ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2016; 2(2): 625-627
www.allresearchjournal.com
Received: 18-12-2015
Accepted: 19-01-2016

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Callogenic response of various parts of *Momordica tuberosa* (Cogn) Roxb

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Abstract
Callogenic response of various parts of explant such as leaf, internode, node, shoot tip and cotyledons of *Momordica tuberosa* was tried on MS medium with varied concentrations of auxins (NAA and 2,4-D) and cytokinins (BA, IBA, BAP). The green compact callus was produced in all the explants in all the combinations except cotyledons. Maximum fresh weight (580±8.82 mg) was observed in the callus derived from internodes on MS+0.8 mg/l BA+0.8 mg/l 2, 4-D). Earlier response (7 days) was observed in the leaf and shoot tips on MS+0.9 mg/l IBA+0.4 mg/l NAA. Cotyledons showed delayed (21 days) response on MS+BAP+2, 4-D, where the callus was white compact which turned green.

Keywords: Cucurbitaceae, *Momordica tuberosa*, Callogenesis. MS (Murashige and Skoog), BA (Benzyl adenine), IBA (Indole -3butyric acid), BAP (6-Benzyl amino-purine), IAA (Indole -3acetic acid), NAA (Naphthalene acetic acid) 2,4-D (Dichlorophenoxy acetic acid).

1. Introduction
Momordica tuberosa (Cogn) Roxb belongs to the family Cucurbitaceae is a weed found growing in the crop fields of Tamilnadu, Andhra Pradesh and Karnataka during the months of August to December. The fruits are used as vegetables by local people. The juice of the leaves are used to cure whooping cough, tubers were reported to have antiovulatory activity. The paste of tuber is used to apply boils, ulcers and snake bite.
Identification and screening of plant organs suitable for callus growth are prerequisite for secondary metabolite production. Hence present investigation was undertaken to study the potentiality of various organs in *Momordica tuberosa* and to determine the most suitable concentration and combination of growth regulators for excellent callus induction.

2. Materials and Methods
2.1 Explant material
Node, internode, leaf and shoot tips of *Momordica tuberosa* were collected from their natural habitats of Veethampatty, Tamilnadu during the rainy season of November 2009. Cotyledons were excised from the moisture seeds. As the explants were very fragile and delicate they were surface sterilized using 75% alcohol for one minute and immersed in 0.1 % mercuric chloride for 4 minutes and rinsed through with sterile distilled water.

2.2 Medium and culture conditions
The basal medium used in the present investigation was full strength Murashige and Skoog, (1962) medium with various combinations of auxins (NAA and 2,4 D) and cytokinins (BA, IBA, BAP) as given in the Table 1. The pH was adjusted to 5.8 by adding 0.1 N Lactic acid or 0.1 N NaOH. Inoculated tubes were incubated at 25±1 ^{0}C under cool white fluorescent lamps of intensity 2000 lux for 12 hours photoperiod. Ten replicates were tried for each concentration and each combinations. The percentage frequency, morphology of callus, maximum fresh weight and the time duration for each explant were tabulated.

Table 1: Showing the concentration of growth regulators on callus formation on various explants of *momordica tuberosa.*

S. No	Explant	Hormones	Concentration
1.	Node	BA + 2, 4 – D	0.6 mg/l-0.9 mg / l
2.	Internode	BA + 2, 4 – D	0.5 mg/l-0.9 mg / l
3.	Leaf	BA + 2, 4 – D	0.6 mg/l-0.9 mg / l
4.	Shoot tip	IBA + NAA	0.2 mg/l-0.9 mg / l
5.	Cotyledon	BAP + 2, 4 – D	0.7 mg/l-0.9 mg / l

3. Results and Discussion

Difference in the composition and culture medium can result in variation in callus induction. Various concentrations and combinations of auxins (NAA and 2, 4 -D) and cytokinins (BA, IBA and BAP) were applied in MS to induce callus in *Momordica tuberosa* and the results are given in Table 2. Of the five explants such as shoot tip, leaf, node, internode and cotyledons, maximum percentage (42%) green compact callus was produced from the internodal segments on MS+0.8 mg/l BA+0.8 mg/l 2, 4–D with a maximum fresh weight of 580±8.82 mg within 14 days. This is the first report on the callogenic response of internodal segments among the members of Cucurbitaceae.

Low concentration of BA and 2,4-D with MS *ie.,* (MS+0.8 mg/l BA+0.8 mg/l 2, 4–D) induced high frequency of (40%) of green compact callus with 571±10.85 mg from the nodes in *Momordica tuberosa.* Whereas in *Momordica charantia* 1.0 mg/l BAP+1.0 mg/l 2,4–D induced high frequency of callus (93.75%) (Munsur *et al*., 2009) [4]. Similar results are obtained from the nodal segments of *Benincasa hispida* and C*ucurbita maxima* (Haque *et al.,* 2008) [1]. In *Trichosanthes dioica* higher concentration of BA (17.76 - 44 µm) produced callus from the nodal segments (Kumar *et al*., 2003) [3].

In the leaves of *Momordica tuberosa* a combination of MS+0.8 mg/l IBA+0.4 mg/l NAA induced earlier production of callus within 7 days when compared to MS+0.8 mg/l BA+0.8 mg/l 2, 4–D which produced only callus after 14 days. In both the cases the amount of callus was same 525±7.07 mg. (Saima malik *et al*., 2007) reported that MS+1.0mg L^{-1} BAP and MS+1.5mg L^{-1} NAA+1.0mg L^{-1} 2,4-D induced hard, green and compact callus from the leaves of *Momordica charantia* whereas (Thiruvengadam *et al.,* 2006) [8] obtained friable calli from the leaves of this speices on MS+1.0mg/l 2,4-D. In *Citrullus colocynthsis* MS+1.5mg/l 2,4-D+1.0mg/l BAP induced callus from the leaves (Savitha *et al*.,2010).

In the cotyledons of *Momordica tuberosa* a combination of MS+0.7 mg/l BAP+0.9 mg/l 2, 4–D produced white green compact callus within 21 days. But compared to all other explants callus induction was delayed in the cotyledons with maximum fresh weight 536±8.61 mg. In *Cucumis melo* MS+0.5 µm 2,4-D+0.5µm BAP induced callus from the cotyledons (Kim *et al*.,1988) [2].

In *Benincasa hispida* young cotyledons produce calli on MS 1-6µm 2,4-D after 6 weeks (Thomas and Sreejah, 2004) [9].

In *Momordica charantia* MS+1.0-1.5 mg/l 2,4-D+1.0-2.0 mg/l BAP induced callus from the cotyledons (Saima malik *et al*.,2007).

As far as shoot tips are concerned a combination of MS+0.9 mg/l BAP+0.9 mg/l 2, 4-D induced maximum callus 536±8.61 mg within 7 days. In *Cucurbita maxima* MS+2.5 mg/l 2,4-D+0.2 mg/l BAP induced callus from the shoot tip whereas in *Beninicasa hispida* MS+0.2 mg/l 2,4-D+3.0 mg/l BAP induced callus form the shoot tip (Haque *et al*.,2008) [1].

4. Conclusion

From the foregoing account it is clear that BA, IBA and BAP promoted cell division when added together at lower concentration with an auxin (*ie*, NAA and 2,4-D). All the parts of the *Momordica tuberosa* are capable of producing callus which can be further manipulated for both regeneration and secondary metabolite production.

5. Acknowledgment

I am grateful to our Managing Trustee Sri G. Rangaswamy, our Secretary, Smt. R. Nandini I have great pleasure in thanking Head of the Department of Botany for the constant encouragement and motivation to take up this work and for providing all the facilities. I place a heartfelt gratitude and indebtedness with sincere thanks to my guide, Dr. (Mrs.) S. Poornima, M.Sc., M.Phil., Ph.D., Associate Professor, Department of Botany, P.S.G.R. Krishnammal College for Women, Coimbatore.

Table 2: Showing the effect of growth regulators on callus formation on various explants of *Momordica tuberosa*

S. No	Explant	MS + Hormones	Concentration (Mg/L)		% Frequency of Callus	Fresh Weight in mg	Time duration	Response
1	Node	BA+2,4-D	0.6 0.7 0.8 0.9	0.6 0.7 0.8 0.9	15±2.65^{a} 30±4.60^{b} 40±4.41^{c} 38±7.29^{d}	501±12.29^{a} 552±19.27^{b} 571±10.85^{c} 564±6.18^{dbc}	14 days	Green compact Callus
CD (P<0.05)					7.52	19.50		
2	Internode	BA+2,4-D	0.5 0.6 0.7 0.8 0.9	0.5 0.6 0.7 0.8 0.9	16±2.33^{a} 27±3.56^{b} 30±4.13^{cb} 42 ± 5.75^{d} 40 ±3.97^{ed}	480±7.07^{a} 535±10.23^{b} 562±13.75^{c} 580±8.82^{d} 578±6.65^{ed}	14 days	Green compact Callus
CD (P<0.05)					6.04	14.20		
3	Leaf	BA+2,4-D	0.6 0.7 0.8 0.9	0.6 0.7 0.8 0.9	16±2.23^{a} 25±2.87^{b} 30±4.13^{c} 28±5.35^{dbc}	480±6.66^{a} 501±11.98^{b} 525±7.07^{c} 520±7.07^{dc}	14 days	Greenish brown compact Callus
CD (P<0.05)					4.75	12.7		
	Leaf	IBA+NAA	0.6 0.7 0.8 0.9	0.2 0.3 0.4 0.5	13 ± 1.10^{a} 15±2.76b^{a} 18±3.31^{ca} 20±3.01^{dc}	425±3.26^{a} 485±7.07^{b} 510±13.14^{c} 525±7.07^{d}	7 days	Greenish brown compact Callus
CD (P<0.05)					7.52	19.50		

4	Shoot tip	BAP+2,4-D	0.7 0.8 0.9	0.7 0.8 0.9	10±2.56[a] 12±3.20[ba] 15±2.42[cb]	360±9.09[a] 385±9.09[b] 405±6.95[c]	7 days	Green white compact Callus
CD (P<0.05)					4.23	11.9		
5	Cotyledon	BAP+2,4-D	0.7 0.7 0.7	0.7 0.8 0.9	5±2.32[a] 7±1.85[b] 10±2.15[c]	425±3.26[a] 485±7.07[b] 536±8.61[c]	21days	Whitish green Compact callus
CD (P<0.05)					2.76	10.3		

The results are statistically analysed and the results are presented in Table 2

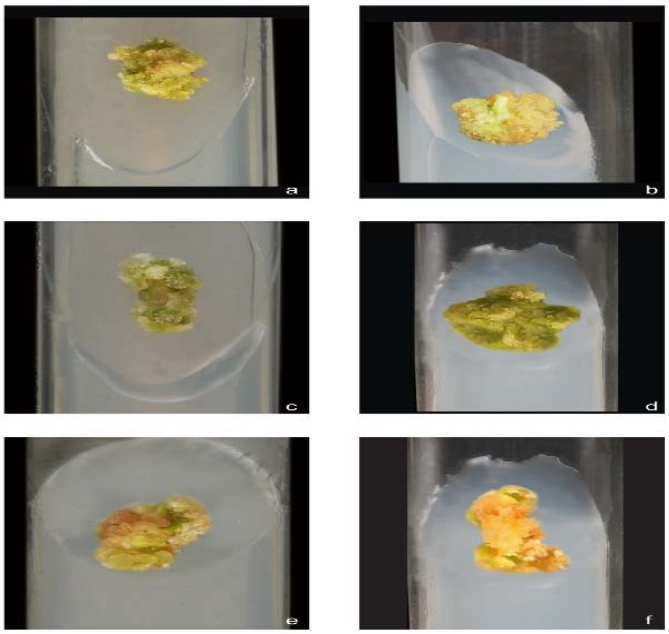

Plate 1: A and B - Node; C And D - Internode; E and F – Leaf

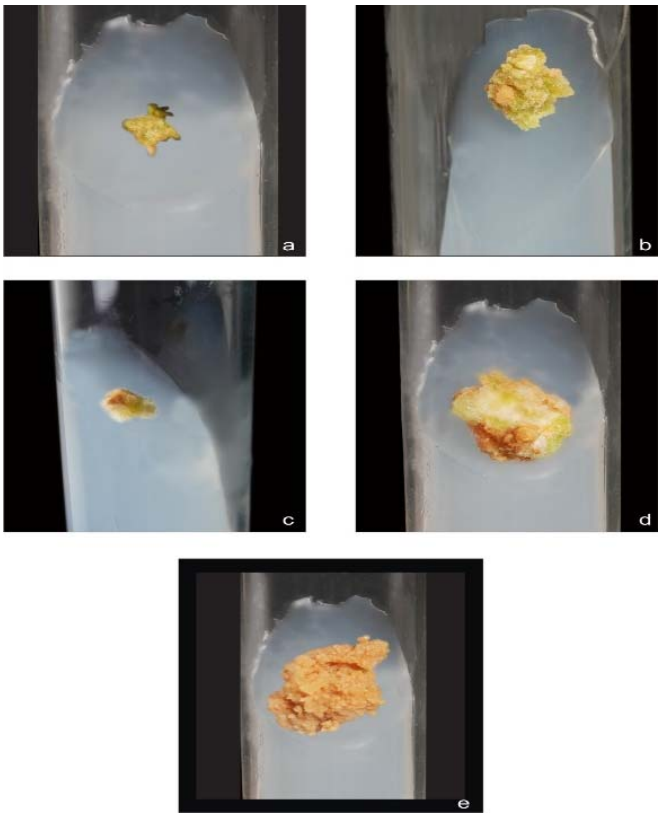

Plate 2: A and B - Shoot Tip; C, D and E – Cotyledon

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