**STUDENT STUDY PROJECT (2018-2019)**

## Name of the Topic:

## Invitro Plant regeneration of Momordica cymbalaria Fenzl.

**Under the Guidance of**

**R. Shyamala Chandra**

**Asst.Prof of Biochemistry**

**V.Spoorthi**

## DEPARTMENT OF BIOTECHNOLOGY

## Kakatiya Government College , Hanamkonda.

## Names of the Students

| S.No | Name of the Student | H.T.No | Group |
|---|---|---|---|
| 1 | E.Manogna | 006-17-3004 | BTBC 3rd Year |
| 2 | K.Anjali | 006-17-3012 | BTBC 3rd Year |
| 3 | M.Pavani | 006-17-3013 | BTBC 3rd Year |
| 4 | M.Sushma | 006-17-3015 | BTBC 3rd Year |
| 5 | P.Arun Raj | 006-17-3019 | BTBC 3rd Year |
| 6 | S.Ravinder | 00617-3021 | BTBC 3rd Year |

- *Invitro* plant regenaration of *Momordica cymbalaria*Fenzl. and assessment of genetic fidelity using ISSR markers.

**Abstract**

This investigation was conducted to describe the plant regenerationfromdifferent explants of*Momordica cymbalaria* Fenzl.,an important medicinal cucurbit. Leaf, shoot tip and nodal explants were successfully callused onMurashige and Skoog's medium(MS) supplemented with different concentrations of auxins 2,4-D(2,4-dichlorophenoxyacetic acid), IBA(indole-3-butyric acid)and cytokininBAP (6- benzylaminopurine)alone. Maximum percentage of callus (95%) was obtained from leaf explants at 1.0mg/l 2,4-D. Leaf derived callus highly responded for the shoot regeneration(13.33 shoots) on half strengthMS medium containing 1.5mg/l BAP incombination with 1.5mg/l IBA.Shootlets were rooted on half strength MS media supplemented with 0.2- 2.0mg/l naphthalene acetic acid (NAA )and indole-3-butyric acid(IBA).The highest number of roots were obtained in 0.5mg/l NAA.The rooted plantlets were successfully hardened and transferred to the green house with 90% of survival.

- **Keywords :** Callus induction, Organogenesis, Plantlets, *Momordica*

**Introduction:**

The plant *Momordica cymbalaria*Fenzl.belongs to the family Cucurbitaceae, it is a herbaceous, trailing herb, arising from a small perennial tuber, originating in the tropical regions of India and South East Asia.The plant has also been named *Luffa tuberose Roxb.* or *Momordica tuberosaRoxb.* It is commonly known as kasarakaya ,Karchikai, Athalakkai, Kakroland kadavanchi(Anon 1962). In India, the plant is not cultivated by the farmer as a regular crop, it occurs naturally in the boundary of fields as a weed plant, in the rainy season.

The plant is recognized in traditional medicine, used for the treatment of diabetes mellitus, rheumatism, diarrhea, skin diseases and ulcer(R. Jeyadevi et al.2012). The fruits are used as a vegetable, and the leavesare also used as leafy vegetable(kirtikar&Basu1993).The fruits are reported to contain citric acid , maleicacid, vitamin C and high crude fiber content (parvathi&kumar 2002).The plant extracts contain saponins , terpinoids and polyphenols like querticin.Querticinreportedantiulcer property (P.Barathidasan et.al 2010).

The methonolic extracts of aerial parts of *Momordica cymbalaria* shows anti cancer property (P.Jeevananatham et.al2011).The plant part extracts shows anti-diabetic(Firdous M et al 2009), hypolipidemic(Yeddula Ezra et al 2014),anti-diarrheal and anti-microbial (Vrushabendra swamy et al 2008), anti-ulcer, neuroprotective (Dhasan PB et al2010), cardioprotective, anti-ovulatory and abortifacient (Raju koneri et al 2008), hepatoprotective, nephroprotective (Kumar P et al 2011), anti-cancer (Nagarathana PKM et al 2016), anticonvulsant (Maniyar YA et al 2015), anti-inflammatory( Jeevanantham et al.2011) activities.

This species is under threat due to some factors they are,the fruits of this plantshad only few seeds that remain dormant and low germination(T D Nikam et al, 2009),habitat losses and other innate factors might have been the reason for their poor germination and low seed viability and also contamination of the plant tubers with soil bacteria, fungus and insects.It can be overcome by micropropagation a biotechnological approach. *Invitro* regeneration is an efficient tool of *ex-situ* conservation of plant diversity because with this technique the endangered species and desired species can be quickly propagated ( Saheli et al 2014;Ozel et al 2015).

It is very economical and easy method for the multiplication of the desired plants.*Invitro* cultures can be alternative to field grown plants for the production of therapeutically valuable compounds. *Invitro* regeneration is applied to produce plants for commercial micropropagation (Shivanandhan*et al.* 2011). T D Nikamet al.( 2009) has previously reported successful indirect organogenesis from *Momordica cymbalaria* using different explants.The major objective of the current research to achieve successful and efficient plant regeneration via adventitious, indirect organogenesis from leaf,internode and shoot tip explants of *Momordica cymbalaria*and assessement of clonal fidelity by using ISSR markers was reported.

## Materials and methods

### Plant material and surface sterilization

*Momordica cymbalaria* plants were collected from Jammikunta crop fields, Telangana state during monsoon season. The plants were maintained and grown in the medicinal harbour of Biotechnology department, Kakatiya university, Telangana state, India. Young leaves and internodes were collected and used as explants. The explants were rinsed under running tap water for 15 mins , washed with 10% tween 20 (liquid soap) for 5mins,then washed 3 times with double sterile distilled water . Then the explants were surface sterilized with 0.1% bavostein( an fungicide) for 5 mins followed by 0.1% mercuric choloride( $Hgcl_2$)for 3 mins in sterile conditions.Then the explants were again washed with sterilized double distilled water to vanish the mercuric chloride.These sterilized explants were inoculated on culture medium.This whole process was carried out under the laminar air flow chamber.

## Culture conditions:

MS medium (Murashige and skoog 1962) supplemented with vitamins, 100mg/ lmyoinositol, 30 g/l sucrose and 7g/l agar and the pH of the medium was adjusted to 5.6 - 5.8with 0.1 N NaOH, or 0.1N HCl after addition of the plant growth harmones i.e., Auxins and Cytokinins with different concentrations (0.5- 2.5 mg/l).After heating the media it is suspended in the culture test tubes and autoclaved at 121°C for 15 mins. All the cultures were maintained in a sterilized culture room and incubated at 26±28°C and relative humidity of 60±10%.An 16 hr photoperiod and 8hr dark conditions are provided.

## Callus induction

Explants including leaf (0.5×0.5 cm), shoot tip(1 cm in length), internode (1 cm in length) were exised from *invivo* germinated plant and sterilized and placed horizontally on MS medium containing tubes. In this experiment the effect of auxins and cytokinins, both are alone and in combinations studied for callus initiation and its proliferation. Auxins such as 2,4-Dichlorophenoxy acetic acid (2,4-D) (0.2,0.5,1.0,1.5,2.0,2.5mg/l), Indole butyric acid(IBA) (0.2, 0.5,1.0, 1.5, 2.0,2.5 mg/l) and cytokinin i.ebenzyladenine (BAP) ( 0.2, 0.5,1.0, 1.5, 2.0, 2.5, mg/l) were used. Dataofrespondedfrequency (%) of callus and morphology of callus recorded after 45 days of culture.

## Shoot organogenesis from callus

Well established green compact callusfrom all explants were further cultured on cytokinins such as BAP (0.5- 3 mg/l) and KN(0.5- 3.0mg/l) supplemented MS medium with auxin IBA (1.5 mg/l) for shoot regeneration. Obtained cultures were transferred on same fresh medium for further elongation of shoots. The dataon frequency percentage of response, mean number of shoots, mean number of shoot length of cultures were recorded after 4 weeks of subculture.

## Acclimatization

The well rooted healthy plantlets were picked up from the culture tubes without any damage to the roots and washed in sterile distilled water for several times to vanish the traces of medium. The plantlets were subsequently transferred to theplastic cups( 6 cm in diameter) containing black soil and sand (2:1)[Fig.3 (e,f)]. The plastic cups were covered with transparent polythene bags to maintain the controlled growth conditions and humidity of about 75-80%, 16 hrsphotoperiod, 26±2°C and 60 $\mu mol^{-2} s^{-1}$ light intensity. The plantlets were regularly irrigated with sterile water every two days for three weeks. Well established plantlets were finally transferred into the greenhouse in the fourth week.

## Formationof adventitious roots

*Invitro*regenaratedshoots( 2-4 cm ) obtained from micropropagatedplantlets were cultured on half strength MS medium supplemented with auxins IBA, NAA (0.2, 0.5, 1.0, 2.0 mg/l) for adventitious roots. Root inducing percentage, mean number of roots and mean length of roots were recorded after 3 weeks of transfer onto the rooting media.

## Statistical analysis

All the treatments consisted of 30 explants and each was repeated three times.The data on frequencyof explants responded for callus, average number of shoots and length of shoots and roots were recorded regularly at weekly intervals.The results were analysed by SPSS software.

## Results and discussion:

Depending on plant species, the most important part of the success obtained in the plant regeneration studies through tissue culture was based on the concentration and combinations of plant growth regulators(Murashige.T 1990). To achieve an successful regeneration in *Momordica cymbalaria* various concentrations of Auxins and Cytokinins were used.

## Callus induction:

Callus initiation was observed after 2weeks of incubation from the cut margins of leaf , node, shoot tipexplants cultured on MS medium supplemented with auxins 2,4-D, IBA,(0.2-2.5mg/l), cytokininBAP(0.2-2.5mg/l) alone. Explants were not responded for callus initiation when they are cultured on hormone-free basal MS medium. Similar results were observed in *M.charantia* (Malik et al. 2007).The percentage of explants responded frequency and morphology of callus was observed depending on the various concentrations of plant growth regulators used. Among all, 2,4-D gave the best callus induction and proliferation followed by BAP,IBA,in all explants. 2,4-D showed high percentage of callus induction (96%) at 1.0mg/l. Leaves are the best explants source for callus induction and shoot regeneration (Neelam singh et al 2011).

2,4-D at low to high concentrations produced white friable nodular callus to green nodular callus [Fig.1(a-c)].Similarly IBA at low to high concentrations produced off white friable callus to light green compact callus and responded forrhizogenesis[Fig.1 (d-f)].Similar results were reported by Neelam singh et al(2011) in *Naringicrenulata*.BAP at low to high concentrations produced light brown friable callus to dark brown friable callus[ Fig. 1(g-i)]. IBA induced high frequency of green compact callus among all tested concentrations of 2,4-D, IBA and BAP and this callus is used for shoot regeneration studies.

Morphogenetic green compact callusof leaf,internode and shoot tipwas shifted to shoot regeneration medium fortified alone with BAP (0.5-3.0mg/l) and KN (0.5-3.0mg/l).There was no initiation of shoot budsobserved with only cytokinins. The present investigation observed that theBAP and KN arenot beneficial incallus organogenesis or differentiationof shoots from callus.Similar reports were observed byArya et al 2008. The combination of cytokinin and auxin gives the best results of organogenesis in plants (Salvi et al (2012),Ahmed et al (2010) and Dode et al (2003).

Theadventitious shoot buds were observed onBAP andKN (0.5-3.0mg/l )in combination with IBA (1.5mg/l) (Fig 2(a)). These initiated shoot buds when transferred to elongation medium continued to grow and formed mature shoots after two subcultures for 3 to 4 weeks at a concentration of BAP and KN(0.5-3.0mg/l) combined with IBA(1.5mg/l)(Table 2).

**Figure.1: Initiation of callus from different plant**

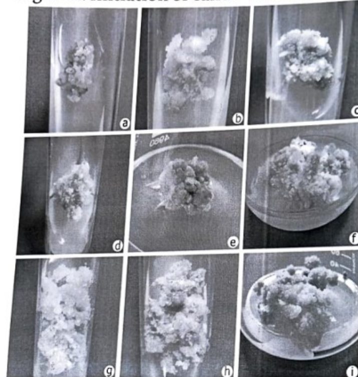


Table 2: Effect of BAP and KN in combination with IBA(1.5mg/l)

| Harmones | Regeneration Frequency | | | Mean No. of Shoots/Callus | | | Mean length of Shoots | | |
|---|---|---|---|---|---|---|---|---|---|
| BAP+IBA | Leaf | Node | Shoot tip | Leaf | Node | Shoot tip | Leaf | Node | Shoot tip |
| 0.5+1.5 | 64±0.57 | 61.00±0.57 | 69±0.00 | 2.33±0.33 | 3.33±0.33 | 2±0.00 | 4.9±0.10 | 2.20±0.11 | 6.50±0.00 |
| 1.0+1.5 | 77.3±0.66 | 68±0.57 | 76±0.57 | 6±0.0 | 5±0.00 | 2.33±0.33 | 3.43±0.06 | 3.40±0.15 | 7.70±0.11 |
| 1.5+1.5 | 92.33±0.66 | 89±0.00 | 84±1.0 | 13.33±0.66 | 8±0.00 | 3.67±0.33 | 4.26±0.14 | 6.16±0.16 | 9.96±0.14 |
| 2.0+1.5 | 81.67±0.33 | 76.33±0.66 | 80±0.57 | 10.33±0.33 | 7.67±0.33 | 3.67±0.33 | 3.66±0.06 | 4.56±0.14 | 8.00±0.10 |
| 2.5 +1.5 | 74.67±0.66 | 70±0.57 | 72.33±0.33 | 6.67±0.33 | 5.33±0.33 | 3±0.00 | 5.6±0.17 | 3.23±0.23 | 7.03±0.03 |
| 3.0+1.5 | 61.33±0.66 | 60±0.00 | 60.67±0.33 | 4.33±0.66 | 4±0.00 | 2.67±0.66 | 5.3±0.03 | 4.13±0.06 | 7.90±0.10 |
| KN+IBA | | | | | | | | | |
| 0.5+1.5 | 48.33±0.66 | 40.67±0.66 | 0.00±0.00 | 1.0±0.00 | 2.67±0.33 | 0.00±0.00 | 5.60±0.20 | 7.23±0.14 | 0.00±0.00 |
| 1.0+1.5 | 52±0.00 | 43±0.57 | 0.00±0.00 | 1.33±0.33 | 3±0.00 | 0.00±0.00 | 5.66±0.20 | 6.96±0.03 | 0.00±0.00 |
| 1.5+1.5 | 54.67±0.33 | 51±0.00 | 46±1.0 | 2.67±0.33 | 4.67±0.33 | 2.33±0.33 | 3.60±0.05 | 4.23±0.23 | 6.30±0.10 |
| 2.0+1.5 | 61±0.57 | 57.67±0.66 | 58.33±0.57 | 2.67±0.33 | 5.33±0.66 | 3.33±0.33 | 3.50±0.17 | 5.63±0.06 | 5.36±0.06 |
| 2.5+1.5 | 67.67±0.33 | 60.33±0.33 | 65±1.7 | 5±0.00 | 6.33±0.66 | 4.67±0.33 | 4.10±0.05 | 5.13±0.03 | 2.83±0.16 |
| 3.0+1.5 | 78.67±0.66 | 70.67±0.66 | 68±0.00 | 7.67±0.33 | 7±0.00 | 5±0.00 | 5.26±0.12 | 5.53±0.06 | 3.56±0.06 |

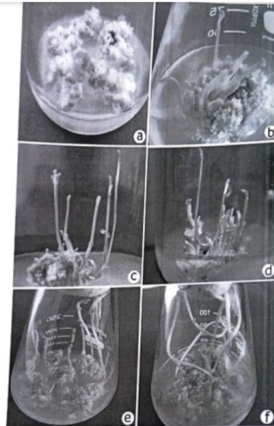
## Effect of BAP and KN in combination with IBA(1.5mg/l):

Alone cytokinins are not showed any shoot bud proliferation. When cytokinins are combined with IBA initiation of shoot buds were observed.The results clearly indicated that low concentration of IBA with cytokinins was greatly favoured for shoot bud proliferation. Similar results were observed by SongulGorel (1998) in *Amygdaluscommunis L.*The percentage of shoot bud initiation was decreased with the combination of the highest BAP(3.0mg/l) and IBA (1.5mg/l) (Table.2). Whereas KN at low to high concentration (0.5-3.0mg/l)with IBA(1.5mg/l) showed increased percentage of shoot bud initiation(Table 2). The shoot growth rate was decreased with the highest concentration of cytokinins (BAP&KN) in combination with IBA(1.5mg/l). The best results were obtained at BAP(1.5mg/l) incombination with IBA(1.5mg/l).

The highest percentage of shoot bud induction after 4 weeks of culture was 92% from leaf, 89% from internode whereas 84% from shoot tip on MS medium fortified with 1.5mg/l BAP in combination with 1.5mg/l IBA (Table 2). The highest number of shoots obtained per explant was 13.33±0.66 from leaf, 8±0.00 from internode, 3.67±0.33 from shoot tip with 1.5mg/lBAP+1.5mg/l IBA (Table 2). Whereas 5.660±0.20 from leaf , 7.23±0.14 from internode ,6.30±0.10 from shoot tip explants fortified with 3.0mg/l KN +1.5mg/l IBA.

**Effect of auxins(NAA, IBA) in root formation:**

*In vitro* grownmicro shootletsfrom all the explants were excised and shifted to half strength rooting media containing IBA (0.5-2.0mg/l) and NAA (0.5-2.0mg/l). Among these two auxins, NAA showed best results (92 %) for rooting. The number of induced roots were less in IBA in compared with NAA. Most of the shoots were produced roots within 3-4 weeks after shifted to rooting medium.




## Table.3: Effect of NAA and IBA on rooting

| Plant growth regulators | % of response | No.of roots | Length of roots |
|---|---|---|---|
| NAA (mg/l) | | | |
| 0.2 | 84 | 6±0.00 | 5.23±0.88 |
| 0.5 | 90 | 7.67±0.33 | 6.80±0.03 |
| 1 | 80 | 4±0.66 | 3.46±0.057 |
| 2 | 77 | 2.33±0.33 | 4.26±0.06 |
| IBA(mg/l) | | | |
| 0.2 | 70 | 3±0.00 | 5.13±0.08 |
| 0.5 | 82 | 4.67±0.33 | 3.66±0.00 |
| 1 | 91 | 5.33±0.33 | 4.33±0.05 |
| 2 | 76 | 5±0.00 | 3.60±0.01 |

## Conclusion:

We demonstrated an efficient regeneration protocol through indirect organogenesis by using leaf, node and shoot tip explants of *Momordica cymbalaria*. The combination of BAP (1.5mg/l) and IBA(1.5mg/l) showed best results for shoot regeneration. Induced shoots were shifted to half strength MS medium fortified with NAA and IBA(0.2-2.0mg/l) for rooting. Then plantlets were acclimatized in pot mixture containing black soil, sand (2:1).