

***IPOMOEA PLATENSIS* – MULTIPLICATION OF A NEW ORNAMENTAL SPECIES IN ROMANIA**

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Abstract

The Ipomoea platensis species belongs to the Convolvulaceae family. It grows as a vine and is widespread in tropical and subtropical areas. This morning glory is a caudiciform species, with tuberous roots, which also grow on the surface of the soil, thus having a good resistance to drought. In Romania's climatic conditions this species is flowering if fertilized but the capsule (bud) aborts usually before seed maturation, therefore the vegetative propagation is the only way to multiply it. In this research we investigated its optimum vegetative multiplication (ex vivo and in vitro). For ex vivo multiplication the best results were obtained using tap water without growth hormones. For in vitro multiplication we used nodal segments and leaf fragments. The nodal segments used as explant produced shoots, while the leaf fragments developed only callus. The explants were placed on MS medium containing cytokinines (BAP, KIN and TDZ) and auxins (IAA and IBA) in different concentrations. Optimal proliferation was observed when shoots were cultivated on MS medium supplemented with 0.5 mg/l IBA and 0.2 mg/l KIN. The concentration and type of cytokinine had an influence on the multiplication of I. platensis species, from nodal segments. Thus, Kinetin in 0.2 mg/l concentration induces the formation of a higher number of shoots compared to the cytokinines BAP in 0.3 mg/l and TDZ in 0.1 mg/l concentration. Consequently, the easiest way to multiply this species is ex vivo, but the highest ratio of multiplication is obtained in vitro, from nodal segments.

Key words: Convolvulaceae, morning glory, micropropagation, ex vivo, in vitro.

INTRODUCTION

Ipomoea platensis Ker-Gawl. is synonym with: *Ipomoea platense*, *Ipomoea digitata* var. *septempartita* Meisn.; *Ipomoea lineariloba* Peter; *Ipomoea platensis* var. *erecta* Hassl.; *Ipomoea platensis* var. *quinque-partita* Hassl.; *Ipomoea platensis* var. *subnovem-partita* Hassl.; *Ipomoea platensis* var. *subseptem-partita* Hassl. (<http://www.catalogueoflife.org>). This specie was first described by John Bellenden Ker Gawler in 1818. It belongs to the *Convolvulaceae* family which includes 50 genera and 1800 species (Orfila and D'Alfonso, 1995). According to some authors, the genus *Ipomoea* comprises 500 species (O'Donell, 1953; 1959), while other authors consider that it would comprise approximately 600-700 species (Meira et al., 2012). Given the large number of species of this genus, there is a great variability in their phenotype. Thus, we encounter annual and perennial species, in the form of shrubs and semi-shrubs, plants with voluble and climbing stems, with tuberous, caudiciform roots, etc. The species under study,

as described by Orfila and D'Alfonso (1995), is native to Argentina, Paraguay and Uruguay, and is widespread in tropical and subtropical areas. It is a caudiciform species, with tuberous roots, which also grow on the surface of the soil, thus having a good resistance to drought. Prefers semi-shaded areas and not direct sunlight. The stem is voluble (4 - 5 m), the leaves are palmate, showing 5-9 lobes (Figure 1c), and the flowers are arranged in cymose inflorescences with 2 - 6 flowers in inflorescence. The caudex (tuberous root together with the stem) can reach up to 60 cm, and the voluble stems reach up to 4.0 m. The flowers are pink infundibuliform (Figure 1b, 1c). Propagation can be done by seeds, but unlike other species of the *Ipomoea* genus, it can be propagated very easily by stem cuttings or root separation. If it is multiplied by seeds, the flowering takes place after 3 years (in the conditions of our country and protected over the winter), and if it is multiplied vegetative, flowers appear already in the following year. This species is totally different from the well-known ornamental species of the *Ipomoea*

genus, widespread in Romania. Due to the fact that the tuberous roots also grow on the soil surface, the species is also very suitable for pot cultivation (Figure 1a), providing a support system or can be grown even in the form of bonsai, because it behaves very well when pruning. This species of morning glory can be used to develop new ornamental genotypes of *Ipomoea* and for other utilizations (medicinal, agrotechnical etc.). It should be mentioned that this is the first detailed description of the species *I. platensis* in the literature of our country.

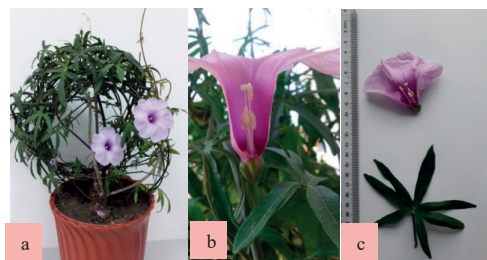


Figure 1. *Ipomoea platensis* in the pot (a); morphological flower characteristics (b and c) (original)

We decided to analyse *ex vivo* and *in vitro* multiplication because in the last three years it was impossible to obtain seeds from this plant. The objective of this study was to identify the proper way to multiply *Ipomoea platensis*.

MATERIALS AND METHODS

Ipomoea platensis is one of the species found in the germplasm collection of the Plant Breeding department of the Faculty of Horticulture Cluj-Napoca, Romania. Opposed to other common species of *Ipomoea* that multiply only by seeds, this one multiplies both by seeds and by stem cuttings. In the present research we tested the *ex vivo* and *in vitro* multiplication capacity of this species. For *ex vivo* propagation stem cuttings were used and for *in vitro* culture the initiation was performed from leaf fragments and nodal segments. The biological material was obtained from the potted mother plant.

Ex vivo multiplication of plants

For *ex vivo* multiplication, the stem cuttings were detached at 1-2 internodes and the foliar surface was reduced. The cuttings were placed

in tap water, water with two concentrations of auxins and perlite substrate obtained four experimental variants: V1 - tap water; V2 - water + 1.5 mg/l indolyl butyric acid (IBA); V3 - water + 3.0 mg/l indolyl butyric acid (IBA); V4 - perlite substrate moistened only with water. The cuttings were maintained at room temperature (22°C), periodically being carried out observation on the root system formation.

In vitro multiplication

The initiation of *in vitro* culture was made from leaf fragments and nodal segments. Disinfection of the explants was carried out by sterilization in ethyl alcohol 65% for one minute followed by immersion in solution of 3% dichloroiso-cyanuric acid Na₂ salt (commercial product Chlorom) to which were added a few drops of Tween 20. The sterilization time was 15 minutes after which the explants were washed three times with sterile distilled water. Leaves were cut along the main rib to a size of 0.5-1.0 cm and placed with the abaxial surface down on the medium. The nodal segments were sectioned to a length of about 0.5 cm.

Explants were inoculated on basic medium MS (Murashige and Skoog, 1962) supplemented with cytokinins and auxins in two concentrations with the following variants: V1 MS + 1.0 mg/l IBA (Indole-3-butyric acid) + 0.5 mg/l BAP (6-Benzyl-Amino-Purine) and V2 MS + 0.2 mg/l IAA (Indole-3-acetic acid) + 0.1 mg/l TDZ (N-phenyl-N'-1,2,3-thiadiazol-5-ylurea).

Sucrose was also added to the medium in a concentration of 3% and agar 0.65%. The pH was adjusted to 5.8 and finally, media were sterilized by autoclave at 121°C for 20 minutes. For multiplication and elongation, the induced shoots were subcultivated on the MS culture medium with 0.5 mg/l IBA and 0.3 mg/l BAP and MS with 0.5mg/l IBA and 0.2mg/l Kin (Kinetine).

All cultures were incubated in growth chambers at a temperature of 23 ± 2°C, with a photoperiod of 16/8 hours and light intensity of 3000 lux provided by cool-white fluorescent light.

Determination of the number of shoots and measurements of their length were performed after six weeks of *in vitro* culture. All data were assessed using ANOVA and Tukey's HSD multiple-range test (p<0.05).

RESULTS AND DISCUSSIONS

Ex vivo multiplication of plants

Multiplication by stem cuttings is the most common method to propagate plants. The most important aspect in this context is the stage of stem maturity. In case of *I. platensis* the stem cuttings must be herbaceous. In our experiment the cuttings formed roots after 10 days in water and 15 days in perlite substrate. In the variants with two concentrations of IBA the stem cuttings were unrooted. According to these results, for statistical analysis we took in consideration only the variants with water and perlite. The results are presented in Figure 2. The highest rooting ratios were obtained in the water variant.

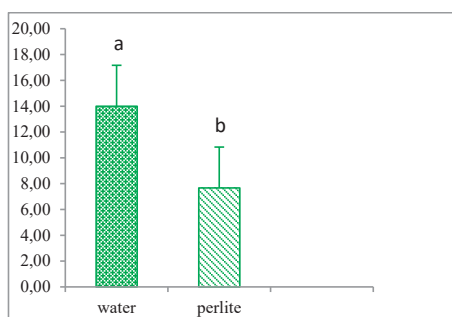


Figure 2. Number of rooted cuttings of *I. platensis* (Values followed by the same letter are nonsignificantly different according to Tukey's test - $P \leq 0.05$)

The obtained cuttings formed 3-4 roots within 3-4 weeks, and after planting in a well-aerated soil they formed a thickened root which then turned into caudex, which assures better resistance to drought and also can be used as a decorative element (Figure 3).



Figure 3. The *ex vivo* rooting of *I. platensis* (original)

Results of *in vitro* multiplication

In our experiences, the culture was initiated from leaf fragments and nodal segments. The explants were inoculated on two media variants with two types of auxines and two of cytokinins.

Leaf fragments and nodal segments formed callus (Figure 4) on the V1 initiation medium (MS + 1.0 mg/l IBA + 0.5 mg/l BAP), although many plant species have the capacity to regenerate shoots from leaf fragments.

In the case of *Ipomoea batatas* species, Gosukonda et al. (1995) reported 25% regeneration of leaf fragments grown on medium with TDZ at a concentration of 0.1 and 0.2 mg/l.

Induction of caulogenesis, i.e. the formation of buds and stems, is stimulated by the presence of cytokinins in the culture medium. It is often necessary to associate cytokinins with auxins. An important role in the neoformation of buds is the nature and concentration of the growth regulators used.



Figure 4. Callus formation from leaf and nodal segments (original)

Since on the first variant of the medium plants have been callused, we tested a new variant that has been supplemented by 0.2 mg/l AIA + 0.1 mg/l TDZ.

In our experience on this culture medium, no multiple shoots have been obtained although TDZ is a cytokinin considered as one of the most active for inducing shoots in tissue cultures. Although no multiplication of shoots was observed, the explants had a vigorous growth reaching an average length of 3.4 cm (Figure 5).

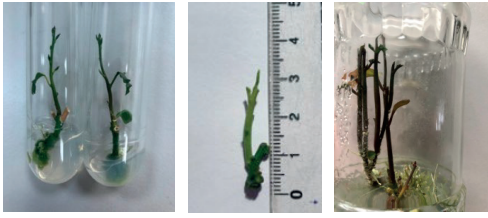


Figure 5. Development of shoots from nodal explant in *Ipomoea platensis* (original)

The BA's superiority over TDZ in the case of multiplication of axillary shoots in *I. batatas* cultivars, where no multiple shoots were formed on TDZ-supplemented media (Mohamed et al., 2007; Dewir et al., 2020).

Other studies reveal that TDZ induces the regeneration of shoots in many species (ornamental and woody) recalcitrant to *in vitro* propagation. The studies made by Pop et al. (2016) on the *Lisianthus* and *Vitis* species, led to the formation of a large number of shoots using a concentration of 1.0 mg/l TDZ and 0.5 mg/l AIA. Morphogenesis induced by TDZ probably depends on the endogenous level of growth regulators being able to modulate also the endogenous level of auxines.

The TDZ medium obtained shoots were subcultivated for multiplication on two MS basic culture media supplemented with 0.5 mg/l IBA and cytokinins 0.3 mg/l BAP and 0.2 mg/l Kin.

With regard to the number of shoots/explant, the superiority of the medium supplemented by 0.5 mg/l IBA and 0.2 mg/l Kin is ascertained by an average number of 4.3 shoots obtained while on the medium supplemented by 0.3 mg/l BAP the number of shoots obtained was significantly lower (Figure 6).

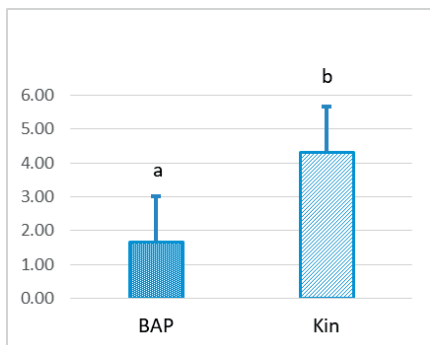


Figure 6. The influence of cytokinin on shoots number

Mengs et al., (2018) reported that using cytokinin combinations in higher concentrations, namely BA 0.5 mg/l and Kin 2.0 mg/l, they obtained callus shoots in the *Ipomoea batatas*.

Our study demonstrates that the development of 3-4 shoots is influenced by the growth hormones used.

The cytokinins used also had an influence on the length of the shoots obtained. Thus on the culture medium supplemented with kin 0.2 mg/l the shoots had an average length of 5.6 cm with significant differences from the other two variants (TDZ and BAP) (Figure 7).

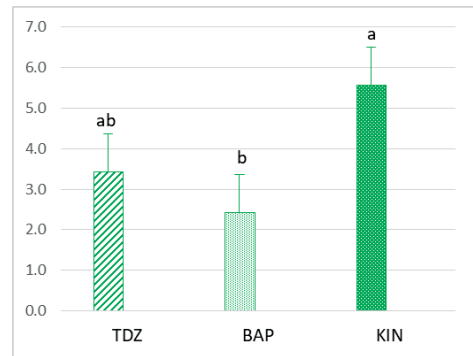


Figure 7. The influence of cytokinin on length of shoots

The formation of a smaller and shorter number of shoots on the BAP medium at a concentration of 0.3 mg/l and 0.5 mg/l auxine IBA is due to callus formation at the base of the explants.

Some studies report the formation of a large number of shoots at 3.0 mg/L BA in combination with 0.5 mg/L NAA, with BA playing an important role in inducing and elongating shoots (Cheruvathur et al., 2015; Fira et al., 2016) and protocorm formation in *Phalenopsis* sp. (Cordea et al., 2019).

In our experiments, the concentration of BAP although very low, respectively 0.3 mg/l BAP, shoots have been callused. The callused tissue developed on the surface of the nutrient medium forming an amorphous mass of thin-walled parenchymal cells without a certain anatomical structure.

From some explants a mixed callus was formed, containing both regenerative and non-regenerative callus. During six weeks of cultivation the callus became green with a

compact structure and meristemoid centers from which 1-2 shoots were differentiated (Figure 4).

In the case of *Ipomoea mauritiana*, massive callus proliferation was observed with the use of the additional MS basic medium with a higher concentration of BAP i.e. 1.0 mg/l in combination with 0.2 mg /l IAA (Islam and Bari, 2013).

It is well known that morphogenesis and *in vitro* growth are regulated by exogenously applied growth phytohormones and the balance between endogenous hormones.

The response of explants to different growth phytohormones also depends to a large extent on the genotype and species.

In our experiments, root formation was recorded in shoots grown on the basic MS medium supplemented by 0.5 mg/l IBA and 0.2mg/l Kin (Figure 5). The roots began to form after a three-week *in vitro* culture period. Similar data were also reported in studies by Islam and Bari (2013) in the *Ipomoea mauritiana* species, where root formation took place on the basic medium with 0.2 mg/l IBA, without other phytohormones.

CONCLUSIONS

In order to *ex vivo* multiplication of the *Ipomoea platensis* species the best rooting variant was obtained by keeping the cuttings in water without any auxines.

In the case of *in vitro* experiments, according to the results obtained, the MS culture medium supplemented by 0.5 mg/l IBA and 0.2 mg/l Kin was the best for multiplication and growth of shoots.

The BAP medium (0.3 mg/l) led to the proliferation of callus at the base of the explants and the formation of a very small number of shoots. This medium variant can be used to induce caulogenesis. Auxine IBA in combination with kinetine determines the formation of the root system by obtaining plants that can be acclimatized *ex vitro* and used in the perpetuation of the species *I. platensis*.

To our knowledge, this is the first report on multiplication and micropropagation of *Ipomoea plantensis* in Romania.

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