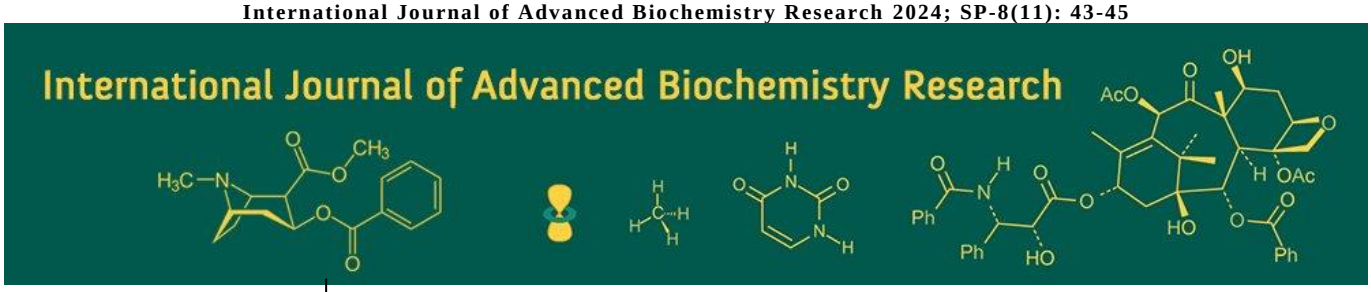

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(11): 43-45
www.biochemjournal.com
Received: 22-09-2024
Accepted: 26-10-2024

Vaishnavi Bhad
PG Scholar, Department of Agricultural Biotechnology, Dr. PDKV, Akola, Maharashtra, India

DR Rathod
Associate Professor, Department of Agricultural Biotechnology, Dr. PDKV, Akola, Maharashtra, India

AG Deshmukh
Assistant Professor, Department of Agricultural Biotechnology, Dr. PDKV, Akola, Maharashtra, India

Dipika Padole
Assistant Professor, Department of Agricultural Biotechnology, Dr. PDKV, Akola, Maharashtra, India

VS Kale
Associate Professor, Department of Vegetable Science, Dr. PDKV, Akola, Maharashtra, India

Vaishnavi Zade
PG Scholar, Department of Agricultural Biotechnology, Dr. PDKV, Akola, Maharashtra, India

Corresponding Author:
Vaishnavi Bhad
PG Scholar, Department of Agricultural Biotechnology, Dr. PDKV, Akola, Maharashtra, India

In vitro rhizogenesis study of spine gourd (*Momordica dioica* Roxb.)

Vaishnavi Bhad, DR Rathod, AG Deshmukh, Dipika Padole, VS Kale and Vaishnavi Zade

DOI: https://doi.org/10.33545/26174693.2024.v8.i11Sa.2796

Abstract
Momordica dioica Roxb. ex Willd. is a perennial, dioecious plant (2n = 28) from the Cucurbitaceae family. Traditional methods of propagation, such as using seeds, stem cuttings, and rhizomatous/tuberous roots, are insufficient for large-scale cultivation as a vegetable crop. This study presents an enhanced and efficient micropropagation technique for wild female M. *dioica* using nodal explants. Shoot proliferation was achieved by subculturing *in vitro* raised shoots on Murashige and Skoog (MS) medium supplemented with concentrations of 6-benzylaminopurine (BAP) 2 mg l^{-1} in combination with L- Glutamic acid (LGA) 2 mg l^{-1}. *In vitro* produced shoots then transformed on half strength MS media containing different concentrations of auxins IBA and IAA form which IBA 2mg l^{-1} produce roots in 12.88 days with root length 9.45 cm and 4.22 number of roots per shoots.

Keywords: *Momordica dioica, in vitro,* micropropagation

Introduction
The genus Momordica is native to tropical Asia, particularly the Indian subcontinent, and is widely distributed across India. In India, the primary species include M. balsa mina, M. charantia, M. cymbalaria, and M. *dioica*. M. *dioica*, commonly known as kokrol or kakoda (spine gourd or teasle gourd), is a perennial, dioecious climber that produces a highly nutritious edible vegetable. The fruits of the spine gourd are rich in proteins (Maharana and Tripathy 1996) [8], lectins, triterpenes such as ursolic acid and momordic ursenol, as well as vitamins, iodine, flavonoids, and glycosides. (Ali and Srivastava 1998; Singh *et al*. 2009) [2, 10] In traditional Ayurvedic medicine, both the vegetable and its fruits are often prescribed for diabetic patients. They are also used as an astringent, febrifuge, antiseptic, anthelmintic, and spermicide. Additionally, M. *dioica* possesses several therapeutic properties, including antioxidant, hepatoprotective, antibacterial, and anti-inflammatory, ant lipid per oxidative, hypoglycaemic, and analgesic effects (Bawara *et al*. 2010) [3]. Aberoumand (2010) [1]. Studies have further analysed the composition and mineral content of M. *dioica* fruits, concluding that they provide an excellent source of essential nutrients
The gender of *Momordica dioica* plants raised from seeds can only be identified after flowering. In central and northern India, the fruits are typically collected from dry deciduous forests and are available for just 25 to 35 days each year following the rains. Many growers are reluctant to cultivate this plant because the sex of the plant whether it will be male (non-fruit-bearing) or female (fruit-bearing) is determined only after flowering. Therefore, it is crucial to develop a protocol for the propagation of selected female plants.
The increasing pressure of population growth, deforestation, intensive agricultural practice and environmental degradation have resulted in the destruction of this plant's natural habitats. The rapid loss of germplasm poses a serious threat to its biodiversity. M. *dioica* exhibits considerable genetic variability in nature. To improve productivity and ensure a reliable supply of planting material, the development of appropriate non-conventional technologies is essential (Sinha 1997) [11]. High-frequency plantlet regeneration from cotyledon-derived callus of M. *dioica* has been reported. Thiruvengadam *et al*. (2007) [12] described somatic embryogenesis and plant regeneration from petiole-derived callus of this species. However, direct regeneration from nodal segments offers an advantage over callus-derived

regeneration, as it ensures true-to-type cloning of selected elite genotypes. Therefore, the present study focuses on developing a highly efficient regeneration and micro propagation system to clone mature female plants of M. *dioica* from nodal segments.

Materials and Methods

Collection, surface sterilization, and culture of nodal explants

Fresh shoot sprouts were collected from mature, field-grown female plants, with 2-3 cm long shoots containing 1-2 nodes used as explants. Explants were wiped with 70% ethanol. Washed with DDW water for 5-10 min. then wash of tween 20 was given for 5 min. Then explants were treated with fungicide bavistin 1% and antibiotic streptocyline 0.1% for 30 min and 25 min respectively followed by immersion in a 0.1% (w/v) $HgCl_2$ solution (disinfectant, Hi-Media, India) for 3 minutes. The explants were then rinsed 6-8 times with sterile water. The sterilized explants were cultured on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing LGA 2 mg/l and BAP 2 mg/l. Analytical grade agar (0.8%) (Qualigens Fine Chemicals, India) was used to solidify the medium, and the pH was adjusted to 5.8 ± 0.02 using 1 N NaOH or 0.1 N HCl before autoclaving at 121°C and 15 psi for 15 minutes. The cultures were incubated in a controlled environment with a temperature of 22 °C, 60% relative humidity, and a light intensity of 40 µmol m^{-2} s^{-1} PPFD under a 12-hour photoperiod

Multiplication of the cultures

The shoots were multiplied and regenerated *in vitro* through two methods: (i) Repeated transfer of mother explants: After excising *in vitro*-regenerated shoots, the mother explants were subcultured on fresh medium. This involved repeatedly transferring the cultures to MS medium supplemented with 2.0 mg L^{-1} BAP and 2.0 mg L^{-1} LGA over 3-4 passages. (ii) Amplification of *in vitro*-raised shoot clumps: The cultures were further multiplied by subculturing the shoot clumps onto MS medium containing 2.0 mg L^{-1} BAP and 2.0 mg L^{-1} BAP.

Rooting of *in vitro*-produced shoots

During the transfer of shoot clumps to fresh medium, elongated and thicker shoots (5-6 cm in length) were carefully excised, with 2-3 shoots taken from each clump. These shoots were then cultured on half strength MS media for root induction, with the addition of 200 mg L^{-1} activated charcoal and the following concentrations of auxins: (i) IBA (0.2, 0.5, 1.0, 2.0mg L^{-1}) ((ii) IAA (0.2, 0.5, 1.0, 2.0, mg L^{-1}).

These cultures were maintained under diffused light at 15 µmol m^{-2} s^{-1} PPFD. Additionally, experiments were conducted to induce ex vitro rooting. Shoots were treated for 4 minutes with IBA at concentrations of 25 mg ml^{-1},

Table 1: Effect of different plant hormones on *in vitro* rooting of spine gourd

Treatment	Half MS media supplemented with (IBA, IAA). Mg L^{-1}	No. of days required for root initiation	Number of roots per shoot	length of root per shoot (cm)	Percentage of rooting (%)
T_1	½ MS control media	20.73	0.99	5.33	42.70
T_2	½ MS+IBA (0.2 mg l^{-1})	18.13	1.39	6.20	45.61
T_3	½ MS+IBA (0.5 mg l^{-1})	16.87	1.79	7.18	56.04
T_4	½ MS+IBA (1.0 mg l^{-1})	13.20	4.13	9.45	77.22
T_5	½ MS+ IBA (2.0 mg l^{-1})	12.88	4.22	9.75	78.02
T_6	½ MS+ IAA (0.2 mg l^{-1})	18.50	1.44	5.53	43.26
T_7	½ MS+ IAA (0.5 mg l^{-1})	17.47	1.50	6.61	48.00
T_8	½ MS+ IAA (1.0 mg l^{-1})	13.67	2.59	8.43	67.48
T_9	½ MS+ IAA (2.0 mg l^{-1})	14.60	2.17	7.74	66.59
	SE(m)±	0.18	0.07	0.13	0.37

Result and Discussion

Root induction

There is a general principle regarding the efficiency of hormones and nitrogen content in culture media. When the goal is to promote rooting response, nitrogen supplements are typically added in lower concentrations. (Driver & Suttle, 1987) [4]. Reduced concentrations of macro and micronutrients are employed to enhance rooting response while minimizing callusing at the cut end. The rooting effects of various plant hormones on the *in vitro* rooting response of spine gourd (*Momordica dioica* Roxb.) were investigated by incorporating IBA and IAA, into the half MS basal medium. The roots were induced in 12.88 days, along with the greatest number of roots per culture 4.22 and root length 9.75 cm was recorded in treatment T5 (½ MS + IBA at 2.0 mg/l) (see Table 1, Figure 1). Additionally, the highest rooting percentage i.e. 78.02 % was observed in this treatment. Overall, better rooting was achieved on half-strength MS medium supplemented with IBA (2 mg/l), which was associated with highest rooting percent and the highest number of roots. Ghive *et al*. (2006) [5] reported well-developed roots using a medium of MS + AdSO4 (80 mg/l) + IBA (1 mg/l). Jadhav *et al*. (2015) found that MS medium supplemented with 0.5 mg/l NAA was optimal for root initiation, while the highest root length was observed with MS + 1.0 mg/l IBA. Consistent with these findings, our results also demonstrated a similar pattern in rooting response with IBA, and IAA for the rooting response.

Fig 1: Rhizogenesis of spine gourd.

Conclusion

In this investigation, the combination of BAP (2 mg/l) and LGA (2 mg/l) effectively induced vigorous and healthy shoot growth, achieving the highest shoot length. The use of half-strength MS medium supplemented with IBA (2 mg/l) produced excellent rooting responses. This protocol represents a viable and commercial approach for the mass multiplication of spine gourd.

References

1. Aberoumand A. A comparative study of nutrients and mineral molar ratios of some plant foods with recommended dietary allowances. Adv J Food Sci Technol. 2010;2:104-108.
2. Ali M, Srivastava V. Characterization of phytoconstituents of fruits of *Momordica dioica*. Indian J Pharm Sci. 1998;60:278-279.
3. Bawara B, Dixit M, Chauhan NS, Dixit VK, Saraf DK. Phyto-pharmacology of *Momordica dioica* Roxb. ex. Willd: A review. Int J Phytomedicine. 2010;2:1-9.
4. Driver JA, Suttle GRL. Nursery handling of propagules. In: Bonga JM, Durzan DJ, editors. Cell and tissue culture in forestry. Vol 2. Boston: Martinus Nijhoff; 1987. p. 320-335.
5. Ghive D, Raut NW, Ghorade RB. Tissue culture studies in spine gourd (*Momordica dioica* Roxb.). Int J Plant Sci. 2006;1:266-268.
6. Hoque A, Hossain M, Alam S, Arima S, Islam R. Adventitious shoot regeneration from immature embryo explant obtained from female × female *Momordica dioica*. Plant Tissue Culture Biotechnol. 2007;17:29-36.
7. Jadhav S, Parmar LD, Chauhan RM. Response of spine gourd genotypes (*Momordica dioica* Roxb.) to micropropagation. Agrotechnology. 2015. Available from: http://dx.doi.org/10.4172/2168-9881.S1.016.
8. Maharana T, Tripathy P. Agrotechniques of *Momordica dioica*: Growing spine gourd in pots. Indian Hortic. 1996;Jan-Mar:16-17.
9. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 1962;15:473-497.
10. Singh D, Bahadur V, Singh DB, Ghose G. Spine gourd (*Momordica dioica*): An underutilized vegetable with high nutritional and medicinal values. Acta Hortic. 2009;809:241-248.
11. Sinha SK. Global change scenario: Current and future with reference to land cover changes and sustainable agriculture in the South and South-East Asian context. Curr Sci. 1997;101:846-854.
12. Thiruvengadam M, Rekha KT, Yang CH. Somatic embryogenesis and plant regeneration from petiole-derived callus of spine gourd (*Momordica dioica* Roxb. ex Willd). Funct Plant Sci Biotech. 2007;Global Sci Books:200-206.