WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 7, 1821-1828. Research Article ISSN 2277– 7105

IN-VITRO CONSERVATION OF MOMORDICA CYMBALARIA HOOK. F.

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Article Received on
19 Feb. 2018,

Revised on 12 March 2018,
Accepted on 01 April 2018

DOI: 10.20959/wjpr20187-11723

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ABSTRACT

Momordica cymbalaria is a tuberous and monoecious species. It is rare and endemic medicinal plant found to be distributed at few localities in Maharashtra, A.P. and Karnataka. Due to its food and medicinal value the species is extensively exploited by the local people hence it is the time to protect the species by means of conservation. In the present study *In vitro* culture technique is used as a conservation strategy for *M. cymbalaria*.

KEYWORDS: In-vitro culture, stem culture**,** M. cymbalaria, conservation.

INTRODUCTION

The Cucurbits are among the most ancient plants used by human beings. About 30 species are cultivated for food in the form of fruits, vegetable and oils. Many species are also used in medicinal and therapeutics. Most of the cucurbits are growing in wild habitat. Due to tremendous applications the cucurbit species are exploited in large quantity. Restricted distribution, low seed germination and ecological as well as environmental threats are also the reasons behind rarity and endangeredness of wild cucurbits in general and *Momordica cymbalaria* in particular. As a result cucurbits are attracting the attention of researchers for conservation as well as cultivation aspects.

The extensive work on distribution, taxonomy and cytology of Indian cucurbits have been done by Nayar and Singh (1998), Dutt and Saran (1998). As far as Kolhapur and nearby districts are concerned *Ex situ* conservation of wild cucurbits has been undertaken by (Chavan, 2000; Shirgave, 2003). A survey of wild cucurbits of entire Bijapur district is

carried out by Mulimani, (2007). The survey revealed some important information regarding ecological status of individual cucurbit species. Being one of the rare and unevenly distributed species, special attention is paid for propagation of *Momordica cymbalaria.* The food and medicinal value the species is extensively exploited by the local people hence it is the time to protect the species by means of conservation. In the present study *In vitro* culture technique is used as a conservation strategy for *M. cymbalaria*. In the present investigation stem explants were used on the modified MS media with different proportions of hormones.

MATERIAL AND METHODS

Extensive field visits were made for the collection of plant from different localities of Solapur district. Plants were collected and maintained in the Botanical garden of Shivaji University as a source of explants. Culture tubes (150mm X 25 mm), conical flasks, pipettes, measuring cylinders, volumetric flasks, Petri dishes, were soaked in 1N HCl for 48 Hrs. and then washed with solution of labolene and rinsed thoroughly with distilled water. All the glass wares wrapped in aluminum foil and autoclaved at 1.06 Kg/ cm^2 pressure and 121 ^oC temperature for 30 min. Sterilization of culture media was done by autoclaving the medium. Explant such as stem of *M.cymbalaria* was first washed under running tap water for 30 min to remove soil and dust particles. Then the explants were washed with solution of labolene and rinsed thoroughly with distilled water and kept ready for explants sterilization. All chemicals used in the experiment were of analytical grade (Himedia) Murashige and Skoog (1962) media. Each experiment with three replicates was arranged and then result was recorded on the basis of physical appearance of the explant. It is clear from the results that 0.15% of mercuric chloride was more effective than other concentrations the explants remain green and show positive response. The lower concentration of $HgCl_2$ shows contamination of explant and higher concentration of $HgCl_2$ affects the explant tissue adversely and it becomes brown. All the cultures were incubated in a growth room at relative humidity of 60% at the temperature of 24 ±2 ^oC under white fluorescent light (2000lux 16watt) provided by Phillips fluorescent tubes (TL40W/a cool day light). For the hardening of plantlets, plastic cups (5cm diameter) filled with autoclaved hardening mix of soil, sand and compost was used in various proportions. The explants were kept in culture room for two weeks to avoid desiccation after the plantlets were then transferred to 50% shade house for further hardening and then finally brought to field for general cultivation.

RESULTS AND DISCUSSION

There are very few reports on *in vitro* culture of stem in Cucurbits (Kageyama *et al.*, 1990). The stem segments of *Momordica cymbalaria* were cultured on MS- I medium supplemented with Auxins and cytokinins singly and in combinations along with coconut milk.

The cytokinins BAP and Kinetin at low concentration show only swelling or the explant remains green. The BAP (3.0mg/l) stimulates callus formation (45%) from stem explants (Shanmugapriya R., 2016) (Table No. 1).

The effect of 2, 4 - D (3.0mg/l) shows (61%) callus initiation. The effect of 2, 4-D on callus induction from stem has been reported in *Solanum tuberosum* by Wang and Huang (1975) Mahender *et. al.* (2009). The 2, 4-D (3.0mg/l) and NAA (3.0mg/l) along with coconut milk (20%) was found to be influence high rate of callus induction and proliferation (Shirgave, 2003). The NAA with coconut milk was found to be more effective in callus induction and proliferation (Table No. 2). The role of NAA on callus formation has been demonstrated (Das *et al*., 2001). The BAP (3.0mg/l) also gives stimulating effect (56 %) for callus induction along with coconut milk (20%). The growth regulator, Kinetin along with coconut milk and without CM induced only the swelling of explants in culture.

The combined effect of BAP (3.0mg/l) and 2, 4-D (3.0mg/l) was proved to be the best combination for callus induction; proliferation and multi shoot induction (78%) for stem explant. (Table No. 3) The similar kind of growth regulators has been tried for *Citrus sp.* (Grinbalt, 1972) and in *Momordica dioica* by Shirgave, (2003).

The stem explants were also cultured on MS I medium supplemented with NAA (3.0mg/l) and 2, 4- D (3.0mg/l) was found to be one of the best combination (75%) for callus induction and proliferation and also shows multishoot induction. The change in the concentration leads to altered response of explants in cultures. (Table No. 4).

Among the various combinations BAP (3.0mg/l) and 2, 4-D (3.0mg/l); NAA (3.0mg/l) and 2, 4- D (3.0mg/l) were proved to be significant for callus proliferation and multishoot induction (Yafeng, *et al*., 2008; Rahman, *et al.*, 1993). Thus it clear from the experiments that the growth regulator level in the explants and their synthesis may determine the morphological nature of explant in culture.

Table No. 1: Effect of NAA, 2, 4-D, BAP and Kinetin on in vitro culture of stem explant of *M. cymbalaria* (Culture period 4 weeks).

Growth regulator in mg/l	% cultures showing response			
	NAA	2,4-D	BAP	Kinetin
0.1	----------	----------	----------	----------
0.5	----------	15 ± 0.1■	----------	----------
1.0	11 ± 0.1■	10.4 ± 0.0●	9± 0.2●	5 ± 0.0■
2.0	14± 0.3●	58± 0.1●Δ	25±0.0■●	11± 0.1■
3.0	15± 0.2●	61.2±1.5●Δ	45±2.0●Δ	19 ± 0.2 ■
4.0	7.4 ± 0.5■	11.5 ± 0.2■	5 ±0.1●	----------
5.0	----------	----------	----------	----------

-------- No response, ■ Remains green, ● Swelling, Δ Callus induction

Table No. 2: Effect of NAA, 2, 4-D, BAP and Kinetin on *in vitro* culture of stem explant of *M. cymbalaria* (Culture period 4 weeks).

Growth regulator in mg/l	% cultures showing response			
	Coconut milk			
	NAA			
	10%	15%	20%	25%
0.1	----------	----------	----------	----------
0.5	----------	----------	----------	----------
1.0	4 ± 0.1●	11.0 ± 0.1●	14.5 ±0.2●	10.1 ± 0.1●
2.0	7.4 ±.0.4●Δ	21.0 ±0.1●Δ	23±0.1●Δ	11.5 ± 0.0●Δ
3.0	11±0.2●Δ	24.0 ± 0.1●Δ	36.0 ±0.2●Δ	14 ± 0.3●Δ
4.0	5.5 ± 0.0●	9.4 ± 0.3●	11 ±0.2●	9 ± 0.3●
5.0	----------	----------	----------	----------
	2,4-D			
0.1	----------	----------	----------	----------
0.5	2.7± 0.0■	4.5 ± 0.0■	11.± 0.1■	6.5 ± 0.1■
1.0	4.7± 0.0●	7.4 ± 0.0●	14.4 ± 0.2●	10.4 ± 0.0●
2.0	23 ± 0.1●Δ	28 ± 0.1●Δ	42.8 ± 0.4●Δ	27.8 ± 0.6●Δ
3.0	27.2 ±1.3●Δ	33.6 ±0.2●Δ	65 ±1.0●Δ	47 ±0.2●Δ
4.0	10.4 ± 0.0■	14 ± 0.3■	16.7 ± 0.7■	11.7 ± 0.0■
5.0	----------	----------	----------	----------
	BAP			
0.1	----------	----------	----------	----------
0.5	----------	----------	----------	----------
1.0	3.2± 0.2●	5.4±0.0●	18±0.0●	7±0.4●
2.0	7±0.4●Δ	14.5±0.2●Δ	42.3±0.6●Δ	10.4±0.0●Δ
3.0	11±0.4●Δ	32±1.2●Δ	56±2.4●Δ	18.0±0.4●Δ
4.0	4.5±0.0●	9.5±0.1●	21±0.2●	3.6±0.0●
5.0	----------	----------	----------	----------
	Kinetin			
0.1	----------	---------	----------	---------
0.5	----------	----------	----------	----------

1.0	2.5 ± 0.0■	4.3 ± 0.0■	7.5 ± 0.2■	2.7 ± 1.0■
2.0	11.3± 0.2●	15.4 ±0.1●	21 ± 0.2●	6.9± 0.0●
3.0	12.5 ± 0.2●	14.4 ±1.8●	18.5± 0.1●	12.2 ± 0.1●
4.0	----------	----------	----------	----------
5.0	----------	----------	----------	----------

-------- No response, ■ Remains green , ● Swelling , Δ Callus induction**.**

Table No. 3: Influence of BAP in combination with 2, 4-D on *in vitro* culture of stem explant of *M. cymbalaria*(Culture period 4 weeks).

Growth regulators(mg/l)		%age of cultures showing response	Morphological nature of explant / callus
BAP	2,4-D		
0.1	1.0	----------	No response
0.5	1.0	----------	No response
1.0	1.0	5±0.1	Remains green
2.0	1.0	9±0.2	Remains green
3.0	1.0	18±0.2	Swelling
4.0	1.0	10±0.1	Remains green
5.0	1.0	----------	No response
0.1	2.0	----------	No response
0.5	2.0	4±0.1	Remains green
1.0	2.0	8±0.2	Remains green
2.0	2.0	63±1.5	Swelling,CI
3.0	2.0	72±00	Swelling,CI
4.0	2.0	31±0.1	Swelling
5.0	2.0	9±0.3	Remains green
0.1	3.0	5.5±0.0	Remains green
0.5	3.0	8±0.2	Remains green
1.0	3.0	11±0.1	Swelling
2.0	3.0	71±0.0	Swelling,CI
3.0	3.0	78±2.0	Swelling,CI,MS
4.0	3.0	30±0.1	Swelling
5.0	3.0	11±0.1	Remains green
0.1	4.0	4±0.2	Remains green
0.5	4.0	4.5±0.2	Remains green
1.0	4.0	9±0.2	Remains green
2.0	4.0	25±2	Swelling
3.0	4.0	41±0.1	Swelling,CI
4.0	4.0	32±0.1	Swelling
5.0	4.0	7±0.4	Remains green

Table No. 4: Influence of NAA in combination with 2, 4-D on *in vitro* culture of stem explant of *M. cymbalaria*(Culture period 4 weeks).

Growth regulators (mg/l)		%age of cultures showing response	Morphological nature of explant / callus
NAA	2,4-D		
0.1	1.0	----------	No response
0.5	1.0	----------	No response
1.0	1.0	2.3±0.1	Remains green
2.0	1.0	15±0.2	Swelling
3.0	1.0	14±0.1	Swelling
4.0	1.0	11±0.1	Swelling
5.0	1.0	---------	No response
0.1	2.0	2.7±0.1	No response
0.5	2.0	9.2±0.0	Remains green
1.0	2.0	14.8±0.2	Swelling
2.0	2.0	58±1.3	Swelling,CI
3.0	2.0	71±0.2	Swelling,CI
4.0	2.0	21±0.1	Swelling
5.0	2.0	6.8±0.0	Remains green
0.1	3.0	7±0.3	Remains green
0.5	3.0	7.6±0.2	Remains green
1.0	3.0	11±0.1	Swelling
2.0	3.0	75±0.2	Swelling,CI
3.0	3.0	75±0.2	Swelling,CI, MS
4.0	3.0	23±0.1	Swelling
5.0	3.0	5±0.0	Remains green
0.1	4.0	5±0.1	Remains green
0.5	4.0	6.3±0.1	Remains green
1.0	4.0	10.4±0.0	Remains green
2.0	4.0	42±0.2	Swelling,CI
3.0	4.0	50±1.0	Swelling,CI
4.0	4.0	29±0.1	Swelling
5.0	4.0	6±0.1	Remains green

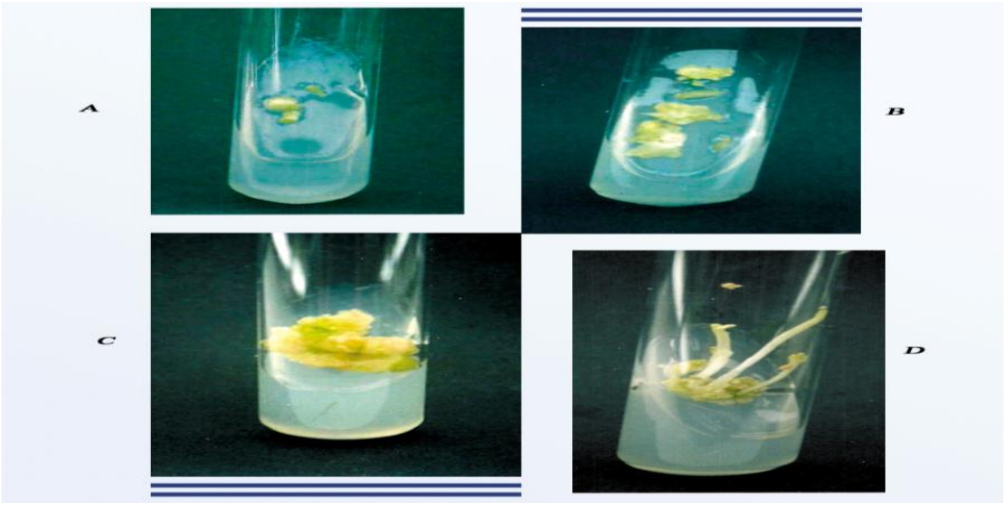

Photo Plate No. - I: *In vitro* culture of Stem explant of *M. cymbalaria.*

A. Stem segment cultured on nutrient medium at an early stage.

B. White compact callus initiates on stem segment cultured on MS 1 containing BAP (3 mg/l) + 2, 4-D (3 mg/l)

C. Stem segment produced profuse callus after four weeks of culture on MS 1 containing BAP (3 mg/l) + 2, 4-D (3 mg/l)

D. Multishoot obtained from stem segment on MS 1 containing NAA (3 mg/l) + 2, 4-D (3 mg/l).

CONCLUSIONS

As far as conservation is concerned popularly, there are two approaches i.e. regeneration and micropropagation. In the present investigation stem explants were used on the modified MS media with different proportions of hormones. In the *Invitro* culture technique each and every part of the species gives different response as per the difference in the hormone composition of media. Therefore, occurrence, quantity, growth rate and nature of callus, as well as occurrence, number and growth rate of multishoot obtained from different explants are studied. In this comparison, **stem segment** are showing more fast response than **leaf segment, but** showing little poor response than **apical bud, axillary bud** as there is presence of active meristematic cells in the apical and axillary bud. These explants are ideal.

ACKNOWLEDGMENTS

We are thankful to U.G.C. for funding the minor research project, entitled "*in- vitro* culture techniques in *Momordica* species."

ABBREVIATIONS

IAA -Indole Acetic Acid, 2, 4-D - 2,4-Dichlorophenoxy Acetic Acid, IBA - Indole Butyric Acid, NAA - Naphthalene Acetic Acid, CI- Callus Induction, CM- Coconut Milk, MS- Murashige and Skoog's Media (1962).

REFERENCES

1. Chavan, N. S. (2000). Ex-situ conservation of wild verities of cucurbits; Minor project submitted to Shivaji University, Kolhapur.
2. Das, S., Jha, T.B. and Jha, S. In vitro propagation of cashew nut: Plant Cell Rep., 2001; 15: 615-619.
3. Dutt, B. and Saran, S. Cytogenetics. In: Cucurbits. Nayar, N. M. and More, T. A. (eds), 1998; 33-38.

4. Grinbalt, W. Differentiation of Citrus stem in vitro. J. Am. Soc. Hort. Sci., 1972; 97: 599-603.
5. Kageyama, K., Yabe, K. and Miyajima, S. Somatic embryogenesis and plant regeneration from stem, leaf and shoot of Melon (Cucumis melo L.) Pt. Tissue Cult. Lett., 1990; 7: 193-198.
6. Mahender Aileni, Srinivasa Reddy Kota, Venugopal Rao Kokkirala, Pavan Umate and Sadanandam Abbagani., Efficient in Vitro Regeneration and Micropropagation of Medicinal PlantMomordica tuberosa Roxb,. Journal of Herbs, spices and medicinal plants, 2009; 15 (2).
7. Mulimani, M.B. (2007). Studies on economically important wild cucurbits of Bijapur district. Ph.D. thesis, Shivaji Univ., Kolhapur.
8. Murashige, T. and Skoog, F. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol Plant, 1962; 15: 473–497.
9. Nayar, N.M. and Singh, R. Taxonomy, Distribution and Ethnobotanical uses. In: Cucurbits. Nayar, N. M. and More, T. A. (eds.) Cucurbits, 1998; 1-18.
10. Rahman, A.H.M.M., M. Anisuzzaman, Ferdous Ahmed, A.K.M. Rafiul Islam and A.T.M. Naderuzzaman, Study of Nutritive Value and Medicinal Uses of Cultivated Cucurbits, Journal of Applied Sciences Research, 1993; 4(5): 555-558.
11. Shanmugapriya R. Callogenic response of petals and tuber parts of medicinal plant. International Journal of Applied Research 2016; 2(7): 615-617.
12. Shirgave P. D. (2003). In vitro culture of Momordica dioica Roxb. Ex. Wild. A Ph.D. thesis, Shivaji University, Kolhapur.
13. Wang, P.J., and Huang. L.C. Callus cultures from potato tissues and the exclusion of potato virus X from plants regenerated from stem tips. Can. J. Bot. 1975; 53(22): 2565-2567.
14. Yafeng, Z., Jiehong, Z., Tao, W., Jiashu, C. Shoot regeneration and the relationship between organogenic capacity and endogenous hormonal contents in pumpkin; Plant Cell Tiss Organ Cult. 2008; 93: 323–331.