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Tissue Culture: A New Era in Vegetable Crop Micro Propagation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Plant tissue culture, emerging as a transformative technology, wields significant influence in agriculture by catering to the escalating global demand for plants. Particularly noteworthy are its remarkable advancements within vegetable crops, firmly establishing plant tissue culture as a leading frontier of innovation. Presently, its application holds immense promise and offers a glimpse into the future of agricultural practices. The multifaceted roles of plant tissue culture in vegetable crops encompass augmenting varietal production, safeguarding endangered species, and facilitating the large-scale production of secondary metabolites, plant-based vaccines, and antibodies through cell suspension culture. The effectiveness of plant tissue culture hinges on

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various factors, including the availability of nutrients, endogenous auxin synthesis, organic compounds, and environmental conditions. Additionally, it tackles the challenge of supplying planting materials for species that cannot be efficiently propagated through traditional seed methods, effectively meeting the ever-growing worldwide agricultural requirements [1]. Primary goal is to assist budding plant biotechnologists and amplify the global influence of the plant tissue culture industry.

Keywords: Plant tissue culture; vegetable crops; secondary metabolites; cell suspension culture.

1. INTRODUCTION

At the heart of plant biotechnology lies plant tissue culture, a method involving the controlled cultivation of various plant components in sterile environments. Coined as "plant tissue culture," this technique enables the precise manipulation of plant materials' growth and development outside their natural habitats. First introduced by German plant physiologist Gottlieb Haberlandt in 1902, he is revered as the pioneer of this field [2] His visionary idea laid the groundwork for advancements in plant biotechnology, enabling researchers to conduct controlled experiments on plant growth, development, and genetics within laboratory settings.

Tissue culture techniques, including protoplast fusion, anther culture, and microspore culture, expedite the generation of homozygous plants compared to traditional breeding methods [3]. Within the domain of vegetable crops, plant tissue culture assumes a central role, pivotal in the advancement of enhanced plant varieties, preservation of endangered species, and the mass production of valuable compounds such as secondary metabolites, plant-derived vaccines, and antibodies via cell suspension cultures. Particularly advantageous for species with constraints in traditional seed-based propagation, this technique effectively addresses the persistent challenge of meeting the rising global demand for plant materials [4].

2. PRINCIPLE OF PLANT TISSUE CULTURE

2.1 Totipotency

Ability of a cell to regenerate into a complete plant. **Vochting (1878)**. “All *in vitro* methods of plant propagation relay on the phenomenon of totipotency of plant cells.Totipotency may be defined as the capacity of plant cell, tissue or organ to regenerate into a whole plant” [5]. Each somatic cell has the same genetic constitution as the zygote and thus potential of expressing all the properties of an organism

2.2 Micro-propagation

The General Technique of Micro-propagation: In the realm of in vitro culture techniques, two main challenges demand resolution: maintaining aseptic conditions to prevent microbial intrusion and fostering optimal growth and development of cultured cells/organs. This is achieved by providing essential nutrients, growth regulators, and suitable growth conditions.

The initiation involves extracting a small plant fragment, the 'explant,' from a robust mother plant [6]. Explants like shoot tips, leaves, stems, lateral buds, and flower tissues are commonly used. Their choice depends on research goals and species. The explant undergoes careful sterilization with chemicals like mercuric chloride and is placed in a designated culture medium [7].

Growth can take two paths: direct shoot proliferation or initial callus formation followed by shoot differentiation. Growth regulators, like auxins and cytokinins, influence development patterns. Manipulating these regulators guides growth toward specific goals [8].

Cultures begin proliferating within 4 to 12 weeks. Subculturing, transferring tissue to fresh medium, is vital due to nutrient depletion. Callus cultures are subcultured every 3 to 4 weeks, while suspension cultures are done more frequently [9].

Multiplication rates vary but can yield thousands to millions of plants from one explant annually. This holds promise for research, conservation, and commercial horticulture.

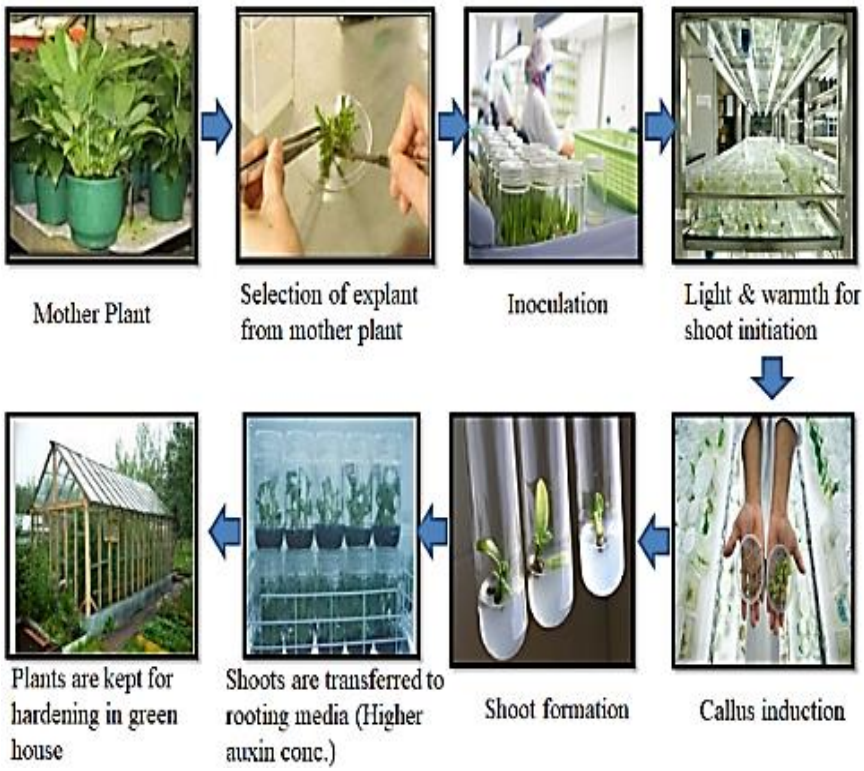

Plate 1. General technique of micro-propagation

List 1. Current role of tissue culture in crop improvement

Meristem and bud culture	Micro propagation for commercial purposes, genetic conservation, genetic transformation and exchange of material
Zygotic embryo culture	interspecific crosses
Anther and isolated microspore culture	Haploid and double haploid production
Cell and tissue culture	in vitro selection, somaclonal variation, somatic embryogenesis, artificial seeds
Protoplast culture	fusion for somatic hybridization

2.3 Techniques of Plant Tissue Culture

Meristem tip culture, Embryo culture, Anther culture, Protoplast culture, Ovule culture, Cell culture, Callus culture

2.4 Meristem Tip Culture

- In this method, the **meristem tip consisting of one or two pairs of leaf primordial are cultured in a cultured medium**
- Rapid multiplication of the plants, which are otherwise not easily propagated by vegetative means, is also possible through meristem tip culture
- Plants produced are **free from pathogens** and can be stored for longer period and in a smaller space

2.5 Anther Culture and Doubled haploids

- Anther culture is used to **produce haploid plants**
- In this technique, **anthers are excised and cultured onto required and standardized culture medium**
- The stage, at which the anther is excised, depends on different species

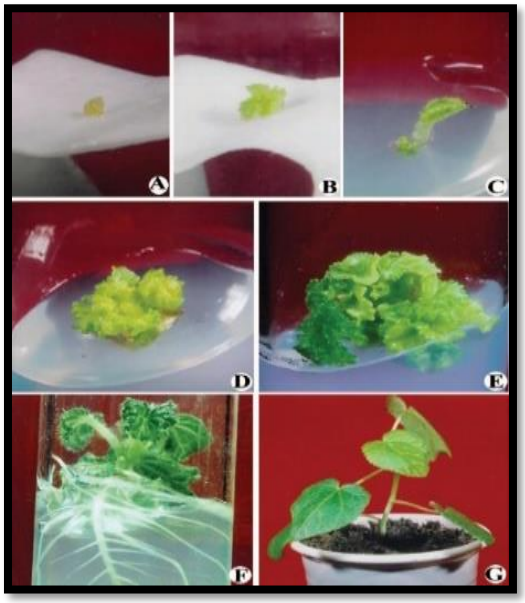

Fig. 1. Different stages of meristem culture in okra (*Abelmoschus esculentus*) Anisuzzaman et al. [10]

A. *Growth of isolated meristem (five days old) on filter paper bridge in liquid medium*
B. *Developed (21 days old) meristem in liquid medium*
C. *And D. Shoot initiation from developed meristem in semi-solid medium*
E. *Shoot multiplication from nodal segment of mericlones*
F. *Rooting in meristem-derived plantlet*
G. *Acclimatization of plantlet in soil*

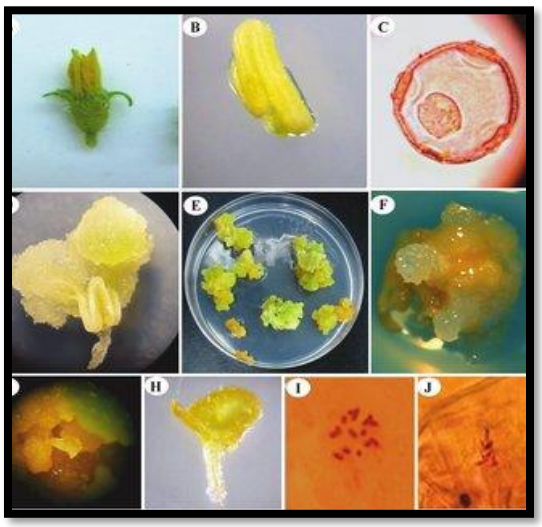

Fig. 2. Callus induction and gametic embryogenesis in cultured anthers of cucumber (*Cucumis sativus* L.) Abdollahi et al. [11]

(A) *Suitably sized (10–15 mm) male flower bud for cucumber anther culture*
(B) *Swollen anther of cucumber on induction medium*
(C) *Cucumber microspore in late uninucleate stage proper for anther culture*
(D and E) *Callus induction from anther of cucumber*
(F) *Globular stage embryo of cucumber*
(G) *Torpedo stage embryo of cucumber*
(H) *Cotyledonary stage embryo of cucumber*
(I) *Chromosome preparation of a root tip cell of a diploid cucumber seedling*
(J) *A cucumber haploid cell prepared from callus-derived embryo with 7 chromosomes*

Table 1. Generation of haploid (n) plants via anther/microspore culture in horticultural crops

Crop species	Mode of haploid development
Asparagus officinalis (Asparagus)	Direct/indirect androgenesis
Beta vulgaris (Beet root)	Direct/indirect androgenesis
Brassica oleracea (cole crops)	Direct/indirect androgenesis
Cucumis sativus (cucumber)	Indirect androgenesis
Lycopersicon esculentum (Tomato)	Direct/indirect androgenesis
Raphanus sativus (Radish)	Direct androgenesis
Solanum tuberosum (Potato)	Direct/indirect androgenesis
S. melongena (Brinjal)	Direct/indirect androgenesis

2.6 Embryo Culture

- Embryo techniques are primarily useful in case of making distant crosses in hybrid production
- It is also useful to overcome seed dormancy
- In this technique the embryo rescued at immature stage before the degeneration is initiated

Interspecific hybrid	**Hybrid plants recovered by**
Lycopersicon esculentum × *L. peruvianum* *Phaseolus vulgaris* × *P. angustissimus* *Vigna pubescens* × *V. unguiculata* *Solanum melongena* × *S. torvum*	Embryo rescue

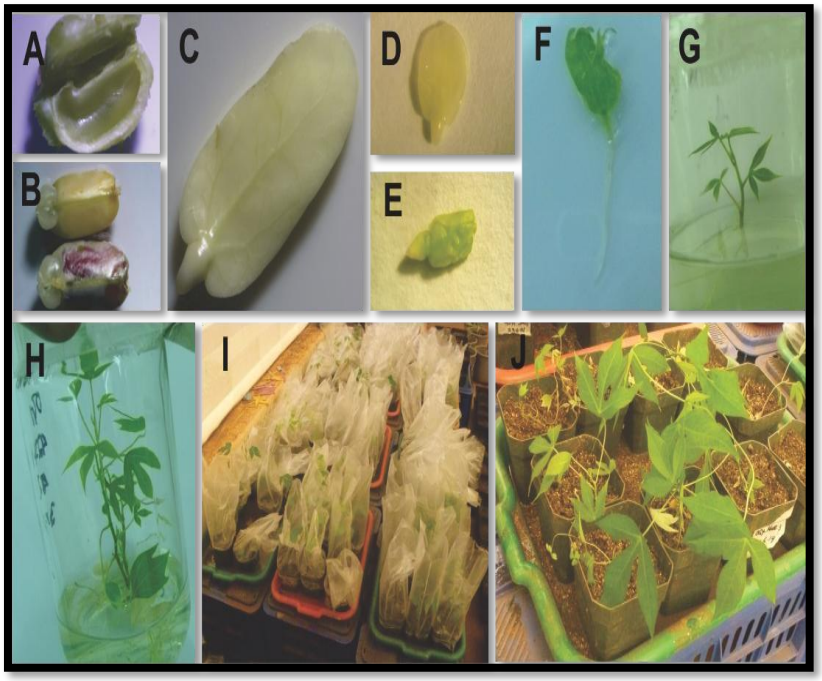

Fig. 3. Embryo rescue at 42 days after pollination (DAP) and regeneration of cassava plantlets Buttibwa et al. [12]

A. and B: *Opened and unopened cassava locules containing one embryo each for extraction*
C. *Freshly extracted embryo*
D. E and F*: Embryo germination after three days, 1 week, and 2 weeks of culture, respectively*
G. and H: *cassava plantlets after 4 and 16 weeks of culture, respectively, from the time embryo rescue was done*
I. and J*: cassava plantlets from embryos rescued of being hardened*

2.7 Cell Culture

- Cell suspension culture are obtained by homogenizing a piece of callus into liquid medium and shaking with shaker until medium becomes cloudy with suspended cells
- This technique is useful to plant breeders because now it is possible to induce desirable variability in an individual cells, which can be into a full plant regenerated [13].

2.8 Protoplast Culture

- In protoplast culture, the rigid **cell walls are first removed** either **mechanically or enzymatically** to expose the protoplast
- The **exposed protoplast is then cultured onto a suitable culture medium**

❖ **Advantages of protoplast culture are** :

- A large homogeneous population of plants can be obtained from a small sample
- Fusion of two protoplast of two different plant species is possible, which may bring a greater variability in plants

2.9 Ovule Culture

- In this technique, **unfertilized ovules are excised and cultured into medium**
- This technique has potential application to produce hybrid seeds in wide genetic crosses, which is rather difficult through conventional means
- It is also efficient method to obtain plants in self-incompatible species

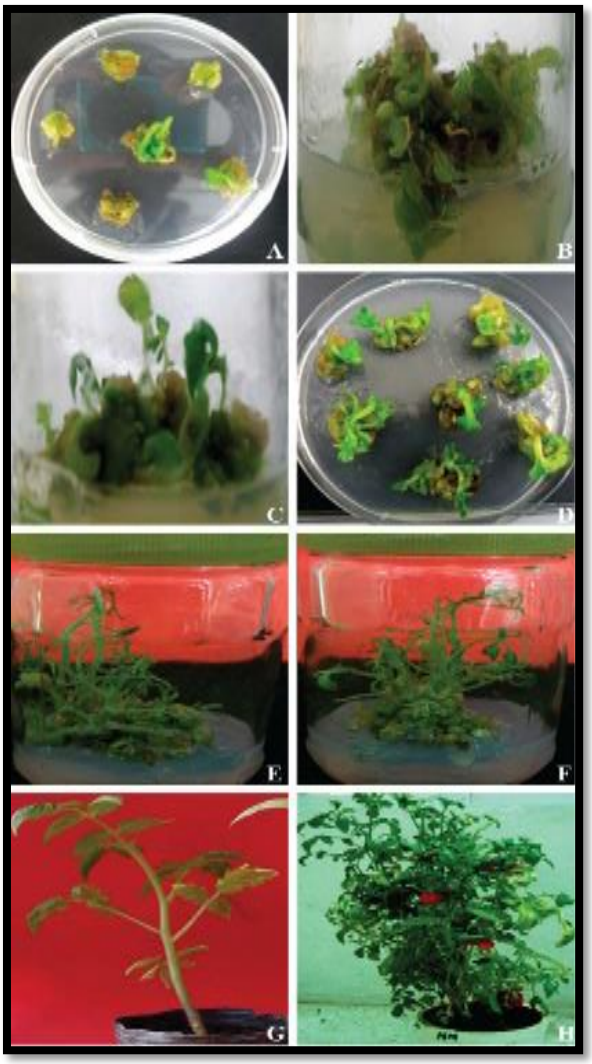

Fig. 4. Callus culture

- **A callus is an unorganized mass of parenchymatous cells**
- In callus culture, the stimulation of cell division and multiplication is required and thus culture medium is accordingly supplemented with necessary regulators
- For callus culture, auxins are required frequently followed by cytokinins
- In callus culture, organogenesis occurs in two stages. In first stage, formation of meristems takes place and in second stage, active growth of stem buds and roots takes place

2.10 Factors Affecting Plant Tissue Culture

Several factors play a significant role in influencing the success and outcomes of tissue culture processes. Here are the key points regarding the factors affecting plant tissue culture:

Growth Media, Environmental Factors, Explant Sources, Genetics

Growth Media in Plant Tissue Culture

Importance of Growth Media

Growth media in plant tissue culture create a controlled setting for manipulating plant tissue growth. Researchers can induce specific responses, like shoot or root formation, by adjusting the media composition, which includes a balanced mix of nutrients, hormones, and supplements [14]. These formulations are crucial for successful in vitro propagation techniques, providing the required conditions and nutrients for plant cell and tissue growth.

Basic Media: Several fundamental growth media are employed in in vitro propagation, including White medium, MS [15] medium, Gamborg's B5 medium, and Chu-N6 medium. These media serve as a foundation for growing plants under controlled conditions.

Composition of Growth Media

- Inorganic Nutrients, Minor Salts, Carbon and Energy Sources, Vitamins, Growth Regulators, Organic Supplements

Applications of Micropropagation

- Enhanced Multiplication Rates
- Challenging Species Propagation
- Multiplication of F1 Hybrid Parents
- Propagation of Unique Lines
- Maintenance of Special Genotypes

Disadvantages

- Specialized Equipment/Facilities Required
- More Technique Expertise Required
- Protocol is Not Optimized for All Species
- Relatively Expensive to Set Up
- Plant Produce May Not Fit Industry Standards

2.11 Role of Tissue Culture in Vegetable Crops

Potato

Potatoes are typically propagated via tubers, a process with a low multiplication ratio ranging from 1:4 to 1:15. However, through tissue culture, numerous clones can be generated from a single seed or explant. This method enables the selection of desirable traits, reduces the space needed for field trials, and eliminates plant diseases through meticulous selection and sterile techniques. In their study, Walia et al. [16] found that “the highest number of shoots per explant (15.50) was observed in T_{10} (MS medium + 0.75 mg/l BAP + 0.25 mg/l kinetin), while the lowest (4.50) was in T_1 (MS basal medium)”. Additionally, the longest shoot length (8.13 cm) was recorded in T_{10}, and the shortest (4.11 cm) in T_1. The results reveal that a proper balance between two cytokinins may trigger more efficient shoot multiplication response. Srivastava et al. [17] found that Kufri Giriraj exhibited strong performance in stem elongation (10.4 cm), leaf length (3 cm), number of micro tubers (14), and micro tuber weight (3.18 g). Rooting efficiency was notable across all varieties except for Kufri Megha. Rabbani et al. [18] demonstrated that the highest shoot length (8.96 cm) was achieved with treatment T_5, which utilized MS medium supplemented with 4 mg/l GA3.

Tomato

Tomato, a vital dietary staple globally, faces declining production due to various pests and diseases. Conventional breeding methods struggle to keep pace with demand. Tissue culture techniques offer a rapid solution for large-scale propagation and the development of pest-resistant germplasm [19]. Jawad et al. [20] found that using MS medium supplemented with 0.5 mg/l IBA + 0.5 mg/l IAA led to 100% rooting in

both shoot tip and nodal explants. In rooting phase, cytokine is not always necessary. The auxin can perform rooting alone or in combination with another auxin. Sharma et al. [21] concluded that "highest shoot regeneration (50.01%) and number of shoots/explant (1.55) was obtained on medium containing 2.0 mg/l of BAP and 0.5 mg/l of IAA (BI_{15}) as compared to the other combinations".

Chilli

Chilies are versatile crops used globally as both vegetables and condiments. To meet demand, faster propagation is crucial. Tissue culture offers a solution for asexual multiplication, providing disease-free, uniform plants quickly, boosting yield and quality [22]. Kaaby et al. [23] discovered that hypocotyls exhibited the highest fresh weight, averaging 160.58 mg. Regarding the interaction between explant type and IAA concentration, the highest fresh weight recorded was 247.26 mg for hypocotyls cultured on medium supplemented with 2 mg/l IAA. Nowaczyk et al. [24] studied that "the propagation rate of Capsicum was significantly increased when IAA was added in a low concentration along with the optimum concentration of BAP". Fatima et al. [25] reported that "maximum calli (83%) of chili induced in cotyledon explant on MS medium containing 2, 4-D (1 mg/L) through in vitro plant regeneration. No embryogenesis could be observed in calli when sub-cultured on auxin free medium. Highest shoot regeneration 5 potential (53%) was observed in cotyledon leaf explant induced calli on MS medium containing BAP and Kin (3 mg/L + 1 mg/L)".

Brinjal

Brinjal suffers significant yield losses from the fruit and shoot borer (FSB), despite repeated insecticidal sprays. Conventional breeding struggles due to sexual incompatibilities and challenges in obtaining fertile progenies. Plant tissue culture offers a solution by providing pathogen-free materials and preserving germplasm efficiently [26]. Mir et al. [26] noted 100% callus formation in both cotyledon and hypocotyl explants on medium supplemented with BAP and IAA. They also found lower callus formation in root explants compared to cotyledon and hypocotyl explants.

Puja et al. [27] concluded that the highest percentage of root regeneration (91.06%) and number of roots (11.08) were observed on MS medium containing 1.00 mg/l of IBA. Kaur et al. [28] noted the earliest callus induction at 6.33 days (D) and the highest percentage of callus induction (90%) when both epicotyl and hypocotyl explants were inoculated in MS medium fortified with 0.5 mg/l NAA + 2.0 mg/l BAP. They also observed the same maximum percentage of callus induction (90%) with 1.0 mg/l NAA + 1.0 mg/l BAP.

Cole Crops

Brassica vegetables, economically important and diverse, include Brassica oleracea, which comprises numerous vegetable and fodder varieties. Breeding programs aim to enhance agronomic and nutritional traits. In vitro regeneration offers a rapid method for producing genetically identical plants, crucial for improving crop characteristics efficiently. Rahman et al. [29] found that apical meristem exhibited superior response, with a callus size of 7.32 cm^2 and weight of 3.52 g, when treated with a combination of 1.0 mg/l NAA + 1.0 mg/l 2,4-D. Boullani et al. (2017) showed that explant size between 1 to 1.5 cm gave maximum number of shoots/ explant in globe artichoke. Ravanfar et al. [30] confirmed that highest percentage of shoot formation (76.66%) in treatment containing 0.1 mg/l TDZ + 0.1 mg/l NAA in broccoli. Munshi et al. [31] found that maximum rooting (98%), number of roots/shoot (8.4), length of roots (6.5 cm) in half strength MS with 0.5 mg/l IBA and minimum days to rooting with four concentrations (0.5, 1.0, 1.5 and 2.0 mg/l) of IBA in cabbage.

Cucurbits

Crops belongs to Cucurbitaceae are generally known as cucurbits or gourds. The conventional propagation methods for large-scale production of sex-specific plants in cucurbits face significant limitations. These challenges have prompted scientists to turn to tissue culture, given its potential for efficient clonal propagation. Biotechnological advancements, particularly in plant tissue culture, play a crucial role in enhancing plant species, as emphasized by Dhumal et al. [32]. Sultana et al. [33] found that leaf disc explants exhibited early response, with callus induction occurring at 4.67 days (D), and achieved a higher percentage of callus induction (91.50%) when cultured in cucumber media supplemented with 2.0 mg/l 2,4-D. Amin et al. [34] observed the earliest shoot initiation at 7.25 days (D), along with the maximum shoot length (3.20 cm) and number of leaves per shoot

(5.00) in ivy gourd under treatment T_4 (MS + 3.0 mg/l BAP + 0.1 mg/l NAA). Patel et al. [35] recorded the highest shoot length (5.2 cm) and number of shoots (10) in spine gourd with the treatment containing 0.5 mg/l NB6 + 0.5 mg/l BAP. Haq et al. [36] concluded that the highest percentage of shoot regeneration (91.66%) and the maximum number of shoots per explant (4.8) were observed in MS medium supplemented with 1.0 mg/l BAP. Additionally, the shortest time for shoot induction (7 days) was recorded in MS medium supplemented with 1.0 mg/l BAP and MS medium supplemented with 1.5 mg/l BAP from nodal segments in pointed gourd. Khatun et al. [37] discovered that the highest percentage of root induction (100%), number of roots per shoot (12), and the longest roots (7.0 cm) were achieved with MS medium supplemented with 1.0 mg/l IBA in watermelon [38-40].

3. CONCLUSION

Plant tissue culture is a highly promising field with a strong present and an even brighter future. The global biotech industry, valued at approximately 50 billion dollars, dedicates 10 % to plant tissue culture products. Commercialization of this technique, coupled with its acceptance among global nursery traders, has driven industry growth. Innovative in vitro screening aids in isolating and regenerating improved cell lines, leading to plants with enhanced traits. This approach holds potential for tailored agricultural advancements, creating more resilient and productive crops. Plant tissue culture has evolved from an experimental pursuit into a pivotal industry shaping agriculture and biotechnology.

4. FUTURE THRUST

Need to develop and standardized the protocol for different vegetable crops

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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