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# In- Vitro Rooting and Hardening of Excised Shoots of Momordica Cymbalaria Hook. F.

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***Abstract:***
*In vitro culture technique is now a days becoming a popular tool for the conservation of rare, endemic and endangered plants. In the family Cucurbitaceae in- vitro propagation of different members has been reported by several researchers. In the present research work in- vitro rooting and hardening is tried for excised shoots of Momordica. cymbalaria. Naphthalene Acetic Acid (NAA) and Indole Butyric Acid (IBA) were tried separately. IBA found more suitable for this purpose.*

***Key Words:*** *Momordica cymbalaria, Rooting, Hardening, IBA, NAA*

**ABBREVIATIONS:**
IBA - Indole Butyric Acid, NAA - Naphthalene Acetic Acid, 2-4-Dichloro Phenoxy Acetic Acid, BAP- Benzyl Adenine Purine, MS-Murashige and Skoog's Media(1962).

## I. INTRODUCTION:

*Momordica cymbalaria* is a rare, wild and medicinally potential plant of cucurbitaceae family. It is endemic to certain parts of Maharashtra, Andhra Pradesh and Tamil Nadu states of India. The fruits are nutritious and utilized as vegetable (Chittapur, 2015). As the species is endemic and threatened, an attempt has been made by Madhale and Chavan (2009) to establish a protocol for its in-vitro culture. They have studied callus initiation and multishoot induction using Benzyl Adenine Purine (BAP) and Naphthalene Acetic Acid (NAA) as well as NAA and 2-4-Dichloro Phenoxy Acetic Acid ( 2-4-D) in Combination. Multishoot Induction is further studied for in-vitro Rooting and hardening. Therefore in the present piece of work an attempt has been made to study using NAA and Indole Butyric Acid (IBA) separately.

## II. MATERIAL AND METHODS:

The plant material of *M. cymbalaria* is collected from the different habitats Solapur dist. of Maharashtra and maintained in the botanical garden of Shivaji University, Kolhapur. Multishoot induction was achieved by following protocol standardized by Madhale and Chavan (2018). Further, excised shoots were cultured on half strength of MS medium containing NAA and IBA(0.1 to 4.0 mg /liter) along with 0.5 % activated charcoal for rooting. Hardening is studied as described by Gamborg and Philips (2004). For the process of hardening plastic cups ( 5cm diameter and 6 cm height ) were filled with hardening mixture (soil + sand + compost in 1:1:1 proportions) were used. The rooted shoots were carefully removed from tubes and transplanted in hardening mixture. These cups were kept in culture room for two weeks to avoid shock due to environmental changes and desiccation. After that these cups were transferred to shade net with 50% light cut off. Finally plantlets were transferred to field.

## III. RESULTS AND DISCUSSION:

The growth regulator NAA and IBA were used separately in the concentration of 0.2 to 0.3 mg/l to study rooting in excised shoots. Positive response of rooting started in the concentration of 0.5 mg/lit. and 1.0 mg /liter in case of NAA and IBA respectively. In case of NAA and IBA positive response gradually increased up to 2.0 mg/liter concentration and started lowering. The response is three fold in IBA than NAA. (Mahipal *et al.*, 2016 and 2017).

The growth regulator IBA was found to be most effective in root induction. (Mahipal *et al.*, 2016). At low concentration of IBA shows 8% rooting induction. The gradual increase in IBA concentration increases the percentage of rooting in culture. IBA 2.0mg/l was found to be most effective for rooting. (M. Chabukswar and Deodhar, 2005) More than 2.0 mg/l concentration of IBA gradually decreases the percent of rooting. (Table No.1) The IBA exhibited best results than the NAA. (Phlomina and Rao, 1999; Segio *et al*., 2000; Johnson, 2002; Vasanth *et al.*, 2002; Biljana, *et al*., 2004).

Root induction in case of IBA started after 4 days of culture. It continues almost up to 15 days. Later on the rooted shoots were transplanted in hardening mixture. The plantlets in hardening mixture were kept for 5 days under laboratory conditions. The hardening process took 20 days. The plantlets were hardened previously under laboratory conditions and then under 50% shed net at 80% humidity and 25-30$^0$C temperature. The hardened plantlets were then transferred to the field for further establishment.

The success rate of hardening process is 80%. No adverse morphological effects were observed except slight wilting. Root: shoot ratio is also depicted in the table. Interestingly it is high in case of 2.5 mg/liter IBA, where root induction is comparatively less. Therefore, 2.0 mg/liter IBA is the best for root induction and growth.

**Table No.-1:** Effect of NAA and IBA on rooting of excised shoots of *M. cymbalaria*

| Growth regulator in mg/l | % cultures showing response | | | |
|---|---|---|---|---|
| | NAA | | IBA | |
| | % of root induction | Ratio of root/shoot | % of root induction | Ratio of root/shoot |
| 0.2 | ..... | ..... | ..... | ..... |
| 0.5 | 8.0 ±0.0 | 1.0±0.0 | ..... | ..... |
| 1.0 | 14.5±1.0 | 2.0±0.0 | 08±0.0 | 3±0.0 |
| 1.5 | 10.0±0.0 | 2.0±0.2 | 18±0.0 | 5±0.8 |
| 2.0 | 18.0±2.5 | 3.0±0.0 | 68±2.5 | 10±2.2 |
| 2.5 | 9.0±0.0 | 1.0±0.0 | 23.2±1.9 | 11±2.0 |
| 3.0 | 2.0±0.6 | 1.0±0.2 | 10.0±0.0 | 8±0.0 |

**(culture period 4 weeks)**

***In- vitro* rooting and hardening of excised shoots of *M. cymbalaria***

A) Profusely root initiation
B) Rooting initiation - I stage
C) Hardening of excised shoots

## IV. CONCLUSION:

The above experimental results indicate that, IBA is superior than NAA in root induction. The significance of IBA in root induction has been reported in *Cucumis sativus* by Shirgave and Chavan, 2001; Misra and Bhatnagar, 1995. The success rate of hardening process is 80%. Overall sixty five days are required to establish new plantlet in the field. Among this period thirty days are required for multishoot development (time up to the excision). 15 days require rooting initiation and 20 days require to hardening process.

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