

Rapid *in vitro* Multiplication, Callogenesis and Indirect Shoot Regeneration in *Ipomoea Mauritiana* – A Rare Medicinal Plant in Bangladesh

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Abstract

Giant potato is a type of morning glory plant belongs to the family Convolvulaceae. It grows as a vine and it has a large glabrous twinner with tuberous root. In India tubers of *Ipomoea mauritiana* that are used as Vidari and many of the Ayurvedic industries use Vidari in popular Ayurvedic nutraceuticals products. The climbing plant produces fruits and seeds but during growing season seeds are mostly failed to germinate. In vitro propagation by tissue culture is invariably needed and under the present investigation efforts are made to develop the protocol for its direct and indirect methods of in vitro regeneration. Nodal and shoot tip explants were cultured on MS medium containing different concentrations (0.5 mg/l–2.0 mg/l) of BAP, Kn and IAA used alone and in different combinations. The media having 1.0 mg/l BAP with 0.5 mg/l IAA proved most ideal showing 95% direct shoot regeneration and highest number of shoot per culture was 6.0. In case of callus induction MS medium supplemented with various concentrations (0.5, 1.0, 2.0 mg/l) of 2, 4-D used alone and in combinations with different concentrations (0.5, 1.0, 2.0 and 3.0 mg/l) of BAP or Kn. Profuse callus induction (85%) was achieved in the media having 1.0 mg/l 2,4-D + 1.0 mg/l BAP in intermodal explants. But in case of callus regeneration higher concentration of BAP (1.0 mg/l) and a lower concentration of NAA and IAA (0.2 mg/l) proved most suitable and highest 30% shoot regeneration was obtained from callus in the media having 1.0 mg/l BAP with 0.2 mg/l IAA.

Keywords: *Ipomoea mauritiana*; In vitro propagation; Medicinal plant; Plant growth regulators

Introduction

Giant potato is a type of morning glory plant belongs to the *Ipomoea* genus. The origin of *Ipomoea mauritiana* is unknown but it is naturalized in many parts of the world including Africa, Australia, Tropical America and it is there all over the tropics. Within India, it has been recorded in the moist tropical region. It grows as a vine. Leaves are 10-15 cm long, deeply palmately divided into 5-7 segments and each lobe is complete with a midrib and lateral veins. Flowers are few to many in axillary or terminal cymes and they are pink or reddish purple with a darker center giving funnel form corolla. Capsules are ovoid and 8-13 mm long. Its underground tuberous root is the most important part for its commercial use.

In India the root has been used from ancient Sanskrit times. It is considered tonic, alterative, aphrodisiac, demulcent, galactogogic and cholagogic [1]. It is recommended for emaciation in children and put into a compound decoction which is nutritive, diuretic, expectorant and useful in fevers and bronchitis [2]. In India tubers of *Ipomoea mauritiana* that are used as Vidari and many of the Ayurvedic industries use Vidari in popular Ayurvedic nutraceutical products [3, 4]. Vidari is also an important component of the popular ayurvedic formulation *Chyavanaprasha* [5] and used in more than 45 formulations of Ayurveda [6].

In Bangladesh *Ipomoea mauritiana* grows as a perennial vine mostly climbing on some big trees but their underground tuberous root is very valuable in herbal market of the country. The plant has been marked as one of the threatened medicinal plant species in the country deserving priority in advance research for exploring the modern avenues of biotechnology particularly towards its development and conservation. Under present investigation efforts have been made to develop *in vitro* regeneration protocol of Bangladeshi cultivar of *Ipomoea mauritiana* for its micro propagation and conservation.

Materials and Methods

Young leaf, shoot tip, node and internode explants were collected from six months old field grown plants of *Ipomoea mauritiana*. Surface sterilization was done separately according to the explant types with Tween-20 and 0.1% HgCl₂. Explants were then cut into appropriate size (1.0-2.0 cm) and cultured in the Murashige and Skoog (MS) medium containing 3.0% sucrose (w/v), 0.8% agar and different concentrations and combinations of plant growth regulators (PGR) for induction of direct and indirect organogenesis. Both auxins (NAA, IAA, IBA and 2,4-D) and cytokinins (BAP and Kn) supplements were used in the media either single or in combinations. The pH of the medium was adjusted to 5.8 ± 1.0. Cultures were grown at 25°C temperature and 16 h/8h light period.

Results and Discussion

In vitro multiplication of plants from nodal and shoot tip explants

Nodal and shoot tip explants were inoculated from actively growing shoots of field grown plants and cultured on MS medium containing different concentrations (0.5 mg/l – 2.0 mg/l) of BAP, Kn and IAA under single application. BAP and Kn (1.0 mg/l) were also used in combination with NAA or IAA (0.2, 0.5 mg/l) under combined

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application. After four weeks of culture, in case of BAP, Kn and IAA, the nodal and shoot tip explants sprouted and developed into 1-4 vigorously growing shoots and attained a length of approximately 4-6 cm. The development of 3-4 shoots from a single node indicated that the original bud might have been induced to divide and produce more than one shoots being influenced by growth hormones. The results are presented in Figure 1. Highest percentage of shoot induction was 95% recorded in the media having 1.0 mg/l BAP with 0.5 mg/l IAA (Figure. 1) and the highest number of shoot per culture was 6.00 ± 0.23 and highest length of shoot per culture was 7.5 ± 0.15 cm. Different stages of shoot formation was shown in Plate 1 showing shoot growth after 2 weeks of culture (A), shoot growth after 2 subcultures (B,C), shoot elongation after 8 weeks of culture (D,E,F). Maximum shoot elongation was reported by Majumder et al. [7] in another medicinal plant *Scoparia dulcis* in the same media composition on MS with 1.5 mg/l BAP + 1.0 mg/l IAA. Highest 90% shoot regeneration was also reported by Verma and Singh [8] in another medicinal plant *Acorus calamus* L in this media combination. But combination of BAP with IAA was proved superior to the combination of BAP with NAA in shoot regeneration. Base callusing was observed in many concentrations.

Callogenesis

Aseptic leaf and internodal explants of *Ipomoea mauritiana* were inoculated on MS medium supplemented with various concentrations (0.5, 1.0, 2.0 mg/l) of 2, 4-D used alone. The hormone 2,4-D (1.0 mg/l) was also used in combinations with different concentrations (0.5, 1.0, 2.0 and 3.0 mg/l) of BAP or Kn for callus induction. Cultures were maintained under 16 h light and 8 h dark regime and data on different parameters were recorded and presented in Figure 2. Callus proliferation was not noticed in all media formulations. The rate of callus formation was slow in the first two weeks but within four weeks they showed a very good growth and covered the entire surface of explants. The highest percentage (85%) of callus induction was observed in the media having 1.0 mg/l 2,4-D + 1.0 mg/l BAP in nodal explants (Plate 2. A,B,C). Enhancement of callus growth was observed when 1.0

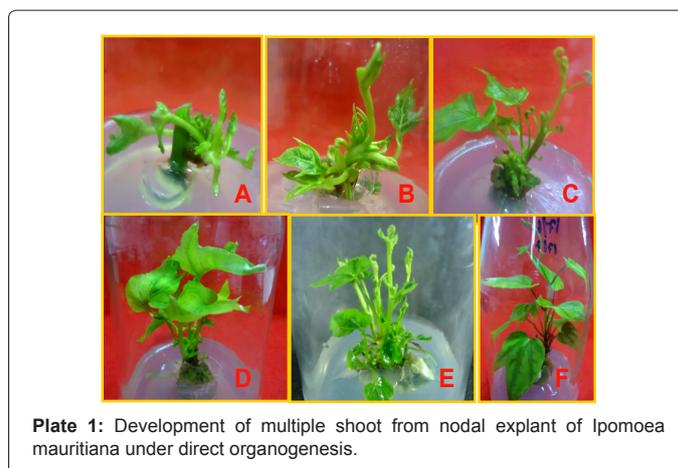


Plate 1: Development of multiple shoot from nodal explant of *Ipomoea mauritiana* under direct organogenesis.

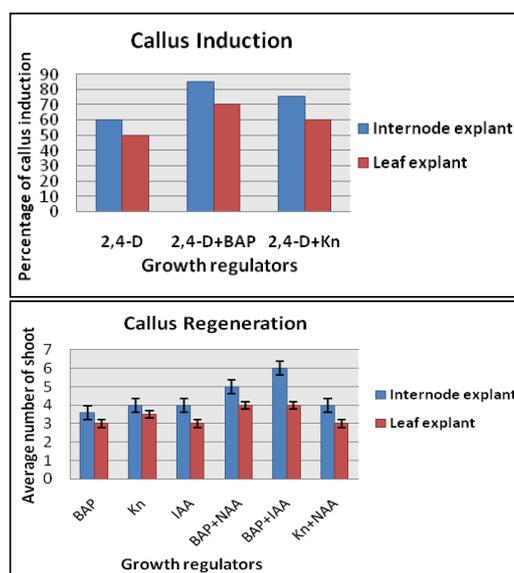


Figure 2: Percentages of callus induction and average number of shoot regeneration from internode derived callus under different concentrations of phytohormones.

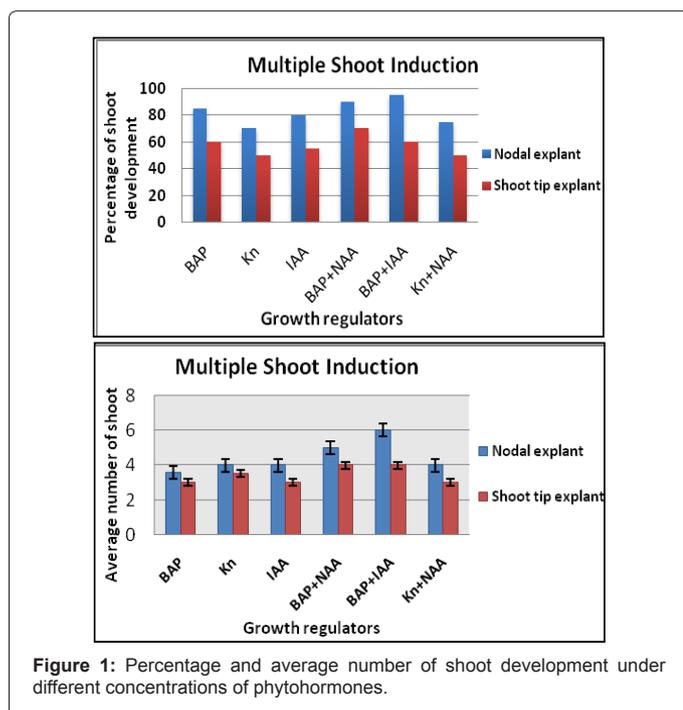


Figure 1: Percentage and average number of shoot development under different concentrations of phytohormones.

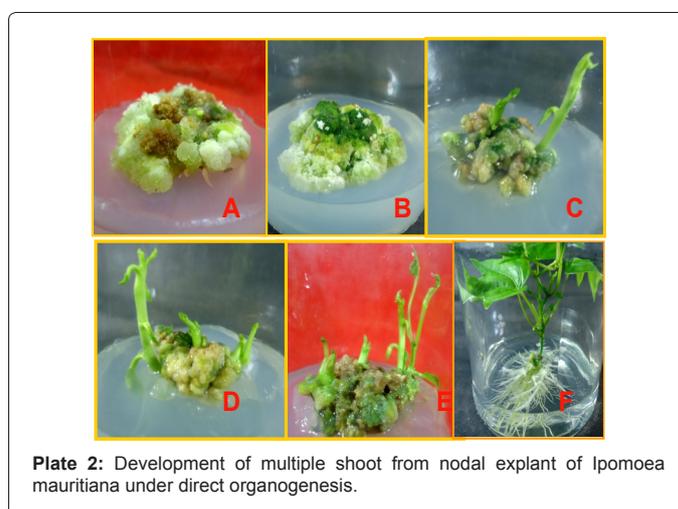


Plate 2: Development of multiple shoot from nodal explant of *Ipomoea mauritiana* under direct organogenesis.

mg/l 2,4-D was used with lower concentrations of BAP or Kn (0.5, 1.0 mg/l). Within six weeks of culture the callus turned green in colour and compact in texture and meristemoid like structures were also developed on the callus indicating its organogenic nature. Better callus induction was obtained by Wong and Taha [9] when the leaf and stem explants cultured on 1.0 mg/l 2,4-D and 1.0 mg/l BAP giving 100% response in an medicinal plant *Allamanda cathartica*. Leaf explant appeared to be best of all for callus length and weight when 1.0 mg/l 2,4-D + 0.25 mg/l BAP was used in potato reported by Haque et al. [10]. Similarly, Pathi et al. [11] observed the compact granular organogenic callus formation (85% frequency) on MS medium supplemented with 2.5 mg/l 2,4-D and 1.5 mg/l BAP under light conditions in *Zea mays* L.

Shoot regeneration on callus

Copious callus proliferation was observed when the basal medium supplemented with 1.0 mg/l BAP in combination with 0.2 mg/l IAA. Percentage of callus induction and regeneration was calculated and presented in Figure 2. The figure shows that highest callus induction was observed in 2,4-D + BAP combination but highest callus regeneration was recorded in BAP + IAA combination. The frequency of shoot regeneration was further enhanced when MS was supplemented with 1.0 mg/l BAP and 0.2 mg/l of IAA and after about six weeks of culture 3-4 adventitious shoots were developed from the callus. Highest 30% shoot regeneration was obtained from callus in the media having 1.0 mg/l BAP with 0.2 mg/l IAA and highest number of shoot regeneration per culture was 6.00 ± 0.28 in the same medium (Plate 2. E). An average of 2-3 shoots per callus were recorded after six weeks of culture. It could be concluded that a higher concentration of BAP (1.0 mg/l) and a lower concentration of NAA and IAA (0.2 mg/l) proved more suitable for shoot regeneration in organogenic callus. Saggoo and Kaur [12] studied on important medicinal plant *Aloe vera* L and used 1.0 mg/l BAP and 0.2 mg/l NAA as the regeneration medium for callus. Etedali et al. [13] used 1.0 mg/l BAP and 0.2 mg/l NAA in the regeneration medium for an ancient crop plant *Vicia ervilia*. Narasimhulu and Reddy [14] obtained highest shoot regeneration from callus derived from epicotyl, hypocotyl, leaves and cotyledons in the medium containing 1.0 mg/l BAP and 0.2 mg/l of NAA in *Arachis hypogaea* L. In our experimental studies rooting was developed from microshoots in half strength MS medium supplemented with different concentrations (0.1, 0.2 and 0.5 mg/l) of IBA used alone or in combination with two concentrations (0.5, 1.0 mg/l) of BAP and Kn. Roots were initiated on these shoots at all concentrations after a culture period of three weeks. Highest 90% of the plantlets were rooted when MS was supplemented with 0.2 mg/l IBA alone (Plate 2. F).

Ipomoea mauritiana is very important and threatened medicinal plant failed to draw the attention for any advanced research. Establishment of complete tissue culture protocol covering its direct regeneration, callus induction and callus regeneration would provide

ready avenues for pursuing advance research in the improvement and conservation of the plant. Profuse callus induction protocol particularly, would initiate cell suspension culture exploring the potentials for isolation of secondary metabolites from cell extract.

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