UNIVERSITY OF WEST-HUNGARY
Faculty of Agricultural- and Food Sciences
Mosonmagyaróvár
Environment Institute

Plant Production Institute
„Using Precision Agricultural Methods"
Doctoral School

Head of Doctoral School:
Prof. Dr. Neményi Miklós
University Professor
corresponding member of Hungarian Academy of Sciences

Program leader:
Prof. Dr. Reisinger Péter CSc
University Professor

Theme consultant:
Prof. Dr. Máthé Ákos DSc
University Professor

STUDY OF THE INDIAN TOBACCO (*LOBELIA INFLATA* L.) PRODUCTIONS, SPECIFICALLY THE PLANT GROWS IN HUNGARY

Written by:
Vojnich Viktor József

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1. INTRODUCTION AND OBJECTIVES

There are 3 million smokers in Hungary. According to a representative survey (HAJDUNÉ, 2011), every third smoker wants to give up their habit. Therefore there are 1 million people, who are interested in a method that could help giving up their habit without mental or emotional difficulties. The subject of my dissertation, Indian tobacco (*Lobelia inflata*), a plant native to North America, can help in the fight against smoking.

Indian tobacco is a plant of significance containing pharmacologically active substances. Studies were carried out in the US in the 1960s and 1970s with the aim of producing the main alkaloid of the plant, lobeline.

Drug of the plant, *Lobeliae herba* is an anticonvulsant, antiasthmatic, emetic, expectorant, respiratory stimulant, and sedative substance, therefore is used for treating several diseases. It came into view in accordance with the recent studies focusing on the central nervous system, drug abuse and multi-drug resistance. Its use in anti-smoking products is also known.

In Hungary, the research on increasing the *in vitro* active substance production of *L. inflata* was started at the laboratory of the Department of Pharmacognosy, Semmelweis University in the 1980s, led by Professor Dr Éva Szőke. These studies focused on active substance production of *in vitro* plants, polyacetylene detection and studying the growth characteristics and special metabolism of genetically transformed and non-transformed *in vitro L. inflata*

cultures with the aims to increase their biomass and active substance production. The experiments on the possibilities in cultivating the plant in Hungary (introduction) were led by Professor Ákos Máthé at the Department of Botany, Faculty of Agricultural and Food Sciences, University of West Hungary.

The basis of my doctoral research is the GVOP project funded by the European Union and carried out between 2003 and 2006, entitled: "Optimizing the production of active substances in *Lobelia inflata* cultures (*in vivo* and *in vitro*) with the aim of producing anti-smoking products."

The goals of my research work, based on the previous experimental results, were:

- optimizing the cultivation and active substance production of *L. inflata* in Hungary, *in vivo* and *in vitro*, mainly trough modifying their nutrition (Magnesium and Nitrogen ground fertilizer treatments)
- providing a comparative evaluation of the following factors between plants propagated *in vivo* and *in vitro* and raised in open field:
    - biomass production
    - production of active substances, with a focus on total alkaloids and lobeline
- examination of the possibility of growing *L. erinus* in Hungary and optimizing its active substance production

2. MATERIALS AND METHODS

The open field experiments of my studies were carried out in Mosonmagyaróvár, at the botany garden of the Faculty of Agricultural and Food Sciences, University of West Hungary in 2010, 2011 and 2012). The *in vitro Lobelia inflata* cultures were studied in Budapest, at the laboratory of the Department of Pharmacognosy, Semmelweis University.

My work included the measurement of growth and specific morphological characteristics, e.g. height (in cm) and leaf surface area (in cm^2) of the plants in Mosonmagyaróvár. Total alkaloid content (in mg/100 g) and lobeline content (in µg/g) of the *L. inflata* plants were measured at the laboratory of Semmelweis University (Budapest).

Plants in the experiments

Indian tobacco seedlings produced with sowing and micropropagation were used for the experiments. The seeds were purchased from the Canadian company, Richters (Ontario, Canada). In the later phases of the experiment, the fruits and seeds of my own plants were collected and used for propagation. The *in vitro* propagated plants were produced with cloning technique at the biotechnology laboratory of the Department of Pharmacognosy, Semmelweis University.

Raising the sown plants in a glasshouse

The plants were propagated generatively, by sowing. Sowing was carried out into soil in a tray in a climate controlled glasshouse. Sowing was carried out in January each year. Germination took two weeks. Thinning took place once, in April or May. Time between sowing and transplanting was 3 to 4 months. Temperature in the glasshouse was between 20 and 30°C. Transplanting took place 3 to 5 weeks after thinning.

Hardening the *in vitro* propagated seedlings

The *in vitro Lobelia* "seedlings" produced at the laboratory of the Semmelweis University were hardened in the glasshouses of the Department of Botany, Faculty of Agricultural and Food Sciences, University of West Hungary in Mosonmagyaróvár. Each year, the beginning of the glasshouse raising period took place in April. Most of the plants developed a seedstalk while they were in the glasshouse. By the time of transplanting, the seedlings hardened significantly, and some of them reached a height of 20 cm. I tried to transplant similarly developed plants into the open field. No genetically transformed plants were used in the *in vitro* experiments. The *in vitro* seedlings were transplanted to open field after 6 weeks of hardening.

Transplanting

The *L. inflata* seedlings were transplanted with their soil balls into hand-dug, 9-10 cm deep holes in the experimental field.

Dimensions of the experimental areas: In 2010: 47.16 m^2 (1.2 m^2 for each patch); in 2011: 47.52 m^2 (1.8 m^2 for each patch); in 2012: 53.55 m^2 (1.8 m^2 for each patch).

Number of treatments: In 2010, 3 treatments, in 2011, 4 treatments and in 2012, 6 treatments were applied, they were based on the experiences of the previous year. There was also a control group each year (Table 1).

Plants per plot: In 2010: 27 plants (21 were propagated by sowing and 6 *in vitro*). In 2011: 40 plants (28 were propagated by sowing and 12 *in vitro*). In 2012: 39 plants (28 were propagated by sowing and 11 *in vitro*).

Table 1. Treatments in the experiment (2010–2012)

Year	**Treatments**						
	Control	**50 kg/ha N**	**100 kg/ha N**	**150 kg/ha N**	**50 kg/ha Mg**	**100 kg/ha Mg**	**150 kg/ha Mg**
2010	X	X	X	-	X	-	-
2011	X	X	X	-	X	X	-
2012	X	X	X	X	X	X	X

Treatment was carried out 1 day prior to transplanting. N (NH_4NO_3, 34%) and Mg ($MgSO_4$, 2%) fertilizers were spread into the soil.

No chemicals or herbicides were applied, only mechanical weed control.

Measuring the plants took place 3 or 4 times a year (Table 2); the number of the leaves was counted, the length and width of the leaves, the dry weight and the height of the plants were measured.

Table 2. Dates of *Lobelia inflata* data collection (2010–2012)

	2010	**2011**	**2012**
sowing in the glasshouse	15.01.2010	22.01.2011	16–17.01.2012
***in vitro* seedlings transferred into the glasshouse**	28.04.2010	12.04.2011	24.04.2012
transplanting into raising trays	01–05.05.2010	27.04–02.05.2011	14–16.05.2012
transplanting into open field soil	15.06.2010	26–27.05.2011	04–05.06.2012
dates of measurement	08.07.2010 17.07.2010 24.07.2010 01.08.2010	22.07.2011 29.07.2011 07.08.2011	31.07.2012 15.08.2012 22.08.2012 30.08.2012
dates of harvest	05–06.08.2010	09–10.08.2011	30.08.2012

Plant care

There were no pathogens or pests during the glasshouse raising phase. In 2010, in the open field trials Spanish slug (*Arion vulgaris*) and Roman snail (*Helix pomatia*) caused significant damage. There

were no other damages caused by pests in the next two years. No chemicals or herbicides were applied, only mechanical weed control (hoeing). Hoeing took place every three weeks.

Biometric evaluation methods of the results: The open field trials were carried out in randomized block design. The results were evaluated and p value (significance level) was calculated with Tukey test. Microsoft Excel 2007/2010 and IBM SPSS v19 softwares were used for data evaluation.

Evaluation methods of the active substances: Total alkaloid content was determined with spectrophotometry, and lobeline content was determined with HPLC (High Performance Liquid Chromatography).

***In vitro* micropropagation experiments**

The open field nutrition experiments were repeated among *in vitro* circumstances in 2012, in the laboratory of the Department of Pharmacognosy, Semmelweis University. Combinations of $MgSO_4$, NH_4NO_3 and KNO_3 were applied on Murashige and Skoog (MS) medium.

Overwintered *L. inflata*

In 2011 and 2012, the overwintered *L. inflata* plants were also transplanted to open field. The plants overwintered in the experimental area, always in rosette form. In 2011, 12 plants, while in

2012, 6 individual plants overwintered. Data were collected on the possibilities of biennial cultivation. The observations focused on morphology and chemical content.

Self-sown *L. inflata*

In 2012, self-sown *L. inflata* plants were also transplanted to the experimental area. The observations focused on morphology and chemical content. The self-sowing phenomenon was recognized in the open field, before the seedlings were transplanted. The self-sown seedlings started to develop in April, under natural conditions.

Lobelia erinus

Parallel trials were carried out in 2011 with Trailing lobelia (*Lobelia erinus* L.) and Indian tobacco. The plants were propagated by sowing, were raised in glasshouse and after a period of hardening, they were transplanted to open field. The focus was on the active substance content.

3. RESULTS AND DISCUSSION

The results on the possibility of cultivating *Lobelia inflata* in Hungary and the fertilization experiments of the plant are presented in an order based on the method of propagation of the plants.

Sown *L. inflata*

Based on the three-year study on growth characteristics, Nitrogen treatment had a favourable effect on sown and glasshouse raised *L. inflata* plants. In 2010 and 2011, the 50 kg/ha N, while in 2012 the 100 kg/ha N ground fertilizer treatment had the best effect.

The favourable effect of ground fertilizers was not equivocal in the case of average leaf surface are (cm^2), since a different treatment resulted in the largest average leaf surface area each year (in 2010, the 50 kg/ha N, in 2011, the 50 kg/ha Mg and in 2012, the 100 kg/ha N fertilizer treatment).

The above ground dry biomass weight value (g) of the sown *L. inflata* was the highest in case of nitrate treatments (in 2010, the 50 kg/ha N and in 2011, the 100 kg/ha N treatment). In case of the roots, highest dry weight values were measured in case of the 100 kg/ha N fertilizer (2010).

The effect of fertilizers was seen in the active substance content of the above ground biomass, as highest lobeline content (in μg/g) was measured after the 50 kg/ha N (2010) and the 50 kg/ha Mg (2011) treatments. The lobeline content of the root was highest (630 μg/g) in the 50 kg/ha Mg fertilizer treatment group (in 2010).

In the total alkaloid content (mg/100 g) increasing experiments, the active substance content of the above ground part of the plant and the root was measured separately. The above ground part had the following results: in 2010, the 50 kg/ha Mg treatment group, while in 2011, the control group had the highest values. In case of the roots, the

highest alkaloid content was measured in the 50 kg/ha Mg treatment group in 2010 (986 mg/100 g).

In vitro propagated *L. inflata*

The above ground dry weight values of *in vitro* propagated *L. inflata* were as follows: in 2011, the 100 kg/ha Mg, while in 2012, the 150 kg/ha N fertilizer treatment showed the best results. In case of root dry weight, the control group (2012) had the highest values. (Height and leaf surface area measurements were carried out on Indian tobacco plants only.)

The highest lobeline content was measured in the above ground parts of the *L. inflata* plants after the following treatments: 100 kg/ha Mg in 2011, and 150 kg/ha N in 2012. In the measurement involving the roots, the 50 kg/ha N treatment had the most significant effect.

The total alkaloid content of the above ground parts of *L. inflata* was highest in the 50 kg/ha Mg treatment group in 2011, while in the control group in 2012. In case of the roots, the effect of the 100 kg/ha N and the 50 kg/ha Mg treatment showed very similar results. In 2012, in the high dose treatment groups (150 kg/ha N and 150 kg/ha Mg) the total alkaloid content of the roots was above 1000 mg/100 g; no other treatment had this high value.

Overwintered sown *L. inflata*

In 2011 and 2012, the active substance content of overwintered sown plants were also measured. These plants had higher values in all characteristics (height, dry weight value, lobeline content, total alkaloid content) in 2011 than in 2012.

Self-sown *L. inflata*

The active substance content of the self-sown *L. inflata* plants was measured in 2012. These plants had higher lobeline and total alkaloid content than the overwintered specimens. Compared to the sown *L. inflata* data of 2011, the self-sown plants had higher total alkaloid content, but lower lobeline content.

Lobelia erinus

In 2011, another *Lobelia* species was examined as well. These plants had the lowest lobeline content and the highest total alkaloid content, compared to sown *L. inflata* plants.

In vitro (laboratory) nutrition experiments

$MgSO_4$ treatment

The open field nutrition experiments were repeated among *in vitro* circumstances, on Murashige and Skoog medium in 2012.

The most significant effect on the dry weight value of the above ground parts was observed after the 1480 mg/L and the 185 mg/L $MgSO_4$ treatments, while in case of the dry weight of the root, after the 185 mg/L $MgSO_4$ treatment.

In case of the lobeline values of the above ground parts of the plant, the 740 mg/L $MgSO_4$ treatment, and in case of that of the roots, the 185 mg/L $MgSO_4$ proved to be the most effective.

In case of the total alkaloid determination of the above ground parts of the plant, the 370 mg/L $MgSO_4$ treatment, and in case of that

of the roots, the 185 mg/L $MgSO_4$ treatment showed be the highest values.

NH_4NO_3 and KNO_3 treatments

Nitrate treatments had the following effect on dry weight: in case of the above ground parts, highest values (1.002 g) were measured on Murashige and Skoog medium with a KNO_3 content decreased to 50% (treatment D1), while in case of the root, the control treatment group (treatment A1) had the highest values (1.331 g).

The highest lobeline content was measured in the above ground parts (347 μg/g) and in the roots (279 μg/g) of plants that were raised on the Murashige and Skoog medium with decreased KNO_3 content (D1).

In case of the total alkaloid content, both the above ground parts (831 mg/100 g) and the roots (915 mg/100 g) had the highest values in the group raised on the Murashige and Skoog medium with decreased KNO_3 content (D1).

4. NEW SCIENTIFIC RESULTS

1. In the trials of the nutrition demand of *Lobelia inflata*, it was seen that the 50 kg/ha Mg and 50 kg/ha N ground fertilization regimes were the most beneficial for the sown and traditionally raised plants. The highest total alkaloid content (490 mg/100 g) of the above ground part was measured in 2010 and the highest

lobeline content (445 μg/g) of the above ground part was measured in 2011, both in the 50 kg/ha Mg treatment group.

2. These were the first results of laboratory experiments with the applied treatments ($MgSO_4$, NH_4NO_3 and KNO_3). The *in vitro* propagated plants raised on the Murashige and Skoog (MS) medium with decreased KNO_3 content (treatment D1) had the highest total alkaloid (831 mg/100 g) and lobeline (347 μg/g) content in the above ground parts of the plants. The highest dry weight value (1.094 g) was observed after the 1480 mg/L $MgSO_4$ treatment.

3. This case was the first observation of self-sown plants in the experimental area in Hungary, which suggests a possibility of open field planting of *L. inflata*.

4. This trial was the first comparison of active substance content of the self-sown and the sown *L. inflata* plants of 2011. The total alkaloid content (819 mg/100 g) of the self-sown plants was higher, but lobeline content (386 μg/g) was lower than those of the planted individuals.

5. This was the first study of growing *Lobelia erinus* as a medicinal herb in Hungary. I found that *Lobelia erinus* produces more total alkaloid (559.9 mg/100 g), but less lobeline (4.92 μg/g), than *L. inflata.*

5. PUBLICATIONS

Journals

Vojnich, V. J. - Máthé, Á. - Gaál, R. - Tüű, Sz.: (2011) Botanical and chemical variabiltiy of Indian tobacco (*Lobelia inflata* L.). Acta Agronomica Óváriensis. Vol. 53. Number 2. 37-48.

Vojnich, V. J. - Máthé, Á. - Szőke, É. - Gaál, R.: (2012) Effect of Mg treatment on the production of Indian tobacco (*Lobelia inflata* L.). Acta Horticulturae (ISHS). 955:125-128.

Vojnich, V. J. - Máthé, Á. - Szőke, É. - Bányai, P. - Kajdi, F. - Gaál, R.: (2013) Effect of Nitrogen and Magnesium nutrition of Indian tobacco (*Lobelia inflata* L.). Journal of Central European Agriculture. Vol. 14. Number 2. . 77-85.

Bányai, P. - **Vojnich, V.J.** - Máthé, Á. - Kursinszki, L. - Szőke, É.: (2014) Enhancement of lobeline production of *in vitro* micropropagated *Lobelia inflata* L. by magnesium treatment. In vitro cellular and developmental biology - Plant [**IF 1.162**]

Abstract

Vojnich, V.J. - Máthé, Á. - Szőke, É. - Vass, M. - Gaál, R.: (2011) Effect of Mg treatment on the production of Indian tobacco (*Lobelia inflata* L.). Magnesium Research. Vol. 24. Number 2. 73-74. [**IF 1.519**]

Bányai, P. - **Vojnich, V.J.** - Máthé, Á. - Kursinszki, L. - Szőke, É. (2013): Effect of magnesium and nitrate treatment on the lobeline production of *in vitro* propagated Indian tobacco (*Lobelia inflata* L.). Magnesium Research. Vol 26. Number 3. 140. **[IF 1.556]**

Vojnich, V.J. - Máthé, Á. - Bányai, P. - Szőke, É. (2013): Effects of magnesium treatments on *in vitro* micropropagation of Indian tobacco (*Lobelia inflata* L.). Magnesium Research. Vol 26. Number 3. 136-137. **[IF 1.556]**

International and native conference oral presentations

Vojnich, V. J. - Máthé, Á. - Szőke, É.: (2010) The introduction and application possibilities of Indian tobacco (*Lobelia inflata* L.) in Hungary. XVI. Young Science Forum. Keszthely, Hungary, 25. March 2010.

Vojnich, V. J. - Máthé, Á. - Szőke, É.: (2010) The examination of the production of Indian tobacco (*Lobelia inflata* L.), a species that can be introduced in Hungary. XXXIII. Óvári Science Day. Mosonmagyaróvár, Hungary, 7. October 2010.

Vojnich, V.J. - Máthé, Á. - Szőke, É. - Tóth, L. - Vass, M. - Kajdi, F. - Gaál, R.: (2011) Effect of Mg treatment on Indian tobacco (*Lobelia inflata* L.). V. International Scientific Symposium, Farm Machinery and Process Management in Sustainable Agriculture. Lublin, Poland, 23-24. November 2011.

Vojnich, V. J. - Csapó, Z. - Bányai, P. - Máthé, Á.: (2012) Comparison of the alkaloid production of in vivo and in vitro propagated Indian tobacco (*Lobelia inflata* L). Debrecen, Hungary, 9. March 2012.

Vojnich, V.J. - Máthé, Á. - Szőke, É. - Csapó, Z.: (2012) Effect of Nitrogen and Magnesium treatments on the production of Indian tobacco (*Lobelia inflata* L.). XVII. International Ph.D. Students Conference. Brno, Czech Republic, 21-22. November 2012.

Vojnich, V.J. - Máthé, Á. - Szőke, É. - Bányai, P.: (2013) Effects of magnesium treatments on in vitro micropropagation of Indian tobacco (*Lobelia inflata* L.) XIII. Hungarian Magnesium Symposium. Budapest, Hungary, 18. April 2013.

Vojnich, V.J. - Máthé, Á. - Szőke, É. - Bányai, P.: (2014) Comparison of the alkaloid of in vitro propagated Indian tobacco (*Lobelia inflata* L.) under both laboratory and open field conditions. XX. Youth Scientific Forum. Keszthely, Hungary, 23-24. May 2014.