IN VITRO PROPAGATION OF HORSERADISH TREE (*MORINGA OLEIFERA* LAM.)

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Abstract

Moringa oleifera is the most widely cultivated species of the moringaceae family, owing to it's to high nutritional and therapeutic values for mankind. Micro propagation, which is a part of plant tissue culture helps to produce large number of plants population in a short time, in order to obtain genetically uniform plants for propagation. The in vitro nodal bud culture establishment was found to be the maximum at BAP @ 2.0 mg/l and induction of multiple shoots, nodal explants were cultured on Murashige and Skoog (MS) agar medium supplemented with various concentration of 6-Benzyl Adenine Purine (BAP) and maintained at 25±2°C in an incubation chamber. Multiple shoots were observed from the nodal explants cultured on MS medium containing 2.0 mg/l BAP at an average of 4.5 shoots per explant and a mean shoot length of 4.84 cm. For *in vitro* root induction, the multiple shoots were cultured on ½ MS medium supplemented with IBA 2.0 mg/l which produced the maximum number of roots (5.4 roots per shoot) with the highest mean root length of 6.3 cm. This study describes the micropropagation protocol for mass production of genetically uniform progenies of moringa which can be used in breeding programme.

Keywords: Moringa, *in vitro*, MS medium, growth regulators

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Introduction

The horseradish tree, the miracle tree also called as ben oil tree / Malunggay, *Moringa oleifera* Lam. Syn. (*M. pterygosperma* Gaertn), is a member of monogenetic family Moringaceae (Suresh Chand *et al.*, 2019), one of the best known species of this family. This plant is native to western sub-Himalayan region of India, Pakistan, Asia and Africa. Now distributed in the Philippines, Cambodia, Americas and the Caribbean islands. It is introduced throughout the Pacific and the Gulf of Mexico, and has been shown to have high adaptation in tropical regions, in extreme climate its growth is slow. Most of the plants have been used in a traditional medicine for the treatment of variety of disorders such as respiratory diseases, hypertension, diabetes, anemia and cancer (Avila Trevino *et al.*, 2016).

The World Health Organization (WHO) has socialized moringa as an alternative food to overcome malnutrition, especially for pregnant and lactating women (Rudiyanto *et al.*, 2011). Moringa has gained significant importance due to the fact that its seed contains 18 to 20 essential amino acids required by the human body. Additionally, it is one of the plant species with a higher seed oil content ranging from 30 to 40% (Cartes and Delaveau, 2014).

Moringa is a drought tolerant fast growing evergreen or deciduous tree that mostly grows up to a height of 10 to 12 meter. The leaves are bipinnate or more commonly tripinnate, up to 45 cm long, alternate and spiral on the twigs (Roloff *et al.*, 2009). Moringa is traditionally propagated by limb cuttings or by seeds. Seed propagated plants show reduced growth rate and vary in phenotype as well as genotype. Vegetative propagation (limb cuttings) is a necessity to obtain uniformity in yield and quality. But propagation by this mode is very slow as the plants need to be cut to a length of 1.0 - 1.5 meter for use as propagule. Trees grown from such limb cuttings are known to have much shorter roots or poor root system (Jahn, 1991). One of the approaches to multiply genetically uniform *Moringa oleifera* species is through tissue culture or micropropagation technique (Stephenson and Fahey, 2004).

Recent applications of micropropagation include not only multiplication and clonal propagation, but also the conservation of germplasm, raising of virus free plant, and achievement of somatic embryogenesis as well as production of synthetic seed, genetically engineered plants, secondary metabolites and other natural derived products from cell suspension and hairy root cultures. As a result, *in vitro* propagation methods are the best alternative for the propagation to achieve genetically uniform plans. This study was intended to optimize *in vitro* propagation protocol of *Moringa oleifera* variety PKM 1.

Materials and methods

Seeds of Moringa cv. PKM 1 were collected from the Department of Vegetable Science, TNAU, Horticultural College and Research Institute, Periyakulam, Tamil Nadu, India.

Raising source of aseptic explant for *in vitro* culture

The seeds were soaked overnight in water and treated for 15 min with 0.1% Tween-20, followed by 20% sodium hypochlorite for 10 min. Then the seeds were rinsed with double distilled water, followed by 70% ethanol for 30 seconds. Later washed thrice with sterile double distilled water to remove traces of ethanol from seeds. Then the seed coats were removed and the decoated seeds were inoculated onto sterilized MS basal medium (Murashige and Skoog, 1962) under laminar airflow chamber. The seed cultures were incubated at 25 ±2°C with relative humidity of 55-65%, light intensity of 2000 lux, under 16 hour light and 8 dark photoperiod.

In vitro culture establishment for shoot induction

The seeds of PKM 1 moringa germinated within 20 days of inoculation and the nodal segments (0.5-1.0 cm) from *in vitro* grown seedlings were excised and cultured onto MS nutrient medium containing 30 g sucrose and 8 g of agar (Hi-media) per litre of media. The pH of the media was adjusted to 5.8. This medium was sterilized in an autoclave for 20 min at 121°C and 15 psi pressure.

In vitro establishment of cultures for shoot induction and mass multiplication

For *in vitro* shoot induction, the experiment was done with five treatments of BAP at different concentrations *viz*., 0.5, 1.0, 1.5, 2.0, 2.5 mg/l and the MS basal medium served as control. For *in vitro* multiple shoot induction, there were nine treatments with BAP at 1.0 and 2.0 mg/l alone and in combination with NAA at 0.2, 0.6, 1.0 mg/l and the MS basal medium served as control treatment.

In vitro root induction from the shoots

For *in vitro* rooting of the shoots cultured, IBA (1.0, 1.5, 2.0 mg/l) and IAA (1.0, 1.5, 2.0 mg/l) at different concentrations were used along with control ½ MS basal medium. The *in vitro* shoot

characters *viz.*, number of shoots and shoot length (cm) were recorded at fortnight intervals and the rooting parameter *viz.* number of roots and length of the roots were recorded 30 days after inoculation in *in vitro* rooting media.

Hardening of rooted plants

Well rooted shoots were transferred for primary hardening to a sterile cocopeat media. The plants were watered and covered with polythene bags with randomly punched holes for better humidity and kept at 26°C for establishment. After development of new flushes, the polythene bags were removed and the plants were taken to shade nethouse for secondary hardening.

Statistical analysis

Statistical analysis was done for the observations recorded in every experiment using SPSS software with completely randomized design. In this study, 10 explants per treatment for each of three replication were used for culture establishment.

Results and Discussion

***In vitro* establishment of cultures for shoot induction**

In the *in vitro* shoot induction from aseptic nodal explants, the explants cultured in the treatment medium (T_5) BAP at 2.0 mg/l responded the best with the maximum number of shoots (4.5) and the shoot length (4.84 cm) (Table 1). This result is in accordance with Shittu *et al.* (2016) who reported that BAP plays vital role in *in vitro* proliferation of nodal segments of moringa, which induced axillary shoots. Sathya Naryana and Varghese (2007) noticed that among the cytokinin, the use of BAP is favoured because of its cost effectiveness and ease to handle. According to Islam *et al.* (2005), BAP was found to be the best for shooting response in *Moringa oleifera*. Thidarat *et al.* (2011) found that the use of BAP at 1 to 2 mg/l gave 100% shoot multiplication rate in moringa. Similarly, findings of Saini *et al.* (2012) indicated that 1 to 2 mg/l of BAP yielding the maximum shoot multiplication rate of 90-94% and number of shoot/explant (8.3 to 9.0 shoots). In contrast, Thiyagarajan and Venkatachalam (2012) observed that increase in concentration of BAP above 3.0 mg/l, leds to decrease in shoot bud induction in *Stevia rebaudiana.*

***In vitro* establishment of cultures for shoot multiplication**

For shoot multiplication, the treatment BAP 2.0 mg/l (SM_6) was observed to be the best with 3.9 number of shoots, with a mean length of 4.4 cm (Table 2).

Mamo and Feyissa (2019) reported that BAP alone and NAA alone resulted in better shoot regeneration than the different concentrations of BAP and in combination with NAA. Drisya *et al*. (2019) observed that the highest response (96%) was observed in 2.5 µM BA and reported that BA seemed to be suitable cytokinin to induce shoot proliferation in *Moringa oleifera* and was in conformity with *Moringa peregina* (Khateeb *et al*., 2013). Huma Fathima *et al.* (2016) found better shoot induction in combination of 0.1 mg/l BAP with 0.5 mg/l NAA in *Moringa concanensis*.

***In vitro* root induction from the shoots**

For *in vitro* root induction and development, half MS basal medium was used as control treatment and IBA at three concentrations (1.0, 1.5, 2.0 mg/l) and IAA three concentrations (1.0, 1.5, 2.0 mg/l) were used along with ½ MS basal medium. Well rooted shoots (5.4 number) were observed in the ½ MS with IBA at 2.0 mg/l (R_4) and the mean root length was 6.3 cm (Table 3). Root formation was noticed on hormone free ½ MS basal medium (R_1).

In this study IBA induced the maximum number of roots per shoot as compared to IAA. The effect of auxin (IBA, IAA) on rooting of shoots was observed. In contrast with the result, Adugna *et al.* (2020) found that the number of roots decreased when the concentration of IBA was increased (2.0 mg/l) in *Moringa stenopetala*. This indicates that low level of auxin is required for root induction.

An increased concentration of auxin leds to suppress the root induction due to a supra optimal concentration. According to the findings of Ridzuan *et al.* (2020), it was observed that MS media fortified with 1.0 mg/l IBA was the most effective in *in vitro* root promotion in *Moringa oleifera*. Based on the findings of the current study, it was found that the addition of 2.0 mg/l IBA to the ½ MS medium led to improved root formation. Some researchers showed that 100% rooting efficiency and maximum number of roots in the medium without any growth regulators (Islam *et al.*, 2005).

This was similar with Forster *et al.* (2013) and further supported by the research of Stephenson and Fahey (2004), who discovered that ½ MS media was the best rooting response. It may be due to the endogenous hormone level already present in the rooted shoots. Similar with the report of Divakaran *et al.* (2006). Suresh Chand *et al.* (2019) ended up the finding that *in vitro* regenerated shoots of moringa had the most roots (86± 0.33%) in enriched WPM with IBA (2.46 µM) followed by

7.33±0.33 % roots formed in enriched WPM with IBA at 1.23 µM.

Hardening

Plantlets were removed carefully from the media without damaging roots and transferred to the sterile cocopeat media. Then covered with randomly punched polythene bags to induce humidity.

References

1. Adugna, A. Y., Feyissa, T. and Tasew, F. S. 2020. Optimization of growth regulators on in vitro propagation of Moringa stenopetala from shoot explants. BMC biotechnology, 20(1), 1-10.
2. Al Khateeb, W., Bahar, E., Lahham, J., Schroeder, D. and Hussein, E. 2013. Regeneration and assessment of genetic fidelity of the endangered tree Moringa peregrina (Forsk.) Fiori using Inter Simple Sequence Repeat (ISSR). Physiology and Molecular Biology of Plants, 19, 157-164
3. Avila-Treviño, J. A., Muñoz-Alemán, J. M., Pérez-Molphe-Balch, E., Rodríguez-Sahagún, A., and Morales-Domínguez, J. F. 2017. In vitro propagation from bud and apex explants of Moringa oleifera and evaluation of the genetic stability with RAMP marker. South African Journal of Botany, 108, 149-156.
4. Cartes, P. and Delaveau, C. 2014. In vitro culture of Moringa oleifera, multipurpose specie for Chile. J. Biotechnol Biomater., 3(5): 307
5. Chand Suresh., Pandey, A. and Verma, O. 2019. In vitro regeneration of Moringa oleifera Lam.: A medicinal tree of family Moringaceae. Indian Journal of Genetics and Plant Breeding, 79(03), 606-613.
6. Divakaran, M., Babu, K. N. and Peter, K. V. (2006). Conservation of Vanilla species, in vitro. Scientia horticulturae, 110(2), 175-180.
7. Drisya Ravi, R. S. Siril, E. A. and Nair, B. R. 2019. The effect of silver nitrate on micropropagation of Moringa oleifera Lam. an important vegetable crop of tropics with substantial nutritional value. Physiology and Molecular Biology of Plants, 25, 1311-1322.
8. Förster, N., Mewis, I., and Ulrichs, C. 2013. Moringa oleifera establishment and multiplication of different ecotypes in vitro. Gesunde Pflanzen, 65(1), 21-31.
9. Huma Fatima, Anjum Perveen and Muhammad Qaiser. 2016. Micropropagation To Rescue Endangered Plant Moringa Concanensis Nimmo (Moringaceae). Pak. J. Bot., 48(1): 291-294
10. Islam, S., Jahan, M. A. A. and Khatun, R. 2005. In vitro regeneration and multiplication of year-round fruit-bearing Moringa oleifera L (No. RESEARCH).
11. Mamo, M., and Feyissa, T. 2019. In vitro regeneration of Moringa Oleifera lam. from leaf explants. SINET: Ethiopian Journal of Science, 42(2), 42-49.
12. Rasool, S., Hameed, A., Azooz, M. M., Siddiqi, T. O. and Ahmad, P. 2013. Salt stress: causes, types and responses of plants. In Ecophysiology and responses of plants under salt stress (pp. 1-24). Springer, New York, NY.
13. Ridzuan, N. I., Abdullah, N., Vun, Y. L., and Supramaniam, C. V. 2020. Micropropagation and defence enzymes assessment of Moringa oleifera L. plantlets using nodal segments as explant. South African Journal of Botany, 129, 56-61
14. Roloff, A., Weisgerber, H., Lang, U. and Stimm, B. 2009. Moringa oleifera Lam., 1785. Sea, 10(10).
15. Rudiyanto, Purwito, S.P, M.Si, Agus & Efendi, Darda and Ermayanti, T. 2021. Growth response of four accessions of Moringa oleifera Linn shoots cultured on various basic media. IOP Conference Series: Earth and Environmental Science. 741. 012054. 10.1088/1755-1315/741/1/012054.
16. Saini, R.K., Shetty, N.P., Giridhar, P., 2012. Rapid in vitro regeneration method for Moringa oleifera and performance evaluation of field grown nutritionally enriched tissue cultured plants. 3 Biotech 2, 187–192.
17. Sathyanarayana, B. N. and Varghese, D. B. 2007. Plant tissue culture: practices and new experimental protocols. IK International Pvt Ltd.
18. Shittu, H. O., Akinboluji, R. J., Odenore, V. D., Igiehon, E., Aghogban, O. N., and Eke, C. R. 2016. In vitro callogenesis of Moringa oleifera Lam. using seed explant. African Scientist, 17(1), 33-40.
19. Stephenson, K. K. and Fahey, J. W. 2004. Development of tissue culture methods for the rescue and propagation of endangered Moringa spp. germplasm. Economic botany, 58(1), S116-S124.
20. Thiyagarajan, M. and Venkatachalam, P. 2012. Large scale in vitro propagation of Stevia rebaudiana (Bert) for commercial application: pharmaceutically important and antidiabetic medicinal herb. Industrial Crops and Products 37,111-117

Table 1: Impact of different concentration of BAP on establishment of nodal explants

Treatments	Treatment details (mg/l)	Mean number of shoots	Mean shoot length (cm)
T_1	MS Basal:(Control)	1.16^{f}	2.05^{f}
T_2	MS+ 0.5 BAP	2.01^{e}	2.34^{e}
T_3	MS+ 1.0 BAP	3.67^{b}	4.13^{b}
T_4	MS+ 1.5 BAP	2.87^{d}	2.59^{d}
T_5	MS+ 2.0 BAP	4.50^{a}	4.84^{a}
T_6	MS+ 2.5 BAP	3.22^{c}	3.56^{c}
	S.Ed	0.0332	0.0401
	CD at 5%	0.1025	0.1235

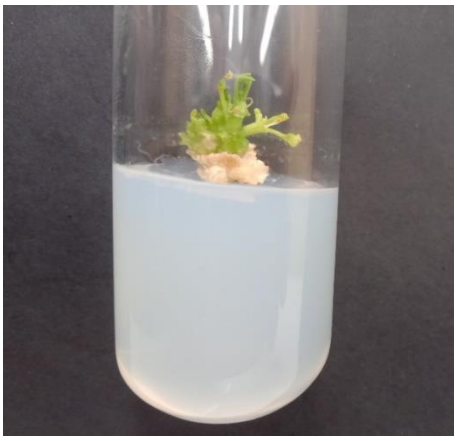
Table 2: Impact of BAP and NAA on *in vitro* shoot multiplication

Treatments	Treatment details (mg/l)	Mean number of shoots	Mean shoot length (cm)
SM_1	MS Basal:(Control)	1.05^{h}	2.00^{g}
SM_2	MS+ 1.0 mg/l BAP	1.63^{g}	2.29^{f}
SM_3	MS+ 1.0 mg/l BAP + 0.2 mg/l NAA	3.17^{b}	3.86^{b}
SM_4	MS+ 1.0 mg/l BAP + 0.6 mg/l NAA	2.75^{c}	3.45^{c}
SM_5	MS+ 1.0 mg/l BAP + 1.0 mg/l NAA	2.22^{f}	2.40^{f}
SM_6	MS+ 2.0 mg/l BAP	3.93^{a}	4.46^{a}
SM_7	MS+ 2.0 mg/l BAP + 0.2 mg/l NAA	2.81^{c}	3.35^{c}
SM_8	MS+ 2.0 mg/l BAP + 0.6 mg/l NAA	2.49^{e}	3.19^{d}
SM_9	MS+ 2.0 mg/l BAP + 1.0 mg/l NAA	2.63^{d}	2.98^{e}
	S.Ed	0.041	0.046
	CD at 5%	0.122	0.138

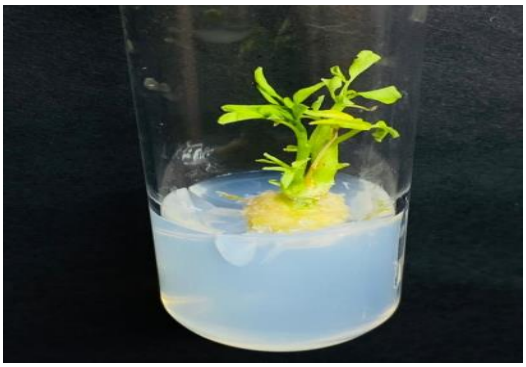
Table 3: Impact of IBA and IAA on *in vitro* rooting of shoots

Treatments	Treatment details(mg/l)	Mean number of roots	Mean root length (cm)
R_1	½ MS Basal:(Control)	3.21e	2.02^{f}
R_2	½ MS + 1.0 mg/l IBA	4.87c	4.43^{c}
R_3	½ MS + 1.5 mg/l IBA	5.10b	4.45^{b}
R_4	½ MS + 2.0 mg/l IBA	5.40a	6.33^{a}
R_5	½ MS + 1.0 mg/l IAA	2.62f	1.42^{g}
R_6	½ MS + 1.5 mg/l IAA	3.34e	2.62^{e}
R_7	½ MS + 2.0 mg/l IAA	3.92d	3.26^{d}
	S.Ed	0.0607	0.0718
	CD at 5 %	0.184	0.218

a) Bud break of inoculated nodal explant



b) Multiple shoot proliferation (BAP @ 2.0 mg/l)



c) Growth of the multiple shoot

d) *In vitro* rooting of shoot

e) Plantlet under primary hardening