HORTICULTURAL SCIENCE
KERTÉSZETI TUDOMÁNY
AGROINFORM Publishing and Printing House Ltd. Hungary-1996

Rewiew Article

Novel tendencies of automation and the rationalization of *in vitro* explant manipulation in micropropagation industry

Fári M.,[1] Kertész T.,[2] Franklin de Melo, N.,[3] and Andrásfalvy A.[4]

[1]*Agricultural Biotechnology Center, Institute for Plant Sciences, Laboratory for Cell Biology & Tissue Culture, H-2100, Gödöllő, Szent-Györgyi A. u. 4., Hungary. Present address: EMBRAPA/CPATSA, BR428 Km 152 Zona Rural, Caixa Postal 23, Petrolina-PE, 56.300-000, Brazil,*
[2]*Budapest, Arany János u 43, H-1221, Hungary,*
[3]*CPATSA/EMBRAPA, BR428 Km 152 Zona Rural, Caixa Postal 23, Petrolina-PE, CEP 56.300 000, Brazil,*
[4]*Agricultural Biotechnology Center H-2100, Gödöllő, Szent-Györgyi A. u. 4., Hungary.*

Key words: Automation, micropropagation, trends, rationalization

Introduction

Micropropagation of horticultural plants is a practically accepted profitable industry. The *in vitro* cloning is a significant procedure mainly in floriculture and nurseries of arboriculture (i.e. fruit growing) all over the world, in advanced as well as in developing countries, in the temperate climate as well as in the tropics and subtropics. At present, several thousands of small enterprises produce a couple of hundred thousand specimens of *vitro plants*. The yearly turnover of the cloned nursery material amounts about half a billion of US$ (60-70 % of it bring the orchids, cut flowers, potted ornamentals).

Present and future plant propagation cannot be imagined without micropropagation, meanwhile the nowadays technologies are generally characterised by at least two considerable disadvantages: (1) the production costs are often relatively high, and (2) the biological value of the planting material is sometimes variable (*Aitken-Christie*, 1992).

At present, the 50 to 75 %-of production costs is labour (*Rajeevan* and *Pandey*, 1986; *Donnan*, 1986; *Pachauri* and *Dhawan*, 1989; *Pierik*, 1990). One of the most efficient possibility of improving economy in short-term is to pay low salaries, i.e. the transfer of the micropropagation into developing countries. Other possibilities, in medium – and long-term conception, the improvement of technology by automation and robotization (*Standaert de Metsenaere*, 1991) and the rationalisation of labour-consuming phases of the micropropagation (*Fári* and *Kertész*, 1995) may reduce the ratio of labour demand and consequently, the costs of production.

Trends of mechanisation of in vitro mass propagation

Modern micropropagation industry developed a number of new tools and devices, moreover, automation and elements of certain „high tech" procedures are already on the way of realisation (*Aitken-Christie*, 1991, *Table 1*).

The automation of micropropagation has been attempted along three distinct lines (*Zandvoort* and *Holdgate*, 1991; *Aitken-Christie*, 1991, *Table 1*):

(1) The mechanization of the procedures of traditional tissue culture requiring manual labour (preparation of the culture medium; cutting, dissection and transfer of vitroplants or tissues; transplantation or potting of explants),

(2) The development of in vitro procedures on liquid media,

(3) The development of alternative culturing systems.

Automation of the traditional tissue culture methods

(i) Preparation, dosing and filling of media.

This sole phase began its intrusion into the commercial laboratories. The first highly productive, continuously filling automatic doser of liquid or gelifying media was a Hungarian development (*Fári*, 1984; *Fári*, 1987). The system was able to fill single use plastic containers for tissue culture (Veg-Box) 720 units per hour (*Fári* et al, 1985;

Table 1. Exemples of the international efforts made in the automation of the plant micropropagation industry

1.) AUTOMATION OF THE TRADITIONAL METHODS IN TISSUE CULTURE:	
Preparation of media and the filling process	
PROPAMATIC (Hungary)	*Fári et al* (1984, 1987b)
Tasman Forestry Ltd (New-Zeland)	*Gleed* (1990)
Semi-automation Systems	
CLONMATIC (Hungary)	*Fári et al* (1987a)
PermX System (Holland)	*Anonymus* (1990)
Cutting and transplantation of the inocula	
Browns System	*Brown* (1988)
Llillys System (Japan)	*Takayama et al* (1990)
C.I.G. Ventures Ltd (Australia)	*Schonstein & Jonson* (1986)
University of Waseda (japan)	*Miwa* (1987)
Toshiba Co (Japan)	*Fujita* (1989)
Agrisystem (Holland)	*Anonymus* (1992)
CLONER (Hungary)	*Fári & Kertész* (1995)
Acclimation and potting of the plantlets	
2.) THE LIQUID SYSTEMS	
Cultivation in liquid culture media	
University of Gent (Belgium)	*Maene & Debergh* (1985) *Vandershaege & Debergh* (1988)
The System of Shoot Hedging (New Zeland)	*Aitken-Cristie & Jones* (1987)
Tisserats System (USA)	*Tisserat & Vandercook* (1985)
Youngs System	*Young* (1989)
Mist System (GB)	*Weathers et al* (1987, 1988)
Systems of bioreactors	
Takayama's Group	*Takayama* (1988)
Preil's Group	*Preil et al* (1990, 1992)
3.) ALTERNATIVE METHODS	
Cultivation of axillary meristems	
The *VITROMATIC* system (Israel)	*Levin* (1985); *Levin et al* (1988)
In vitro culture system based on autotrophy	
The Concept of Kozai (Japan)	*Kozai et al* (1988)

Balogh et al, 1990) by means of the CLONMATIC device developed to move containers, to fill the medium, cover and expediate the containers automatically, all under aseptic conditions *(Fig. 1)*. This CLONMATIC line has been completed with a 50 liter medium kitchen and sterilizer of batch system and all that with digital control by the Dutch PPS b.v. company (*Zandvoort* and *Holdgate*, 1991). A continuous sterilizer unit has been developed also by the Tasman Forestry Ltd (New Zealand, Gleed, 1990).

(ii) Cutting and transfer of explants: For nodal explants the first mechanized and automated system has been planned by *Schonstein* and *Jonson* (1986). On the Waseda University, in Japan, *Miwa* (1987) was one of the first in construction of a robot model for micropropagation. The prototype facilitated the sterile transfer of an explant in 60 secunds in a completely automated system. The robotized cutting and transfer system of the Toshiba Company (*Fujita*, 1989) identified the objects (nodes of the vitroplant) by three dimensional image analysis. A partially mechanized cutting and manipulating system has been developed by *Brown* (1988), and an entirely mechanized system for cutting has been described for the purpose of cutting bulbs of lilies raised in vitro by *Takayama* et al (1990). Robotization of cutting and manipulation of explants is still confronted with several problems. The safety of sterilizing and the quality of explants does not satisfy yet the requirements of the users, moreover, the system is extremely expensive.

(iii) Potting and acclimation of sterile explants: At the end of the phase of sterile cultivation the rooted plantlets have to be transplanted to be fit for the usual horticultural or nursery technologies. We cannot ignore the adopted practices of the existing nurseries. There are several

exemples of complete automation in the manipulation of plantlets up to the final preparation to sale (in the Nederlands, USA, France, etc.). It is expected mainly that

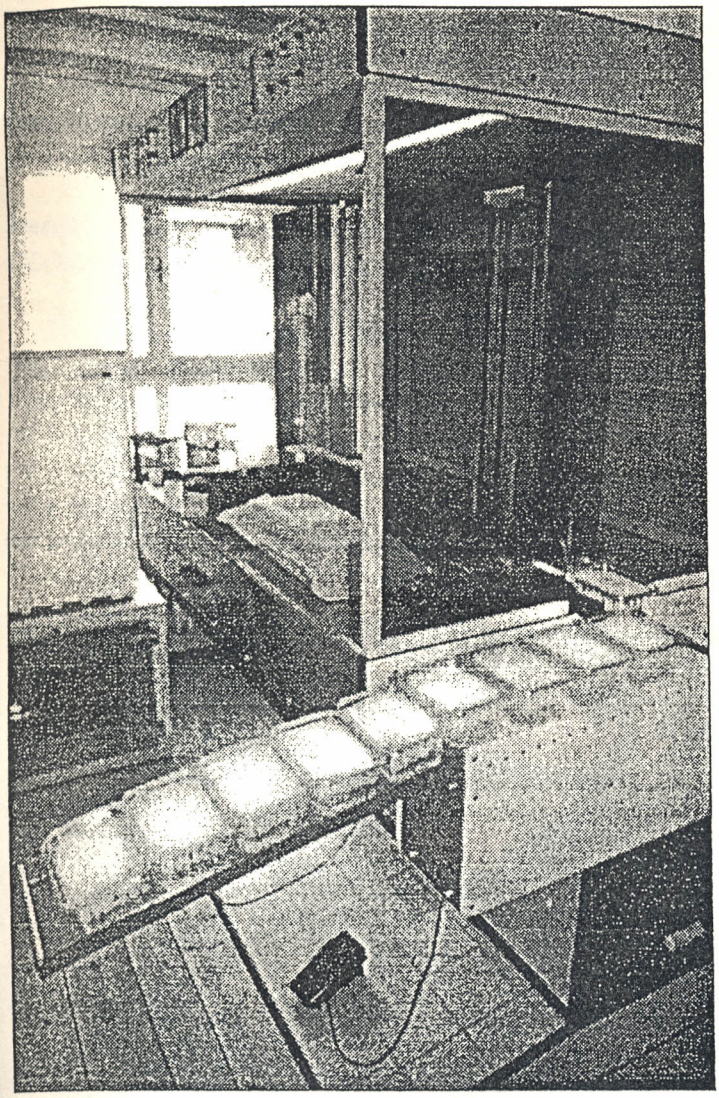

Fig. 1. Clonmatic, semi-automated medium filling machine

the transplantation should be performed quickly without the need of trained labour and the survival of the transplants must be maximal.

Attempts of automation in systems based on liquid media

(i) Culture systems with liquid media: Most of the attempts are bound to utilize the advantages, practical and physiological ones, which are connected with the liquid media. Therefore, this methods deserve special attention. The advantages conspicuous in relation to the semi-solid or gelified culturing media are praized by *Zandwort* and *Holdgate* (1991). *Maene* and *Debergh* (1985), *Vandershaege* and *Debergh* (1988), as well as *Aitken-Christie* and *Jones* (1987) explored several systems of that type. *Aitken-Christie* and *Davies* (1988) achieved 30 to 40 % reduction of costs in an experiment of 18 moths by the mechanization of their „hedging system" developed for that purpose. Supplying and dosing of the liquid medium has been controlled by a computer in the system of *Tisserat* and *Vandercook* (1985). The net gain of the method was the reduction of man labour to the fifth. Another idea of *Young* et al (1989) offered solution to the cultivation of species threatened with vitrification in liquid culture by using swimming baskets for the vitroplants. Microporous membrans were tried by other people (*Hamilton* et al, 1985; *Kong* and *Chin*, 1988; *Matsumoto* and *Yamaguchi*, 1989). *Weathers* et al (1988) dealt with spraying of the substrate, so the „nutritive fog" proved its utility in vitro cultures of *Nephrolepis*, *Musa* and *Cordyline*. Bulbe plant species were multiplied well in a fermentor of the experiments of *Takayama* (1990). The fermentor as a tool of micropropagation has been studied in details as one of the first by *Preil* et al (1990). It seems that the technique of multiplication based on the idea of bioreactors proved to be applied preferably in species developing storage organs (*Lilium longiflorum*, *Solanum tuberosum*, *Gladiolus* sp., *Colocasia esculenta*, etc.).

Alternative culturing systems

(i) Culturing of meristem glomerules: The system has been developed under the name PBInd. (Izrael) VITROMATIC (*Levin*, 1985; *Levin* et al, 1988). The meristem aggregates are cultured either in a fermentor or, quite simply, in a series of batches where these meristem aggregates multiply. Subsequently, the divided and homogenized explants have to be placed on a special substrate matrix where the explants are rooted. The experiments aimed to learn about the physiology of the meristems comprized also retardants (*Ziv*, 1989; 1990).

(ii) Culturing of shoot bunches and nodes: Populus and *Pinus radiata* nodes are cut and cultured automatically in the experiments of *McCown et al* (1988) and *Aitken-Christie* et al (1988). *Populus* tolerated well the liquid phase media, but *Pinus radiata* nodes were difficult to grow in submers cultures.

(iii) Somatic embryogenesis and the synthetic seed: Stewart et al (1958) made the historical discovery, so the *in vitro* somatic embryogenesis focused the attention of researchers to the possible production of synthetic seed. Beyond doubt, it would be a colossal hit to concentrate the benefits of the *in vitro* technique and all tricks of up to date biotechnology, „genetic engineering", in one integrated system producing clones derived from single cells, immediately. Those aspects and the present state of research were summarized by *Redenbaugh* et al (1993). Recently, promising results are published on model plants *(Euphorbia pulcherrima)* by *Preil* and *Beck* (1991). The authors synchronized and optimized the embryogenesis successfully in the bioreactor. Those results are pledges of claims in developing efficient industrial multiplication systems with other species too.

(iv) Autotrophic cultur of plant tissues and organs: Kozai, (1988) explored the possibilities of omitting carbohydrates of the substrate in order to suppress the heterotrophic nutrition, and how the athmospheric carbondioxid may substitute

sugars of the medium. In an appropriately planned complex system there will be hope to create conditions for autotrophic nutrition and multiplication even without the maintenance of the aseptic status. As proved on model species (*Rosa* sp., *Lycopersicon esculentum* etc.) it was feasible that the discontinuance of sterility did not impaire essentially the growth and multiplication.

Designing of micropropagation bench: novel initiatives by CLONER Rationalized Micropropagation Bench (Fig. 2)

The Cell-and Tissue Culture Laboratory of the Agricultural Biotechnology Center (ABC), Gödöllő, Hungary, carried out experiments with the goal of reducing the cost of labour in commercial micropropagation. During the last five years, these work has been mainly focused on the improvement of the process of manipulating the explants, because this phase requires 30–40 % of the total cost of labour

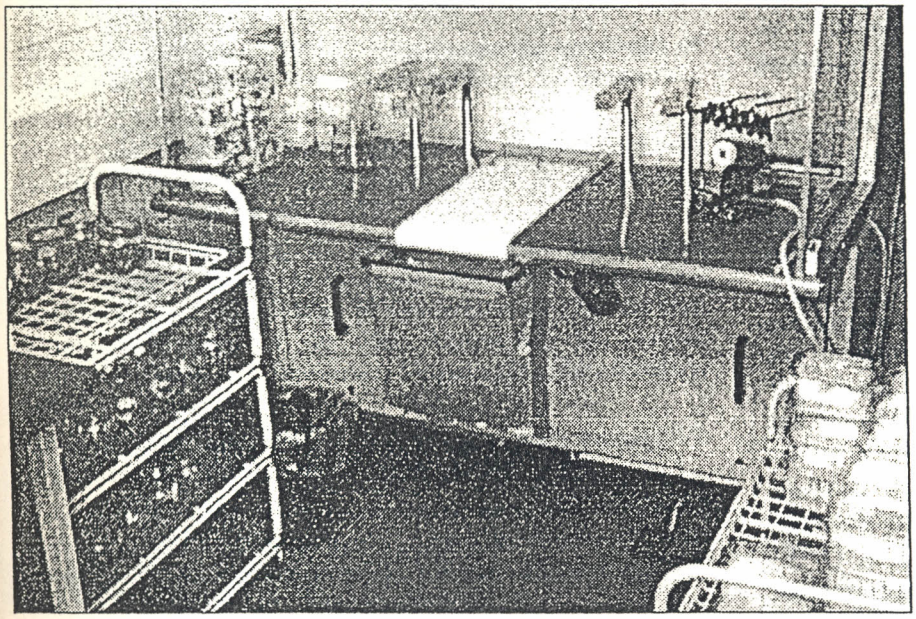

Fig. 2 CLONER, rationalized micropropagation bench

(*Standaert-de Metsenaere*, 1991), and it has key importance in commercial micropropagation (*Aitken-Christie*, 1991).

The following criteria have been investigated:

– How could we reduce, efficiently, the labour of explant preparation by simplifying the process?

– How the new processes could be integrated into the up-to-date micropropagation technologies, on a reasonable basis?

The team of ABC designed the CLONER rationalized micropropagation bench *(Fig. 2)* which is a simple and labour-saving new type of laminar air flow cabinet (*Fári* and *Kertész*, 1995). The CLONER comprises two inventions, the *Sterile Inoculation Device -STI and the Opening and Closing Device -OCD* (*Fári* and *Kertész*, 1993a and 1993b). This system has been characterized carefully by the biological as well as by the economical criteria of micropropagation.

Sterile Inoculation Device -STI

Continuous sterile, electric powered working surface to assist in the cutting process of the explants and in the automated collection of the by-products (calli, pieces of leaves, shoot-buds, roots etc.). The prototype was designed for a commercial type laminar air flow box. This device can easily fit into any kind of air flow micropropagation boxes.

Opening and Closing Device -OCD

Mechanically driven tool to assist in the opening and closing process of tissue culture vessels. The prototype was designed to open high (vaulted lid) type Veg-Box plastic tissue culture containers developed in Hungary (*Fári* et al, 1987; *Balogh* et al, 1990), but can be modified to open other plastic containers and glass jars. This device can easily fit into any kind of air flow micropropagation box. According to pilot-scale experiences, the operation process with the CLONER – on the routine level – could improve dramatically the efficiency of labour by 25-100 % and subsequently, could considerably reduce the total cost of production.

The cost-analysis of the operation of the CLONER bench proved, that the reduction of labour of the micropropagation process is also possible by relatively simple tools. It seems that designing and/or rationalizing of the micropropagation technology has considerable possibilities for future development.

Acknowledgements

This project has been granted by the National Committee for Technological Development of Hungary (OMFB, No 91-97-16-0335/91) to the Agricultural Biotechnology Center.

Literatures

Aitken-Christie, J. and Jones, C. 1987. Towards automation: radiata pine shoot hedges in vitro. Plant Cell Tissue Organ Culture 8: 185- 196.

Aitken-Christie, J., Singh, A.P. and Davies, H.E. 1988. Multiplication of meristematic tissue: a new tissue culture system for radiata pine. In: Hanover, J.W., Keathley, D.E. (Eds) Genetic Manipulation of Woody Plants. Plenum Press, New York, 413-432.

Aitken-Christie, J. 1991. Automation. In: Micropropagation. Technology and Application, Debergh, P.C. and Zimmermann,

R.H. (Eds.), Kluwer Academic Publ. , Dordrecht, Boston, London, pp. 363-388.

Balogh Z., Fári M. and Förstrer H. 1990. Tool and process to cultivate organisms on agar-free support systems *in vitro*. (Eszköz és eljárás organizmusok *in vitro* nevelésére agarmentes hordozóközegen., *in Hungarian*.) Hungarian patent, No. 207529.

Brown, F. 1988. Apparatus for and methods of cutting and/or moving plant tissue. Patent PCT/GB87/00918.

Donnan, A. 1986. Determining and minimizing production costs. In: Zimmerman, R.H., Griesbach, R.J., Hammerschlag, F.A., Lawson, R.H. (Eds). Tissue culture as a plant production system for horticultural croips. Martinus Nijhoff Publ. Dordrecht, NL. 167-173.

Fári M. 1984. Device for plant micropropagation (Berendezés növények mikroszaporítására, in Hungarian). Hungarian Patent, No. 15742/ 84.

Fári M., Németh J., and Andrásfalvy A. 1985. Thin PVC-foil covering (TPFC), an efficient method for culture and pre-acclimation of *in vitro* plant cultures. Acta Horticulturae, 212: 371-374.

Fári M. 1987. Development of technical and biological basis of micropropagation toward automation and industrialization. In: Moet Hennessy (Ed.) Electronics and Management of Plants, Paris, 55-56.

Fári M. and Kertész T. 1993a. Device for opening and closing of micropropagation vessels (Nyitó-záró szerkezet, *in Hungarian*). Hungarian Invention, No: OTH 27724.

Fári M. and Kertész T. 1993b. Device for Continuous Sterile Inoculation (Folyamatos steril munkaasztal, *in Hungarian*). Hungarian Invention, No: OTH 27725.

Fári M. and Kertész T. 1995. CLONER. Rationalized Micropropagation Bench, an Innovation Dévoted to Commercial Micropropagation. Proceedings of Conference on Plant In vitro Culture in Memory of the 50th Anniversary of Gottlieb Haberlandts Death, 1-3 September, Mosonmagyaróvár, Hungary, p. 44.

Fujita, N. 1989. Applicdation of robotics to mass propagatiion system. In Vitro Cell Dev. Biol. 25(3): 22A-47.

Gleed, J. 1990. Overcoming constraints to successful commercial production of tissue cultured *Pinus radiata*. In: Abstracts Conifer Biotechnology Group 5th meeting, Keynote presentations July 1990, Shell Research Ltd., Sittingbourne, UK: 5.

Hamilton, R., Pederson, H., Chin, C.K. 1985. Plant tissue culture on membrane rafts. Biotechniques 3: 96.

Kong, Y. and Chin, C.K. 1988. Culture of asparagus protoplasts on porous polypropylene membrane. Plant Cell Reports 7(1): 67-69.

Kozai, T. 1988. Autotrophic (sugar free) tissue culture for promoting the growth of plantlets in vitro and for reducing biological contamination. Int. Symp. Application of biotechnology for small industries development in developing countries. Bangkok, Thailand, September 1988: 1-21.

Levin, R. 1985. Process for plant tissue culture propagation. European Patent No. 0132414A2.

Levin, R., Gaba, V., Tal, B., Hirsch, S., DeNola, D. and Vasil, I.K. 1988. Automated plant tissue culture for mass propagation. Bio / Technology 6: 1035 - 1040.

Maene, L. and Debergh, P. 1985. Liquid medium additions to established tissue cultures to improve elongation and rooting in vivo. Plant Cell Tissue Organ Culture 5: 23-33..

Matsumoto, K. and Yamaguchi, H. 1989. Onwoven materials as a supporting agent for in vitro culture of banana protocorm - like bodies. Tropical Agriculture 66(1): 8-10.

McCown, B.H., Zeldin, E.L., Pinkalla, H. A. and Dedolph, R.R. 1988. Nodule culture: A developmental pathway with high potential for regeneration, automated micropropagation, and plant metabolite production from woody plants. In: Hanover, J.W., Keathley, D.E. (Eds) Genetic Manipulation of Woody Plants. Plenum Press N.Y. 149-166.

Pachauri, R. K., Dhawan, V. 1989. Cost analysis of micropropagated plants. In: Dhawan, V. (Ed) Applications of Biotechnology in Forestry and Horticulture. Plenum Press N.Y. 275-282.

Pierik, R.L.M. 1990 Commercial micropropagation in Western Europe and Israel. In: Debergh, P.C., Zimmerman, R.H. (Eds) Micropropagation of horticultural crops. Kluwer Ac. Publ. Dordrecht.

Preil, W., Florek, P., Wix, U. and Beck, A. 1988. Towards mass propagation by use of bioreactors. Acta Hort. 226: 99-106.

Rajeevan, M.S. and Pandey, R.M. 1986 Economics of mass propagatopn of papaya through tissue culture. In: Withers, L.A., Anderson, P.G., (Eds) Plant tissue culture and its agricultural applications. Butterworths London 211-215.

Schonstein, D.N. and Johnson, B.J., 1986. Method and apparatus for dividing plant material. Australian Patent PCT/AU086/00136.

Standaert-de Metsenaere, R.E.A. 1991. Economic consideration. In: Micropropagation. Technology and Application. Debergh, P.C. and Zimmerman, R.H. (Eds.), Kluwer Academic Publ., Dordrecht, Boston, London. pp. 123-140.

Stewart, F.C., Mapes, M.O., and Mears, K. 1958. Growth and organised development of cultured cells. II Organisation of cultures grown from freely suspended cells. Am. J. Bot. 45: 705-708.

Takayama, S., Swedlund, B., and Miwa, Y. 1990. Automated propagation of microbulbs of lilies. In: Vasil, I.K. (Ed) Cell culture and somatic cell genetics of plants. Vol. 8. Academic Press.

Takayama, S. 1990. Mass propagation of plants through shake- and bioreactor-culture techniques. In: Bajaj, Y.P.S. (Ed), Tissue culture in forestry and agriculture. Vol. 15. Springer Verlag.

Tisserat, B. and Vandercook, C.E. 1985. Development of an automated plant culture system. Plant Cell Tissue Organ Culture 5: 107-117.

Vanderschaeghe, A.M., Debergh, P.C. 1988. Automation of tissue culture manipulations in the final stages. Acta Hort. 227: 399-401.

Weathers, P.J., Cheethan, R.D. and Giles, K.L. 1988. Dramatic increases in shoot number and lengths for Musa, Cordyline and Nephrolepis using nutrient mists. Acta Hort. 230: 39-44.

Young, R.E., Hale, A., Camper, N.D., Keese, R.J. and Adelberg, J.W. 1989. An alternative mechanized plant micropropagation approach. ASAE/CSAE Paper No. 896092, ASAE, St Joseph, M.I. 49085-9659, USA.

Zaandwort, E. and Holdgate, I. 1991. In: Intern. Congr. of Plant Biotechnology in Horticulture, Geneve, Suisse.

Ziv, M. 1989. Enhanced shoot and cormlet proliferation in liquid cultured gladiolus buds by growth retardants. Plant Cell Tissue Organ Culture 17: 101-110.

Ziv, M. 1990. Morphogenesis of gladiolus buds in bioreactors. Implication for scaled up propagation of genotypes. In: Nijkamp, H.J.J., Plas, L.H.W. van der, Aartrijk, J. van (Eds) Progress in Plant Cellular and Molekular Biology. Kluwer Ac. Publ. Dordrecht, NL 119-124 .

Az automatizálás tendenciái és az inokulum manipulálás észerúsítésének új lehetőségei a mikroszaporításban

Fári Miklós, Kertész Tamás, Natoniel Franklin de Melo és Andrásfalvy András

Összefoglalás:

A növényi mikroszaporítás költségeinek 60-70%-át a magasan képzett kézimunka-hányad jellemzi, ami a módszerek iparszerű alkalmazását korlátozza. A szakterület műszaki és biológiai alapjainak fejlesztésében és automatizálásában Magyarország a nyolcvanas években nemzetközileg élenjáró volt és kereskedelmi sikereket is könyvelt el. A fejlesztések hazai irányítását 1991-1995 között az MBK Sejt -és Szövettenyésztési Laboratóriuma folytatta az OMFB és az FM támogatásával. A nemzetközi fejlesztések fő koncepciója a nyolcvanas évek végétől a totális automatizálás-robotizálás volt. Ez a fejlesztési koncepció gyakorlatilag nem váltotta be a hozzá fűzött reményeket, elsősorban a magas műszaki költségek miatt. Magyarországon – az MBK irányításával – egy gyökereiben más koncepció alapján indult el a munka 1991-ben. A fő célkitűzés olyan muszaki megoldások keresése volt, amelyek alacsony árfekvésben, a meglévó laborstrukturák érintetlenül hagyásával akarták csökkenteni a kézimunkaerő szükségletet és/vagy növelni a termelést. Modellkísérletek után ennek egyik lehetséges módja az inokulum manipulálás rutin munkafázisainak felgyorsítása volt, új műszaki megoldások és eszközök kifejlesztésével. A kísérletek eredménye a CLONER – Racionalizált mikroszaporító oltófülke névre keresztelt rendszer. A CLONER két új műszaki eszközt alkalmaz, melyekkel a steril inokulum manipulálás rutin munkafázisai fele annyi időt vesznek igénybe, mint a korábbi megoldások. Az új megoldások lehetővé teszik a steril inokulum manipulálás teljesítményének 25-100%-os növelését, amelyek együttesen a végtermék termelési költségének 15-25 %-os csökkenését eredményezhetik. A CLONER két új eleme: (1) Steril doboz nyitó-záró berendezés és (2) Folyamatos steril inokuláló munkaasztal.