

Thesis of Doctoral (PhD) Dissertation

BIOECONOMIC ASPECTS OF PROPAGATION AND PRODUCTION OF BIOTECHNOLOGICAL PROPAGATED GIANT REED

Gabriella Antal

Supervisor:

Prof. Dr. Miklós Gábor Fári, DSc

Professor



UNIVERSITY OF DEBRECEN

Károly Ihrig Doctoral School of Management and Business

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1. PRELIMINARIES, AIMS AND HYPOTHESES OF THE RESEARCH

The University of Debrecen has been conducting biological, genetical, agronomic and biotechnological researches with perennial, primarily herbaceous plant species (“bio-generation” species) are suitable for bioindustrial processing that can be grown on marginal lands. The term of “bio-generation” includes new generation species which can be propagated biotechnological methods (FÁRI et al., 2014). During the last decade, one of the priority R&D areas of the predecessor of the Department of Botany Plant, Physiology and Biotechnology (Faculty of Agricultural, Food Sciences and Environmental Management, University of Debrecen) were developments of biotechnological propagation technologies of giant reed ecotypes, testing biological properties of ecotypes, researches and developments of plant nursery and cultivation practices. Giant reed (*Arundo donax* L.) is a perennial rhizomatous herbaceous grass, it does not produce viable seeds. It is a well-known ornamental species and it has been considered promising dedicated bioenergy plant, but it can be propagated by only vegetative methods with high propagation costs.

The University of Debrecen has achieved several practically useful results through the cooperation of Hungarian and foreign research teams and private companies. Our field trials of biotechnological propagated giant reed have been established in the USA, Italy, Spain, China, Romania, Ukraine and Slovakia besides Hungary from 2008 to 2013. After this period numerous plantations were established in Hungary and foreign countries also in large-scale. I joined the researches as a horticultural engineering bachelor student in 2008, in addition, I had the chance to take part in the R&D works on biological, biotechnological, cultivation practices, propagule production and plant nursery technologies of giant reed as a master and PhD student. I worked as a consultant and carried out researches in the laboratory and greenhouse at MOP Biotech Ltd. (Nyíregyháza, Hungary), a biotechnological company dealing with propagule production, from 2012 to 2014.

As the Hungarian literature of giant reed is based on non-domestic production and utilisation data, I think the scientific presentation of my researches conducted on biotechnological propagation and production of giant reed ecotypes from 2010 to 2016 could usefully contribute to the industrial production of giant reed. My researches also focused on the economic analysis of efficient propagation technologies, production and utilization of giant reed (*Figure 1*).

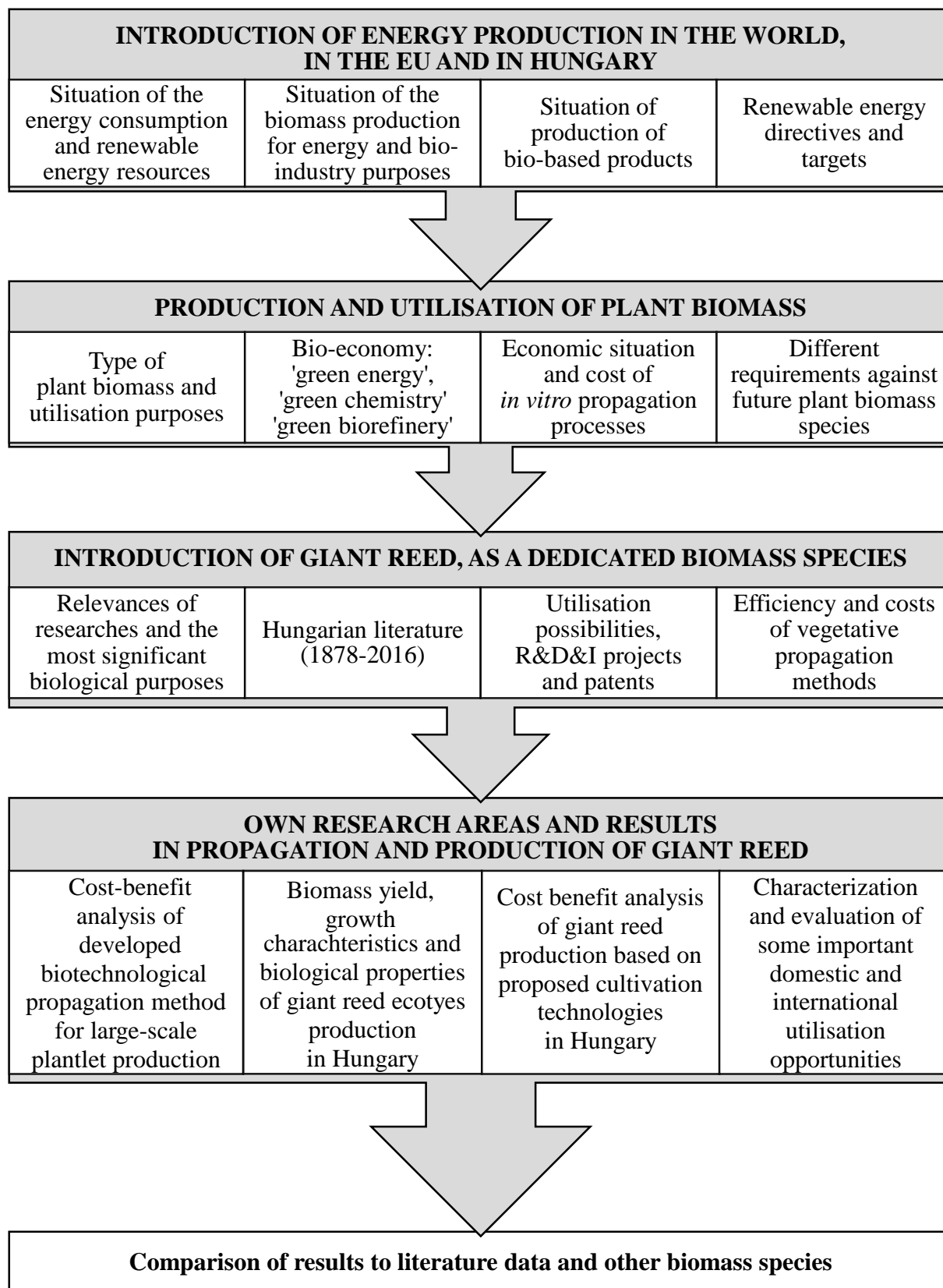


Figure 1: Introduction of my PhD dissertation and my researches (2010-2016)

Due to the development of biological and biotechnological basic researches and challenges in climate change in the last decades, one of the areas of bio-economy or bio-based economy is the development of more efficient, renewable primarily biomass feedstock production and

utilisation has been considered an increasingly researched area. The largest biotech companies in plant genetics areas have been conducting researches with dedicated, woody or herbaceous biomass plants that produce feedstock's for the future bio-industries and green biorefineries. Like many other economically significant plants (oil palm, banana, pine trees, coffee, cocoa, bamboo, etc.), one group of the biomass plants can be propagated only vegetatively by division of clumps (perennial grasses, Jerusalem artichoke), by cuttings (eucalyptus, poplar, willow, miscanthus, giant reed, sugar cane), or by biotechnological methods (*in vitro* micropropagation, somatic embryogenesis).

My scientific researches (2010-2016) focused on the aspects of the biotechnological propagation of giant reed, as a vegetatively propagated, promising rhizomatous perennial grass. The *main and specific aims* of my primer researches introduced in my dissertation are listed as follows:

The introduction of giant reed production as an energy plant derived from somatic embryos and the analyses of biomass yield from 2010 to 2016 under small-scale plantation at the Experimental Garden of Future Biomass Crops at University of Debrecen in accordance with the following sub tasks:

- Researches of cultivation elements and harvesting (soil preparation, propagation, establishment of plantation, agronomic and cultivation elements, irrigation, fertilization, plant protection) to determine an optimum cultivation system in Hungary,
- Assessment of multiannual biomass yields on non-irrigated split-plot fields compared with other potential energy plants: *Miscanthus x giganteus* (miscanthus) and 'Burton SC' maize hybrid,
- Crop safety test with prime attention to cold resistance of giant reed ecotypes,
- Comparison of yield per hectare of giant reed from different climates, with different plant density and biomass yield data in literature,
- Development and assessment of propagation technologies (traditional vegetative and *in vitro* propagation) of giant reed ecotypes originated from Hungary with higher cold resistance.

Economic and cost-benefit analysis of biotechnological propagule production, biomass production and utilization prospects of giant reed derived from somatic embryos.

Based on my researches (2012-2015), based on Hungarian and international literatures, reports from companies dealing with the propagule production of giant reed, own collected data from MOP Biotech Ltd., the sub tasks can be listed as follows:

- Comparison and assessment of efficiency of technologies in giant reed propagule production,
- Cost-benefit analysis of a new biotechnological propagule production that is deemed the most efficient and suitable for large-scale production,
- Introduction of elements in cultivation practices that are recommended for giant reed production in terms of expected production costs, cost-benefit analysis of giant reed production in Hungary,
- Assessment of critical elements of production and utilization prospects for giant reed compares to literatures and other biomass plant data.

Hypotheses and questions to be answered during my researches:

1. The giant reed meets the biological, ecological, environmental and ecological requirements of future biomass species.
2. The application of new biotechnological methods (somatic embryogenesis) results lower cost propagation and production of giant reed.
3. The biomass yields of giant reed production in Hungary exceed the yields of another perennial herbaceous grass, miscanthus biomass production.
4. The utilization of giant reed as bioenergy or bio-based feedstock (biogas, bioethanol) results positive values compared to other biomass plants.

2. DATABASE AND METHODS

Intensity and time dimension of literatures dealing with giant reed and R&D can be well described by current literature databases. Based on Google Scholar database, the number of publications dealing with giant reed is over 16 000 of which approximately 1 420 publications have the scientific name of the plant ('giant reed' – 'Arundo donax') in their titles (Until 18.08.2018). Between 2006 and 2017, more than 14 800 scientific papers published which are related to giant reed and almost 927 publications appeared with the plant's name in their titles. Approximately 230 literatures related to the plant were presented in my dissertation from almost 300 references.

Literatures introduced in the last and current century indicate that giant reed can be used for several purposes (combustion, bioethanol-, biogas-, biochemical-, paper-, viscose-, cellulose production, woodwind musical instruments, stakes for plants, fishing rod, construction materials, ornamental or aromatic species, etc.). It has been considered one of the most promising lignocellulose plants in biomass industry, thanks to high biomass production of hectare, favourable chemical composition, positive energy balance of cultivation and adaptability of different kind of soils and conditional. To produce and spread bio-industrial products with higher added value from giant reed, economical production of the plant is also necessary, including propagule production and acquisition as one of the key issues.

2.1. Small-scale field trials of biotechnological propagated continental and subtropical giant reed ecotypes and their biomass yields (2010-2016)

The University of Debrecen is provided experimental field of 10 000 m² to the Department of Plant Biotechnology where perform biological, cultivation and breeding researches with several perennial, herbaceous, semi-shrub biomass plants. Within the framework of the University of South Carolina (USA) and the University of Debrecen (Hungary) joint research program, has been developed *in vitro* propagation techniques of giant reed and miscanthus based on methods of MÁRTON-CZAKÓ (2002a, b, 2007a, b). From *in vitro* propagation of two different giant reed ecotypes and miscanthus uses somatic embryogenesis were propagated and acclimatized seedlings were planted in the Experimental Garden of Future Biomass Crops at University of Debrecen.

Biomass species in the Experimental Garden of Future Biomass Crops at the University of Debrecen were planted in hard, calcareous chernozem, slightly alkaline ($7.93 \text{ pH} \pm 0.025$) soil with relatively high humus ($3.14\% \pm 0.407$) and significant CaCO_3 (4.67 ± 0.00) content. A subtropical ecotype – originated from the USA – and a continental ecotype – originated from Hungary were the basic plant material of the experiments (*Figure 2*). *In vitro* propagule production was completed by the Márton-Czakó methods under a multiannual cooperation between the employees of a micropropagation company, PRO TEAM Nonprofit Ltd. (Újszentmargita, Hungary) and the Department of Plant Biotechnology (University of Debrecen). Establishment of biotechnologically produced giant reed ecotypes took place according to the following planting form and system shown in *Figure 2.*, by a method that is used in arable vegetable production.

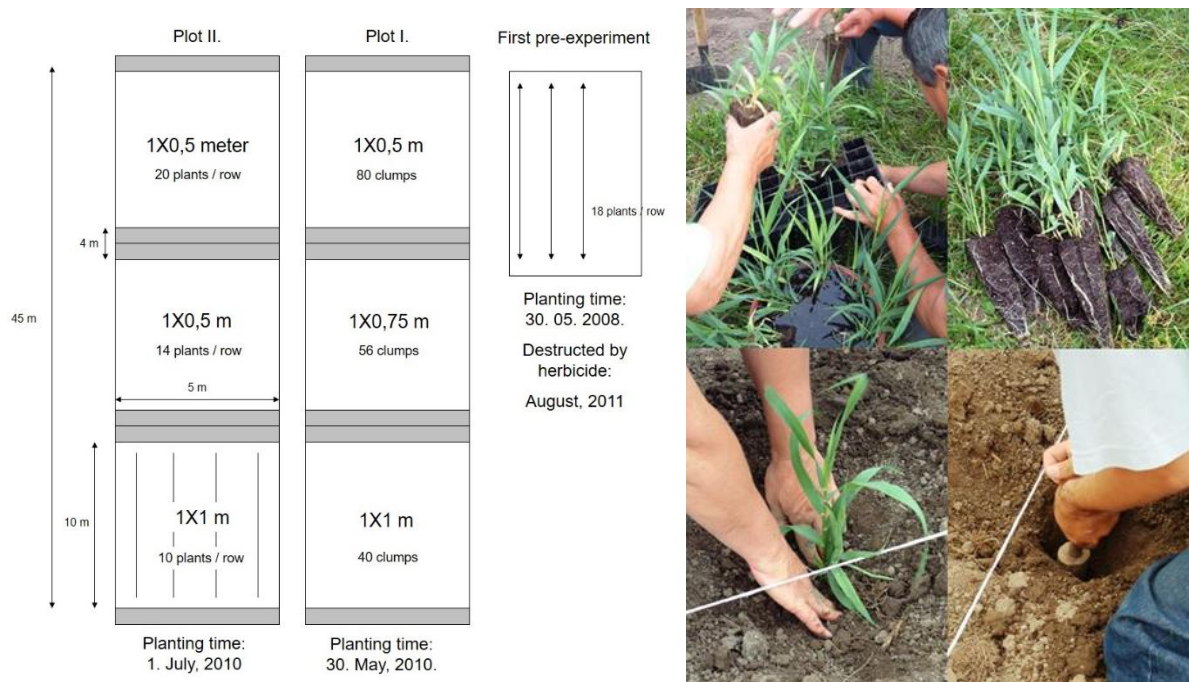


Figure 2: Small-scale field trials of giant reed and establishment of plantation derived from somatic embryogenesis in the Experimental Garden of Future Biomass Crops at the University of Debrecen (2008-2016)

Source: own photos and composition, Antal, 2012

Warmer adapted (subtropical) giant reed ecotypes (from South Carolina, Columbia, USA) were planted in the plot I. at the end of May in 2010. After than a month, at the beginning of July in 2010, the continental adapted giant reed plantlets (from Újszentmargita, Hungary) in the plot II. were planted. The aims of establishment at different planting date were to optimise

the proper planting time and to examine the effects of planting time on the biomass yields. Tillage was conducted in the autumn of 2009 with deep ploughing (40-50 cm) on an uncultivated grassland. Seedbed preparation was conducted in the spring, immediately before planting (2010) by rotary cultivator. 4 rows per bed, 1 m rows spacing and 3 different densities (1 m, 0.75 m and 1x0.5 m) were prepared and each bed was surrounded by a 1-meter-long border (as buffer zone). Weed control, fertilization and irrigation did not be carried out in the cultivation years, only it took place mechanical weed control at twice in the first two years.

I determined the biological potential, growth properties and biomass yields (2010-2016) of the subtropical ecotype (plot I.) and the continental ecotype (plot II.) of giant reed based on the main weather conditions and soil sampling data.

To avoid edge effect, I evaluated 10 plants from each variety and spacing by random sampling from the middle rows. We measured the fresh and dry matter (g), number of shoots per clumps, average height of shoots (cm), ratio of leaf-stem of plants (%) and I calculated the biomass yield per hectare. Based on ongoing monitoring for 6 years I determined the optimum planting and harvesting time and I examined the morphological and growth characteristics and the cold resistance of the ecotypes. I calculated biomass yields per year and hectare for each plant density based on the dry matter content of harvesting feedstock and estimated biomass yield per hectare (*Figure 3*).

During the multi-annual experiment, we examined the effects of winter and early-spring frosts on plant growth and biomass yields. We have not been replanted the frost damaged plants, so the biomass yield per hectare was determined by reducing plant density plantations from the third year. Frozen plants were not replaced, so biomass yields per hectare were calculated by the reduced number of plants from the fourth year. I compared the annual biomass yields of plot I. and II. with miscanthus planted in a similar system in the immediate vicinity of the plantation and with the biomass yields of 'Burton SC' maize hybrid planted in a less and a more productive soil.

I compared own data from my small-scale field trial with biomass yields from international and domestic literatures. I also calculated biomass yields of the Hungarian and foreign conditions depending on plantation years, which were the bases of my economic calculations.



**Figure 3: Sixth years old giant reed and miscanthus plantations
(Debrecen, Hungary – October, 2015)**

Source: own photos and composition. A) miscanthus plantation; B) giant reed plantation; C) miscanthus (left side) and giant reed (right side) plantations; D) miscanthus (front) and giant reed (behind) plantations

Descriptive statistical methods, including the mean, standard deviation and one-way ANOVA were used. The results were evaluated with Microsoft Office Excel and SPSS 13.0 (SPSS Inc., Chicago, IL) programs. I applied the above mentioned statistical test if their basic conditions were fulfilled. The significant differences between the species and treatment were determined by Tukey's or Scheffe test at a probability level of 5%.

2.2. Vegetative propagation methods of winter-frost tolerant, new Hungarian giant reed ecotypes

The main objectives of my propagation researches to select frost-tolerant giant reed ecotypes ('Longicaulis' varieties) in continental climate, to propagate the selected lines by different vegetative methods (stem cuttings, micropropagation, somatic embryogenesis), to evaluate the efficiency of different propagation methods. The plant materials were two-year-old overwintered stem of high giant reed clumps that collected in Hungary, near Lake Balaton

(Balatonkenese, Balatonlelle, Balatonföldvár) in late October, 2013. In spite of winter (2012/2013), the collected stems of clumps could survive and develop shoots from second years old nodes. The complex stem cutting and *in vitro* propagation methods were conducted based on Antal et al. (2014c) (Figure 4).

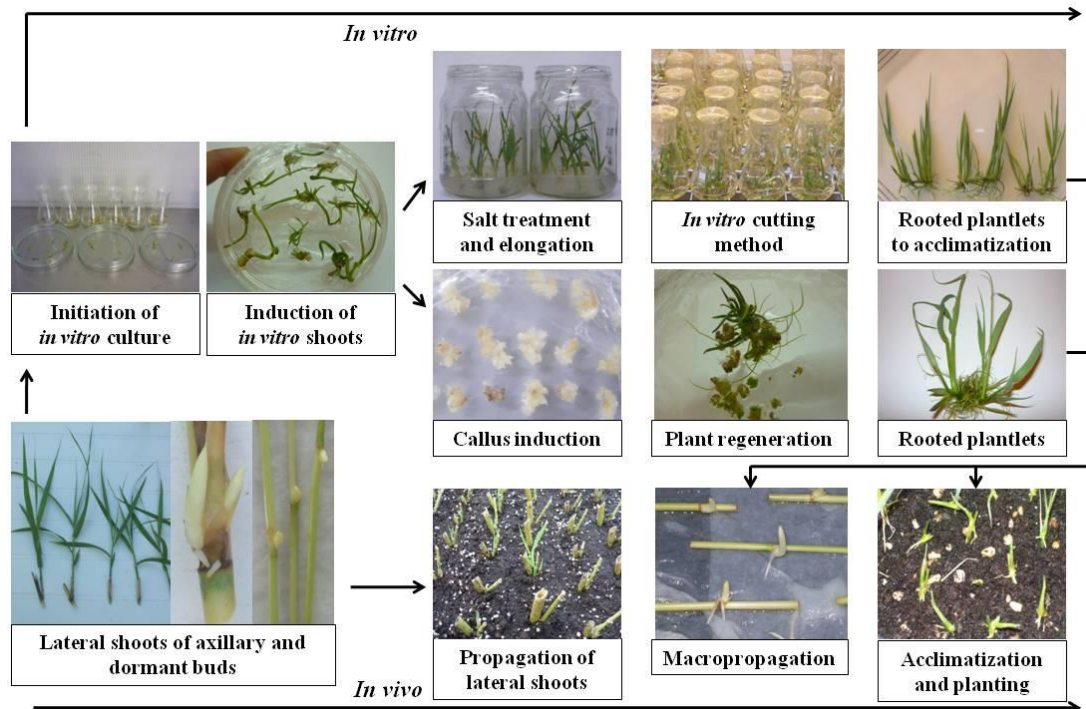


Figure 4: Micro- and macropropagation methods of frost-tolerant giant reed ecotypes

Source: own composition, based on Antal et al., 2014c

Our propagation researches were conducting in laboratories of Department of Plant Biotechnology and greenhouse of Agricultural Research Centre, at the University of Debrecen. The development of propagation systems and establishment of plantation of propagated different kind of ecotypes and striped leaf variants of giant reed are supported by the TÁMOP-4.2.2.A-11/1/KONV-2012-0041 project. More than 2 years research working on micro- and macropropagation methods resulted the selected ecotypes and striped leaf-variants that can be also important in the breeding of new varieties, in the following biological, genetic and agronomical researches (Antal et al., 2018).

2.3. Methodology of economic analysis for the propagation, cultivation and utilization of giant reed

My aim is to compare the efficiency and cost-effectiveness of different propagation technologies of giant reed that I have known and I have developed during my researches with data available in literature and with the technologies of companies dealing with propagule production. Based on economic calculation of PILU et al. (2012, 2013) and CORNO et al. (2014) in production of giant reed one of the most significant direct cost is the propagule cost. More profitable production of giant reed needs to reduce the propagule cost lower than 0.5 EUR of rhizome propagation cost or 0.35 EUR of micropropagation cost of plantlet.

The purposes of my researches were to introduce the improved and known different propagation methods (stem cuttings, rhizome propagation, micropropagation, somatic embryogenesis) and compare some performance indicator and efficiency of methods. To carry out my researches I used for technologies for the propagule production from foreign literature, collected data from MOP Biotech Ltd., my own data and reports of Italian and Hungarian private companies.

The economic calculation of propagation and production of giant reed was conducted the followings. Considering of continental climate in Hungary, I conducted cost-benefit analyses of the most efficient biotechnological methods by characterizing the main phases of somatic seedling technologies (laboratory and nursery). Based on key performance indicators, basic material per unit (750 000) and maximum number of plant output per year (2 million) I calculated the costs for production and represented the expenses by nature, output conditions (yield, sales price, production value), and specified the output and its efficiency. I compared the results for annual costs for production, production value and incomes in a production cycle, cost price for propagule production and cost-benefit ratio with other technologies for propagule production. During my researches, I concluded that there is no evaluable large-scale industrial data of biotechnological propagation of giant reed that analyses in details the production cost and economic assessment.

Based on my multi-annual research I determined the cultivation elements of giant reed production is recommended in continental climates, such as Hungary, depending on the production costs with respect to each operation and the 20-year-old production period of the plantation. Recommended items in cultivation practices and their costs are introduced on the

basis of a publication by MOP Biotech Ltd., but the detailed description and nomination of plantation did not get permission. Production values and incomes are calculated depending primarily on the method for combustion (dry chippers) in terms of different revenues (8000-16 000 HUF) can be reached in Hungary, lack of proper bioindustry and processing plants.

I classified the most important risk factors for the market launch and large-scale production of giant reed according to different criteria (biological, ecological, environmental and economical), on which I prepared a summary table of advantages and disadvantages of production. Several possibilities for the utilization of giant reed as green and dry bioindustrial raw material were also analysed by the evaluation of literature data (combustion, biogas and bioethanol production) compared to other biomass species.

3. KEY FINDINGS OF THE DISSERTATION

3.1. The main results of small-scale field trials of biotechnological propagated continental and subtropical giant reed ecotypes and their biomass yields comparison with other biomass plants (2010-2016)

Giant reed can produce different biomass yields per hectare under different geographical and production conditions. The annual biomass yield of the plant is affected by several factors (microclimate, soil, agronomic techniques, harvesting period and method and so forth). The aggregate values of available biomass yield used in my dissertation shows a high standard deviation and they present the yearly average biomass data of countries with a warmer climate and a longer growing season. Regarding multiannual yield, giant reed can produce a biomass yield of 23-39.4 dry tonnes/ha which is well above the yield reached on our field experiments in Debrecen (Hungary). *Figure 5.* shows the estimated biomass yields of giant reed ecotypes per hectare from two different climatic areas, under continental conditions (Debrecen, Hungary), produced biotechnologically methods, in case of harvest in February.

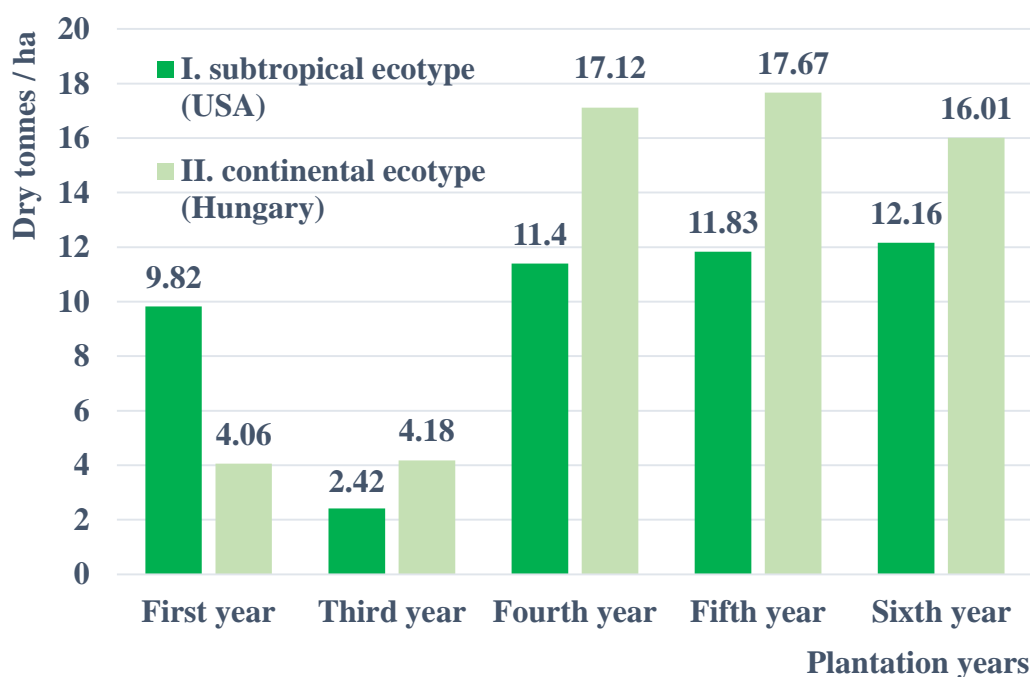


Figure 5: Estimated biomass yield of giant reed ecotypes harvesting in end of the winter (Debrecen – February, 2010-2016)

Source: own composition and data

Based on my own field experiments, giant reed ecotype from warmer climate can produce a biomass of 16.37 dry tonnes/ha by a six-year average on calcareous chernozem soil without irrigation and fertilization, while the continental ecotype from Hungary can produce a biomass of 18.05 dry tonnes/ha annually under winter harvest time and different plant density. Produced biomass yields refer to the dry biomass per hectare harvested in end of winter (February).

It can be concluded that in 1x1 meter plant spacing (10 000 plants per hectare) is enough to establish giant reed plantation. Based on our field experiment, already in the first year in more dense plant spacing (13 334 or 20 000 plant per hectare) the biomass productions did not increase, but the establishment costs are greatly influenced.

I did not list biomass data for the second year on *Figure 5.*, because yields cannot be compared due to different harvesting period. High biomass yields in the second year (49-50 dry tonnes/ha) derived from the fact that green biomass harvested at the end of the growing season, in October 2011, before the plant derived its nutrition's into the rhizomes in the soil. The moisture content of the seemingly dry stocks was high, ranging between 40-45%.

Due to the experiments carried out and processed literature data can be determined that the cold temperature might be a limiting factor in production of giant reed. Temperature below -20°C for 2 weeks in February (2012) resulted in serious frost damages in giant reed plantation due to lack of snow cover. Apical, lateral and secondary lateral buds of the rhizomes damaged. 56.82% of ecotype from warmer climate and 82.04% of ecotype from Hungary were sprouting after winter season. In the third year after the frost damage, the subtropical ecotype produced an average of 2.42 dry tonnes/ha while the continental ecotype produced an average of 4.18 dry tonnes/ha regardless of plant density during harvesting in spring of 2013. Frost damage in 2012 affected biomass yields in the following years. An average of 55.59% of the subtropical ecotype while an average of 74.40% of the continental ecotype could regenerate which proportions remained constant later. Because of the damaged plants were not replenished, the biomass yield per hectare was determined by a decreased plant density from the fourth year. Those giant reed types/varieties need to be produced and grown in Hungary and in regions with a temperate climate with similar winter frost conditions that better tolerate extreme winter.

Based on ongoing field experiment that I have been carrying out for years, it can be concluded that calculated in the first two years the biomass yield of giant reed significantly exceed the yields of miscanthus (8.08 and 33.14 dry tonnes/ha, respectively). Due to the frost damage, in the sixth cultivation year giant reed could achieve again the biomass yields of miscanthus. Without frost damage, giant reed would have produced more biomass than miscanthus every cultivation year based on dry matter of remaining clumps, so in the production of the giant reed needs to utilize climate-adapted varieties or ecotypes.

Many literatures confirmed the genetic diversity of giant reed has been very low among different climate and continent, such as expected for an agamic-reproducing plant. Based on the literature and our experiments, there exist ecotypes with different morphological and biological properties, which were formed effects of climate, as evidenced by the marked differences between our investigated two types of giant reed originating from different climates. As a polyploid plant, these changes could be epigenetic properties. Interesting to mention that in the case of these lines the frost tolerance and the accompanying morphological markers remained stable in the regenerated plants in subsequent somatic generations in spite of the long term embryonic phase. The study of epigenetic inheritances, genetic and molecular methods, is an interesting new field of giant reed researches depending on the different propagation techniques and ecotypes.

The Hungarian Fundamental Law states that genetically modified organism are banned in the country, so it is not possible to produce so called cold-tolerant transgenic giant reed varieties. But it can be established *in vitro* tissue culture from selected cold-tolerant variants, and with natural or artificial frost treatments or/and mutant selection of giant reed lines can be produced more tolerant varieties, which could be propagated by biotechnological methods in large-scale (Antal et al., 2014c, chapter 3.2.).

3.2. The main results of vegetative micro- and macropropagation methods of winter-frost tolerant, new Hungarian giant reed ecotypes

Based on Antal et al. (2014c) could be developed a successful combined agamic propagation protocols from the overwintered shoots of ‘Longicaulis’ ecotype of giant reed grown under temperate climate conditions exposed to winter-frost.

Using our developed propagation methods can be calculated that from one investigated clump with *in vitro* cutting method with lateral shoots of axillary and dormant buds can be produced almost 6500-8700 rooted *in vitro* plantlets need for acclimatization which by further stem cutting method can be produced almost 25 000-35 000 developed plantlets. By combining our micro- and macropropagation methods based on propagation by stem cuttings, 400 to 450 plants can be produced from one *in vitro* shoot during 11 to 12 months of growth. *In vitro* micropropagation of giant reed conducted mostly with the dividing of shoot nodules with high concentration of growth regulators. By application of the developed micropropagation system resulted higher efficient propagation method. By shortening the internodes with 0.2% NaCl pretreatment to produce more nodes in one shoot and more plants in one tissue vessel after micropropagate the stems of *in vitro* shortened shoots.

Another technique is the propagation by somatic embryogenesis. The embryogenic/organogenic callus can be maintained longer time and by plant regeneration can be produce plantlet every period of the year. The embryogenic/organogenic calli are excellent plant material for mutant selection, which can be a perspective breeding technique of giant reed lack of application of genetically modified methods for Hungary and foreign countries. But long time and continuous maintenance of *in vitro* tissue culture could be lead to a decrease genetic potential and somatic variation of plant material. By investigating new *in vitro* agamic propagation protocol for winter-frost tolerant ‘Longicaulis’ giant reed ecotype, it has continued after 70 years of the Martin Krickl’s work to selection of winter-tolerant giant reed varieties in University of Debrecen (Antal et al., 2014c). Besides the produced ecotypes, can be produced and selected different kind of leaf-variants – different colour longitudinal striped – and albino plants, which were planted in the Biomass Experimental Garden of University of Debrecen (*Figure 6*). The new variants with different morphological properties can be important in the breeding of new ornamental horticultural varieties and can be utilise as plant

material in the biological researches of giant reed (photosynthesis, genetic and molecular biological examination, etc.) (Antal et al., 2018).



Figure 6: 3 years old leaf variants of striped giant reed derived from somatic embryogenesis (Debrecen, Hungary – July, 2015)

Sources: own photos, Antal et al., 2018

3.3. Economic assessment of somatic seedling propagation and production of giant reed

3.3.1. The main results of efficiency and cost-benefit analyses of biotechnological propagated giant reed

Generally the giant reed plantation is established from rhizomes, but numerous literature reports about the possibilities of *in vitro* propagation methods. Based on my researches, the investigated somatic seedling propagation technology (MOP Biotech Ltd.) is one of the most efficient biotechnological propagation method for giant reed propagation. *In vitro* somatic seedling production is divided into 2 main phases: laboratory (31 weeks) and nursery phases (7-8 weeks). During propagation I distinguished the produced plant materials according to stages of development and sales opportunities: embryogenic micro-clusters as plant material (*Figure 7A*); *in vitro* rooted shoots developed in cluster – knotty/cluster seedlings for acclimatization (*Figure 7B*); individual plantlet in the propagation tray is suitable for establishment of plantation (*Figure 7C, D*).

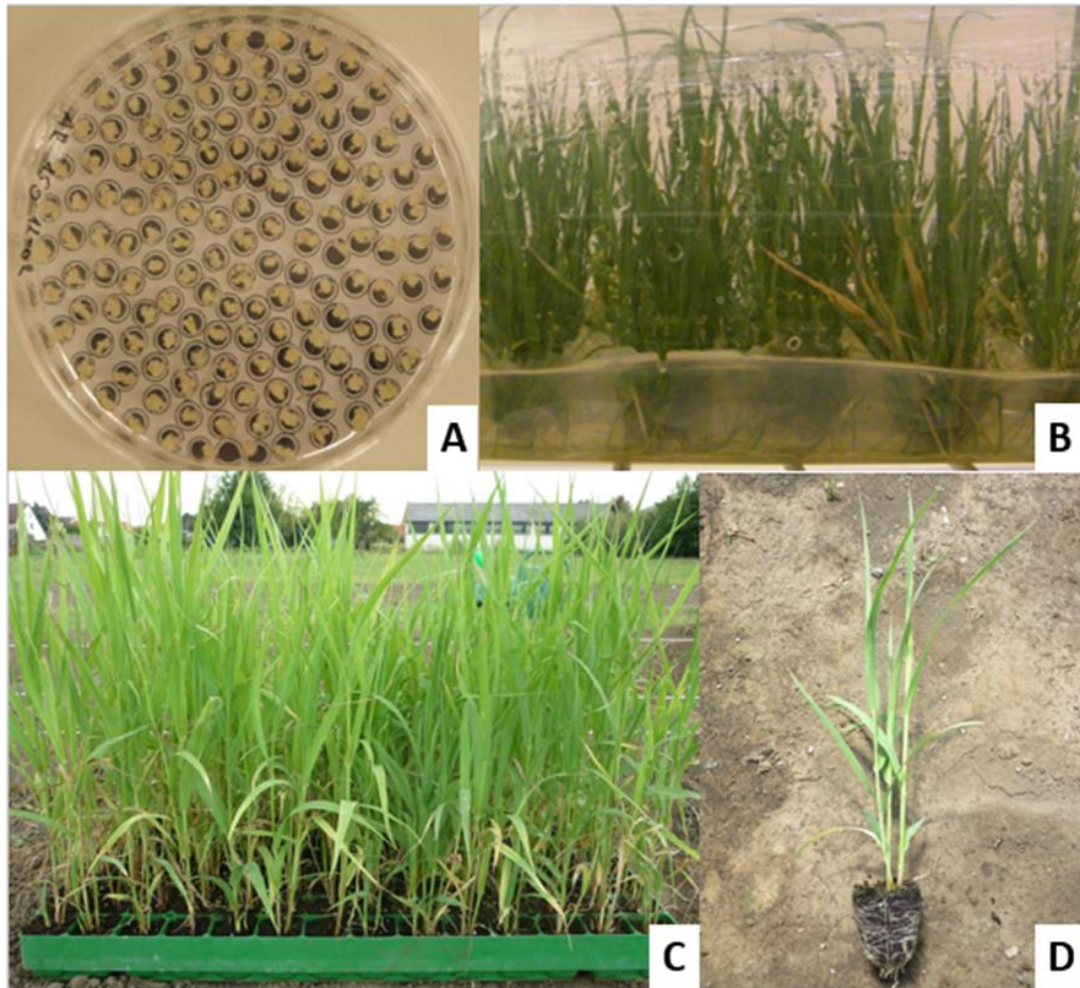


Figure 7: Process of somatic seedling technology of giant reed with the main development phases of biotechnological propagation (MOP Biotech Ltd.)

Source: own photos and composition, 2014. Notes: 1. phase in the Laboratory: A) 150 embryogenic micro-clusters (approx. 2 mm diameter) in Petri-dish as plant material. B) in vitro rooted shoots developed in cluster (8-10 cm high knotty/cluster seedlings) for acclimatization; 2. phase in the Nursery: C, D) individual plantlet in the propagation tray is suitable for establishment of plantation (25-30 cm high plantlet)

Figure 8 shows the giant reed propagation technology with the production capacity of 2 million plants taking optimum acclimatization and plant nursery periods and climatic conditions in Hungary into account.

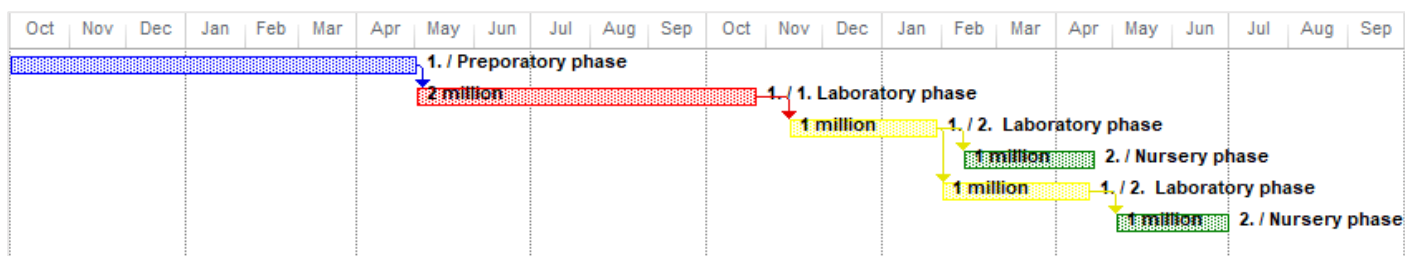


Figure 8: Somatic seedling technology for 2 million/year plants production in Hungary

Source: own composition, 2014. Comment: Blue, red and yellow bars refer to the main stages of the laboratory phase while the green bars show the nursery process under plastic tunnel. The width of the bars represents the nursery period in months.

1. phase: Laboratory

The base plant material includes a Petri dish with embryogenic micro-clusters (*Figure 7A*), of which roughly 750 000 knotty/cluster seedling are suitable for acclimatization can be produced in the first stage of the propagule production during the 8.5-month growing cycle. Considering the maximum capacity of space, working hours and devices, as much as 1 million knotty/cluster seedling (2 million knotty/cluster seedling in 2 cycles) are suitable for acclimatization can be produced on 11 *in vitro* culture shelves annually. This means from 1 embryogenic micro-cluster can be produced 5000 knotty/cluster seedling in one year by somatic embryogenesis. The value of plant production output per unit plant material is multiple of other biotechnological propagation technique which are available in the literature.

Under climatic conditions in Hungary, six permanent workers can produce 2 million knotty/cluster seedling plants/year in a laboratory of 100 m² in the laboratory phase (1 million/cycle). This means that one person can produce 340 000 knotty/cluster seedling per year, which significantly exceed the annual labour capacity of a traditional micro-propagation laboratory. The efficiency of investigated somatic seedling technology is 10 times more than e.g. somatic embryogenesis propagation and regeneration method of Dan et al (2013, 2014) can be produced 35-40 000 plants per person per year. Using the investigated technology, before the phase of acclimatization one person one day can transfer 13 650-15 000 knotty/cluster seedlings to the last culture vessel and medium. The efficiency of this phase is almost 10 times (between 6.8 and 18.75) more than Tóth (2005) described data compared to the efficiency of transfer with scalpel (800-1100 pieces/day) or with scissor (1500-2000 pieces/day).

Approximately 95 000 plants per m² are suitable for acclimatization can be produced in a laboratory cluster culture cycle by the appropriate choice of technological elements (culture medium composition, culture vessels, culturing method and time, etc.) and physical factors for propagation. The high efficiency is linked to the fact that knotty/cluster seedlings derived from somatic embryos can be more easily and quickly taken apart with tweezers, because the plants don't need to be chopped with scalpels or scissors reducing labour time of transferring cultures. Depending on the place of the purchase of the equipment, the total production cost for the production of 2 million plants per year is between 25 527 191 HUF (Chinese purchase) and 32 624 208 HUF (European purchase).

Material cost accounts for 23.56% of production of which 32.80% is the high-value laboratory equipment, 12.81% is the culture vessels, 37.95% is the other laboratory instruments and equipment and 16.51% is the culture medium components. Labour cost accounts for 66.45%, other direct production cost (rents, overhead costs, etc.) for 8.70% and other overhead cost accounts for 1.29% of 2 million plant production.

Production value and profitability for *in vitro* somatic seedling production of giant reed are shown in *Table 1*. Net profit of 37 412 809 HUF can be gained at the sales price of 31.47 HUF from the sales of 2 million knotty/cluster giant reed seedlings, that are suitable for acclimatization. Direct cost of plant production is 12.60 HUF/plant and cost-benefit ratio of production is 146.56 HUF (*Table 1*).

Table 1: Production value and profitability for somatic seedling production technology of giant reed (2013-2014)

The place of purchase of high-value assets	1. phase/ 'A' production type		1. phase/ 'B' production type	
	China	Europe	China	Europe
Number of produced plants / yield (pcs)	750 000		2 000 000	
Sales price (HUF/plant)	31.47			
Production value (HUF)	23 602 500		62 940 000	
Direct production cost (HUF)	16 211 756	23 318 773	25 197 191	32 304 208
Margin requirement (HUF)	7 390 744	283 727	37 742 809	30 635 792
Overhead cost (HUF)	150 000		330 000	
Total production cost (HUF)	16 361 756	23 468 773	25 527 191	32 634 208
Net profit (HUF)	7 240 744	133 727	37 412 809	30 305 792
Cost related profitability (%)	44.25%	0.57%	146.56%	92.87%
Direct cost price (HUF/plant)	21.62	31.09	12.60	16.15

Source: own data and composition, 2014

2. phase: Nursery

Knotty/cluster seedling produced in the laboratory phase that are ready for acclimatization can be transplanted in heated plastic tunnel from mid-February is taking climatic and light conditions in Hungary into account. Initiating of culturing in the laboratory is recommended to adjust to the date of transplantation in the plastic tunnel (*Figure 8*). Nursery time that consists of the acclimatization phase of bare-root cluster plants from the laboratory (14-21 days) and development of individual plants in propagation trays (35-42 days) requires 7-8 weeks depending on temperature and light conditions (*Figure 9A, B, C*). Establishment of giant reed plantation can be performed on a continuous basis until August in Hungary, as based on the developed technology in nursery, well-acclimatized and adequately rooted plants may be overwintered till plantation either outside or under the plastic tunnel (*Figure D*).

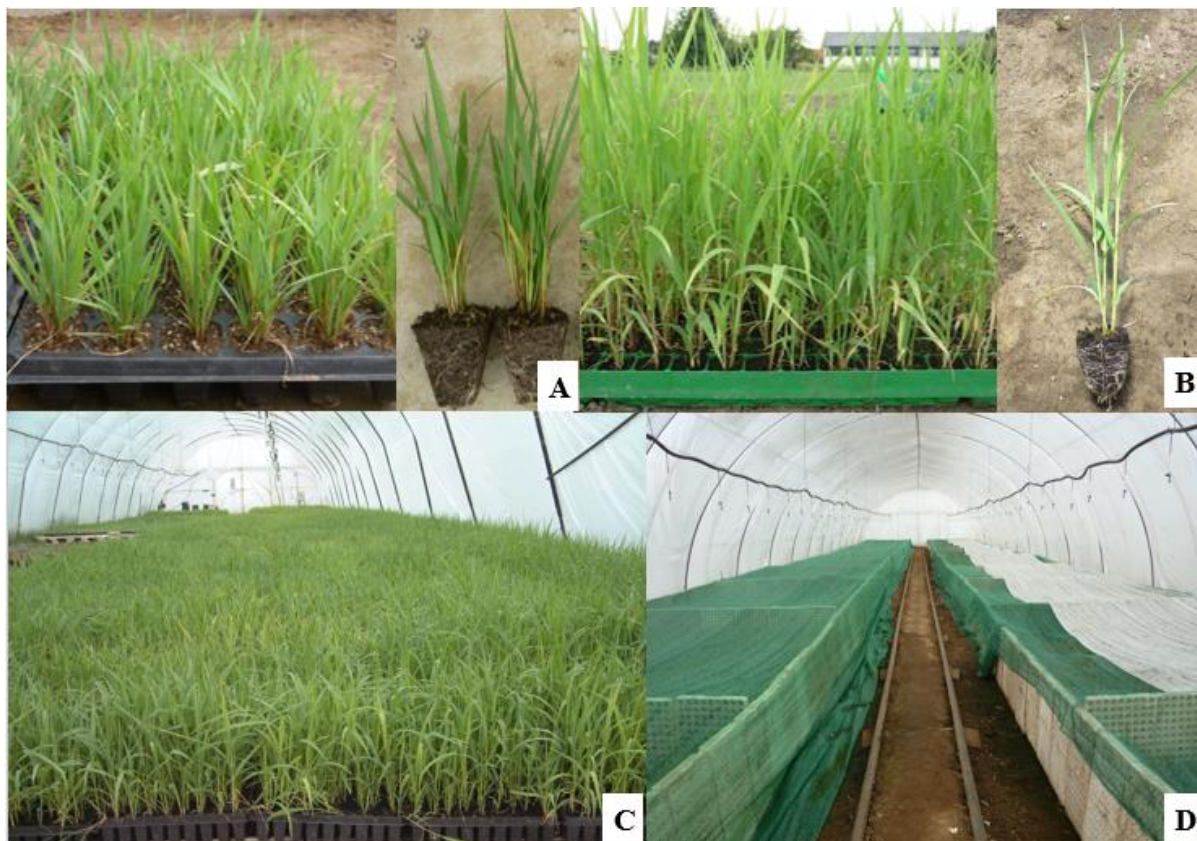


Figure 9: Nursery phases of somatic seedling technology of giant reed

Source: own photos and composition, 2014. Comment: A) Knotty/cluster-plantlet in propagation tray B) Individual-plantlet in propagation tray. C) Individual plantlets in propagation tray (30 cm³) are suitable for establishment of plantation. D) Plantlet wintering in under large plastic tunnel in Hungary.

Table 2. shows the production value, production costs, revenues for somatic plantlet throughout the total propagule production in the case of 750 000 and 2 million plants produced.

Table 2: Production values and revenues for throughout propagule production

	Individual somatic plantlets are suitable for establishment of giant reed plantation	
Yield (plant/year)	750 000	2 000 000
Sales price (HUF/plant)	47.47	47.47
Production value (HUF)	35 606 025	94 949 400
Direct production cost (HUF)	28 211 756	57 197 191
Margin requirement (HUF)	7 394 269	37 752 209
Overhead cost (HUF)	300 000	480 000
Total production cost (HUF)	28 511 756	57 677 191
Net profit (HUF)	7 094 269	37 272 209
Cost related profitability (%)	24.88%	64.62
Direct cost price (HUF/plant)	37.62	28.60

Source: own data and composition, 2014

The investigated private company sold the knotty/cluster seedlings and individual somatic plantlet in foreign market (Italy, Spain, China, etc.). Total production cost for 2 million somatic plantlet per year was 57 197 191 HUF. Net profit for plants sold at 47.47 HUF (0.15 EUR) was 37 752 209 HUF per year. Cost price for *in vitro* somatic seedling technology depends on the output of the plant and capacity of production. Cost price for giant reed propagule production may be reduced to 28.60 HUF (0.09 EUR) by an efficient biotechnological method. We have information from a company based in Pescara, Italy about the propagule cost for its plants from its own *in vitro* micropropagation technology was 0.35 EUR and cost of rhizomes propagule was 0.5 EUR (Corno et al, 2014; Pilu et al., 2013; I12). But the detailed description of technology, cost and profitability indicators are unknown. By using the investigated somatic seedling technology may be reduced the propagule cost by the half (0.12 EUR – 37.62 HUF) and by the third (0.09 EUR – 28.60 HUF). It may be one-fifth of the cost of rhizomatous propagule (0.5 EUR). Currently, in Hungary Arundo Cellulóz Framing Ltd. (Újfehértó) and related companies have been dealing with *in vitro* propagation of giant reed. The plantlets from *in vitro* propagation sold at 74-75 HUF (0.24 EUR) in 2014-2015 (I13) and technology is protected by numerous patents according to available information.

3.3.2. The main results of expected costs for giant reed production depending on the recommended cultivation practices

A production of perennial plants is entirely different from arable crops. In economic terms, the lifecycle of a plant lasts from the production or purchase of propagule till the destruction of the plantation. Based on our researches of multiple years and processed literature data suggest that giant reed production is possible not only in the southern countries in Europe, but also in Hungary due to its temperature and light conditions. Based on literature obtained so far, the lifecycle of the plant may cover more than 20 years. During these period the cost of establishment, the price of propagule and the annual harvest with additional costs are the greatest expenses and operations (*Table 3*).

Table 3: Expected cost for giant reed production per hectare during the twenty-year-old lifecycle divided into operations in Hungary

Type of operation	Number of operation (pcs)	Cost of operations (HUF/ha)	Total 20 years (HUF/ha)	Ratio of operation (%)
Soil preparation	1	33 000	33 000	1.19%
Propagule	1	500 000	500 000	17.99%
Establishment	1	50 000	50 000	1.80%
Weed control (manual, mechanical and chemical)	5	14 400	72 000	2.59%
Fertilization	5	25 000	125 000	4.50%
Harvesting, transportation, storage	20	100 000	2 000 000	71.94%
Direct production cost (HUF)	-	-	2 780 000	100%

Source: based on MOP Biotech Ltd. data, 2012-2014, own composition. Comment: data refer to plantation that established in 2012. Cost for machinery refers to contract work. The table includes neither the cost for the destruction of the plantation nor the land leasing.

Based on calculations from 2012 to 2014, direct production cost for biotechnology-derived giant reed plantations is 2 780 000 HUF per hectare for the lifecycle of 20 years. The rates of costs during the lifecycle of 20 years are listed as follows: soil preparation (1.19%), costs for

propagule and plantation (19.79%), weed control (2.59%), fertilization (4.50%), harvesting, storage and transportation (71.94%). The calculations suggest that it was not the cost for propagule and plantation which amounted to the highest during giant reed production, but the harvesting and additional cost per year if biotechnology-propagated somatic seedling plants are used (Table 4).

Table 4: Expected production values and revenues for giant reed production depending on sales price in Hungary

Yield (dry tonne/ha/20 year)	388		
Sale price (HUF/dry tonne)	8 000	12 000	16 000
Production value (HUF/ha/20 year)	3 104 000	4 656 000	6 208 000
Direct production cost (HUF/ha/20 year)	2 780 000		
Margin requirement (HUF/ha/20 year)	324 000	1 876 000	3 428 000
Margin requirement (HUF/ha/year)	16 200	93 800	171 400
Overhead cost (HUF/ha/20 year)	300 000		
Total production cost (HUF/ha/20 year)	3 080 000		
Net profit (HUF/ha/20 year)	24 000	1 576 000	3 128 000
Net profit (HUF/ha/year)	1 200	78 800	156 400
Cost related profitability (%)	0.78%	51.17%	101.56%
Direct cost price (HUF/ dry tonne)	7 165		

Source: own composition based on MOP Biotech Ltd. data, 2012-2014, without financial support.

Comment: data refer to plantation that established in 2012. It can be calculated in the first year with 8 dry tonnes / ha biomass yield, from second year with 20 dry tonnes / ha biomass yield

In Hungary, the consumption of household sector is not significant therefore biomass power plants are expected to be the greatest market outlet of the plant. Absolutely dry giant reed chops that might decrease at 8000 HUF (25-26 EUR) ranged from 12 000 to 16 000 HUF (38-52 EUR) (2012-2014). Total cost of giant reed production was 3 080 000 HUF while net profit per hectare was around 1 200-156 400 HUF depending on the revenue. Direct cost price was 7 165 HUF/dry tonne/ha (26 EUR). It is concluded that current low price of energy does not

enable efficient production to combustion contributed also by the absence of the domestic processing industry (*Table 4*).

Based on economic calculations carried out in similar years by Arundo Italia Ltd., total cost of giant reed production exceeded the Hungarian data, but the costs for harvest and transportation are not significant due to the proximity of the market outlets. The company establishes giant reed plantations, particularly for biogas, but also for bioethanol production with the suitable facilities. The calculation of 20 years giant reed cultivation, total direct production cost amounts 14 350 EUR (717.5 EUR/ha), from propagule cost is 3 850 EUR/ha (26.83%), cultivation and maintenance cost of plantation 10 500 EUR/ha (73.17%) with 35 dry tonnes/ha biomass yield. In North-Italy, direct production cost amounts 700-1000 EUR annually, direct cost price has been changing between 13 and 20 EUR (Corno et al., 2014; I12).

Currently, global giant reed production utilise as biogas and bioethanol production, but other forms of its use were also reported. The purposes of giant reed utilisation need to produce higher-value added products, such as biofuels, biogas, basic chemical components, bio-plastic raw materials and fodder, feed or pharmaceutical components. However, absence of biorefinery industry, it has not yet spread the production of giant reed in large-scale, in spite of numerous advantageous properties of giant reed or other dedicated biomass species.

4. NEW AND NOVEL RESULTS OF THE DISSERTATION

1. It was the first scientific dissertation in Hungary which is conducting researches with giant reed, in the research area of biotechnological propagation, biomass production and utilisation of giant reed.
2. Based on hundreds of literature relating to this scientific field I concluded that one of the main obstacles is the propagule production out of the factors (biomass yield, production cost, sales price, costs for transportation, manufacturing and storage, and the existence of market outlets and their proximities) that have a profound effect on giant reed production as feedstock in bioindustry.
3. The costs for propagule production may be reduced by biotechnology for giant reed somatic seedling technology. Compared with the costs for rhizome propagule (0.5 EUR) and plantlet derived from micropropagation (0.385 EUR), propagule may be produced by the technology concerned at a cost price of 0.09 EUR in Hungary. A more competitive presence can be introduced on the market by homogenous products (plantlet) in large quantity produced with high efficiency.
4. Based on the six-year-old field experiments for giant reed production in Debrecen, I demonstrated that those varieties/ecotypes should be grown and produced in the future that are adapted to the climate conditions. New molecular breeding methods of biotechnology should be used to produce new varieties.
5. Based on the six-year-old field experiments for giant reed production in Debrecen, I demonstrated that ecotypes from a subtropical climate (USA) could produce a biomass yield of 16.37 dry tonnes/ha/year in calcareous chernozem soil without irrigation and fertilization while ecotype from a continental climate (Hungarian) could produce a biomass yield of 18.05 dry tonnes/ha/year at 3 average plant density.

5. PRACTICAL USE OF THE RESULTS

Based on my research it can be concluded that giant reed might be one of the most promising biomass plants in terms of production, ecological, environmental, biological and economics not only on a global scale, but also in Hungary.

Due to lack of efficient biotechnological methods and expertise in production in Hungary so far, large scale production has not been possible, there has not been a cheap propagule that is suitable for large-scale production and adapts to the Hungarian climate. Based on foreign initiatives for giant reed production, propagule was produced by the costly and non-efficient division of rhizomes and stem cuttings at a cost of 0.5-1 EUR. One of the greatest benefits of biotechnological propagation is the virus-free, homogenous propagule that can be produced at any part of the year in large-scale purposes. Thanks to the investigated methods, one person can produce 340 000 knotty/cluster seedling per year, which significantly exceed the annual labour capacity of a traditional micro-propagation laboratory. By the development of micropropagation technologies, Italian professionals could further decrease the cost of propagule which can be reduced by half by somatic seedling technology concerned (cost price for propagule production may be less than 0.1 EUR). Due to developments in biotechnological propagation methods, it can be produced million homogenous virus-free plantlet, which contribute to large-scale giant reed production.

However, large-scale utilisation of cost-efficient giant reed biotechnological propagation technologies itself not resulted the large-scale production of giant reed, because of the absence of biomass factories and biorefinery industry. My researches can be concluded that the highest costs in giant reed production are not the cost for propagule (17.99%) now, but harvest, transportation and storage (71.94%) per year.

The ‘future biomass plants’ and their production must be scientifically planned. It is expected that ‘there is only one right way or plant’ approach cannot be pursued. There are only few formally recommended dedicated bioenergy species. It is impossible to plan and invest if there is a lack of production data, scientific results are uncertain and there is no suitable processing industry. In my opinion, in the future, in the case of more efficient green energy production and plant biomass-based biorefinery industry, the giant reed can also be produced to utilize with numerous other biomass plant species.



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Candidate: Gabriella Antal
Neptun ID: LWZP6H
Doctoral School: Károly Ihrig Doctoral School of Management and Business
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