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MICROPROPAGATION TO RESCUE ENDANGERED PLANT *MORINGA CONCANENSIS* NIMMO (MORINGACEAE)

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Abstract

Efficient micropropagation was developed for an endangered plant; *Moringa concanensis* Nimmo. The plant has many medicinal properties. It is an antioxidant, anti-diabetic and a powerful tonic alternative. A high frequency and maximum number of shoots were produced in MS supplemented with the combination of Benzylaminopurine (BAP) and α-Naphthalene Acetic Acid (NAA) (0.10mg/l +0.05mg/l). Rooting was achieved by the inoculation of regenerated shoots on half strength MS without addition of hormone.

Key words: *Moringa concanensis*, Micropropagation, Endangered medicinal plant, Tissue culture.

Introduction

Moringa concanensis Nimmo; commonly known as Muhua by the local people of Sindh, belongs to the family Moringaceae. It is an important medicinal plant. It grows wild in Pakistan (Tharparkar) and India (Rajhistan). In Pakistan it is rarely present in some areas of the Mithi, Nagarparkar, Diplo, Chachro, Digri) of Tharparker (Sindh province) (Manzoor *et al*., 2007). Plant is threatened with prolong drought and poor soil conditions. Now it is an endangered species in Tharparker and likely to be extinct from this area. *M. concanensis* is a small tree with alternate, bipinnate leaves having small pedicelate yellow flowers with pink or red veins. The fruit is a linear pod, 35–40 cm long (Qaiser, 1973). The plant is used in pharmaceutical and cosmetic formulations. It has a long history of application in traditional medicine. All plant parts such as stem, leaves, seeds and flowers are very important in local and Unani traditional medicine. They are powerful antioxidants. The roots of this species are used most commonly in medicine as substitute for *M. oleifera*. They are used to relieve spinal cord pain. The leaves of the plant are used to prevent sore eye, reduce blood pressure, and treat diabetes, jaundice and some skin tumors. The flower is given to cure thyroid problem and the dried powdered seeds of the species is used internally for intestinal worms (Anbazhakan *et al*., 2007). Plant tissue culture offers recovery of disease free clones and preservation of valuable germplasm**.** The present study was performed to establish an efficient and reliable protocol for the conservation of *M. concanensis* through tissue culture.

Materials and Methods

Explant cleaning and treatment: Healthy plant twigs, having 8-10 leaves and stem length of 4-6 cm were collected from the Botanical Garden, Centre for Plant Conservation, University of Karachi and selected as the source of explant.

The twigs were systematically washed under running tap water. The materials were then given a quick rinse with 70% ethanol followed by washing with a dish washing liquid for 3-4 minutes. Later they were sterilized with 10% commercial bleach (Sodium hypochlorite solution, NaOCl) for 15 minutes in a laminar air flow chamber (Techno Scientific Lahore, Pakistan), to avoid any contamination. Finally the inoculums were thoroughly rinsed thrice with sterile distilled water.

Initiation: Nodal segments which were taken as explant were cultured on MS (Murashige & Skoog, 1962), supplemented with 3% (w/v) sucrose and solidified with 0.8% agar (w/v). The pH was adjusted to 5.8 before sterilization by autoclaving at 121 ^{o}C for 20 minutes.

Multiplication: After successful initiation the shoots were transferred in MS supplement with two cytokinin [6-Benzylaminopurine (BAP) and Kinetin (Kin)] and combination of two cytokinin with an auxin NAA (alpha Naphthalene acetic acid) for shoot multiplication. In the first set of experiment, 5 different concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 mg/L) of each hormone were tested. In the second set, 0.05mg/l of NAA (auxin) was combined with different concentrations (0.05, 0.10, 0.20, 0.30, 0.40 and 0.50 mg/L) of each cytokinin.

The shoots were also cultured on hormone free MS media, which were used as control in experiment. Each treatment was replicated three times. Number of shoots formed and the length of shoots were determined after 40 days.

Rooting: The isolated shoots (3-4cm) with three to four pairs of leaves were transferred to MS medium in half strength without addition of hormones. Full strength MS and MS medium supplement with the hormone NAA and IBA (Indol-3 butyric acid) in different concentrations (0.10, 0.20, 0.30, 0.40 and 0.50 mg/L) for production of healthy roots were also tested.

Acclimatization: Well-developed plantlets with 4-6 leaves and roots were removed from the culture medium, washed gently under running tap water and transferred to the soil for acclimatization. The plantlets were enclosed in plastic bags to avoid dryness and maintain humidity. The plastic bags were removed after a week and the plants were transferred to the nursery and finally to their respective habitat.

Results and Discussion

The results indicated that the MS medium supplemented with BAP in the concentration ranging from 0.1 mg/L - 0.3 mg/L, produced large number of shoots in addition to longer shoots of an average height of 8.16 cm (Table 1, Fig. 1). However, in the case of kinetin, nodal explants responded best in 0.3 mg/L Kin and showed maximum number and the highest shoots of 4.5cm long with 5.66 shoots/explants (Table 2, Fig. 2). The combination of cytokinin and auxin, that is the combination of 0.1 mg/L BAP and 0.5 mg/L NAA were considered as the best hormone for better proliferation with 19.66 shoots with an average length of 8.16 cm (Table 3, Fig. 3). The control medium also showed multiple shoot development but they were smaller in size (Table 1. Fig. 4).

Among the 4 treatments given to the shoots of *M. concanensis* for root development, the half strength MS without addition of any hormone proved to be the best media for root formation and produced around 5 roots of 13.3 cm in length (Table 4).

Well rooted plantlets were transferred to plastic bags with soil-soil with manure and peat moss, acclimatization observed in all cases (Fig. 5). About 50% of plantlets showed normal growth after 15 days of acclimatization.

Table1. Effect of BAP on nodal explants of *M. concanensis*.

S. No.	Concentration of BAP (mg/L)	Average number of shoots[a]	Average shoot length[a] (cm)
1.	MS	6.33 ± 1.33 abc	4.43 ± 0.23 bc
2.	½ MS	2.83 ± 0.16 c	2.86 ± 0.46 c
3.	MS + 0.1 BAP	16.0 ± 2.5 a	6.86 ± 0.69 a
4.	MS + 0.2 BAP	5.0 ± 1.15 bc	8.16 ± 0.60 a
5.	MS + 0.3 BAP	14.33 ± 3.9 ab	6.0 ± 0.77 ab
6.	MS + 0.4 BAP	12.00 ± 4.3 abc	4.26 ± 0.37 bc
7.	MS + 0.5 BAP	7.0 ± 4.04 abc	4.26 ± 1.42 bc

Table2. Effect of Kinetin on nodal explants of *M. concanensis*.

S. No.	Concentration of Kin (mg/L)	Mean number of shoots[a]	Mean length of shoot [a] (cm)
1.	MS + 0.10 Kin	3.83 ± 0.66 b	3.5 ± 0.23 ab
2.	MS + 0.20 Kin	4.66 ± 0.66 ab	3.03 ± 0.82 ab
3.	MS + 0.30 Kin	5.66 ± 0.17 a	4.5 ± 1.44 a
4.	MS + 0.40 Kin	1.76 ± 0.14 c	0.9 ± 0.66 b
5.	MS + 0.50 Kin	0.33 ± 0.33 d	0.75 ± 0.75 b

Values with same letters in same column are not significantly different ($p<0.05$) Using Duncan's multiple range test,
[a] values are means ± standard error

Table3. Effect of combination of hormones on nodal explants of *M. concanensis*.

S. No.	Media concentration (mg/L)	Average number of shoots [a]	Average length of shoots [a] (cm)
1.	MS + 0.05 NAA + 0.05 BAP	5.33 ± 1.45 cde	8.33 ± 1.87 a
2.	MS + 0.05 NAA + 0.10 BAP	19.66 ± 2.60 a	8.16 ± 1.30 a
3.	MS + 0.05 NAA + 0.20 BAP	19.33 ± 2.96 a	8.00 ± 1.52 a
4.	MS + 0.05 NAA + 0.30 BAP	20.66 ± 1.20 a	6.16 ± 1.87 a
5.	MS + 0.05 NAA + 0.40 BAP	14.75 ± 5.08 ab	5.00 ± 1.95 b
6.	MS + 0.05 NAA + 0.50 BAP	7.33 ± 0.33 cd	5.66 ± 1.45 a
7.	MS + 0.05 NAA + 0.10 Kin	11.00 ± 1.15 bc	5.00 ± 1.73 b
8.	MS + 0.05 NAA + 0.20 Kin	10.33 ± 0.88 bcd	6.33 ± 1.57 a
9.	MS + 0.05 NAA + 0.30 Kin	10.50 ± 1.44 bcd	5.83 ± 1.45 a
10.	MS + 0.05 NAA + 0.40 Kin	5.0 ± 0.57 cde	4.73 ± 1.43 b
11.	MS + 0.05 NAA + 0.50 Kin	0.00 ± 00	00 ± 00

Table 4. Effect of different growth regulators on the *In vitro* rooting of *M. concanensis*.

S. No.	Media composition (mg/L)	Mean length [a] (cm)	Mean number of roots [a]
1.	½ MS	13.33 ± 2.90 a	5.16 ± 0.44 a
2.	MS	10.66 ± 2.33 ab	6.3 ± 0.88 a
3.	MS + 0.1 NAA	8.00 ± 1.15 bc	3.06 ± 0.47 b
4.	MS + 0.2 NAA	5.83 ± 0.16 cd	1.96 ± 0.26 bc
5.	MS + 0.3 NAA	5.16 ± 0.60 cd	1.00 ± 0.00 c
6.	MS + 0.4 NAA	1.53 ± 0.86 d	0.66 ± 0.33 c
7.	MS + 0.5 NAA	2.00 ± 00 d	0.33 ± 0.33 c
8.	MS + 0.1 IBA	4.60 ± 0.20 cd	2.0 ± 0.57 c
9.	MS + 0.2 IBA	3.26 ± 1.63 d	1.33 ± 0.66 c
10.	MS + 0.3 IBA	7.70 ± 0.15 bc	3.00 ± 0.57 b
11.	MS + 0.4 IBA	3.13 ± 0.59 d	1.66 ± 0.66 bc

Fig. 1. Shoot formation in MS with 0.3mg/L BAP.
Fig. 2. Shoot formation in MS with 0.3mg/L Kinetin.
Fig. 3. Shoot formation in MS with 0.1 mg/L BAP and 0.5 mg/L NAA.
Fig. 4. Shoot formation in MS without hormone.
Fig. 5. Acclimatized plant.

Results of many micropropagation studies are quite similar to our findings. Goswani *et al*. (2013), while working on nodal explant of *Citrus limon* L, observed maximum shoot formation on low level of BAP. Similarly Norrizah *et al*. (2008) in their studies, found that 0.5 mg/L of BAP appeared as the best for regeneration of *Pogoestemon cablin* shoots from nodal segment. They also reported that the high concentration of BAP resulted

in reduced height and number of shoots and shoot became stunted in the concentration of 2 mg/L of BAP.

It was observed that the most effective hormone found during study was a combination of BAP and NAA. The effect was also analyzed by Daneshvar *et al*. (2013), they found maximum shoot proliferation in the combination of 0.2mg/L BAP with 0.15mg/L NAA in *Aloe vera* L. Duhoky & Rasheed (2010), also explained increased role of cytokinin in the presence of auxin in *Gardenia jasminoides.* A combination of BAP and NAA was also recognized effective in shoot regeneration of *Lathyrus sativus* (Barik *et al*., 2005). Among the four different treatments given to *M. concanensis* shoots for root induction, ½ M S and full MS were found to be most appropriate for root development. Our results were in agreement with Marfori (2011) who also found best rooting of *M. olifera* in a hormone free medium.

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