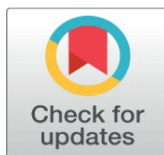


OPTIMIZATION OF TISSUE CULTURE FOR MOMORDICA DIOICA TO ENHANCE MEDICINAL APPLICATIONS

Priya Dwivedi¹, Ashwini A. Waoo²  ¹ Department of Biotechnology, Faculty of Life Science and Technology, AKS University, Satna, (M.P.), INDIA, 485001

Corresponding Author

Ashwini A. Waoo,
ashwiniwaoo@gmail.com

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ABSTRACT

Momordica dioica, a traditional medicinal plant of the Cucurbitaceae family, is sought after for its various medicinal properties including antibacterial, anti-tumorigenic, analgesic, anti-diabetic, anti-inflammatory, and anti-allergic activities. Plant tissue culture allows micropropagation and regeneration. Standard culture protocol established for *Momordica dioica* includes Root, leaf, fruit, and apical meristem explants on MS basal medium with BAP-2,4D. This plant has diverse biological activity, good cytotoxicity on cancerous cells, and versatile uses in health, medicine, and food due to its numerous beneficial properties. Further work is in progress to evaluate the chemical constituents present in this plant.

Keywords: *Momordica Dioica*, Plant Tissue Culture, Media, Cucurbitaceae, Thidiazuron, IAA, etc.

1. INTRODUCTION

Momordica dioica is a perennial, dioecious climber and creeper plant belonging to the *Cucurbitaceae* family. Globally known as Spiny gourd; in India, also called Kankro, Kartoli, Kantola, Ban karola, or Janglee karela, consumed as vegetables (Talukdar & Hossain, 2014). *Momordica dioica* has around 80 species; in India, there are six types, four dioecious and two monoecious (Joseph *et al.*, 2005). This plant showcases various properties, including traditional medicinal and scientific aspects (Bhawar *et al.*, 2010). *Momordica dioica*, a plant from the Indo-Malayan region, is valued for its nutritional benefits and medicinal properties, traditionally used to treat various ailments like eye illnesses, poisoning, fever, and diabetes (Vaidya *et al.*, 2003). Research optimized the culture media for *Momordica dioica*'s rapid growth, emphasizing the importance of plant growth regulators in creating a regeneration medium (Kapadia *et al.*, 2018). For instance, various combinations and quantities of hormones, such as 6-benzyl Amino Purine (BAP), 2, 4-dichlorophenoxyacetic acid (2, 4-D), 6-furfuryl-amino Purine (Kinetin), and thidiazuron (TDZ), are utilized in shoot multiplication media. Similar to this, alternative auxin concentrations, such as 1-Naphthalene Acetic Acid (NAA), are utilized to optimize the media for *Momordica dioica* micro-propagations, (Dar *et al.*, 2021). Commercial farming of this

vegetable has not been tried due to its dioecious nature; seed impermeability makes cucurbit seeds dormant for prolonged periods (Baratakke *et al.*, 2013; Dwivedi & Waoo, 2021). Male and female plants in a 1:15 ratio in nature. Plant population declining, conservation is vital. Plant biotechnology is useful for duplication and conservation (Ashis Ghosh *et al.*, 2005). Female *Momordica dioica* is chosen for regeneration due to its medicinal properties like anti-diabetic, analgesic, antifertility, nematocidal, and more (Dwivedi & Waoo, 2020). A study explores the anticancer properties of *Momordica dioica* extract on breast and colon cancer, and lung cancer cells (Li *et al.*, 2012). Cancer, caused by abnormal cell growth, is a leading cause of death globally. Natural products from fruits and vegetables, along with plant-derived agents like taxanes, possess anticancer properties (Talib *et al.*, 2021).

2. MATERIAL AND METHODS

The tissue culture of *Momordica dioica* was done on Murashige and Skoog medium with standardized hormonal treatment for better growth.

Collection of Explant:

The *Momordica dioica* was collected from mature plants in the Satna Vindhya region, within Satna's forest range. The collected explants were kept in plastic bags and stored at 25°C for 2-4 hours to maintain viability before processing.

Surface sterilization

Surface sterilization in tissue culture requires meticulous steps including explant preparation, washing, treatment with Laboline detergent and Bavistin for (15-20 min) separately, and then surface sterilization with 0.1% HgCl₂ and 70% ethanol, and multiple washings to ensure aseptic conditions under the laminar chamber.

Preparation of media:

Synthetic media nourished *Momordica dioica* in tissue culture, using Murashige and Skoog (MS) medium with 3% sucrose, calcium chloride, and 0.7% agar at pH 5.8 (Murashige & Skoog, 1962). Stock solutions for MS medium components were prepared, supplemented with growth regulators, dispensed into culture bottles, and sterilized via autoclaving (IM & M, 2012).

Initiation of culture from different explants;

Micropropagation aims to mass multiply a valuable species near extinction. The study examines various explants for efficient protocol development in *Momordica dioica* (Rai *et al.*, 2012).

1. *In vitro* seed germination of *Momordica dioica*

Surface-sterilized *Momordica dioica* seeds were inoculated on MS medium and 1/4 strength MS medium with sucrose and agar. Incubated in the dark for 2 days, then exposed to fluorescent light under a 16/8-hour photoperiod (Shekhawat *et al.*, 2011).

2. To standardize the Medium and Growth Regulators for shoot induction

For shoot induction experiments, 5-10 cm in vitro seedlings were used. Cotyledonary and axillary nodes were excised as they are highly regenerative (Zimik & Arumugam, 2017). MS medium with varying cytokinin concentrations (BAP 1.0-2.0 mg/l, Kinetin 8-10 mg/l) and different auxin levels (NAA 1.5-2.0 mg/l, IAA, TDZ) on agar-induced shoots. pH adjusted to 5.8 before autoclaving (Yasmin *et al.*, 2022).

3. Shoot initiation and apical meristem of mature plants;

Fresh shoots, with meristematic tissue, were harvested and sectioned for plant propagation (Thorat *et al.*, 2018). Active meristematic cells were chosen from nodal segments for shooting, then inoculated onto initiation media and incubated at 25°C. (Murray *et al.*, 2012).

Table 1: Different Media Used for Shoot Initiation Experiment

Treatment NO.	Medium + Growth hormones mg/l
1	MS + 1.0 BAP mg/l
2	MS + 1.0 BAP mg/l+TDZ
3	MS + 2.0BAP + 0.5 KN mg/l+TDZ
4	MS+1.0BAP + 1.0NAA mg/l+TDZ
5	MS+2.0BAP + 1.0IAA mg/l+TDZ

4. To Standardize Multiplication of Shoots on Different Medium

Shoots, aged 20-25 days, isolated and subcultured for axillary multiplication utilizing MS medium with BAP and KN at 1.0-2.0 mg/L, along with auxins IAA and NAA at 0.1-0.5 mg/L (De G Alvarez et al., 2006). A series of experiments optimized shoot multiplication with weekly evaluations of key parameters like initiation percentage, multiplication rate, shoot height, and callus formation (Table 2).

Table 2: Different media used for Shoot Multiplication Experiment

Treatment NO.	Medium + Growth hormones mg/l
1	MS+1.0 BAP
2	MS+2.0 BAP+TDZ
3	MS+2.0 BAP+1.0 NAA+TDZ
4	MS+2.0 BAP+1.0 IAA+TDZ
5	MS+2.0 KN+0.5 IAA+TDZ

5. To Standardize the Medium and Growth Regulators for Root Induction

Momordica dioica shoots lack roots in multiplication medium; transferred to low-salt rooting medium for induction, effective for various plant species (Thakur et al., 2011). The shoots were separated and inoculated with different auxin concentrations to observe root initiation, length, and number in Table 3.

Table 3: Combinations of Plant Growth Regulators for Root Formation

Treatment NO.	Medium + Growth hormones mg/l
1	MS 1/2 BAP
2	MS 1/2 BAP+TDZ
3	MS 1/2 + 1.0 IBA+TDZ
4	MS1/2 + 1.0 NAA+TDZ
5	MS + 1.0 mg/l IAA+TDZ

6. Hardening and Planting of Tissue Cultured Raised Plants:

The tissue-cultured plantlets were shifted from the lab to the greenhouse gradually. Plantlets with established roots were taken out, pre-acclimatized in diffused sunlight, and washed meticulously before transplantation (Da Silva et al., 2017). The plantlets were transplanted into glass jars with vermiculite and MS salt solution. After 4-5 weeks, they were acclimatized in a greenhouse mist chamber with controlled humidity and temperature.

3. RESULT

Tissue culture experiments identified optimal conditions for growth and development stages for *Momordica dioica* through hormonal treatments and media assays.

Seed Germination

In comparing culture media for seed germination, half-strength MS medium showed the highest effectiveness with 97% germination and 7.6 cm seedlings, surpassing White's and B5 media. This suggests optimal growth for *Momordica dioica* (Jui et al., 2023).

Explant Response to Hormonal Treatments

Seed Explants

The seed explants showed different responses to hormonal treatments. The most effective treatment for growth (97%, 3% contamination) was 2.0 mg/L BAP + 1.0 mg/L IAA and TDZ. Other treatments had lower growth rates: 1.0 mg/L BAP (64%, 56% contamination) and 2.0 mg/L BAP + 0.5 mg/L KN + TDZ (59% growth, 41% contamination).

Shoot Explants

Shoot explants responded best to 2.0 mg/L BAP + 1.0 mg/L NAA and TDZ, showing 80% growth and 20% contamination. While other combinations were less effective.

Axillary and Apical Meristems

Among axillary and apical meristem explants, The best response came from a drug combination of 2.0 mg/L BAP, 1.0 mg/L NAA, and TDZ, with 80% growth and 20% contamination, compared to less successful results from other treatments.

Root Explants

Root explants grew best with 1.0 mg/L NAA and TDZ (85% growth, 15% contamination). Other treatments were less successful: 1.0 mg/L IBA + TDZ (75% growth, 25% contamination) and 2.0 mg/L BAP + TDZ (70% growth, 30% contamination).

Hormonal Treatment Effects

Different explants treated with various hormone combinations showed varying growth rates. Seed explants treated with BAP and IAA had the highest growth rate, shoot explants, and meristem explants had a similar lower growth rate, and root explants responded best to NAA and TDZ. Generally, TDZ-based combinations were more effective than single hormone treatments across all explant types (Table 4).

Table 4: Effects of Various Hormone Combinations on Growth and Contamination.

S. No.	Explant	Hormones mg/L	% of Growth	% of Contamination
1	SEED	1. BAP (1.0)	64%	56%
		2. BAP (1.0) + TDZ	70%	30%
		3. BAP (2.0) + KN (0.5) + TDZ	59%	41%
		4. BAP (1.0) + 1.0 NAA + TDZ	80%	20%
		5. BAP (2.0) + 1.0 IAA + TDZ	97%	3%
2	SHOOT	1. BAP (1.0)	47%	53%
		2. BAP (2.0) + TDZ	58%	42%
		3. BAP (2.0) + 1.0 NAA + TDZ	80%	20%
		4. BAP (2.0) + 1.0 IAA + TDZ	72%	28%
		5. KN (2.0) + 0.5 IAA + TDZ	69%	31%
		1. BAP (1.0)	35%	65%
		2. BAP (2.0) + TDZ	60%	40%
		3. BAP (2.0) + 1.0 NAA + TDZ	80%	20%
		4. BAP (1.0) + 1.0 IAA + TDZ	65%	35%
		5. KN (1.0) + 0.1 IAA + TDZ	55%	45%
4	ROOT	1. BAP (2.0)	60%	40%
		2. BAP (1.0) + TDZ	75%	25%
		3. IBA (1.0) + TDZ	85%	15%
		4. (1.0) NAA + TDZ	55%	35%
		5. (1.0) IAA + TDZ	70%	30%

Shoot Induction

Cotyledonary nodes from 18-day-old seedlings showed 70% success in inducing shoots in 20-25 days with MS medium containing 2.0 mg/L BAP or 1.0 mg/L BAP with 0.5 mg/L NAA and TDZ being the best hormonal treatment. Higher Kinetin and TDZ concentrations above 1.0 mg/L hindered shoot development.

Mature Plant Explants

Explants from non-flowering mature plants sprouted 80% within 10-15 days. Growth started in apical meristems and the first three nodal segments in 7-10 days. Axillary buds near the apical bud were highly responsive. Sterilization with 0.1% HgCl₂ for 4-5 minutes had a 70-80% success rate in preventing contamination, consistent with previous studies on *Momordica dioica*.

4. DISCUSSION

The study on *Momordica dioica* tissue culture had both similar and contrasting observations compared to prior research. An impressive 97% seed germination rate was achieved with an MS half-strength medium, contradicting some past findings. This study's high success implies methodological improvements and highlights the need for more research on germination factors in *Momordica dioica*. Some new combinations showed high effectiveness (Kapadia *et al.*, 2018). Rarely used before, the mix of BAP, IAA, and TDZ led to a growth rate of 97% in seed explants. The cocktail of BAP, NAA, and TDZ resulted in 80% growth for shoot and meristem explants, surpassing traditional approaches. The study revealed new culture conditions for root explants and successful approaches for shoot induction, offering valuable insights for *Momordica dioica*. Optimal hormone combinations, like 2.0 mg/L BAP alone or 1.0 mg/L BAP with 0.5 mg/L NAA and TDZ, were identified for successful tissue culture. The present investigation values mature plant explants, yielding an 80% sprouting rate in 10-15 days, expanding propagation possibilities from juvenile tissues, (Hesami *et al.*, 2023). The effective culture of mature explants is crucial for clonal propagation. A sterilization protocol using 0.1% HgCl₂ for 4-5 minutes prevents contamination (70-80%), (Gu *et al.*, 2022), surpassing earlier methods with variable effectiveness in controlling tissue culture contamination. This study presents improved protocols for *M. dioica* tissue culture and new hormone combinations, highlighting the complexities in plant tissue responses that warrant further exploration for universal protocols (Long *et al.*, 2022).

5. CONCLUSION

This research on *Momordica dioica* tissue culture optimized conditions and protocols, achieving high success rates for seed germination and sprouting. Effective hormone combinations and improved methods offer new possibilities for propagation. Successful sterilization also addressed contamination issues. Future studies can probe the molecular foundations of these hormone combinations.

CONFLICT OF INTERESTS

None

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None

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