Factors affecting in vitro propagation of *Momordica balsamina*: a medicinal and nutritional climber

Gulab Singh Thakur · Mukeshwar Pandey ·
Rohit Sharma · Bhagwan Singh Sanodiya ·
G. B. K. S. Prasad · Prakash Singh Bisen

Published online: 19 April 2011

© Prof. H.S. Srivastava Foundation for Science and Society 2011

Abstract A protocol was developed for multiple shoot induction in a climber with highly medicinal and nutritional values i.e. *Momordica balsamina* (Cucurbitaceae). Effects of various factors on micropropagation were also studied. Addition of BAP at 1.0 mg/l in MS medium stimulated proliferation of shoots with 6–8 coefficients from the axillary buds. Low concentration of activated charcoal (0.2%) promoted shoot elongation and reduced callus formation. The combination of 1.0 mg/l BAP and 1.0 mg/l KN promoted shoot elongation and proliferation. NAA proved to be more effective in inducing higher

number of roots per shoot than IBA. The plantlets raised in vitro were acclimatized in green house and successfully transplanted to natural condition with 85% survival. The present investigation showed that direct regeneration of *M. balsamina* offer a good opportunity to use micropropagation technique as a complementary tool for breeding and other applications.

Keywords *Momordica balsamina* · In vitro propagation · Climber · Momordin

Abbreviations

BAP	6-Benzylaminopurine
NAA	α -Naphthaleneacetic acid
IAA	Indole-3 acetic acid
2,4-D	2, 4-Dichlorophenoxyacetic acid
Kn	Kinetin

G. S. Thakur (✉) · R. Sharma · B. S. Sanodiya · P. S. Bisen
Plant Biotechnology Laboratory,
R&D Division, Tropilite Foods Pvt. Ltd,
Davar Campus, Tansen Road,
Gwalior 474002 MP, India
e-mail: gs.thakur@davars.com

P. S. Bisen
Research and Development Centre,
Bisen Biotech and Biopharma Pvt. Ltd,
Biotech Research Park, M-7, Laxmipuram, Transport Nagar,
Gwalior 474010 MP, India

G. S. Thakur · B. S. Sanodiya · G. B. K. S. Prasad · P. S. Bisen
School of Studies in Biotechnology, Jiwaji University,
Gwalior 474011 MP, India

M. Pandey
Plant Biotechnology Laboratory, Department of Biotechnology,
Jaipur Engineering College and Research Center,
Sitapura, Tonk Road,
Jaipur 302022 Rajasthan, India

P. S. Bisen
Defense Research and Development Establishment (DRDE),
Ministry of Defence, Govt. of India,
Jhansi Road,
Gwalior 474002, India

Introduction

Momordica balsamina also known as ‘Balsam apple’ (African pumpkin), is an important medicinal and nutritional plant of Cucurbitaceae. It is an annual to perennial tendril-bearing herb native to tropical regions of Africa. In India, it occurs naturally in forest, in the rainy season. The leaves, fruits, seeds, and bark are reported to have various medicinal and nutritional importance and called ‘Hidden gift of Nature’ (Thakur et al. 2009). The fruit extract of *M. balsamina* shows anti-HIV property (Bot et al. 2007). ‘Momordins’ is capable of inhibiting the growth of HIV and other viruses (Ortigao and Better 1992). The leaves and fruit extracts of this plant shows antiplasmodial activity and is being used against malaria in African traditional

medicine. The extract of various parts of this plant shows shigellocidal, anti-diarrhoeal, antiseptic, antibacterial, antiviral, antiinflammatory, hypoglycemic and antimicrobial properties (Hassan and Umar 2006; Akinyemi et al. 2005; Jigam et al. 2004).

M. balsamina is a wonder plant for nutraceutical sciences. The leaves are important source of nutrients having 17 amino acids (Hassan and Umar 2006). The wild vegetable could be promoted as a protein supplement for cereal-based diets in poor rural communities. Its high potassium content is a good source for the management of hypertension and other cardiovascular conditions.

The restricted distribution and indiscriminate over exploitation of *M. balsamina* coupled with low seed set, viability, habitat-specific and poor seed germination rate has made it rare in the wild. The micropropagation techniques can play an important role in the rapid multiplication of elite clones and germplasm conservation of *M. balsamina*. The advantage of micropropagation system over conventional seed or vegetative propagation is that it is possible to quickly multiply plants with desirable genotype (Bonga 1987). In vitro propagation using axillary buds has been reported in different

species. The present investigation demonstrated the in vitro regeneration and multiplication potential of this plant and the various factors affecting the in vitro propagation are reported.

Materials and methods

M. balsamina plants were collected from Tropical Forest Region of Jabalpur during monsoon season. The plants were maintained and grown in the Botanical Garden of Tropilite Foods Pvt. Ltd., Davars Campus, Gwalior (M.P.), India. Axillary bud from the actively growing new shoots were excised and used as explant. The explants were rinsed in running tap water for 20 min, cleaned in 10% liquid soap solution for 5 min and then rinsed with double distilled water 2–3 times. The explants were surface sterilized with 0.1% mercuric chloride solution and for 2–3 min in 70% ethanol. Finally, the explants were washed in sterilized double distilled water and cultured on MS medium (Murashige and Skoog 1962) with vitamins, 3% sucrose, 0.8% agar supplemented with different concentrations (0.5–5.0 mg/l) of 6-benzylaminopurine (BAP) and Kinetin (Kn). Activated charcoal (0.1–

Fig. 1 In vitro regeneration of *Momordica balsamina*. **a** Initiation in *M. balsamina* using nodal explant; **b** Multiplication in MS medium supplemented with 1.0 mg/l BAP after 2 weeks; **c** Multiplication in MS medium supplemented with 1.0 mg/l BAP after 4 weeks; **d** Multiplication and elongation in MS medium supplemented with 1.0 mg/l BAP, 1.0 mg/l KN with 0.2% activated charcoal after 2 weeks; **e** Multiplication and elongation in MS medium supplemented with 1.0 mg/l BAP, 1.0 mg/l KN with 0.2% activated charcoal after 4 weeks; **f** Elongated plantlets of *M. balsamina*; **g** Rooting in *M. balsamina*; **h** Rooted plantlets of *M. balsamina*; **i** Hardened plants of *M. balsamina* in pots

0.5%), carbon sources (sucrose, dextrose, lactose and mannose) were added in the media and their effects were observed. All the cultures were incubated at $27\pm 2^\circ\text{C}$ under 2,000–2,500 lux for 16 h/day.

The in vitro regenerated shoots were cultured on MS medium with different strengths to evaluate their effects on shoot elongation. The regenerated shoots were cultured on half-strength MS medium fortified with auxins viz., indole acetic acid (IAA), indole butyric acid (IBA) and α -naphthalene acetic acid (NAA) at different concentrations (0.5–5.0 mg/l) to study their effects on rooting. Plantlets with well developed roots were washed thoroughly with distilled water and cultured in a mixture of peat and sand (2:1) in pots and transferred to green house. Initially high humidity was maintained by covering with transparent plastic bags. After 15 days, plastic bags were removed and survival percentage (%) was recorded after 1 month of transfer.

Results and discussion

Effect of cytokinins on shoot initiation, multiplication and elongation

Effect of BAP

BAP has frequently been reported to induce better shoot multiplication than other cytokinins (Deora and Shekhawat

1995; Ahmed et al. 1990). The bud explants were cultured on MS media supplemented with different concentration of BAP (Fig. 1a). BAP at 1.0 mg/l stimulated proliferation of the bud meristems (Fig. 1b) to form bud clusters and the co-efficient reached 6–8. The maximum survival of explants also occurred in the same medium (Fig. 1c), as compared to higher concentrations of BAP where slow growth was observed in 3 mg/l and 5 mg/l BAP. MS medium supplemented with BAP proved effective for shoot multiplication but it resulted in high callusing (Fig. 1c). The stimulating effect of BAP on bud break and multiple shoot formation has been reported earlier by Bhatt et al. (1995).

Effect of kinetin

In the initiation stage, maximum frequencies of bud break (63%), bud elongation (86%), mean number of regenerated shoots (5.75) and shoot length (4.23 cm) were observed at 1 mg/l Kn (Fig. 2a, b) while at concentration greater or lower than 1.0 mg/l treatment resulted in decline all parameters. In the multiplication stage, maximum frequencies of bud break (72%), bud elongation (88%), mean number of regenerated shoots (3.42) and shoot length (6.41) were observed at 5 mg/l Kn (Fig. 2c, d).

In the present study Kn at different concentration did not show any significant rise in shoot number but it did affect elongation of plantlets.

Fig. 2 Effect of different concentration and combination of cytokinin on (a) Frequency of bud break and bud elongation; (b) Mean no. of shoot and shoot length; (c) Frequency of bud break and bud elongation; (d) Mean no. of shoot and shoot length) initiation and multiplication of explant *M. balsamina*

Fig. 3 Effect of different concentration of MS on shoot elongation

Effects of interaction of BAP and Kn

The combination of BAP and KN did not cause any significant increase in shoot number in the present study. The shoot elongation was prominent (Fig. 1d) in MS medium supplemented with 1.0 mg/l BAP and 1.0 mg/l Kn (10.37 cm) as compared to BAP at 5.0 mg/l (2.75 cm) and Kn at 5.0 mg/l (4.43 cm) (Fig. 2d). It is evident that combination of cytokinins promoted excellent shoot elongation and proliferation (Fig. 1e, f).

Effect of strengths of media

On subculture, the regenerated shoots of *M. balsamina* gave the greatest number of shoot (3.75) multiplication on MS full strengths medium as compared to $\frac{1}{2}$ or $\frac{1}{4}$ strength MS (Fig. 3). However, Maximum elongation of the shoot was observed in $\frac{1}{2}$ MS compared to full and $\frac{1}{4}$ strengths.

Effect of activated charcoal

Different concentrations of activated charcoal were added in the selected media (MS + 1.0 mg/l BAP + 0.2% activated

Fig. 4 Effect of different concentration of activated charcoal

charcoal) in order to reduce/inhibit high callusing. Activated charcoal was taken at 0.1%–0.5%, but 0.2% (Fig. 4) was observed to be most effective to inhibit the callus growth up to 90%. In the present study activated charcoal at very low concentration was most effective to inhibit the callus growth and promote highest shoot elongation.

Effect of auxins on rooting

Auxin supplemented basal medium is generally referred to as rooting medium (Mittal et al. 1989; Kackar et al. 1991). In the present study rooting was induced by reducing medium strength to half. Similar reports have been observed by Kartha et al. (1974).

Effect of IBA

Significant differences in rooting were observed on various concentrations of IBA i.e. 0.5, 1.5, 2.5 mg/l. Maximum roots number (2.67) and root length (3.35) were observed in 1.5 mg/l IBA (Fig. 5). While at concentration lower than 1.5 mg/l decline in all parameters. Similar results were shown in callus culture of *M. dioica*. (Hoque et al. 1995).

Effect of NAA

NAA is another vital plant growth regulator which plays an important role in the rooting of in vitro plants (Fig. 1g). Significant differences in rooting were observed on various concentration of NAA i.e. 0.1, 0.3, 0.5 mg/l (Fig. 5). Maximum roots number (4.75) and root length (5.25) were observed in 0.3 mg/l NAA (Fig. 1g, h).

Acclimatization & hardening

Plants raised in vitro are habituated to survive in a high humid environment which often results in poor cuticle development (Sutter 1985). When these plants are transferred to nature,

Fig. 5 Effect of different concentration of auxin on rooting of *M. balsamina*

they undergo wilting due to excessive desiccation and death (Dietrich et al. 1992) caused by poor stomatal control. In the present study to provide humidity, polybags were used to cover the plantlets. For better field survival the plants were acquainted to the external environment (Bhagyalakshmi 1991) gradually by opening the plants for 1 h daily. The fast growth attained by *M. balsamina* in the present work may possibly be attributed to the porosity of soil maintained by providing sand at the upper layer of pots for better root development. This has in turn facilitated high rate of field survival (85%) of the in vitro raised plants (Fig. 1i). Similar high field transfer success (90%) has been reported in other in vitro raised plants (Quraishi et al. 2004).

Conclusion

The results thus provide evidence that the axillary shoot buds have the potential to regenerate and act as a source of propagules for conservation of the medicinal and nutritive plant *M. balsamina*. Further work to isolate bioactive principles and to elucidate the mechanism of medicinal and nutritive properties of the plant extracts needs to be determined.

Acknowledgements Authors are thankful to the Council of Scientific and Industrial Research (CSIR), New Delhi for the award of Emeritus Scientist to Prof. P.S. Bisen and to the Director, Defence Research & Development Establishment, DRDO, Ministry of Defence, Govt. of India, Jhansi Road, Gwalior, India for extending facilities to PSB.

References

- Ahmed Z, Zaidi N, Shah FH (1990) Micropropagation of *Melia azedarach* from mature tissue. Pak J Bot 22:172–178
- Akinyemi KO, Mendie UE, Smith ST, Oyefolu AO, Coker AO (2005) Screening of some medicinal plants used in south-west Nigerian traditional medicine for anti-*salmonella typhi* activity. J Herb Pharmacother 5(1):45–60
- Bhagyalakshmi N (1991) Short term training course on plant tissue/cell cultures for the production of phytochemicals and for plant improvement. CFTRI, Mysore, pp 151–164
- Bhatt SR, Chnadell KPS, Mallik SK (1995) Plant regeneration from various explants of cultivated piper species. Plant Cell Rep 14:398–402
- Bonga JM (1987) Tree tissue culture applications. In: Maramorosch K (ed) Advances in cell culture. Academic, Florida, pp 209–239
- Bot YS, Mgbojikwe LO, Nwosu C, Abimiku A, Dadik J, Damshak D (2007) Screening of the fruit pulp extract of *Momordica balsamina* for anti HIV property. Afr J Biotechnol 6:047–052
- Deora NS, Shekhawat NS (1995) Micropropagation of *Capparis decidua* (Forsk.) Edgew.—a tree of arid horticulture. Plant Cell Rep 15:278–281
- Dietrich B, Mertinat H, Luckner M (1992) Reduction of water loss during ex vitro acclimatization of micropropagated *Digitalis lanata* clone plants. Biochem Physiol Pflanzen 188:23–31
- Hassan LG, Umar KJ (2006) Nutritional value of balsam apple leaves. Pak J Nut 5:522–529
- Hoque A, Islam R, Joarder OI (1995) In vitro plantlets differentiation in kakrol (*Momordica dioica* Roxb). Plant Tissue Cult 5(2):119–124
- Jigam AA, Akanya HO, Adeyemi DJ (2004) Antimicrobial and antiparasitic effects of *Momordica balsamina*. Niger J Nat Prod Med 8:11–12
- Kackar NL, Solanki KR, Singh M, Vyas SC (1991) Micropropagation of *Prosopis cineraria*. Indian J Biol 29:65–67
- Kartha KK, Gamborg DL, Constabel F (1974) In vitro plant formation from stem explants of rape (*Brassica napus* cv. zephyr) Physiol. Plantarum 31:217–220
- Mittal A, Agrawal R, Gupta SC (1989) In vitro development of plantlets from axillary buds of *Acacia auriculiformis*—a leguminous tree. Plant Cell Tissue Organ Cult 19:65–70
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant 15:473–497
- Ortigao M, Better M (1992) Momordin II, a ribosome inactivating protein from *Momordica balsamina*, is homologous to other plant protein. Nucl Acids Res 20:4662
- Quraishi A, Koche V, Sharma P, Mishra SK (2004) In vitro clonal propagation of neem (*Azadirachta indica*). Plant Cell Tissue Organ Cult 78:281–284
- Sutter EG (1985) Morphological, physical and chemical characteristics of epicuticular wax on ornamental plants regenerated in vitro. Ann Bot 55:321–329
- Thakur GS, Bag M, Sanodiya BS, Debnath M, Bhadouriya P, Prasad GBKS, Bisen PS (2009) *Momordica balsamina*- a medicinal and nutraceutical plant for health care management. Curr Pharm Biotechnol 10(7):667–682