Environmental Engineering and Management Journal

November 2014, Vol.13, No. 11, 2709-2715 http://omicron.ch.tuiasi.ro/EEMJ/



"Gheorghe Asachi" Technical University of lasi, Romania



TISSUE CULTURE AND AGAMIC PROPAGATION OF WINTER-FROST TOLERANT 'LONGICAULIS' ARUNDO DONAX L.

Gabriella Antal^{1*}, Erika Kurucz¹, Miklós Gábor Fári¹, József Popp²

¹Department of Agricultural Botany, Plant Physiology and Biotechnology, University of Debrecen MÉK, Böszörményi út 138., Debrecen, 4032, Hungary ²Faculty of Applied Economics and Rural Development, University of Debrecen GVK, Böszörményi út 138., Debrecen, 4032, Hungary

Abstract

The primary objectives of this study were to identify the ecotypes of the 'longicaulis' giant reed (*Arundo donax* L.) that are winter-frost and cold tolerant in temperate climates on marginal lands and to perform biotechnological research on these ecotypes. We searched for large 'longicaulis' giant reed ecotypes, which wintered successfully in spite of the 2012/2013 winter frosts and sprouted from stem nodes in the spring of 2013 in three locations of the Balaton basin. Starting *in vitro* culture from the buds of the secondary shoot of the full-grown plant on Murashige and Skoog (1962) medium supplemented with 0.3 mg L⁻¹ 6-benzyl-aminopurine (BAP) and 0.05 mg L⁻¹ 1-naphthalene-acetic acid (NAA) was the most satisfactory during October. Our experiments indicated that *in vitro* propagation by stem cuttings can be successfully performed from *in vitro* shoots (node number increased with 0.2% NaCl treatment) on Murashige and Skoog (1962) medium containing kinetin (3 mg L⁻¹) and indole-3-acetic acid (3 mg L⁻¹). 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.

Key words: giant reed, longicaulis ecotype, macropropagation, micropropagation, winter-frost tolerance

Received: February, 2014; Revised final: October, 2014; Accepted: October, 2014

1. Introduction

The giant reed (*Arundo donax* L.) is a tall perennial cane originating from the Indian subcontinent. The giant reed belongs to the family *Poaceae*, and it does not produce any seeds, but it can be propagated vegetatively from the rhizome; therefore, this reed is a well-known ornamental plant worldwide (Perdue, 1958). As a result of the industrial need for renewable energy and biomass, there is an increasing commercial demand for giant reed production (Mariani et al., 2010). The first large-scale giant reed production program was launched in the decade before the Second World War in Italy. The primary objective of establishing Torviscosa, the first Italian "technopolis", was the

large-scale production of cellulose from giant reed to produce viscous artificial silk and paper and for gunpowder manufacturing (Scaini, 2012). During the second half of the 1990s, foreign technical literature regarded the giant reed as one of the most promising plants of the cellulose-based biomass industry (El Bassam, 1996). The first European giant reed research program, which also featured biotechnological propagation methods, was named the "Giant reed (Arundo donax) Network Improvement, Productivity and Biomass Quality" and began in 1997 (Bacher et al., 2001). Over the past ten years, the giant reed was considered one of the most significant "cellulose farming" plants of the biomass industry (Angelini et al., 2005; Lewandowski et al., 2003; Mantineo et al., 2009; Pilu

^{*} Author to whom all correspondence should be addressed: e-mail: antalgabriella87@gmail.com; Phone: +36 52 508 444/ 88486; Fax: +36 52 508 444/ 88486

et al., 2012). Research regarding the biotechnological propagability of the giant reed began in Europe during the last decade of the 20th century. Szilárd Tóth, a Hungarian researcher, was the first to initiate the tissue cultivation of the giant reed in Germany (Tóth and Mix-Wagner, 1998; Tóth et al., 1998a) and continued this work in Hungary at the University of Debrecen (Tóth et al., 1998b; Tóth and Pepó, 1999). The popularity and industrial production and processing of the giant reed are restrained by three primary factors. First, there is a lack of inexpensive and programmable mass propagation methods with guaranteed efficiency (Angelini et al. 2009, Ceotto and Di Candilo, 2010). Second is the restricted breeding opportunity, which results from the lack of generative cycles (Balogh et al., 2012; Takahashi and Takamizo, 2012). Third, there is the unsatisfactory or even lack of experience in large-scale production (Cosentino et al., 2008, Nasso et al., 2011).

According to the most recent studies, it is possible to overcome these three obstacles to production. Currently, the mass propagation of the giant reed (Takahashi et al.; 2010, Cavallaro et al., 2011; Herrera-Alamillo and Robert, 2012) and its genetic transformation (Dhir et al., 2010; Takahashi and Takamizo, 2012) have been thoroughly investigated. In addition, somaclonal breeding, the induction and controlled selection of mutants (Trebbi et al., 2013), phytoremediation potential (Alshaal et al., 2013a, b), and virological and risk background of clonal propagation (Tóth et al, 2011) are also important aspects that should be evaluated. During recent years, giant reed has been considered a promising energy crop because of its high biomass production. A comparison of Arundo donax and Miscanthus x gigantheus in a long-term field experiment in Central Italy (Pisa) showed an higher biomass production for giant reed, with an average biomass production of 37.7 tons of dry matter per hectare compared to 28.7 t ha for miscanthus.

These results were obtained without irrigation and with fertilization each year with 100 kg P₂O₅/ha, 100 kg K₂O/ha and 100 kg N/ha (Angelini et al., 2009; Pilu et al., 2013). Arundo donax cultivation (15 years life span) under these conditions may be the best choice for an energy crop with an annual production cost of approximately 700 Euro/ha. The production cost for maize or sorghum is much higher, being 1 700 euro/ha and 2 100 euro/ha, respectively. Pilu et al. (2013) estimate the cost to be 0.5 Euro/plant for Arundo donax propagules, such as young plantlets in pots. However, the real cost of these materials will be defined when a large-scale reproduction system is used. Additionally, the number of propagules per hectare must be considered.

Reports on field establishment rates vary from approximately 2,500 propagules per hectare to 10,000 - 20,000 propagules per hectare. *Arundo donax* cultivation on a large scale does not currently exist, and the few existing experimental fields have been primarily obtained by the transplantation of rhizome fragments, usually with 10,000 rhizomes per hectare, making this procedure very expensive, with costs of nearly 1 Euro per vital rhizome (Ceotto and Di Candilo, 2010; Pilu et al., 2013).

The land for rhizome production should be large because it has been estimated that from 1 m² of mature culture (three years), only 20 vital rhizomes can be harvested. Therefore, for rhizome production for transplantation, at least three hectares of crop to produce the rhizomes are necessary to transplant a 20 hectare land without losing this capacity each year, with a ratio of 6.6 for new hectares planted and hectares used to produce the rhizomes. However, for maize F1 seed production, this ratio is greater than 1 to 100, which is the main reason for the high cost of large scale giant reed production (Ceotto and Di Candilo, 2010; Pilu et al., 2013). Another strategy to overcome the propagation problem is the use of an in vitro propagation technique as reported by Takahashi et al. (2010).

The goal of our study is to cut the cost to less than 0.5 Euro/plant for *Arundo donax* propagules, such as young plantlets in pots. Experiments in Hungary show that that this reduction can be achieved on a large scale.

2. Objectives

One of the main practical objectives of breeding of the giant reed in areas with temperate continental climate, including Hungary, is to increase winter tolerance. It is not widely known that giant reed breeding began in Germany and Austria in the 1940s with a special focus on increasing cold tolerance (Krickl, 1946). In these locations, giant reed production is unstable primarily because of windy, snowless, and cold winters with temperatures permanently below 10 °C (Antal et al., 2012).

Fundamental of The Law Hungary specifically states that genetically modified organisms are banned in the country. It is not possible to produce cold-resistant transgenic giant reeds. One alternative is the in vitro mutant selection (performed in sterile cultures) of cold-tolerant giant reed varieties. The other alternative is based on the observation that the stems of certain planted or natural giant reed populations can sometimes survive the winter and develop lateral shoots the next year without any external protection (Antal and Fari, 2013).

To exploit both these approaches, tissue cultures must be started from the morphogenic meristems (dividing tissues) of the giant reed. These meristems can grow under sterile conditions, and their sterile (in vitro) maintenance must also be assured. Following the establishment of sterile tissue culture and/or the induction of mutants and the controlled selection of targeted artificial and/or natural frost treatments, the shoots of the more winter-frost-tolerant giant reed lines can be produced high volume either at using microor macropropagation techniques.

3. Materials and methods

3.1. Plant materials

The lateral shoots of a two-year-old overwintered stem of *Arundo donax* were collected in Hungary in mid-June 2013 and late October 2013. Based on the internode length, the angle of the leaf blade and width as well as color of epidermis the ecotypes were classified into the 'longicaulis' group of *Arundo donax* (Balatonkenese: 47.03230 N, 18.103199 E; Balatonlelle: 46.79242 N, 17.724737 E; Balatonföldvár: 46.85233 N, 17.89748 E).

Single-bud stem cuttings, 2 cm long, were prepared from the axillary and dormant buds of the lateral shoot for propagation. Cuttings of larger diameter were cut in half lengthwise. Approximately 250 cuttings were used for the establishment of the *in vitro* cultures and their propagation from green cuttings.

3.2. Establishment of sterile in vitro shoot cultures from the axillary buds of two-year-old giant reed lateral shoots

3.2.1. Sterilization procedure

The stem cuttings were sterilized with 70% (v/v) ethanol for 1 minute, followed by one rinse with distilled water. Then, 2% calcium hypochlorite solution and 0.1% (v/v) Tween 20 detergent were used for 20 minutes for sterilization. The inoculums were soaked in 15% hydrogen peroxide for 6 minutes. The cuttings were rinsed with sterile distilled water three times. The entire sterilization process and *in vitro* culturing occurred in the laminar box. A total of 250 cuttings were sterilized in four replications.

3.2.2. Culture media and culture vessels

Murashige and Skoog (1962) culture medium was used, which contains micro- and macroelements, including vitamins (MS, Duchefa Biochemie, The Netherlands) and 3% sucrose, pH 5.78. 0.2% Phytagel (Sigma-Aldrich Kft., Hungary) was used to solidify the culture medium. Following sterilization, the explants were grown on solid medium by adding 0.3 mg L⁻¹ 6-benzyl-aminopurine (BAP) and 0.05 mg L⁻¹ 1-naphthalene-acetic acid (NAA) (Duchefa Biochemie, The Netherlands) (Takahashi et al., 2010). After sterilization of the culture media (121 °C for 20 minutes), 100 mg L⁻¹ cefotaxime (Teva Gyógyszergyár Kft., Hungary) was added with a cellulose acetate syringe filter (0,22 µm, Labsystem Kft., Hungary) to eliminate the bacteria. The explants were grown in 100 mL Erlenmeyer flasks and gamma sterilized transparent polypropylene Petri dishes 10 cm in diameter (Sarstedt Kft., 4 mL medium per explant per Petri dish) at 25 °C and 40 µmol m⁻² s⁻¹ illumination for 16 hours (Polylux XL® S30W/830, Tungsram, GE Lighting Hungary Kft., Hungary). The sterilization efficiency was evaluated after seven days.

To maintain sterile cuttings, passages to hormone-free, solid medium occurred every four weeks with 100 mL Erlenmeyer flasks as described in the methods above.

3.3. Modifying the internode length of in vitro shoots with NaCl treatment

Elhawat et al. (2013) examined the dwarfing effect of culture media containing various concentrations of NaCl on *in vitro* shoot cultures of *Arundo donax*. Their research mostly focused on determining the salt resistance of the plant and the selection of NaCl-tolerant lines. The desired dwarfing effect on *Arundo donax* can also be achieved with plant growth regulators and other compounds, such as sodium selenate (Domokos-Szabolcsy et al., 2005, 2012).

Our previous experiments showed that plants can adapt to increased NaCl concentrations of 0.5 and 1%, although elongation of the stem stops at 0.5% and the leaves start to yellow (Elhawat et al., 2013). To shorten the internode length and obtain an in vitro shoot, which has as many developed nodes as possible, 0.2% and 0.4% NaCl (Duchefa Biochemie, The Netherlands) was added to the culture medium. The dwarfing pretreatment with NaCl was tested to avoid somaclonal variations, which is a reasonable requirement for a new method not based on plant growth regulators. The in vitro shoots were grown in 13 cm tall culture dishes 720 mL in volume and 9 cm in diameter. A total of 10, 15 and 20 inoculums per concentration were sterilely placed into a culture dish covered using the TPFC method (Fári et al., 1987). The experiment had three replicates, and the evaluation was performed at four weeks.

3.4. Micropropagation of longicaulis giant reed from NaCl-treated stem micro-cuttings

The elongated shoots treated with NaCl were cut into 1 to 2 cm long stem cuttings, basal shoots shoot apices. During the preliminary and experiments, the shoot apex and basal parts should be separated from the stem parts to overcome apical dominance. Notably, the majority of dormant buds were able to sprout by laving stems horizontally. although the dormant buds could be more stimulated to sprout by chopping the internodes of the stems (Fig. 1/A, B). According to the method of Herrera-Alamillo and Robert (2012), the cuttings were grown on liquid culture medium containing 3 mg L⁻¹ IAA (indole-3-acetic acid) and 3 mg L^{-1} kinetin (KIN, 6furfurylaminopurine) (Duchefa Biochemie, The Netherlands). Erlenmeyer flasks were used in our previous experiments (Fig. 1/C); however, a foursided transparent polypropylene (PP) box of 1000 mL volume closed with snap-on caps (PP1000: 7.3 cm tall, 16 cm long and 10.7 cm wide, Korona Kft., Kaposvár, Hungary) was used because of the small culture volume and the inefficient labor use of the Erlenmeyer flasks.



Fig. 1. Micro- and macropropagation of longicaulis *Arundo donax* ecotype. The horizontal position of the nodes are shown with arrows (A). Cultures of the sprouted shoots grown in horizontal position after 14 days of incubation (B). 28-day-old cultures grown in 100 mL Erlenmeyer flasks (C). Sprouting and rooting of 14 day-old stem macro-cuttings. The arrows point to the part of internode above the shoot bud (D). Growth and rooting of stem macro-cuttings after being planted into the soil at 28 days. The arrows point to the part of internode above the shoot bud (E). Growing of 21-day-old acclimatised plantlet in nursery tray obtained from separated and rooted stem micro-cuttings (F)

A total of 70, 50, 45, 40 and 35 inoculums were placed into the PP1000 culture dishes with 100-100 mL culture medium in each. The cultures were grown in the light using the above described method and were evaluated after 90 days, taking the experiments of Herrera-Alamillo and Robert (2012) as a baseline, but the medium was no refreshed.

3.5. Macropropagation of longicaulis giant reed from stem macro-cuttings

The stems produced during the in vitro propagation from stem cuttings were acclimatized in a greenhouse in propagation trays, planted in cells of 60 cm³ in volume per plantlet. Thin plastic cover net were used for one week to protect acclimatized plantlets against water lost maintaining sufficient moisture lever of the nursery trays. Special rooting substrate with high nutrient content (3000 mg l^{-1}) was used (Jó Föld XXL, Pax96 Ltd., Kecskemét, Hungary). The 16-week-old, 70-80 cm tall plants grown in 60x40 cm nursery trays (126 plants per tray), were cut back to 5-10 cm height. The green shoots were immersed in water for 48 hours until processing. Before preparing the cuttings, the leaves were removed from the stems to make the axillary buds of the stem visible, and they were sliced into 1-2-bud cuttings. The cuttings were incubated on wet filter paper for 14 days at 25-27°C in the dark with frequent ventilation (Fig. 1/D). The stem cuttings with small white roots and white axillary buds, which started to elongate, were planted into the soil mixture described above (Fig. 1/E). The cuttings were used as the base material (250 cuttings altogether) and were rooted at the time of both samplings directly after their removal (late July and October) from a greenhouse.

3.6. Statistical analysis

Descriptive statistical methods, including the sum, mean, and standard deviation and one-way ANOVA were used to determine the impact of the treatments. The data were evaluated with OpenStat ver. 17.0. The significant differences between each treatment were determined with Tukey's test at a probability level of 5%.

4. Results and discussion

4.1. Establishment of in vitro cultures from lateral shoots of two-year-old longicaulis giant reed ecotype

The cuttings prepared during the sampling in July 2013 rooted 100%, but it was not possible to establish a sterile shoot culture due to internal infections. Of the 150 cuttings prepared during the sampling in early October rooted 15%. Of these, 15 sterile *in vitro* shoot cultures were prepared with 10% efficiency.

During the sampling in October, the average height of the overwintered two-year-old stems was 292.4 cm (SD \pm 35.5), the average number of nodes that sprouted per stem was 18.4 (SD \pm 4.51) and there were approximately 200 per stock. The average length of the lateral shoots was 25.35 cm (SD \pm 11.22), of which 3-4 single-bud cuttings can be prepared. The shoot apex of one of the two-year-old stems did not freeze during the winter, but continued to grow during the growing season. From each individual, approximately 600-800 propagable buds can be found; therefore, a maximum of 60-80 *in vitro* shoots can be obtained with the currently used external sterilization procedure. At the time the shoot cultures were initiated, one to two shoots were produced on one stem node. Following a four-month long cultivation, a ten-fold propagation rate was obtained without cutting the stems. No morphological deformation was observed on the culture medium containing 6-benzyl-aminopurine (0.3 mg L⁻¹) during the cultivation of the subcultures. Our observations are identical to Takahashi et al. (2010). The sterilization procedure was not highly effective because of infections.

During large-scale production, starting material should be prepared before cutting. These can be maintained with pre-treatments consisting of proper conditioning and fungicide. For the giant reed, must be established using virus-free propagation material produced with *in vitro* methods to avoid aphid-spread viruses, such as the barley yellow stripe virus (Tóth et al., 2011).

An alternative is the change of the initial explants. In addition, immature inflorescent can also be suitable for starting an Arundo culture because they can be sterilized at an efficiency of 90% (Tóth and Mix-Wagner, 1998, Tóth et al., 1998 a, b; Linder and Gallagher, 1998; Tóth and Pepó, 1999; Bacher et al., 2001). However, this method is restricted by the fact that it can only be used once, at the beginning of flowering.

4.2. Modifying the internode length of in vitro shoots with NaCl treatment

It was concluded that the fresh weight of plants decreased in comparison with the control as a result of NaCl treatments (data not shown). The shoot length also significantly decreased in all explant densities (Fig. 2). Furthermore, it was observed that



Fig. 2. Effect of NaCl concentrations and densities of explant on the shoot length of longicaulis giant reed grown *in vitro* (the letters at the top of the column represent the significant differences as a result of NaCl concentrations, which were determined with Tukey's test at the p<0.05% level)

the number of axillary buds significantly increased in all cases when 0.2% NaCl was added (Fig. 3).

In the case of culture vessels containing 15 plants grown on 0.2% NaCl concentration, we noted that significantly more axillary buds developed compared to the control and 0.4% NaCl concentration. It was also observed that the number of buds did not increase adding 0.4% NaCl and the plants could not develop to the same extent as in the case of 0.2% NaCl (Fig. 3). The shortest internode of the control plantlet was 0.3 cm, while it was only 0.1 cm in the case of 0.2% and 0.4% NaCl concentrations. To the contrary the longest internode of the control plants was 4.2 cm, whereas it was 2.4 cm in the case of 0.2 and 0.4 % NaCl concentration (data not shown). In the further stages of cultivation, it was observed that any applied NaCl treatments did cause physiological and morphological not abnormalities in the subsequent development of the plantlets. Meanwhile it was observed that 0.2% NaCl concentration resulted significantly higher number of axillary buds during subsequent in vitro propagation stages based on stem micro-cuttings resulting 30% higher multiplication rate in comparison with the control cultures (Fig. 3).

4.3. Micropropagation of longicaulis giant reed from NaCl-treated stem micro-cuttings

The highest fresh weight was obtained for the PP1000 box containing 50 and 45 initial stem cutting, in which one plant had 2 mL culture medium on average. The average fresh weight of the plants was 0.81 g (SD \pm 0.232). The largest loss was observed for the 50 and 40 inoculums per PP1000 box (12.0% and 12.5%, respectively), in which 5 and 6 stem cuttings did not grow, respectively. The highest germination rate (1.36%) was achieved for the 45 inoculums per box following the three-month-long cultivation without refreshing the culture medium.



Fig. 3. Effect of NaCl-concentrations and densities of explant on the number of shoot buds of longicaulis giant reed grown *in vitro* (the number of shoot buds involves the basal and dormant buds, as well as the shoot apex. Asterisk marks the significant difference examined with Tukey's test at the p<0.05% level

The proportion of rooted plants that developed from 1 axillary shoot was 79.49% for 40 stem cutting per box and 75.15% for the 50 inoculums per box. The number of cases with two rooted axillary shoots increased for the 70 and 45 inoculums per PP1000 box (17 and 10, respectively). Altogether, the number of axillary shoot plants with three shoots did not reach 5%.

The proportion of shoots shorter than 1 cm was 15.91 and 11.48% (14 and 7 shoots) for the 70 and 45 inoculums, respectively. The proportion of 1-3 cm long shoots was nearly identical (55.68% and 55.74%). Altogether, the most optimal combination was obtained with 45 stem cuttings in a PP1000 mL culture box on liquid culture medium containing MS + 3% sucrose + 3 KIN + 3 IAA during a 3-monthlong cultivation without refreshment of the culture medium. With this method, it is possible to obtain 6500 - 7800 plants of axillary shoot origin, which are suitable for acclimatization (Fig. 1/F). According to Herrerra-Alamillo and Robert (2012),the propagation rate can be double or tripled with the multiple changes of the culture medium.

4.4. Macropropagation of longicaulis giant reed from stem macro-cuttings

The majority of shoots produced during *in vitro* propagation from stem cuttings survived acclimatization. Approximately 10% of the shoots from the PP1000 dish containing the 50 initial stem cuttings died. The viable dormant buds of the chopped stem cuttings of the ripened 16-week-old plantlets swelled slightly after being soaked with water. The leaves and shoot apex must be removed after soaking but before rooting to avoid potential infections.

Under the optimal conditions for the rooting of cuttings (dark, warm, and humid place), 128 buds began to grow from 30 stem cuttings of 12.9 cm in length on average (SD \pm 2.32). The average number of buds per stem is 4.27 (SD \pm 1.08). The summer period was more efficient for taking cuttings and rooting them because the external circumstances during the autumn were not optimal for the growth of cuttings (low temperature, short daytime, unfavorable lighting).

5. Conclusions

The primary objective of our present study was to continue the early work of Martin Krickl investigating new *in vitro* agamic propagation protocol for winter-frost tolerant 'longicaulis' giant reed (*Arundo donax* L.) ecotypes.

Our experiments indicated that *in vitro* propagation by stem cuttings can be successfully performed from *in vitro* shoots (node number increased with 0.2% NaCl treatment) on Murashige and Skoog (1962) medium containing kinetin (3 mgL⁻¹) and indole-3-acetic acid (3 mgL⁻¹).

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. To the best of our knowledge, our study is the first report describing a successful combined agamic propagation protocols from the overwintered shoots of longicaulis ecotype of giant reed (*Arundo donax* L.) grown under temperate climate conditions exposed to winter-frost.

Acknowledgments

The work is supported by the TÁMOP-4.2.2.A-11/1/KONV-2012-0041 project. The project is co-financed by the European Union and the European Social Fund.

References

- Alshaal T., Domokos-Szabolcsy É., Márton L., Czakó M., Kátai J., Balogh P., Elhawat N., El-Ramady H., Gerőcs A., Fári M., (2013a), Restoring soil ecosystems and biomass production of *Arundo donax* L. under microbial communities-depleted soil, *Bioenergy Research*, 7, 268-278.
- Alshaal T., Domokos-Szabolcsy É., Márton L., Czakó M., Kátai J., Balogh P., Elhawat N., El-Ramady H., Fári M.G., (2013b), Phytoremediation of bauxite-derived red mud by giant reed, *Environmental Chemistry Letters*, **11**, 295-302.
- Angelini L.G., Ceccarini L., Bonary E., (2005), Biomass yield and energy balance of giant reed (*Arundo donax* L.) cropped in central Italy as related to different management practices, *European Journal of Agronomy*, 22, 375-389.
- Angelini L.G., Ceccarini L., Nasso D.N, Bonari E., (2009), Comparison of *Arundo donax* L. and *Miscanthus x* giganteus in a long-term field experiment in Central Italy: Analysis of productive characteristics and energy balance, *Biomass & Bioenergy*, **33**, 635–643.
- Antal G., Fári M.G., (2013), Spontaneous Viviparia and Nodal Shoot Formation after Winter Season in Giant Reed (Arundo donax L.), Conference, Proc. of the Conference on Plant Biotechnology for the Future Of Agriculture in the Central European Region, Plants for the Future, 30th September – 2nd October, Cluj-Napoca, Romania.
- Antal G., Márton L., Czakó M., Fári M.G., (2012), Inherited Frost Tolerance of Arundo Donax Synplants in Field Test, Pannion Plant Biotechnology Workshops, Advances in Plant Breeding and Plant Biotechnology in Central Europe, Jun 4 – 6 th, Debrecen, Hungary.
- Bacher W., Mix-Wagner G., Sauerbeck G., El Bassam N., (2001), Giant Reed (Arundo donax L.) Network -Improvement Biomass Quality, Final Report, FAIR-CT-96-2028, Participant 9, FAL (Institute of Crop Science), Braunschweig, 1-72.
- Balogh E., Herr J.M., Czako M., Marton L., (2012), Defective development of male and female gametophytes in *Arundo donax* L. (Poaceae), *Biomass* & *Bioenergy*, 45, 265-269.
- Cavallaro V., Tringali S., Patané C., (2011), Large-scale in vitro propagation of giant reed (Arundo donax L.), a promising biomass species, Journal of Horticultural Science & Biotechnology, **86**, 452-456.

- Ceotto E., Di Candilo M., (2010), Shoot cuttings propagation of giant reed (*Arundo donax* L.) in water and moist soil: The path forward?, *Biomass & Bioenergy*, **34**, 1614-1623.
- Cosentino S.L., Copani V., Patanè C., Mantineo M., D'Agosta G.M., (2008), Agronomic, energetic and environmental aspects of biomass energy crops suitable for Italian environments, *Italian Journal of* Agronomy (Rivista di Agronomia), 2, 81-95.
- Dhir S., Knowles K., Pagan L., Mann J., Dhir S., (2010), Optimization and transformation of *Arundo donax* L. using particle bombardment, *African Journal of Biotechnology*, **36**, 6460-6469.
- Nasso D.N., Roncucci N., Triana F., Tozzini C., Bonari E., (2011), Productivity of giant reed (Arundo donax *L*.) and miscanthus (Miscanthus x giganteus Greef et Deuter) as energy crops: growth analysis, *Italian Journal of Agronomy*, **22**, 141-147
- Domokos-Szabolcsy É., Holb I., Prokisch J., Kovács B., Veres Zs., Fári M.G., (2005), Impact of sodiumselenate ont he growth of radish (*Raphanus sativus* L.) plantlets *in vitro*, *International Journal of Horticultural Science*, **11**, 113-115.
- Domokos-Szabolcsy É., Marton L., Sztrik A., Babka B., Prokisch J., Fári M.G., (2012), Accumulation of red elemental selenium nanoparticles and their biological effects in *Nicotinia tabacum*, *Plant Growth Regulation*, 68, 525-531.
- El Bassam N., (1996), Renewable Energy: Potential Energy Crops for Europe and the Mediterranean Region. Giant reed, Miscanthus (Miscanthus spp.) Reu, Technical Series 46. Federal Agricultural Research Centre (FAL), Braunschweigh, Germany. Food and Agriculture Organization of the United Nations. Rome, 67-72, 87-94.
- Elhawat N., Domokos-Szabolcsy É., Alshaal T., Molnár M., Antal G., Márton L., Fári M.G., (2013), *In vitro* salt tolerance comparison of two giant reed ecotypes (*Arundo donax L.*) induced by somatic embryogenesis (in Hungarian), XIX. Plant Breeding Scientific Days, 7th March, Keszthely, Hungary.
- Fári M., Andrásfalvy A., Németh J., (1987), Thin PVC foil covering (TPFC), an efficient method for culture and preacclimatization of *in vitro* plant cultures, *Acta Horticulturae*, **212**, 371–374.
- Herrera-Alamillo M. Á., Robert M.L., (2012): Liquid in Vitro Culture for the Propagation of Arundo donax, In: Plant Cell Culture Protocols, Methods in Molecular Biology, Loyola-Vargas V.M., Ochoa-Alejo N. (Eds.), 877, Springer Science and Business Media, 153-160.
- Krickl M., (1946), About the question of winter hardiness of Arundo donax L. (in German), *Journal TAG Theoretical and Applied Genetics*, **17**, 67-69.
- Lewandowski I., Scurlock J.M.O., Lindvall E., Christou M., (2003), The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe, *Biomass & Bioenergy*, 25, 335-361.
- Linder C.C., Gallagher J.L., (1998), Tissue culture and regeneration of the giant reed, *Arundo donax* L., *American Journal of Botany*, **85**, 89.
- Mantineo M., D' Agosta G.M., Copani V., Patane C., Cosentino S.L., (2009), Biomass yield and energy balance of three perennial crops for energy use in the semi-arid Mediterranean environment, *Field Crops Research*, **114**, 204-213.

- Mariani C., Cabrini R., Danin A., Piffanelli P., Fricano A., Gomarasca S., Dicandilo M., Grassi F., Soave C., (2010), Origin, diffusion and reproduction of the giant reed (*Arundo donax* L.): a promising weedy energy crop, *Annuals of Applied Biology*, **157**, 191–202.
- Murashige T., Skoog F., (1962), A revised medium for rapid growth and bioassays with tobacco tissue cultures, *Physiology Plant*, **15**, 473–497.
- Perdue R.E., (1958), Arundo donax Source of musical reeds and industrial cellulose, *Economic Botany*, 12, 368–404.
- Pilu R., Bucci A., Badone F. C., Landoni M., (2012), Giant reed (*Arundo donax* L.): A weed plant or a promising energy crop?, *African Journal of Biotechnology*, 38, 9163-9174.
- Pilu R., Manca A., Landoni M., (2013), Arundo donax as an energy crop: pros and cons of the utilization of this perennial plant, *Maydica Electronic Publication*, 58, 54-59.
- Scaini M., (2012), Utopia and Fascist Foundation Cities, The case of Torviscosa, In: Utopia e Contro-Utopie, Palmisano A.L. (Ed.), DADA, Rivista di Antropologia Post-Globale, 1, Trieste, On line at: http://www.dadarivista.com/Singoli-articoli/2012utopia/p16.pdf
- Takahashi W., Takamizo T., (2012), Molecular Breeding of Grasses by Transgenic Approaches for Biofuel Production, In: Transgenic Plants - Advances and Limitations, Çiftçi Y.O. (Ed.), InTech, Rijeka, Croatia, 91-116.
- Takahashi W., Takamizo T., Kobayashi M., Ebina M., (2010), Plant regeneration from calli in giant reed (*Arundo donax* L.), *Grassland Science*, 56, 224–229.
- Tóth E.K., Kriston É., Nyerges K., Nardin F., Czakó M., Márton L., Fári M., (2011), Virological investigation of third-generation biomass plants – Arundo donax L. as an example (in Hungarian), *Plant protection*, 47, 451-454.
- Tóth Sz., Mix-Wagner G., (1998), Embryogenic callus Induction of Different Explants of Miscanthus sinensis, Miscanthus x giganteus and Arundo donax genotypes, In: Sustainable Agriculture for Food, Energy and Industry and Strategies Toward Achievement, El Bassam N. (Ed.), James & James Science Publishers, 249-253.
- Tóth S., Mix-Wagner G., Frahnert C., Deuter M., El Bassam N., (1998a), *In-vitro Cultures of Different Explants of Miscanthus sinensis, Miscanthus x* giganteus and Arundo donax genotypes, 10th European Conference of Biomass for Energy and Industry, 8-11 June, Würzburg., Germany, 1062-1066.
- Tóth S., Mix-Wagner G., Frahnert C., Deuter M., El Bassam N., (1998b), *In vitro propagation of different Miscanthus genotypes and Arundo donax plants* (in Hungarian), IV. Plant Breeding Scientific Days. Budapest, Hungary.
- Tóth S., Pepó P. (1999), In-vitro culture of different explants of Miscanthus genotypes and arundo donax (in Hungarian), Scientific Publications of the Agricultural University of Debrecen, 34, 101-111.
- Trebbi D., D'Angelantonio M., Predieri S., Valli F., Tuberosa R., Salvi S., (2013), *Physical mutagenesis of the bioenergy species Arundo donax L.*, XXI. International Plant & Animal Genome, 12-16th January, San Diego, CA, USA.