



## IN VITRO CALLUS INDUCTION OF *ABELMOSCHUS MOSCHATUS* MEDIK. L. BY USING DIFFERENT HORMONE CONCENTRATION

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### ABSTRACT

*Abelmoschus moschatus* medik. L. is an important medicinal and aromatic herbaceous plant of family (Malvaceae). The seeds of this medicinal herb are valued for their demulcent, diuretic and stomachic properties. They are served as well as an antiseptic, a stimulant, a tonic and an aphrodisiac. They are also served as cooling and carminative agents. A present investigation method for in vitro callus induction of *Abelmoschus moschatus* L. (muskdana plant) is limited. Explants were cultured on Murashige and Skoog (MS) basal medium supplemented with 0.1-0.3 mg/l BAP and 1-3 mg/l NAA for callus induction. The good callus induction was found in MS medium supplemented with 0.3 mg/l BAP & 2 mg/l NAA from first leaf explant. First leaf gets better and early response as compared to other explants such as cotyledonary leaf, epicotyl, hypocotyl and root etc.

**Keywords:** *Abelmoschus moschatus* medic, First leaf, Callus, BA, NAA.

### INTRODUCTION

*Abelmoschus moschatus* is commonly known as Musk okra. It is an erect, annual, hirsute herb and 1.5 m tall in height. The leaves are palmately compound, flowers are yellow and musk scented.<sup>1</sup> The seed of this plant are known as Ambrette seed, which contain moisture 11.14 %, protein 2.3 %, starch 13.35 % and fatty oil 14.5 % and Seed coat contains sesquiterpene alcohol and farnesol.<sup>2</sup> Analysis of volatiles oil report stated that flower, leaf and petal contains myricetin-3-glucoside, glycoside of cyanidin, beta-D-glucoside, beta-sitosterol myricetin and its glucoside<sup>3</sup>. The characteristics musk odour is due to the presence of a ketone-ambrettolide and lactones of ambrettillidic acid. Seed oil have been used as a tonic, stimulant, carminative, diuretic, demulcent and stomachic properties. In Phillipines regions, decoction of the root and leaves is taken as an emollient remedy for gonorrhoea and rheumatism. It is used against bite of venomous reptiles, kapha and vata.<sup>4</sup> Seeds oil also applied externally to treat cramp, poor circulation and aching joints. In Arabs, seeds are used as flavoring liquors or scent to coffee. Seed are used as an insecticide or fruit extract or upper part of the leaves shows insecticidal activity. The tincture of seed is used in nervous disability, hysteria and nervous disorders,<sup>5</sup> this has a strong, peculiar and long lasting odour; reminiscent of musk is used in high grade perfumery. Leaves and root mucilage used in venereal disease. Cotyledonary node explants of *A. moschatus* were used for induced regeneration and not for callus induction.<sup>6</sup> Due to its highly medicinal & chemical property it is essential to study this plant for future benefits to meet basic need. In the present study, callus induction would permit secondary metabolite from genetically engineered cells.

### MATERIAL AND METHODS

The seeds of musk okra were obtained from 'Shree Shell', Nagpur. The seeds were maximum germinated with Hot water treatment for 5 min. Explants such as Epicotyl, Hypocotyl, Root, cotyledonary leaf and First leaf were taken and then sterilized with the help of 0.1% HgCl<sub>2</sub> and 70% ethanol for 2 min. followed by washing in distilled water under aseptic condition on laminar air flow chamber. Explants were cut by scissors and inoculated on MS<sup>8</sup> medium supplemented with varying concentration of BAP (6-Benzyl amino purine) and NAA ( $\alpha$ -Naphthalene acetic acid) in the range 0.1 -0.3 mg/l BAP and 1-3 mg/l NAA for callus induction.<sup>7</sup>

**First leaf for callus induction:** First leaf explant were inoculated on MS basal nutrients salt medium including vitamins supplemented with varying concentration of hormone in a medium included 3 gm sucrose and were solidified with 0.8 gm agar. Medium was adjusted at pH 5.8 using 1N NaOH and 1 N HCl. The sterilized explants were cultured under laminar air flow chamber. The cultures were maintained at 25 $\pm$ 2<sup>0</sup> C and 65% humidity in plant growth chamber.

**Nature of callus:** Callus, color, nature and physical condition were counted.

### RESULTS

MS medium supplemented with different hormone concentration of BAP & NAA was tried for callus induction, which is mentioned below:

- 1.0.3 mg/l BAP & 3mg/l NAA
- 2.0.2 mg/l BAP & 2mg/l NAA
- 3.0.1 mg/l BAP & 1mg/l NAA
- 4.0.3 mg/l BAP & 2 mg/l NAA



5. 0.2 mg/l BAP & 3 mg/l NAA

6. 0.1 mg/l BAP & 2 mg/l NAA

7. 0.1 mg/l BAP & 3 mg/l NAA

All other hormone concentration was found to be responsible for the development of callus from various explants, the 0.3 mg/l BAP & 2mg/l NAA were the best

hormone concentration used for callus induction among all the concentration. Explants such as epicotyl, hypocotyl, cotyledonary leaf, root and first leaf were showing some response in the medium but first leaf explant were shown good response. However, First leaf shows early response and maximum callus growth in the medium.

**Table 1:** Effect of BAP and NAA on the first leaf of *A. moschatus*

Sr. no.	Medium	Hormone concentration Taken (mg/l)	No. of explants	Callus formation
1	MS	0.3 mg/l BAP & 3 mg/l NAA	10	++
2	MS	0.2 mg/l BAP & 2 mg/l NAA	10	++
3	MS	0.3mg/l BAP & 2 mg/l NAA	10	+++
4	MS	0.2 mg/l BAP & 3 mg/l NAA	10	+
5	MS	0.1 mg/l BAP & 1 mg/l NAA	10	++
6	MS	0.1 mg/l BAP & 2 mg/l NAA	10	+
7	MS	0.1 mg/l BAP & 3 mg/l NAA	10	+

+++ = Good, ++ = Low, + = Poor, - = Absent

**Table 2:** Effect of BAP and NAA on the epicotyl of *A. moschatus*

Sr. no.	Medium	Hormone concentration Taken (mg/l)	No. of explants	Callus formation
1	MS	0.3 mg/l BAP & 3 mg/l NAA	10	++
2	MS	0.2 mg/l BAP & 2 mg/l NAA	10	++
3	MS	0.3mg/l BAP & 2 mg/l NAA	10	+
4	MS	0.2 mg/l BAP & 3 mg/l NAA	10	++
5	MS	0.1 mg/l BAP & 1 mg/l NAA	10	++
6	MS	0.1 mg/l BAP & 2 mg/l NAA	10	+
7	MS	0.1 mg/l BAP & 3 mg/l NAA	10	+

+++ = Good, ++ = Low, + = Poor, - = Absent

**Table 3:** Effect of BAP and NAA on the hypocotyl of *A. moschatus*

Sr. no.	Medium	Hormone concentration Taken (mg/l)	No. of explants	Callus formation
1	MS	0.3 mg/l BAP & 3 mg/l NAA	10	++
2	MS	0.2 mg/l BAP & 2 mg/l NAA	10	++
3	MS	0.3mg/l BAP & 2 mg/l NAA	10	++
4	MS	0.2 mg/l BAP & 3 mg/l NAA	10	+
5	MS	0.1 mg/l BAP & 1 mg/l NAA	10	++
6	MS	0.1 mg/l BAP & 2 mg/l NAA	10	+
7	MS	0.1 mg/l BAP & 3 mg/l NAA	10	-

+++ = Good, ++ = Low, + = Poor, - = Absent

**Table 4:** Effect of BAP and NAA on the cotyledonary leaf of *A. moschatus*

Sr. no.	Medium	Hormone concentration Taken (mg/l)	No. of explants	Callus formation
1	MS	0.3 mg/l BAP & 3 mg/l NAA	10	++
2	MS	0.2 mg/l BAP & 2 mg/l NAA	10	+
3	MS	0.3mg/l BAP & 2 mg/l NAA	10	++
4	MS	0.2 mg/l BAP & 3 mg/l NAA	10	-
5	MS	0.1 mg/l BAP & 1 mg/l NAA	10	+
6	MS	0.1 mg/l BAP & 2 mg/l NAA	10	++
7	MS	0.1 mg/l BAP & 3 mg/l NAA	10	+

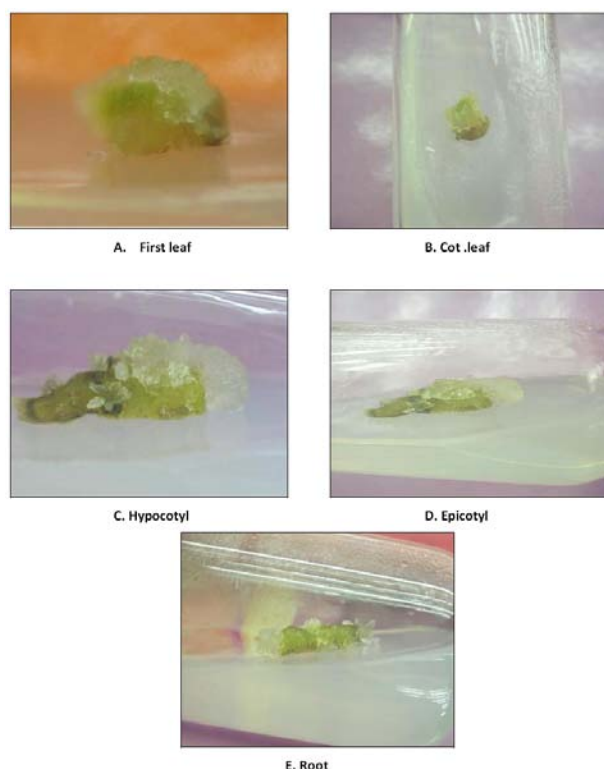
+++ = Good, ++ = Low, + = Poor, - = Absent

**Table 5:** Effect of BAP and NAA on the root of *A. moschatus*

Sr. no.	Medium	Hormone concentration Taken (mg/l)	No. of explants	Callus formation
1	MS	0.3 mg/l BAP & 3 mg/l NAA	10	++
2	MS	0.2 mg/l BAP & 2 mg/l NAA	10	+
3	MS	0.3mg/l BAP & 2 mg/l NAA	10	+
4	MS	0.2 mg/l BAP & 3 mg/l NAA	10	+
5	MS	0.1 mg/l BAP & 1 mg/l NAA	10	-
6	MS	0.1 mg/l BAP & 2 mg/l NAA	10	++
7	MS	0.1 mg/l BAP & 3 mg/l NAA	10	-

+++ = Good, ++ = Low, + = Poor, - = Absent





**Figure 1:** Callus induction from different explants

#### DISCUSSION AND CONCLUSION

Green friable calli were developed from all explants on all seven combinations of BAP & NAA. Callus induction was observed within 8 days and response of callus induction from first leaf was observed between hormone concentrations. As we go to higher hormone concentration to get maximum and early response of callus. Callus was induced more frequently from all the explants except first leaf. There were considerable differences of response between explant types and

hormone concentration in the formation of callus. Finally, the study on callus induction would offer the possibility to select different calli for further investigation on physiological, chemical and pharmaceutical aspects.

#### REFERENCES

1. Verma, DM, Balakrishnan, RR and Dixit, RD. Flora of Madhya Pradesh. Botanical Survey of India, Lucknow, India. 1993 p. 90–191.
2. Srivastava, UC, Chadha, KL and Gupta Rajendra. Advances in Horticulture Vol. 11-Medicinal and Aromatic Plants Malhotra Publ. House, New Delhi, Ambrette seed. 1995 p. 887-897.
3. Rastogi, RP and Mehrotra, BN. Compendium of Indian Medicinal Plants. Vol. II. (1970-1979) Central Drug Research Institute, Lucknow and Publ. and Information Directorate, New Delhi 1991.
4. Agharkar, SP. Medicinal plants of Bombay presidency. Scientific Publ. Jodhpur, India. 1985 First reprint p. 1-2.
5. Lawrence reviews. References Hibiscus Hibiscus abelmoschus Abelmoschus moschatus Medic. 1994.
6. Sharma, R. and Shahzad, A. Thidiazuran (TDZ) induced regeneration from cotyledonary node explants of *Abelmoschus esculentus* (L.) Moench. 2008, p.449-452.
7. Kabir, AH, Sarker, KK, Sharmin, SA, Islam, MS and Alam, MF. Callus induction and plantlet regeneration in *Abelmoschus esculentus* (L.) Moench. 2008, P.193-204.
8. Murashige, T. and Skoog, F. A revised medium for rapid growth and Bioassays with Tobacco tissue culture. Physiology Plant 15, 1962, p. 473-497.

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