (4.56 number of shoot induced) and elongation (2.97 cm) were obtained from the induced shoot cut placed on MS media supplemented with 2.00 mg/l BAP + 6.00 mg/l GA3 and free BAP + 6.00 mg/l of GA3, respectively	cotyledon node explant	[55]

Table 3: Continued.

Species studied	Objectives of the study	Results reported	Culture type	Ref.
12. *Musa* x *paradisiaca* L.	Micropropagation	Shoot initiation was greater on MS basal medium with 3 mg/l BAP for Dwarf and Giant Cavendish and 2 mg/l for Poyo varieties. BAP and IAA at 3 + 0.4, 4 + 0.4, and 3 + 0.2 mg/l for Dwarf, Giant, and Poyo, respectively, were better combinations for high rates of shoot proliferation and elongation. Further multiplication of shoots required up to 5 times subculturing of 1 month each on the same media combination. Better rooting was obtained when the shoots were cultured on MS medium with 2.12 mg/l (NAA) for Dwarf and Giant Cavendish and 1.74 mg/l (IBA) for Poyo	Meristem culture	[32]
13. *Echinops kebericho* Mesfin	Micropropagation & Virus cleaning	100% germination was recorded in fresh seeds and dropped to 65.18% and 22.3% for 3 and 5 months seeds, respectively. After 42.57 ± 0.58 days of culture with 1.0 Kin and 0.5 after 42.57 ± 0.58 days of culture Kin + 0.1 mg/l NAA showed maximum shoot proliferation on shoot induction media and shoot multiplication media, respectively. Better rooting was obtained on 1/3 MS containing 1.5 mg/l NAA with 8.23 roots and 4.82 cm root length and established under greenhouse with 83% survival	Micropropagation using shoot tips	[13]

Table 3: Continued.

Species studied	Objectives of the study	Results reported	Culture type	Ref.
		3% active chlorine for 20 m was found to be a better treatment combination yielding 82.5% ± 5.00% contaminant-free germinated seedlings. For shoot induction, MS + 4.5 mg/l B AP + 0.5 mg/l IAA and MS medium containing 8 mg/l Zeatin were found to be better, resulting in 77.5% ± 5.00% and 67.50% ± 5% induction percentage for nodal and shoot tip explants, respectively.		
14. *Capsicum annuum* L.	Micropropagation, conservation Optimatizion of sterilizing protocol	Maximum shoot multiplication responses were obtained on MS + 3 mg/l BAP + 2 mg/l Kinetin with a mean number of 9.2 ± 0.2 and 8.6 ± 0.00 shoots for nodal and shoot tip explants respectively. Best shoot elongation and rooting responses were reported on MS + 0.5 mg/l IBA resulting mean value of 29.6 ± 0.12 root number, 4.25 ± 0.20 cm root length, and 5.12 ± 0.20 cm shoot height. The plantlets showed 77.5% survival during acclimatization and transplanting	Micropropagation (shoot apices and node)	[23]
15. *Coccinia abyssinica* (Lam.) Cogn.var. 29	Micropropagation and optimation of sterilants	NaOCl (2.0%) for 5 min gave high percentages of survived nodal (79.43 ± 0.6) and shoot tip (74.33 ± 0.58) explants. BAP (3.0 μM) was found to be an optimum concentration for SI, yielding 80% for nodal and 70% for shoot tip explants. The combination of BAP (3.0 μM) with IAA (0.5 μM) was reported to have been obtained as optimum concentration yielding 13.4 and 11.03 shoots per explants for nodal and shoot tip, respectively, for shoot multiplication. ½ MS with IBA (0.5 μM) and IAA (1.5 μM) yielded more than 90% rooting with optimum root number and length	Micropropagation (shoot tip)	[53]

Table 3: Continued.

Species studied	Objectives of the study	Results reported	Culture type	Ref.
16.*Aframomum corrorima* (Braun) Jansen	Micropropagation	Cultures initiated from axillary bud explants of rhizome using 0.5 mg/l thidiazuron (TDZ) in combination with 3 mg/l paclobutrazol (PBZ) gave about 26 shoots/explant (about 12.6-fold than the control) within eight weeks. Shoot multiplication was also from rhizome enhanced when TDZ at 0.5 mg/l was simultaneously used with either 2 mg/l imazalil (IMA) or 3 mg/l N6-benzyladenine (BA) in the culture medium. Subsequent shoot elongation and development of functional roots were reported to have been attained after one to three monthly subcultures on a plant growth regulator (PGR)-free basal medium	Micropropagation	[49]
17. *Solanum tuberosum* L.var. Hunde and var. Ararsa	Micropropagation and determination of the optimum concentration of sucrose for microtuberinduction	Two potato varieties were tested for their microtuber induction under five levels of sucrose (40, 60, 80, 100, and 120 g/l. In both varieties, among the five concentrations of sucrose, MS medium with 60 g/l sucrose exhibited a better response. This medium produced an average value of (1.97 ± 0.02) microtuber number, $(3.60 \pm 0.04$ mm) microtuber diameter, and $(0.08 \pm 0.002$ g) microtuber weight tuber in the variety after 42.57 ± 0.58 days of culture. On the other hand, after 35.67 ± 0.58 days of culture, the mean values of (2.90 ± 0.031) microtuber number, $(2.95 \pm 0.01$ mm) microtuber diameter, and $(0.06 \pm 0.001$ g) microtuber weight were recorded in the variety Hunde	Micropropagation (auxiliary bud)	[35]

Table 3: Continued.

Species studied	Objectives of the study	Results reported	Culture type	Ref.
18. *Ipomoea batatas* (L.) Lam. var. Beletech, Awassa-83 & Belela	Micropropagation	The minimum days for root induction was recorded for variety Beletech (3.167 days) in shoots cultured on media supplemented with 0.5 mg/l IBA with 0.5 mg/l NAA. Variety Awassa-83 induced root within 3.83 days in shoots cultured on media supplemented with 1.0 mg/l IBA and 0.5 mg/l NAA, whereas the variety Belela shoots have induced roots within 3.83 days on media supplemented with 1.0 mg/l IBA and 1.0 mg/l NAA. The maximum number of roots per shoot was recorded on MS media supplemented with a combination of 1.0 mg/l IBA with 0.5 mg/l NAA (11.7) followed by (9.3) on media with 0.75 mg/l IBA with 0.5 mg/l NAA, respectively. Maximum root length was observed for Beletech (3.4 cm) followed by Awassa-83 (3.43 cm) cultured on the media containing a combination of IBA 0.75 mg/l and NAA 0.5 mg/l. Acclimatization with a survival rate was 90% for Beletech and 80% for Awassa and 83% for Belela varieties	Meristem culture	[53]
19. *Brassica carinata* A. Br. var. Holeta & yellow dodola	Micropropagation	The explant from cotyledon was more responsive (95% callusing frequency) as compared to hypocotyl (80.7%) on MS with 0.5 mg/l 2,4-D using yellow dodola. Yellow dododa gave maximum shooting (98%) in MS supplemented with 2 mg/l BAP. The highest rooting percent from a yellow dodola variety was at IBA 0.3 mg/l	callus culture (hypocotyl and cotyledon)	[29]

BAP = 6-benzylaminopurine, B5 = Gamborg medium, $CaOCl_2$ = calcium hypochlorite, CIM = callus induction medium, 2, 4-D = 2, 4-dichlorophenoxyacetic acid, EC = embryonic callus, EGM = embryo germination medium, GA3 = gibberellic acid, Hgcl2 = mercuric chloride, h = hours, IAA = indol acetic acid, IBA = Indole-3-butyric acid, Kin = Kinetin, KOcide = contact protect ant fungicide, MS = Murashige and Skoog, NAA = α-naphthalene acetic acid, m = minutes, NaOCl = sodium hypochlorite, s = seconds, PGRs = Plant growth regulators, RI = root induction, Sol.= solution, SC = sucrose, TDZ = Thidiazuron, v/v = volume per volume, w/v = weight per volume.

bulb weight and overall productivity. As a continuation of these, the recent studies (2021/2022) covered the effect of MS medium strength, sucrose concentration, and pH level on shoot multiplication and root formation [21], *in vitro* propagation protocol for rapid regeneration of high-value crops like sugarcane using a completely randomized design with factorial arrangement [30]. In these studies, treatments with growth regulators had an overall positive impact on all parameters measured but with better performance due to growth regulator types, their concentrations, and combinations for the particular plant species studied [34–39]. BAP, IBA, IAA, and NAA were the predominantly used plant growth hormones and are followed by 2–4 D, TDZ, IMA, and GA3.

3. Discussion

The information provided in Tables 1–3 was assessed and discussed using the following interconnected thematic factors, and these are followed with conclusions and recommendations for standardized research in plant micropropagation in Ethiopia.

3.1. Embryo Morphology, Somatic Embryogenesis, and Networked Gene Regulation on Micropropagation. The production of functional plants in micropropagation can be increased by improving the morphology of somatic embryos during induction and development through culture manipulation. However, this is not always clear from the reports made so far on the Ethiopian plants. The degree of somatic embryogenesis varies with *explants* of particular genotypes used and their physiological states and these have gene expression patterns [63, 64]. The genotypic variation within a given species affects the process of embryogenesis while the culture manipulation improves the morphology of somatic embryos during induction and development where all are influenced by the identity of genotypes in any standardized study.

Auxin and wound-induced callus formation pathways converge to the same gene regulation network and rely on the cooperative action of defined sets of transcription factors [6, 65]. Gain or loss of function of many cell cycle or developmental regulators might result in callus formation [5]. The detailed genetic structure, molecular marker, and allelic types of these networked pathways may differ between species, genotypes, type of *explants* used, and previous environmental growth conditions of parental stocks. These impact the standardization and repeatability of micropropagation. In addition to the detailed genetic structures, phenomics and allelic oscillation can limit the results of any micropropagation studies. Thus, integrating "omics" data at broader levels of systems biology through using genomics and phenomics could result in a better prediction of micropropagation. Thus, phenomics and other omics studies should be pursued on genotypes of interest to enable a standardized protocol development of plant micropropagation.

Reports in the literature show that rooting capacity differs due to endogenous auxins and ranges from very easy to very difficult to root [66–68]. These differential capacities could be due to differences in gene regulation and their networks. Thus, plant micropropagation and regeneration studies in Ethiopia need to take into account this for each genotype using appropriate markers and, when possible, with information on variant genes that respond to endogenous auxins. The seasonal variation and variation in nutrient use efficiency and the age of the stocks or mother *explants* also impact micropropagation and should be defined and stated unlike the various reports summarized in Tables 1–3.

3.2. Reliable Genetic Stability Tests on Micropropagated Propagules. The micropropagation protocols described in Tables 1 and 2 were expected to have a high frequency of organogenesis or embryogenesis, with propagated clones remaining true to their parent types when tested using reliable genetic markers. The methods employed to assess the genetic stability of plant genotypes in the past included morphology [69], secondary compounds, protein electrophoresis [70, 71], and cytology [72, 73]. Other methods focused on chromosomal variation, restriction fragment polymorphism (RFLP), intersimple sequence repeat (ISSR), simple sequence repeat (SSR), and single nucleotide polymorphism (SNP) markers-based analyses. Any of these methods that assess genetic stability were not applied in the studies whose results were summarized in Tables 1–3. The micropropagation reports assumed a low risk of genetic instability and failed to consider the minimum standardized DNA markers [74] like the one developed and reported for *Magnolia sirindhorniae* Noot. & Chalermglin, which confirmed the genetic uniformity and stability of regenerated plants of *M. sirindhorniae.*

When proteins are used as markers, attention should be given to the stages of growth and physiology of the plants used. Factors that control the expression of proteins through differential levels of transcription in various organs do differ even in genetically identical plants and thus need to be taken cautiously. Comparisons based on these characters are rather difficult, particularly when data from different geographical areas [70, 71] and physiological stages are considered. Significant alterations, in both chromosome number and morphology, have been demonstrated as a result of regenerating plants from culture via a callus phase [73]. Observations of these changes in micropropagated *plantlets* have not yet been tested in the plants reported in Tables 1–3. However, one should note that the incidence of gross chromosomal aberrations and their progeny is overemphasized since chromosomal abnormalities are usually eliminated in the plantlets [73]. When required, such tests should be done on different sections of plants since some plants may be chimerical and possess sectors with different ploidy levels higher in cell or callus cultures than in plants regenerated directly from preexisting meristems.

3.3. Culture Environment for Micropropagation and Establishment of Propagules in the Field. The micropropagated plants should be protected from epigenetic variation that results from environmental factors and various stresses including doses of PGR and levels of humidity in culture vessels [75–77]. In most of the reported cases of the present review, optimized establishment of propagules in the field is not included. They did not provide clear and complete information on sources, history, and genotypes of *explants* used. The components of culture medium and their interaction as well as the timing of the subculture period are not provided. The reported *in vitro* micropropagation growth conditions comprised temperature, light, pH, and growth culture vessels, each of which varies among the plants studied.

(i) Temperature: incubation temperature for micropropagation ranges from 20 to 28°C [78]. Some species root more effectively at higher temperatures.

For example, some cultures of grapes root better when exposed to higher temperatures. A study on grape micropropagation for salt tolerance in Ethiopia (Table 1) used a temperature of 27°C. However, it is not clear whether the difference in temperature between reports from other studies and that of the reports from Ethiopia [38] is due to differences in genotypes used and/or other factors. Fluctuations in temperature during day and night influence growth and organogenesis in cultures [79], and this suggests the need to redefine the optimum temperature requirements of the various reports in Tables 1 and 2, together with various sources of light given below.

(ii) Light: the role of light (irradiance, spectral quality, and photoperiod) is probably one of the most neglected and poorly studied factors in plant tissue culture including micropropagation studies and is not limited to those reported in Tables 1 and 2. Light quality should be considered when choosing light sources for culture rooms, and both quality and intensity should be checked periodically. In addition, a laboratory with an appropriate design and regular electric supply is required. In embryo-derived callus cultures of Douglas fir, red light stimulated adventitious bud formation [80, 81], although this is not universal. For example, in tobacco callus cultures, blue light is more effective than red in stimulating shoot growth [82]. The effect of light on callus induction/morphogenesis/somatic embryogenesis differs between species and genotypes used and thus needs to be defined through experiments. In some reported cases, dark pretreatment was considered helpful. This was shown in an experiment on *Cicer arietinum* using *explants* excised from leaflets of immature plants grown under 24 hours of darkness with better callus initiation response than the same *explants* grown under a 16-hour photoperiod [82].

(iii) pH: generally, cell growth in culture is not significantly affected under a broad range of initial pH (4.0–7.0) of the medium. It is difficult to draw meaningful conclusions about the optimum pH of a medium unless pH is constantly monitored and readjusted. The pH of the medium is influenced by factors, such as the genotype of an *explant*, media type, initial pH, and incubation time. Woldeyes et al. [21] reported that both shoot multiplication and root formation of okra were reduced with decreasing pH and resulted in no root formation at pH 5.0. However, the fluctuation of the pH during the experiment was not monitored, and the genotype used was unmarked. The decrease or increase in pH reported elsewhere depends on whether the media used contain ammonium or nitrate as a nitrogen source [83, 84]. Hence, information on a range of pH needs to be defined with nitrogen sources in the media used on genetically marked genotypes in order to optimize and develop a repeatable protocol of micropropagation.

(iv) Growth media culture vessels used: for media cultures, Petri dishes, Erlenmeyer flasks, bottles, magenta vessels, or test tubes are commonly used, and each of them affects growth differently. The volume of both the atmosphere [79] and nutrients in a vessel affects growth rates and morphogenesis and thus should be standardized. Proper ventilation of the atmosphere in the vessels is important since the accumulation of volatiles or ethylene production causes vitrification and inhibits morphogenesis [85] and other physiological processes. Rapid changes in the atmosphere, for example, when vessels are opened, may result in a temporary "gas shock" of the culture [86]. Studies similar to those summarized in Tables 1–3 as well as other studies with similar objectives should evaluate the quality of micropropagation and regeneration in test tubes, magenta vessels, and Petri dishes (with their specified closures that permit air exchange) with constant and variable volumes of media.

3.4. The Problems of Defining Precultures and Physiological Stages of Explants. Immature tissues and organs are invariably more morphogenetically plastic *in vitro* than mature tissues and organs and thus have a high frequency of cultural survival and growth rates *in vitro*. However, the various reports given in Tables 1–3 failed to define and standardize species and genotype-dependent physiological stages for optimum micropropagation. In some studies like the case of noug (*Guizotia abyssinica*), the results obtained were not compared with other similar studies [87]. The differences may be due to the types of genotypes and physiological stages of *explants* used. Cultural conditions of *explants* and background information on environmental growth conditions of parents and other prior treatments of the species to study should not be overlooked unlike studies reported on *Ximenia americana* L. [25] in Ethiopia. These authors [25] used *X. americana* samples from two ecological sources (Boset in the central-eastern Oromia and Gambela regions in Ethiopia) but reported their results without treating the two sources separately.

3.5. Surface Sterilization of Explants. The most common surface sterilants in plant micropropagation culture are calcium and sodium hypochlorite, with calcium salt being less toxic to tissues than sodium salt [88]. However, calcium hypochlorite reacts with carbon dioxide in the atmosphere and is chemically unstable. Most commonly, a dilute solution of sodium hypochlorite (0.25–2.63%) is used as a disinfectant. An emulsifier such as Tween-20 (polyoxyethylene sorbitan monolaurate) is added at the rate of 1 drop per 100 ml of solution. The most commonly used sterilization agents on Ethiopian plants reported in Tables 1 and 2 were ethanol and sodium hypochlorite. The concentrations of sodium hypochlorite used range from 1 to 20% w/v with

exposure times of 5–20 minutes. However, the exposure time and concentration were not correlated. Besides, all hypochlorite should have been completely rinsed out from the tissues, because any trace left behind will interfere with amino acid uptake and metabolism [88]. However, none of the reports summarized in Tables 1 and 2 carried out tests for traces of hypochlorite left after washing with water. There were no comparative assessments of different surface sterilants except in a few cases, like in a study [34] that examined the *in vitro* propagation protocol using a completely randomized design with four replications and a factorial arrangement for the rapid regeneration of apple rootstock and scion node culture.

The results of sterilization using 0.3% mercuric chloride for 7 min scored a higher percentage of no contamination although this also varied among genotypes studied. As a special case of laboratory conditions, bacteria and fungi, may escape surface sterilization of original explants and arise as laboratory contaminants [89]. These factors should have been taken into consideration along with the type of *explants*, disinfectant type, and/or treatment time employed.

3.6. Nutritional and Nonnutritional Composition of Medium. The majority of plant micropropagation media are chemically defined with additional variables of plant growth regulators, the concentration of agar, and methods of preparation [90, 91]. White media [92] contains the nutrients normally required by plant cells, especially for root cultures. However, the amounts of nitrogen and potassium were found to be inadequate to sustain maximum growth of callus and cell suspension cultures [93, 94]. Callus and cell cultures of some species prefer MS, while others grow better in the B5 Gamborg et al. [95] or Erikson [96] media. The need for richer mineral salt mixtures is compensated by adding yeast extracts, protein hydrolysates, amino acids, coconut milk, or other organic supplements. There is a wide use of the MS [97] medium or its modifications. The MS and Erikson [98] media are similar, but the Erikson media contain twice the amount of phosphate and much lower concentrations of micronutrients than the MS medium. The B5 Gamborg et al. [98] medium contains relatively low amounts of ammonium, a nutrient that may repress growth in batch cultures. A number of culture media have been developed and tested to satisfy the needs of a variety of plant cells and tissues for micropropagation. Some of these media differ in degree as dilutions of standard media, while others combine the micronutrient elements of one with the micronutrient elements of another [99].

The Schenk and Hildebrandt (SH) [100] medium resembles B5 Gamborg et al. [98]. However, the amount of mineral salts is slightly higher, and ammonium and phosphate are supplied as $NH_4H_2PO_4$. The salt content of the Heller medium (Heller, 1953) cited in [101] is relatively low. Different concentrations of vitamins, amino acids, Myo-inositol, and phytohormones are usually added to the chemical composition of the various media to meet the optimum growth requirements. There are also numerous other media in use, including Litvay, Anderson, Quoirin-Lepoivre, Durzan, Woody Plant Medium (WPM), Gresshoff-Doy, and Zimmerman media. A major difference between MS and WPM is the level of macronutrients. There is a wealth of literature on various types of media and additives; however, in Tables 1 and 2, the media composition used is mainly MS with no comparison with other media for a likely better performance.

Agar is among nonnutritional media components and sometimes inhibits shoot growth. Substituting agar with liquid or gelrite-solidified media strongly promotes the growth of shoot cultures. A liquid or gelrite-solidified media increases succulence and vitreous growth [102], which is vulnerable to mechanical damage. In some cases, a compromise can be achieved by using a combination of agar and gelrite or using charcoal to reduce the inhibitory effect of agar with the removal of any cellular waste products. This aspect of the work is seldom covered on Ethiopian plants studied so far. Morphogenesis as well as callus growth rates and growth response of the cultures partly depends on the concentration and degree of purification of agar [90, 103]. Agar is a source of many minerals, in particular, sodium and possibly some vitamins and toxins, which may complicate the metabolic and nutritional requirements of micropropagation culture studies. The most interesting agar substitutes studied include positively charged dextran microspheres, plant agar, a starch copolymer, poly acryl amide, silica, and Ficoll, a sucrose polymer. Activated charcoal adsorbs and prevents the browning of tissues [104] and stimulates embryogenesis and rooting. However, the type and purification of agar and activated charcoal added to nutrient media to remove aromatic waste products excreted by cultured tissues are not given in most of the reported cases summarized in Tables 1 and 2.

3.7. Sources of Nitrogen, Carbohydrates, Micronutrients, and Plant Growth Regulators in Plant Micropropagation Culture Media

3.7.1. Nitrogen. Most of the micropropagation studies conducted on Ethiopian plants so far lack comparative studies to identify referred nitrogen sources. The growth rate in the medium containing nitrate is lower than the rate in the medium containing both nitrate and ammonium. On the other hand, the growth on ammonium in excess of 8 mM can be deleterious and is generally considered toxic to plant cells [98]. Most plant micropropagation media incorporate both nitrate and ammonium salts as an inorganic nitrogen source for growth. Embryogenesis of some species like carrot is severely inhibited when grown on media containing nitrate as the sole nitrogen source, while the addition of low concentrations of ammonium stimulates both growth and embryogenesis [105]. An adequate assessment of the suitability of nitrogen sources in a medium and working out the genes involved in nitrogen metabolism and embryogenesis are needed for the required standardization and repeatability.

3.7.2. Carbohydrates. In addition to serving as carbon and energy sources, carbohydrates play an osmoregulatory role (as osmoticum) and are *in situ* regulators of morphogenesis in both a medium and tissue. Thus, many micropropagation cultures, especially those that involve embryo cultures, perform properly only when cultured on a nutrient medium with a high osmotic potential [106]. Sucrose is generally the best carbon and energy source [106] and has been the carbohydrate of choice in the vast majority of reports on shoot induction and micropropagation. In many plant species, sucrose is used at a final concentration of 10 g/L to 30 g/L. However, it is not always the most effective carbohydrate. Fructose, glucose, sorbitol, and mannitol were found to be effective in the shoot initiation and micropropagation of several plants in a stage-specific fashion. Myo-inositol may not be essential but is added since it has been shown to enhance callus growth. Other studies have shown that the generation and establishment of photoautotrophic cultures require cell selection and manipulation of levels of growth regulators and carbon dioxide [107]. Several studies have indicated that the endogenous carbohydrate status of the *explant* at the time of selection is important in the development of adventitious roots. The role of various carbohydrates, their optimum concentration, and endogenous status in *explants* need to be studied to fill the gap left in studies on Ethiopian plants reported in Tables 1 and 2, as well as those that will be studied in the future.

3.7.3. Micronutrients. The optimal requirements of micronutrients, including their components and how they are dissolved, for the species summarized in Tables 1 and 2 need to be revisited. Iron chelates stimulated embryogenesis and root growth, whereas iron dissolved in nonchelated form did not. EDTA, without iron, at concentrations comparable to those used in micropropagation culture media, stimulates nitrate reductase [108] and inhibits ethylene formation and this may be significant because reduced nitrogen stimulates embryogenesis, while ethylene inhibits it thus negatively impacting micropropagation. The optimum level of micronutrients and their combined effect are thus of interest to work out in the plants reported.

3.7.4. Plant Growth Regulators. The effects of auxin type, concentration, and their interactions with each other and with other PGRs influence *in vitro* plant regeneration and micropropagation. Bonneau et al. [109] tested the effect of 200 growth regulator combinations in culture media on the European Spindle Tree (*Euonymus europaeus* L.). The results indicated that only four combinations (IAA-benzyl amino purine and IAA-kinetin) allowed differentiation of somatic embryos depending on auxin exposure time. However, this differs among various species, genotypes, and explants used. Amente and Feyissa [30] used a completely randomized design and factorial treatment arrangements (unlike many other reports) of different levels of BAP for *in vitro* propagation of sugarcane in Ethiopia to enhance the availability of healthy and true-to-type planting materials. However, the claim that the parent plants were true to type was not tested and reported and needs further study.

All levels of hormone treatments used in Ethiopian plant micropropagation culture studies had positive impacts on all parameters measured [30–39]. However, only specific hormone types and concentrations resulted in better performance during plantlet acclimatization. Genotype differences in the species studied evoke varying responses to the same PGRs where hormone type and concentration are considered for effective *in vitro* propagation of respected genotypes/cultivars. The presence of cytokinins, kinetin, and/or zeatin in the induction medium for the expression of somatic embryogenesis and micropropagation needs to be worked out with appropriate design and factorial treatment arrangements for each specific genotype of a given plant species. In addition to this, the role of ABA to stimulate callus formation and embryogenesis and the application of silver nitrate to enhance embryogenesis for micropropagation in species with high ethylene levels need to be studied and documented.

Some plants with relatively high concentrations of auxins induce the regeneration of roots due to interconnected distribution and signaling profiles that stimulate cambial activity and align with root apical meristems [63, 64]. Rooting of adventitious shoots and cotyledon cultures, and the regenerated shoots from callus in some angiosperms, can be promoted by low concentrations of auxins [64, 110]. Hence, the removal of an auxin from the medium, leads to root formation. The role of different auxins and their levels of performance for micropropagation of the various species reported in the present review remains to be thoroughly worked out. The distribution of auxins in various meristems is well established as presented above. The physiological effects of each PGR are determined by the kind of growth regulator and its concentration, the presence or absence of other PGRs, and the genetic makeup and physiological status of the target tissue in a micropropagation. We do not yet have data on these factors in many species, including in those reported in Tables 1 and 2. Other studies that are of interest could be the effect of growth hormones, colchicine concentration, and immersion time [26] for increasing ploidy levels followed by micropropagation in several economically important bulb bearing plants.

3.8. Application of Plant Growth-Promoting Microbes (PGPMs) in Micropropagation. Propagules developed under aseptic conditions are transferred to soil by protecting them from soil pathogens through the application of special antifungal treatments. In addition, growing them in special transplanting media increases their survival, which is influenced by the kind of substrates used. For example, the percentage of survival of *Cicer arietinum* propagules was high (85.4%) in pure vermiculite than in a mixture of vermiculite and perlite (1 : 1) (20.4%). The propagules transplanted into sandy soil and sawdust mixture (1 : 1) did not survive [82]. A consolidated effort is required in optimizing diverse forms of micropropagation from various

sources of explants and such efforts should include specifications of appropriate transplanting media in various ecologies. PGPMs help plants to access nutrients and protect them from diseases, pests, and abiotic stresses. This is done through a variety of processes, including the production of phytohormones, siderophores, phosphate solubilization, and induction of plant intrinsic systemic resistance responses [111]. Mengistie and Awlachew [112] reported indigenous *Bacillus* species as PGPR for different varieties of tomato under *ex vitro* (not through *in vitro* plant micropropagation) and found promising results for the conditions in Northwest Ethiopia.

PGPMs as sustainable plant growth enhancers have the potential of addressing multiple stresses and thus call for them to be integrated with research in plant breeding, micropropagation, and agronomic improvements. Soumare et al. [111] presented an overview of the importance of PGPMs and their potential applications in plant micropropagation where their analysis, based on published articles, revealed that the process of *in vitro* classical tissue culture techniques under strictly aseptic conditions needs to be reviewed. PGPMs can positively impact the growth of *explants* and ensure better survival by sustaining the shock during transplantation into a greenhouse or glasshouse and field [112]. Plants from micropropagation are adversely affected by water stress, because of the low absorption capacity of their roots. Inoculation of these plants with PGPMs in *vitro* is an effective step to deal with low water absorption capacity as well as to boost posttransplant performance of in *vitro* grown plants through increasing nutrient availability and inducing resistance to pathogens.

Mycorrhizal fungi also produce hormones that control plant development and activate signaling pathways during biotic and abiotic stresses. Meixner et al. [113] showed that plants inoculated with AMF (Arbuscular Mycorrhizal Fungi) had higher levels of auxins than noninoculated plants. Fungi, especially AMF, play an important role in water uptake and availability [114], thereby increasing the rate of photosynthesis and osmotic adjustment under environmental stresses [115]. AMF also increases the uptake of micronutrients such as P, Zn, Cu, and Fe. The contribution of both AMF and PGPMs is significant during the acclimatization phase because the weak adventitious root system (without root hair) of in *vitro* plants does not allow optimal absorption of nutrients from the soil during the early stage of the weaning step. The lower survival rate and poor establishment of *in vitro* plants under field conditions may also be due to the fact that the transferred *in vitro* plants do not find their natural microsymbiont partner. Diez et al. [116] showed that *in vitro* mycorrhization with *Pisolithus tinctorius* and *Scleroderma polyrhizum* strains increased the formation of secondary roots. Similarly, Sahay and Varma [117] reported a 90% posttransplantation survival rate of micropropagated tobacco and brinjal plants treated with the endophytic fungus, *Piriformospora indica*. This biopriming has also been reported to increase resistance against pathogen attacks [118]. Nevertheless, certain endophytic fungi can be plant pathogens like the case with *Fusarium equiseti*, which was suspected to cause bamboo blight and culm rot diseases and limit the micropropagation process [119]. The use of the mycorrhization technique can be important for the growth and development of micropropagated *plantlets*; however, prior to this, the process of production of pure fungal inoculum for micropropagation needs to be resolved.

Kargapolova et al. [120] have shown the efficacy of the inoculation with *Ochrobactrum cytisi* in potato micropropagation. A 50% increase in the mitotic index of root meristem cells and a 34% increase in shoot length were reported under *ex vitro* conditions. *In vitro* mycorrhization of micropropagated plants before acclimatization increases survival and resistance to water stress and ensures better mineral nutrition of the plant by enhancing the functionality of the root system [121]. Numerous findings validated the use of *in vitro* mycorrhization techniques in several plant species, such as *Castanea sativa* [122], *Helianthemum* spp. [122], *Citrus* spp. [123], and *Quercus suber* [116]. Research is needed to select efficient, multifunctional, stress-tolerant PGR-producing microbes that have ecological plasticity for use at different stages of micropropagation. Particular attention should be given to mixed-strain consortiums rather than monostrain inoculums to take advantage of functional complementarities to be carried along with plant micropropagation studies in Ethiopia. A great deal of effort should be devoted to the bioformulation of these microbes for suitable applications. The use of nanoformulations may enhance the stability of biofertilizers [124, 125] with respect to heat, desiccation, and UV inactivation. The need for bionanotechnology research inputs in *in vitro* micropropagation culture techniques is obvious.

The use of microbes deserves careful monitoring of endophytic communities, especially for plants used as raw food because some strains that are pathogenic to humans can be stably maintained in cultivated tissues and *ex vitro* plants. In addition, much remains to be learned from PGPMs in order to identify appropriate candidates to develop bioformulations for suitable application in plant micropropagation. Studies on the responses of crops and other economically useful plants to inoculation with symbiotic and nonsymbiotic PGPMs will help identify which plants in the Ethiopian plant bioresources are suitable candidates for microplant *biotization*. Studies to identify and evaluate the capacity of micropropagation studies using PGPMs should be seriously considered as discussed above. Application of inoculants of plant growth-promoting microorganisms (PGPM) on the plants reported so far through micropropagation reduces costs incurred during the whole micropropagation process and transfer to transplanting media.

3.9. Highlights of Advances in Plant Micropropagation Studies. Plant micropropagation benefits from progress in several research fields, including emerging and cutting-edge biosciences. Such integrated advances in science enhance conservation, mass propagation, genetic manipulation, bioactive compound production, and crop improvement [125, 126]. Other areas of plant micropropagation technology that should show advances in Ethiopia include plant

genetic resource management and regeneration of endangered plant species through recombinant DNA technology and genetic engineering for the safe conservation of their genetic stock in gene banks (*cryopreservation*) as suggested by Kumari [126]. Other additional advances should cover epigenetics, transcriptomics, and proteomics-based testing for the plasticity of genome responses as well as for the application of plant growth-promoting microbes in micropropagation research programs.

Advances in plant micropropagation offer economic prosperity by drawing the attention of applied biologists, businessmen, businesswomen, and entrepreneurs. Such prospecting advances should be based on the reorganization of the currently dispersed plant micropropagation and related laboratories into research program laboratories equipped with the necessary capacity and capabilities for conducting research on commercially high-value plants and for the production of phytochemicals for commercial purposes. A laboratory of this type must meet a high standard of cleanliness with regular fumigation, air filtering, standardized electrical wiring safety, power backups, and ventilators, which are seldom found in many existing laboratories in the country.

4. Major Conclusions, Future Prospects, and Recommendations

The objective of this paper is to review and assess the various factors considered in micropropagation in the so far reported studies on Ethiopian plant species, with reference to the results and efforts made in the country, the challenges faced, and the major gaps noted in order to propose recommendations regarding standard micropropagation. The results and growth conditions reported may be difficult to repeat on other genotypes due to the lack of specificity of the genotypes used and their markers.

In order to maintain clarity, repeatability, and enrichment of current biological advances, recent advances in proteomics, transcriptomics, and epigenetic analyses should be anchored in micropropagation studies. In most cases, the plant micropropagation studies reported to date underrate the importance of *explants*, the culture environment, timing (subculture period, dosage of PGR), and interactions between these factors. A proper physiological stage of development for optimum regeneration has to be established and standardized in terms of the species used and the specific genotype selected.

Comparative assessments of surface sterilants require studies on methods of distribution of disinfectants and adequate disinfection of *explants*. In order to determine the most appropriate media and agar type for Ethiopian plants, MS media should be compared with various other media. There should be an adequate assessment of both macronutrients and micronutrients and their combined effects, for specific species and genotypes that are properly identified and marked in order to ensure the genetic stability of micropropagated plants.

Air exchange in culture flasks needs to be studied with appropriate design. It is useful to define the information of light quality and intensity and dark pretreatment on the Ethiopian plants/crops so far reported and others to be dealt with in the future. PGPMs should be applied to select efficient, multifunctional, stress-tolerant, and PGRs-producing microbes that have ecological plasticity for their use in different stages of micropropagation, with particular attention to mixed-strain consortiums to take advantage of functional complementarities.

Micropropagation studies in Ethiopia require a standardization of laboratory design and facilities in order to get the maximum benefit from existing useful plants. Studies on plant micropropagation cultures must be programmatic and have the necessary levels of skilled human resources, technology, infrastructure, and institutional structures to contribute effectively to competitive agricultural research programs. In order to improve crop resistance to biotic and abiotic stresses, as well as to increase food crop nutritional quality, this technology should be developed. Furthermore, these programs should take into account the impact of various systems of agroecological diversity in the country.

Research programs in plant micropropagation and related studies should be focused on the current needs of the country without compromising opportunities that may arise in the near future or beyond. A sound research program should be set up to develop products of economic importance that are sustainable and reap the benefits of the country's abundant plant bioresources. In the use of plant micropropagation, one should consider indigenous plants with high economic potential that are underutilized, while also integrating various scales of indigenous knowledge.

Data Availability

This MS is a critical review based on the published reports that are referred and listed in the reference lists of the MS, Data are thus available as a secondary document.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

The first author designed the study, analyzed the data, and wrote the paper. The second author collected the data and the required literature and contributed to the writing. The third author commented, edited, and proofread the prepared manuscript. The corresponding author responded to the queries raised by the reviewers, edited, and contributed to the overall interpretation of the data collected. All authors read and approved the final manuscript.

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References

[1] O. M. Oseni, V. Pande, and T. K. Nailwal, "A review on plant tissue culture, A technique for propagation and conservation of endangered plant species," *International Journal of Current Microbiology and Applied Sciences*, vol. 7, no. 7, pp. 3778–3786, 2018.

[2] A. Fehér, "Callus, dedifferentiation, *totipotency*, somatic embryogenesis: what these terms mean in the era of molecular plant biology?" *Frontiers of Plant Science*, vol. 10, no. 536, 2019.

[3] K. Kalinowska, S. Chamas, K. Unkel et al., "State-of-the-art and novel developments of *in vivo* haploid technologies," *Theoretical and Applied Genetics*, vol. 132, no. 3, pp. 593–605, 2019.

[4] A. Iwase, N. Mitsuda, T. Koyama et al., "The AP2/ERF transcription factor WIND1 controls cell dedifferentiation in arabidopsis," *Current Biology*, vol. 21, no. 6, pp. 508–514, 2011.

[5] A. Iwase, M. Ohme-Takagi, and K. Sugimoto, "WIND1: a key molecular switch for plant cell dedifferentiation," *Plant Signaling & Behavior*, vol. 6, no. 12, pp. 1943–1945, 2011.

[6] M. Ikeuchi, K. Sugimoto, and A. Iwase, "Plant callus: mechanisms of induction and repression," *The Plant Cell Online*, vol. 25, no. 9, pp. 3159–3173, 2013.

[7] J. L. Zimmerman, "Somatic embryogenesis: a model for early development in higher plants," *The Plant Cell Online*, vol. 5, no. 10, pp. 1411–1423, 1993 Oct.

[8] T. Abrar, H. Temam, and W. Redwan, "Current status and opportunities of plant tissue culture in Ethiopia," *International Journal of Molecular Sciences*, vol. 5, pp. 19–28, 2019.

[9] Y. M. Abebaw, D. C. Tobiaw, B. A. Abate, B. K. Eshete, S. K. Seymour, and K. Tesfaye, "Plant tissue culture research and development in Ethiopia: a case study on current status, opportunities, and challenges," *Advances in Agriculture*, vol. 2021, Article ID 9979549, 12 pages, 2021.

[10] R. Berhanu and T. Feyissa, "Factors affecting in vitro propagation of cassava (*Manihot esculenta.* Crantz) varieties of "Kello" and "Qulle"," *Ethiopian Journal of Biological Sciences*, vol. 12, pp. 25–39, 2013.

[11] W. Ahmed, T. Feyissa, and T. Disasa, "Somatic embryogenesis of a coffee (*Coffea arabica* L.) hybrid using leaf explants," *The Journal of Horticultural Science and Biotechnology*, vol. 88, no. 4, pp. 469–475, 2013.

[12] D. S. Kassahun Bantte, T. Feyissa, and T. Feyissa, "Interaction effects of 6-benzylaminopurine and kinetin on in vitro shoot multiplication of two sugarcane (*Saccharum officinarum* L.) genotypes," *Advances in Crop Science and Technology*, vol. 2, no. 4, 2014.

[13] B. Manahlie and T. Feyissa, "Micropropagation of kebericho: an endandered Ethiopian medicinal plant," *American Journal of Plant Sciences*, vol. 5, no. 26, pp. 3836–3847, 2014.

[14] D. S. Kassahun Bantte, T. Feyissa, and T. Feyissa, "Effec ts of polyvinyl pyrrolidone and activated charcoal to control effect of phenolic oxidation on *in vitro* culture establishment stage of micropropagation of sugarcane (*saccharum officinarum* L.)," *Advances in Crop Science and Technology*, vol. 3, no. 4, 2015.

[15] S. Teshome and T. Feyissa, "In vitro callus induction and shoot regeneration from leaf explants of *Glinus lotoides* (L.)— an important medicinal plant," *American Journal of Plant Sciences*, vol. 6, no. 9, pp. 1329–1340, 2015.

[16] F. K. Gemechu and T. Feyissa, "Hyperhydricity (vitrification) of micropropagated shoots of two grapevine (*Vitis vinifera* L.) cultivars," *International Journal of Current Trends in Pharmacobiology and Medical Sciences*, vol. 1, pp. 71–77, 2016.

[17] S. Teshome and T. Feyissa, "Direct organogenesis of *Glinus lotoides* L.-Antihelmintic herb using nodal segments, Plant Tissue Cult," *Plant Tissue Culture and Biotechnology*, vol. 26, no. 1, pp. 131–141, 2016.

[18] I. Teshome, S. Teshome, T. Soromessa, and T. Feyissa, "Development of an efficient *in vitro* propagation protocol for *Satureja punctata*– a rare aromatic and medicinal plant," *Taiwania*, vol. 61, pp. 41–48, 2016.

[19] A. Jemal, B. Kassahun, and F. Tileye, "Protocol optimization for in vitro shoot multiplication of Jackfruit (*Artocarpus heterophyllus* L.)," *African Journal of Biotechnology*, vol. 16, no. 2, pp. 87–90, 2017.

[20] S. Teshome, T. Soromessa, and T. Feyissa, "In vitro propagation of a threatened medicinal plant *Satureja abyssinica* through nodal explants-Antimicrobial and antifungal herb," *International Journal of BioSciences and Technology*, vol. 10, pp. 20–25, 2017.

[21] G. G. Woldeyes, T. F. Senbeta, A. Y. Adugna, and A. W. Abegaz, "The effect of MS strength, pH and sucrose concentrations on in vitro propagation of okra (*A. belmoschus esculentus* L.) from shoot tip explants," *Research Square*, pp. 1–17, 2021.

[22] Y. Seyoum and F. Mekbib, "In vitro germination and direct shoot induction of Yeheb (*Cordeauxia edulis* Hemsl.)," *Far Eastern Affairs*, vol. 3, pp. 452–458, 2014.

[23] T. Hailu, B. Abera, and J. Daksa, "*In vitro* direct organogenesis protocol for mass propagation of an elite Ethiopian hot Pepper (*Capsicum annuum* L.) Cultivar: mareko Fana," *American Journal of Plant Sciences*, vol. 6, no. 9, pp. 1435–1443, 2015.

[24] L. Gugsa and J. Kumlehn, "Somatic embryogenesis and massive shoot regeneration from immature embryo explants of tef," *Biotechnology Research International*, vol. 2011, pp. 1–7, 2011.

[25] K. Mohamed and T. Feyissa, "In vitro propagation of *Ximenia americana* L. from shoot tip explants: a multipurpose medicinal plant," *SINET: Ethiopian Journal of Science*, vol. 43, pp. 1–10, 2020.

[26] M. G. Hailu, K. T. Mawcha, S. Nshimiyimana, and S. Suharsono, "Garlic micro-propagation and polyploidy induction *in vitro* by colchicine, plant breed," *Plant Breeding and Biotechnology*, vol. 9, pp. 1–19, 2021.

[27] A. Setargie, F. Mekbib, and E. Abraha, "*In vitro* propagation of papaya (*Carica papaya* L.)," *World Journal of Agricultural Sciences*, vol. 11, pp. 84–88, 2015.

[28] G. Genene and M. Firew, "*In vitro* regeneration of disease free enset [*Ensete ventricosum* (Welw) Cheesman] planting materials from bacterial wilt diseased plants using shoot tip culture," *African Journal of Biotechnology*, vol. 15, no. 40, pp. 2192–2201, 2016.

[29] T. A. Getachew, M. Firew, and A. Belayneh, "*In vitro* propagation of Ethiopian mustard (*Brassica carinata* A. BRAUN)," *African Journal of Biotechnology*, vol. 13, no. 48, pp. 4438–4448, 2014.

[30] G. Amente and T. Feyissa, "Effect of growth regulators concentrations on in vitro multiplication of three elite sugarcane (Saccharum officinarum L.) genotypes using shoot tip culture," *Journal of Scientific Agriculture*, vol. 6, 2022.

[31] D. Zinaby, E. Gebre, and Y. Asfere, "Genotype and plant growth hormone interaction effect on *in vitro* propagation of early maturing enset (*Ensete ventricosum* (welw.) Cheesman)

cultivars," *Plant Cell Biotechnology and Molecular Biology*, vol. 22, pp. 89–102, 2021.

[32] G. Mekonnen, M. C. Ejigu, and M. Muthsuwamy, "*In vitro* propagation of banana (*Musa paradiasca* L.) plant using shoot tip *ex plant*," *Food Science Technol*, vol. 9, 2021.

[33] D. Birhan, D. Obssi, and K. Mulugeta, "Protocol optimisation for micropropagation of Ethiopian yam," *African Crop Science Journal*, vol. 29, no. 1, pp. 43–57, 1970.

[34] D. Z. Grea, Y. A. Wuhibe, and G. A. Zewdu, "Establishment of an efficient *in vitro* propagation protocol for rapid regeneration of apple (*Malus x domestica.* Borkh) root stock and scion via node culture," *Plant Cell Biotechnology and Molecular Biology*, vol. 22, pp. 108–123, 2021.

[35] S. T. Hajare, N. M. Chauhan, and G. Kassa, "Effect of growth regulators on *in vitro* micropropagation of potato (*Solanum tuberosum* L.) gudiene and belete varieties from Ethiopia," *The Scientific World Journal*, vol. 2021, Article ID 5928769, 8 pages, 2021.

[36] T. Hailu, T. Haileselassie, and T. Feyissa, "In Vitro regeneration of korarima (Aframomum corrorima (braun) P. C. M. Jansen): A Threatened Spice and Medicinal Herb from Ethiopia," *Scientific*, vol. 2022, 2022.

[37] D. B. Kassaye E, "*In vitro* optimization of the protocol for micropropagation of Plum (*Prunus salicina* L. Var. Methley) from nodal explants," *Biotechnology International*, vol. 8, pp. 137–148, 2015.

[38] G. Kassa and T. Feyissa, "*In vitro* propagation of two grapevine (*Vitis vinifera* L.) cultivars under conditions of salt stress, plant tissue cult," *Plant Tissue Culture and Biotechnology*, vol. 30, no. 1, pp. 47–56, 2020.

[39] K. B. Ayenew B and W. Tefera, "In *Vitro* propagation of Ethiopian ginger (*Zingiber officinale*) cultivars: evaluation of explant types and hormone combinations," *African Journal of Biotechnology*, vol. 11, pp. 3911–3918, 2012.

[40] T. Disasa, T. Feyissa, K. Dagne, and I. J. Biosci, "*In* vitro regeneration of Niger (*Guizotia abyssinica* L. F)," *International Journal of Biosciences*, vol. 1, pp. 110–118, 2011.

[41] B. T. Mulugeta Diro, "*In vitro* aseptic culture establishment of sugarcane (*Saccharum officinarum* L.) varieties using shoot tip explants," *Advances in Crop Science and Technology*, vol. 2, no. 3, pp. 1–6, 2014.

[42] M. Tesfa, B. Admassu, and K. Bantte, "In V*itro* shoot multiplication of elite sugarcane (*Saccharum officinarum* L.) genotypes using liquid shake culture system," *Journal of Biology, Agriculture and Healthcare*, vol. 6, pp. 35–40, 2016.

[43] G. L. Getahun T and T. Feyissa, "Regeneration of plantlets from unpollinated ovary cultures of Ethiopian wheat (*Triticum turgidum* and *Triticum aestivum*)," *African Journal of Biotechnology*, vol. 12, pp. 5754–5760, 2013.

[44] D. Yirssaw, T. Wondyifraw, D. Nigussie, and A. Bekele, "Effects of plant growth regulators on *in vitro* cultured nodal explants of cassava (*Manihot esculenta* Crantz) clones," *African Journal of Biotechnology*, vol. 13, no. 28, pp. 2830–2839, 2014.

[45] A. Mengesha, B. Ayenew, T. Tadesse, and B. Tadesse, "Acclimatization of *in vitro* propagated Pineapple (*Ananas comosuss* L.) plantlets to ex vitro condition in Ethiopia," *American Journal of Plant Sciences*, vol. 4, no. 2, pp. 317–323, 2013.

[46] M. Yifter, D. Sbhatu, F. Mekbib, and E. Abraha, "*In vitro* regeneration of four Ethiopian varieties of sesame (*Sesamum indicum* L.) using anther culture," *Asian Journal of Plant Sciences*, vol. 12, no. 5, pp. 214–218, 2013.

[47] A. Dagnew, S. Shibru, A. Debebe et al., "Micropropagation of banana varieties (*musa* spp.) using shoot-tip culture," *Journal of Agricultural Sciences*, vol. 22, pp. 14–25, 2012.

[48] F. Bekele, "In vitro propagation of anchote (*Coccinia abyssinica*) (lam.) cogn," *African Journal of Plant Science*, vol. 7, no. 6, pp. 253–264, 2013.

[49] W. Tefera and S. Wannakrairoj, "Synergistic effects of some plant growth regulators on *in vitro* shoot proliferation of korarima (*Aframomum corrorima* (Braun) Jansen)," *African Journal of Biotechnology*, vol. 5, pp. 1894–1901, 2006.

[50] M. F. Mulugeta Diro, "Microtuber induction of two potato (*Solanum tuberosum* L.) varieties," *Advances in Crop Science and Technology*, vol. 02, 2014.

[51] T. Shiferaw, T. Feyissa, and L. Micropropagation of Glinus Lotoides, "An endangered medicinal plant," *Advances in Life Science and Technology*, vol. 34, pp. 32–41, 2015.

[52] Y. Yambo and T. Feyissa, "Micropropagation of anchote [*Coccinia abyssinica* (Lam.) Cogn.]: high calcium content tuber crop of Ethiopia," *African Journal of Agricultural Research*, vol. 8, pp. 5915–5922, 2013.

[53] T. Wondimu, T. Feyissa, and G. Bedadav, "Meristem culture of selected sweet potato (*Ipomoea batatas* L. Lam.) cultivars to produce virus-free planting material," *The Journal of Horticultural Science and Biotechnology*, vol. 87, no. 3, pp. 255–260, 2012.

[54] T. Mesfin and T. Feyissa, "Micropropagation of *Plectranthus edulis* (vatke) agnew from meristem culture," *African Journal of Biotechnology*, vol. 13, pp. 3682–3688, 2014.

[55] C. Derso and T. Feyissa, "Micropropagation of yeheb from shoot tip: an endangered multipurpose shrub," *Plant Science International*, vol. 2, pp. 1–12, 2015.

[56] M. Baker and D. Penny, "1, 500 scientists lift the lid on reproducibility," *Nature*, vol. 533, no. 7604, pp. 452–454, 2016.

[57] S. L. Mcarthur, "Repeatability, Reproducibility, and Replicability: tackling the 3R challenge in biointerface science and engineering," *Biointerphases*, vol. 14, no. 2, Article ID 020201, 2019.

[58] A. L. Plant, L. E. Locascio, W. E. May, and P. D. Gallagher, "Improved reproducibility by assuring confidence in measurements in biomedical research," *Nature Methods*, vol. 11, no. 9, pp. 895–898, 2014.

[59] L. P. Freedman, I. M. Cockburn, and T. S. Simcoe, "The economics of reproducibility in preclinical research," *PLoS Biology*, vol. 13, no. 6, Article ID e1002165, 2015.

[60] R. d. Souza, A. Ambrosini, and L. M. P. Passaglia, "Plant growth-promoting bacteria as inoculants in agricultural soils," *Genetics and Molecular Biology*, vol. 38, no. 4, pp. 401–419, 2015.

[61] E. A. P. Lopes, A. D. A. D. Silva, A. C. D. E. S. Mergulhão, E. V. N. D. Silva, A. D. Santiago, and M. D. V. B. Figueiredo, "Co-Inoculation of growth promoting bacteria and glomus clarum in micropropagated cassava plants," *Revista Caatinga*, vol. 32, no. 1, pp. 152–166, 2019.

[62] S. Badiji, Y. Mairon, I. Ndiaye et al., "*In vitro* propagation of the gum Arabic tree (*Acacia senegal.*(L.) Wild): developing a rapid method for producing plants," *Plant Cell Reports*, vol. 12, pp. 629–633, 1993.

[63] J. Immanen, K. Nieminen, O. P. Smolander et al., "Cytokinin and auxin display distinct but interconnected distribution and signaling profiles to stimulate cambial activity," *Current Biology*, vol. 26, no. 15, pp. 1990–1997, 2016.

[64] S. V. Petersson, A. I. Johansson, M. Kowalczyk et al., "An auxin gradient and maximum in the arabidopsis root apex

shown by high-resolution cell-specific analysis of IAA distribution and synthesis," *The Plant Cell Online*, vol. 21, no. 6, pp. 1659–1668, 2009.

[65] K. Lee, O. S. Park, and P. J. Seo, "RNA-Seq analysis of the Arabidopsis transcriptome in pluripotent calli," *Molecules and Cells*, vol. 39, no. 6, pp. 484–494, 2016.

[66] O. Norgren, *Growth Differences between Pinus Sylvestris and Pinus Contorta*, Swedish University of Agricultural Sciences, Skara, Umeå, 1995.

[67] T. T. Kozlowski and S. G. Pallard, "Environmental regulation of vegetative growth," in *Growth Control Woody Plants*Acadamic Press, Cambridge, MA, USA, 1997.

[68] D. E. Parfitt and S. Arulsekar, "Measurement and origin of genetic variation in tissue culture systems," *Cell Tissue Cult. for. Gen. Princ. Biotechnol*, Martinus Nijhoff Publishers, Leiden, Netherlands, pp. 286–297, 1987.

[69] I. R. Denton, R. J. Westcott, B. V. Ford-Lloyd, and Ford-Loyd, "Phenotypic variation of *Solanum tuberosum* L. cv. Dr McIntosh regenerated directly from shoot tip culture," *Potato Research*, vol. 20, no. 2, pp. 131–136, 1977.

[70] M. W. Lassber and Thomas, "Detection of somatic variation," *Isozymes in Plant Genetics and breeding.Plant Genet. Breed*, pp. 207–217, Elsevier Science Publishers, Amsterdam, Netherlands, 1983.

[71] R. H. Potter and M. G. K. Jones, "Molecular analysis of genetic stability," in *In Vitro Methods for Conservation of Plant Genetic Resources*, pp. 71–91, Chapman & Hall, London, UK, 1991.

[72] W. R. Larkin, S. A. Ryan, R. I. S. Brettel, and Scowcroft, "Heritable chromosomal variation in wheat," *Theoretical and Applied Genetics*, vol. 67, pp. 443–455, 1984.

[73] S. Karp A and Bright, "On the causes and origins of somaclonal variation," *Oxford Surveys of Plant Molecular and Cell Biology*, vol. 2, pp. 199–234, 1985.

[74] Y. Cui, Y. Deng, K. Zheng et al., "An efficient micropropagation protocol for an endangered ornamental tree species (*Magnolia sirindhorniae* Noot. & Chalermglin) and assessment of genetic uniformity through DNA markers," *Scientific Reports*, vol. 9, no. 1, p. 9634, 2019.

[75] J. Molinier, G. Ries, C. Zipfel, and B. Hohn, "Transgeneration memory of stress in plants," *Nature*, vol. 442, 2006.

[76] T. J. Oh, M. A. Cullis, K. Kunert, I. Engelborghs, R. Swennen, and C. A. Cullis, "Genomic changes associated with somaclonal variation in banana," *Physiologia Plantarum*, vol. 129, 2007.

[77] Y. Desjardins, J. F. Dubuc, and A. Badr, "*In vitro* culture of plants: a stressful activity," *Acta Horticulturae*, vol. 812, pp. 29–50, 2009.

[78] V. Chalupa, "Temperature," in *Cell and Tissue Culture in Forestry: General Principles and Biotechnology*, J. M. Bonga and D. J. Durzan, Eds., pp. 142–151, Martinus Nijhoff Publishers, Leiden, Netherlands, 1987.

[79] J. M. Dunwell, "Anther culture in *Nicotiana tabacum*: the role of the culture vessel atmosphere in pollen embryo induction and growth," *Journal of Experimental Botany*, vol. 30, no. 3, pp. 419–428, 1979.

[80] M. Seibert, P. J. Wetherbee, and D. D. Job, "The effects of light intensity and spectral quality on growth and shoot initiation in tobacco callus," *Plant Physiology*, vol. 56, no. 1, pp. 130–139, 1975.

[81] P. Norton and White, "The role of specific light qualities in the induction of photosynthesis in isolated cells of Picea Glauca," *Plant Physiology*, vol. 39, 1964.

[82] K. S. Barna and A. K. Wakhlu, "Whole plant regeneration of *Cicer arietinum* from callus cultures via organogenesis," *Plant Cell Reports*, vol. 13, no. 9, pp. 510–513, 1994.

[83] O. L. Gamborg and J. P. Shyluk, "The culture of plant cells with ammonium salts as the sole nitrogen source," *Plant Physiology*, vol. 45, no. 5, pp. 598–600, 1970.

[84] P. Mehta and H. S. Srivastava, "Comparative stability of ammonium- and nitrate-induced nitrate reductase activity in maize leaves," *Phytochemistry*, vol. 19, no. 12, pp. 2527–2530, 1980.

[85] W. K. Coleman, T. J. Huxter, D. M. Reid, and T. A. Thorpe, "Ethylene an endogenous inhibitor or root regeneration in tomato leaf discs cultured *in vitro*," *Physiologia Plantarum*, vol. 48, no. 4, pp. 519–525, 1980.

[86] B. Thoiron, A. Thoiron, J. L. Guiel, U. Luttge, and M. Thellier, "Solute uptake of *Acer pseudoplatanus* cell suspensions during recovery from gas shock," *Physiologia Plantarum*, vol. 46, no. 4, pp. 352–356, 1979.

[87] T. D. Nikam and M. G. Shitole, "In vitro plant regeneration from callus of Niger (*Guizotia abyssinica* Cass.) cv. Sahyadri," *Plant Cell Reports*, vol. 17, no. 2, pp. 155–158, 1997.

[88] A. A. Abdul-Baki, "Hypochlorite and tissue sterilization," *Planta*, vol. 115, no. 4, pp. 373–376, 1974.

[89] R. Shields, S. J. Robinson, and P. A. Anslow, "Use of fungicides in plant tissue culture," *Plant Cell Reports*, vol. 3, no. 1, pp. 33–36, 1984.

[90] P. C. Debergh, "Effects of agar brand and concentration on the tissue culture medium," *Physiologia Plantarum*, vol. 59, no. 2, pp. 270–276, 1983.

[91] L. C. Huang and T. Murashige, "Plant tissue culture media: major constitutents, their preparation and some applications," *Tissue Culture Association Manual*, vol. 3, no. 1, pp. 539–548, 1977.

[92] P. White, *The Cultivation of Animal and Plant Cells*, Ronald Press, New York, NY, USA, 2nd edition, 1964.

[93] P. G. Risser and P. R. White, "Nutritional requirements of spruce tumor cells *in vitro*," *Physiologia Plantarum*, vol. 17, no. 3, pp. 620–635, 1964.

[94] J. Reinert and P. R. White, "The cultivation *in vitro* of tumor tissues and normal tissues of *Picea glauca*," *Physiologia Plantarum*, vol. 9, no. 2, pp. 177–189, 1956.

[95] O. L. Gamborg, R. A. Miller, and K. Ojima, "Nutrient requirements of suspension cultures of soybean root cells," *Experimental Cell Research*, vol. 50, no. 1, pp. 151–158, 1968.

[96] T. Eriksson, "Studies on the growth requirements and growth measurements of cell cultures of *Haplopappus gracilis*," *Physiologia Plantarum*, vol. 18, no. 4, pp. 976–993, 1965.

[97] T. Murashige and F. Skoog, "A revised medium for rapid growth and bio assays with tobacco tissue cultures," *Physiologia Plantarum*, vol. 15, no. 3, pp. 473–497, 1962.

[98] O. L. Gamborg and J. P. Shyluk, "Nutrition, media and characteristics of plant cell and tissue cultures," in *Plant Tissue Cult*, T. A. Thrope, Ed., Academic Press, New York, NY, USA, pp. 21–44, 1981.

[99] C. Bornman, "Regeneration in vitro of economically important crop plants in Nordic countries," *Hered. Supply*, vol. 3, pp. 7–13, 1985.

[100] R. U. Schenk and A. C. Hildebrandt, "Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures," *Canadian Journal of Botany*, vol. 50, no. 1, pp. 199–204, 1972.

[101] O. L. Gamborg, T. Murashige, T. A. Thorpe, and I. K. Vasil, "Plant tissue culture media," *Invitro*, vol. 12, no. 7, pp. 473–478, 1976.

[102] R. C. Cooke, "The use of an agar substitute in the initial growth of Boston ferns *in vitro*," *HortScience*, vol. 12, no. 4, p. 339, 1977.

[103] H. W. Kohlenbach and W. Wernicke, "Investigations on the inhibitory effect of agar and the function of active carbon in anther culture," *Zeitschrift für Pflanzenphysiologie*, vol. 86, no. 5, pp. 463–472, 1978.

[104] G. Fridborg, M. Pedersen, L. E. Landstrom, and T. Eriksson, "The effect of activated charcoal on tissue cultures: adsorption of metabolites inhibiting morphogenesis," *Physiologia Plantarum*, vol. 43, no. 2, pp. 104–106, 1978.

[105] D. F. Wetherell and D. K. Dougall, "Sources of nitrogen supporting growth and embryogenesis in cultured wild carrot tissue," *Physiologia Plantarum*, vol. 37, no. 2, pp. 97–103, 1976.

[106] D. C. W. Brown, D. W. M. Leung, and T. A. Thorpe, "Osmotic requirement for shoot formation in tobacco callus," *Physiologia Plantarum*, vol. 46, no. 1, pp. 36–41, 1979.

[107] W. Barz and W. Husemann, "Aspects of photoauthotrophic cell suspention cultures," in *Plant Tissue Culture*, A. Fujiwara, Ed., pp. 245–248, Japanase Association For Plant Tissue Culture, Tokyo, Japan, 1982.

[108] D. T. Clarkson and J. B. Hanson, "The mineral nutrition of higher plants," *Annual Review of Plant Physiology*, vol. 31, no. 1, pp. 239–298, 1980.

[109] J. Bonneau, L. Beranger-Novat, and N. Monin, "Somatic embryogenesis and plant regeneration in a woody spruce: the European Spindle Tree (*Euonymus eurpaeus* L.)," *Plant Cell Reports*, vol. 13, pp. 135–138, 1994.

[110] R. Amerson Hv and Mott, "Improved rooting of western white pine tissue culture produced shoots," *Science*, vol. 28, pp. 822–825, 1982.

[111] A. Soumare, A. G. Diédhiou, N. K. Arora et al., "Potential role and utilization of plant growth promoting microbes in plant tissue culture," *Frontiers in Microbiology*, vol. 12, Article ID 649878, 2021.

[112] G. Y. Mengistie and Z. T. Awlachew, "Evaluation of the plant growth promotion effect of *Bacillus* species on different varieties of tomato (*Solanum lycopersicum* L.) seedlings," *Advances in Agriculture*, vol. 2022, Article ID 1771147, 6 pages, 2022.

[113] C. Meixner, J. Ludwig-Müller, O. Miersch, P. Gresshoff, C. Staehelin, and H. Vierheilig, "Lack of mycorrhizal autoregulation and phytohormonal changes in the supernodulating soybean mutant *nts1007*," *Planta*, vol. 222, no. 4, pp. 709–715, 2005.

[114] D. Püschel, M. Bitterlich, J. Rydlová, and J. Jansa, "Facilitation of plant water uptake by an arbuscular mycorrhizal fungus: a Gordian knot of roots and hyphae," *Mycorrhiza*, vol. 30, no. 2-3, pp. 299–313, 2020.

[115] I. Soumare, A. Diop, T. Manga, and A. Ndoye, "Role of Arbuscular mycorrhizal fungi and nitrogen fixing bacteria on legume growth under various environnemental stresses," *International Journal of Biosciences*, vol. 7, pp. 31–46, 2015.

[116] J. Díez, J. L. Manjón, G. M. Kovács, C. Celestino, and M. Toribio, "Mycorrhization of *vitro* plants raised from somatic embryos of cork oak (*Quercus suber* L.)," *Applied Soil Ecology*, vol. 15, no. 2, pp. 119–123, 2000.

[117] N. S. Sahay and A. Varma, "A biological approach towards increasing the rates of survival of micropropagated plants," *Current Science*, vol. 78, pp. 126–129, 2000.

[118] S. Harish, M. Kavino, N. Kumar, D. Saravanakumar, K. Soorianathasundaram, and R. Samiyappan, "Biohardening with plant growth promoting rhizosphere and endophytic bacteria induces systemic resistance against Banana bunchy top virus," *Applied Soil Ecology*, vol. 39, no. 2, pp. 187–200, 2008.

[119] B. Tyagi, S. Tewari, and A. Dubey, "Biochemical characterization of fungus isolated during in vitro Propagation of Bambusa balcooa," *Pharmacognosy Magazine*, vol. 13, no. 4, pp. S775–S779, 2018.

[120] K. Y. Kargapolova, G. L. Burygin, O. V. Tkachenko, N. V. Evseeva, Y. V. Pukhalskiy, and A. A. Belimov, "Effectiveness of inoculation of *in vitro*-grown potato microplants with rhizosphere bacteria of the genus *Azospirillum*, Plant Cell," *Plant Cell, Tissue and Organ Culture*, vol. 141, no. 2, pp. 351–359, 2020.

[121] A. Martins, "In *Vitro* mycorrhization of micropropagated plants: studies on *Castanea sativa* mill," in *Mycorrhizae: Sustainable Agriculture and Forestry*, K. F. Z. A. Siddiqui and M. S. Akhtar, Eds., Springer, Berlin, Germany, pp. 321–336, 2008.

[122] M. A. Morte, A. Cano, M. Honrubia, and P. Torres, "*In vitro* mycorrhization of micropropagated *Helianthemum almeriense* plantlets with *Terfezia claveryi* (desert truffle), Agric," *Agricultural and Food Science*, vol. 3, pp. 309–314, 1994.

[123] P. Quatrini, M. Gentile, F. Carimi, F. D. Pasquale, and A. M. Puglia, "Effect of native arbuscular mycorrhizal fungi and *Glomus mosseae* on acclimatization and development of micropropagated *Citrus limon* (L.) Burm," *The Journal of Horticultural Science and Biotechnology*, vol. 78, no. 1, pp. 39–45, 2003.

[124] E. Malusá, L. Sas-Paszt, and J. Ciesielska, "Technologies for beneficial microorganisms inocula used as biofertilizers," *The Scientific World Journal*, vol. 2012, pp. 1–12, 2012.

[125] D. H. Kim, J. Gopal, and I. Sivanesan, "Nanomaterials in plant tissue culture: the disclosed and undisclosed," *RSC Advances*, vol. 7, no. 58, pp. 36492–36505, 2017.

[126] J. Kumari, "Plant genetic resources: advancing conservation and use through biotechnology," *Journal of Biotechnology & Biomaterials*, vol. 5, no. 6, 2015.