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In vitro regeneration of *Mirabilis jalapa* L.

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Abstract

In vitro regeneration of *Mirabilis jalapa* L., an important medicinal plant, has been successfully established from nodal explants. Maximum numbers of shoots (8.1) were obtained using Murashige and Skoog (MS) medium containing BAP (3.0 mg/l). Next to the highest response by nodal explants of *M. jalapa* was shown on MS medium supplemented with 2.0 mg/l BAP +1.0 mg/l IAA + 1.0 mg/l Kn. In it, the mean value of shoot number was 7.3. The highest frequency of root induction (80%) were obtained on MS medium supplemented with IAA (0.5 mg/l) and 40 ml/l coconut water. The rooted plantlets were transferred for hardening following acclimatization and finally were successfully planted in the field.

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Introduction

Mirabilis jalapa L. (Fam.: Nyctaginaceae) is commonly known as 'four o'clock plant' produces beautiful flowers that usually open at around 4 o'clock in the afternoon. It is a popular ornamental plant grown worldwide for the beauty of its flowers (which can be white, pink, yellow, or multicolored), and having a sweet fragrance (Siddiqui *et al*., 1990). It is a perennial herb, which reaches a height of 50-100 cm from the tuberous root. Apart from its ornamental value, it has also medicinal properties and is used in the herbal medicine practices around the world. The plant has successful uses for the treatment of diarrhoea, dysentery, conjunctivitis, edema, swellings, muscular pain and abdominal colics (Daniel, 2006; Holdsworth, 1992). And its leaf extract has antibacterial, antiviral, and antifungal activities. It is also used to control viruses and yeast. *M. jalapa* is rich in many active compounds of which, most important compound found by researchers is a group of amino acid-based proteins, called mirabilis antiviral proteins (Wang *et al*., 2002). This plant also contains alanine, alpha-amyrins, arabinose, beta-amyrins, brassica-sterol, beta-sitosterols, campesterol, stigma-sterol, tartaric acid, trigonelline etc. (Kataoka *et al*., 1992).

Conventionally, *M. jalapa* reproduces via viable seed but low percentage (50%) of seed viability and the hawk-moth populations are the only species that act as its pollinator thus limiting its natural propagation (Rio and Burquez, 1986). In this context, an alternative way for large scale propagation is tissue culture, a well-known biotechnological tool for the mass propagation of rare, endangered and threatened medicinal plants which are facing the danger of extinction. Though *M. jalapa* currently is not under any risk of being threatened in Bangladesh, yet to ensure any mass scale propagation for extracting medicinal ingredients, tissue culture can be implemented. By using plant tissue culture technique, genetically similar plantlets can be produced in relatively short time. Besides, by maintaining genetic stability, tissue cultured plants are also valuable in speeding up conventional breeding and propagation, reducing space, labor requirement and achieving manipulative goals that cannot be carried out via *in vivo* conditions. *In vitro* propagation

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is also a powerful tool for the production of medicinal secondary metabolites as well as for the purpose of conservation (Nadeem *et al*., 2000) and commercialization. There are very limited reports on *in vitro* regeneration of *M. jalapa* (Zaccai *et al.*, 2007; Xu *et al*., 2005). So, to develop an *in vitro* regeneration protocol for this plant is urgent. Therefore, the present study was conducted to develop an efficient protocol for *in vitro* regeneration of *M. jalapa* using nodal explants.

Material and methods

The present experiment was conducted in Bangladesh Council of Scientific and Industrial Research (BCSIR), Chattogram, Bangladesh. Nodal explants with axillary buds of *M. jalapa* were collected from BCSIR research field. Nodal segments (1-2 cm long) without leaves were surface sterilized initially with mild detergent and washed with tap water for 30 min. After that, explants were surface sterilized with 70% (v/v) ethanol for 1 min, followed by 0.1% $HgCl_2$ by gentle shaking for 8-10 min, and rinsed four times with autoclaved distilled water. Isolated explants were cultured on MS media (Murashige and Skoog, 1962) containing BAP, NAA, IAA and Kn singly or in combinations for *in vitro* regeneration of shoots. MS media supplemented with coconut water in combination with hormones were also used for shoot regeneration. *In vitro* regenerated shoots were sub-cultured regularly to fresh medium at an interval of 12 - 15 d for further multiplication. Elongated shoots were separated and cultured on rooting medium for root formation. About 2-3 cm long shoots were separated and cultured on rooting medium containing full strengths of MS without hormonal supplement or with different concentrations of IAA and IBA. All *in vitro* grown cultures were maintained under illumination on a 12 h photoperiod at 25 ± 2°C. The plantlets with sufficient root system were then transplanted to small plastic pots containing sterilized soil for their establishment. Twenty plantlets were transplanted into small plastic pot for hardening. After 30 days, the acclimatized plants were transferred to larger pots containing of sterile peat and sand in a ratio of 1:1 and the acclimatized plantlets were watered and fertilized as needed.

Results and discussion

For *in vitro* regeneration of shoots, nodal explants were used in this experiment. Different concentrations and combinations of BAP, IAA, Kn and NAA were used in MS medium to determine the optimum media composition for initiation and development of multiple shoots from node explants of *M. jalapa.* Among the media components used in this study, the highest (80%) percentage of response was obtained on MS medium supplemented with 3.0 mg/l BAP using nodal explants (Table I). Initiation of shoots were found within 7-11 days of inoculation (Fig. 1a). The mean number of shoots/explants was found 8.1 (Table I). The initiation of multiple shoots (Fig. 1b) was obtained after one week of culture. For further multiplication, regenerated shoots were sub-cultured on the same media. At this stage the shoots were found to proliferate (Fig. 1c) in same media combination (MS+ 3.0 mg/l BAP). Elongation of such shoots were obtained after 40 days of culture (Fig.1e). Ling *et al.*(2009) also reported that different concentrations of BAP (0.25 -20.00 µM) showed best response for callus and shoot induction in *M. jalapa* from leaf explants. Banu *et al.* (2017) observed better response in *Gynura procumbens* (Lour.) Merr. on MS with 3.0 mg/l BAP from nodal explants. Khan *et al.* (2018) reported that best callus induction and multiple shoots formation was found on MS with 2.0 mg/l BAP in *R. serpentina.* According to Gaber (2015), combinations of 3.0 mg/l Kn and 0.25 mg/l NAA showed highest response for multiple shoot induction in *M. jalapa.*

Table I. Responses of explants on MS medium supplemented with different concentrations and combinations of hormone (mg/l) for *in vitro* regeneration of shoots of *M. jalapa*

Hormonal combination (mg/l)				Responses of explants (%)	Days required to get response	Mean no. of shoots/explants
BAP	IAA	Kn	NAA			
0.5	-	-	-	-	-	-
1	0.2	-	-	30	4-6	1.7
1	-	0.2	-	-	-	-
1			0.2	27	9-11	2.5
1.0	-	0.5	-	52	11-15	5.0
2	0.5	-	-	40	8-9	5.5
2	-	-	0.5	60	5-11	5.8
2	1	1	-	70	5-13	7.3
2		1	1	64	8-10	6.5
3	-	-	-	80	7-11	8.1

Fig. 1(a-f). Different stages of *in vitro* regeneration of *Mirabilis jalapa*. a) Initiation of shoot from node explants on MS medium supplemented with 3.0 mg/l BAP, b) Multiple shoot formation from the node explants on the same media after one week of culture, c) Proliferation of multiple shoots after two weeks of culture on MS + 3.0 mg/l BAP, d) Elongation of shoot on the same media after 40 days of culture, e) Root formation on MS supplemented with IAA (0.5 mg/l) and 40 ml/l coconut water, f, g)The regenerated plantlets were successfully hardened and shifted to the plastic pot

Next to the highest response, (Table I) nodal explants of *M. jalapa* also showed better response on MS medium supplemented with 2.0 mg/l BAP +1.0 mg/l IAA + 1.0 mg/l Kn. The percentage of responsive explants was 70% (Table I). Shoot initiation was observed within 5 - 13 days of inoculation for nodal explants. The mean number of shoot was found 7.3 (Table I). Xu *et al.* (2005) also reported that MS medium in combination with 1.0 mg/l of IAA showed good response in shoot induction of *M. jalapa.* Rani *et al.* (2014) also showed that the best shoot elongation of *R. serpentina* was found on MS media supplemented with 3.0 mg/l IAA+ 3.0 mg/l BAP. Khan (2017) demonstrated that best shoot proliferation response was observed from nodal explants in MS medium with a combination of 1.0 mg/l BAP and 1.0 mg/l IAA in *Piper nigrum* L.

Combinations of 2.0 mg/l BAP + 1.0 mg/l Kn + 1.0 mg/l NAA also showed good response from the node explants of *M. jalapa*. In the same media combination as mentioned above, 64% nodal explants showed response with mean value 6.5 shoots (Table I). Gaber (2015) reported combinations of BAP, Kn, NAA with MS medium showed best response for shoot multiplication and callus induction of *M. jalapa*. Goswami *et al.* (2018) found responses on shoot regeneration of *B. juncea* in combination of BAP and NAA using hypocotyl as explants. Banu *et al.* (2017) observed better response in *G. procumbens* on MS with BAP and Kn supplemented medium. Pandiyan and Selvaraj (2012) showed that 1.0 mg/l BAP, 1.0 mg/l Kn and 1.0 mg/l NAA was the most suitable combination for obtaining maximum number of shoots from *Bacopa monnieri* (L.) Pennell.

Root induction is an essential step after elongation of multiple shoots. After 40 days, regenerated shoots were isolated and used for root induction. It was tried in a number of experiments. MS media with various concentrations and combinations of IAA, IBA (mg/l) were tried first for root initiation. But no response was observed. To get better response MS media with various concentrations and combinations of IAA (mg/l) with coconut water (ml/l) were used. Among all combination MS medium with 0.5 mg/l IAA+ 40 ml/l coconut water showed best rooting response, which required 7-14 days for root formation (Fig. 1e). Coconut water contains different types of plant growth regulator and mineral (Yong *et. al*., 2009) which may be responsible for growth of the plant. Mukarlina *et al.* (2010) also reported that coconut water in combination with NAA was found to be effective in root induction in case of *Paraphalaeonopsis serpentilingua* (J. J. Sm.) A. D. Hawkes. Gaber (2015) also reported that auxin was effective for root induction in *M. jalapa*. Jain *et. al.* (2003) reported best rooting of *R. serpentina* by supplemented MS medium with 0.5 mg/l IBA. After sufficient development of roots, rooted plantlets were transferred to plastic pots having soil and kept in culture room (Fig.1 f, g). After hardening, plants were successfully planted into lager plastic pots. For their further growth and establishment the survived plantlets were transferred to small field plots.

Conclusion

It can be concluded that, the present *in vitro* regeneration protocol of *M. jalapa* is efficient and reproducible. This regeneration protocol reported in the present study can be used for the large scale production of this medicinal plant within a short time and to yield important secondary metabolites for pharmaceutical industries with a range of further biotechnological applications.

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