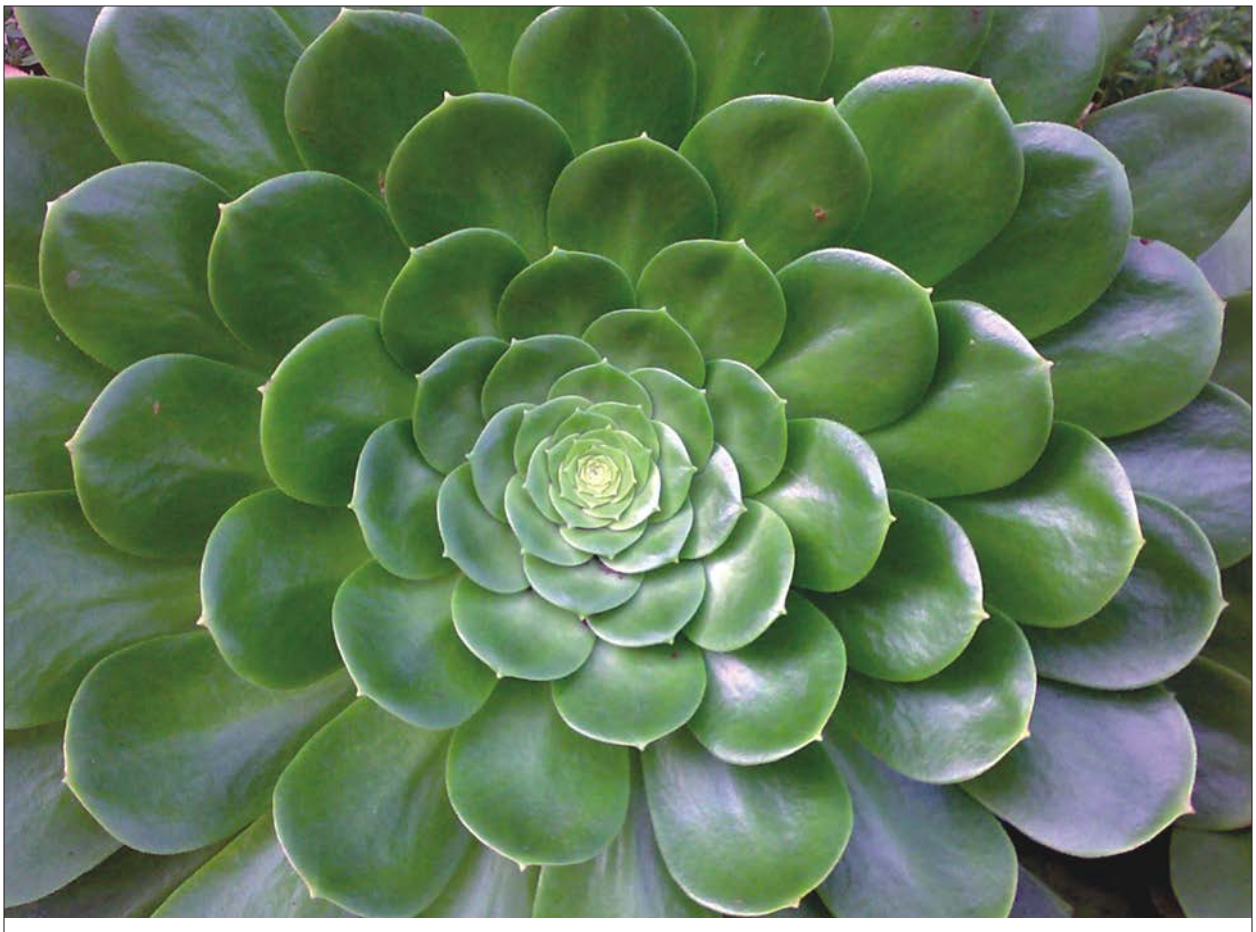

Journal of
Plant Sciences

ISSN 1816-4951

Academic
Journals Inc.

www.academicjournals.com

Journal of Plant Sciences 6 (6): 213-224, 2011
ISSN 1816-4951 / DOI: 10.3923/jps.2011.213.224

Regulating Gene Expression in High-scale Plants Micropropagation

[1,2]Ariel D. Arencibia, [1]Aydiloide Bernal, [1]Leidy Cortegaza, [2]Rolando Garcia, [1]Odalis Nodarse and [1]Ignacio Santana

[1]National Institute for Sugarcane Research, CUJAE 2½ Km, Boyeros 19390, Havana City, Cuba
[2]Faculty of Agronomy and Forestry, University Catholic of Maule, San Miguel 3605, Talca, Chile

Corresponding Author: Ariel D. Arencibia, National Institute for Sugarcane Research, CUJAE 2½ Km, Boyeros 19390, Havana City, Cuba

ABSTRACT

Signaling mechanisms have been elicitated towards the priming and biopriming stages during micropropagation in Temporary Immersion Bioreactors (TIBs) in sugarcane as model plant. CO_2-rich TIBs induces a photomixotrophic condition adequate for the production of natural phenolic metabolites, altogether increasing multiplication rates and functional plants rooting. When combined with *Gluconacetobacter diazotrophicus* inoculations during transplanting, originate a significant improvement of the percentage of plant adaptability to natural conditions. A more efficient micropropagation process has been optimized on the basis of an accurate exploitation of the natural plant physiology.

Key words: Gene expression, priming, biopriming, TIBs, sugarcane

INTRODUCTION

Plant tissue culture refers to growing and multiplication of cells, tissues and organs on defined solid or liquid media under aseptic and controlled environment. Forty years of *in vitro* plant research has delivered many well-developed systems that are routinely applied to scientific and commercial activities, namely: (a) micropropagation of genotypes; (b) production of disease-free material from excised apical meristems; (c) international germplasm exchange; (d) generation of somaclones; (e) rapid disease and pest resistance screening; and (f) germplasm conservation (Snyman *et al*., 2011).

Plant micropropagation procedures are conducted under conditions as natural or similar to those in which the plants will be ultimately grown *ex vitro* (Ahloowalia *et al*., 2004). The individual plant species, varieties and clones require specific modification of the growth media, weaning and hardening conditions. In general, the process of plant micropropagation is universally divided into well-defined stages depending on genotype/specie (Debnath, 2011).

Network discovery is a generic term describing the effort of elucidating the nature of relationships between molecules and associated properties emerging from of a biological network. Multiple types of networks have been described with respect to the types of molecules involved and the dimension of the molecular network (Weitz *et al*., 2007). Similar efforts are under way to construct plant transcriptional regulatory networks, for example those that control flower and root development (Grieneisen *et al*., 2007), photomorphogenesis (Jiao *et al*., 2007; Nemhauser, 2008) or the circadian clock (Zeilinger *et al*., 2006).

Technological advances in biological experimentation are now enabling researchers to investigate living systems on an unprecedented scale by studying genomes, proteomes or molecular networks in their entirety. Whether gene expression analysis provide a rich source of quantitative biological information that allows researchers to move beyond a reductionist approach by both integrating and understanding interactions between multiple components in cells, organisms and processes (Baginsky *et al*., 2010).

This study considers plant micropropagation as a complex physiological network where each step must believe as an independent one, i.e., establishment, multiplication, rooting, adaptation to field conditions, etc. Integration/management of gene expression systems in parts of the entire micropropagation process (network) have been conducted in sugarcane as model plant. Regulations of gene expression towards useful traits increase the productive efficiency during plant micropropagation integrating both basic and applied researches.

Temporary Immersion Bioreactors (TIBs): Bioreactors are vessels designed for large-scale cell, tissue or organ culture in liquid media. A more precise control of the plant growth gaseous exchange, illumination, medium agitation, temperature and pH than the conventional culture vessels is providing by bioreactors increasing the multiplication rates and growth of plant cultures (Levin and Tanny, 2004; Paek *et al*., 2005). The environment of the growth room determines the light, temperature and gases in the bioreactor vessels (Morini and Melai, 2004).

In temporary immersion bioreactors, the cultures are immersed in the medium, for a preset duration at specified intervals in dependence of plant species and genotypes (Adelberg and Simpson, 2002; Debnath, 2011). TIBs demonstrate a positive effect on plant physiology regulating key pathways as photosynthesis, respiration, transport and nutrient assimilation. As consequences both cell development and cell division rates are significantly enhanced (Etienne and Berthouly, 2002). Environmental factors influencing plant physiology, including the presence of systemic microorganisms (benefic, pathogenic and contaminants), during plant micropropagation are show in Fig. 1.

The influence of these environmental factors on plant physiology could be considered similar to those for conventional micropropagation. Nevertheless, key differences with the time and quality

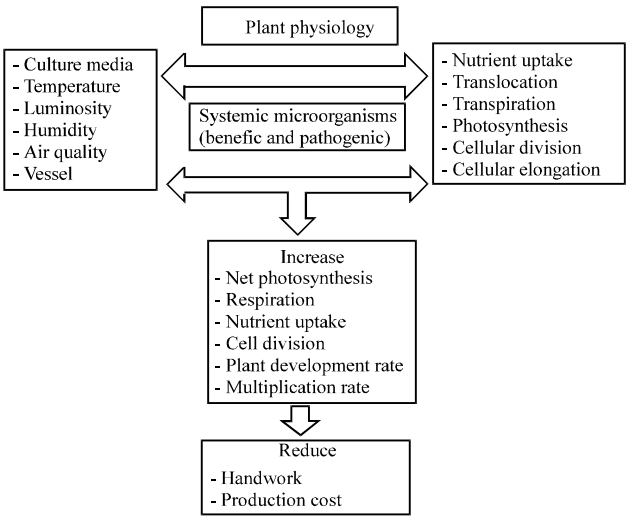

Fig. 1: Factors influencing plant micropropagation in Temporary Immersion Bioreactors

of the exposure are demonstrated throughout the interactions. Other significant difference is the improved availability to manipulate or regulate, the interactions using automatic devices. For superior practical use of bioreactor cultures, research aimed at improving the physical and chemical environments-such as increased air exchange, increased Photosynthetic Photon Flux (PPF) and optimized CO_2 content are necessary (Paek *et al*., 2001; Morini and Melai, 2004).

Prevention of contamination in bioreactors requires a proper handling of the plant material, of equipment during transfers and cultures during production. Culture contamination which is a major problem in conventional commercial micropropagation, is even more acute in TIBs (Leifert, 2000). Only the surface sterilized explants, indexed as diseases-free must be used to initiate cultures in bioreactors. However, despite the precautions taken in initiating cultures, bioreactors can become contaminated from the environmental microbes. It is noticeable that photomixotrophic plant cultures show a significant diminution or even control of environmental contamination which could be also associated with the significant reduction of sucrose in the culture medium and the production of natural bioactive phytomolecules (Sivakumar, 2006; Arencibia *et al*., 2008).

Considering the main advantages and disadvantages, the use of bioreactors has led to the development of suitable technologies for plant propagation. Currently, various plant species as sugarcane, pineapple, banana, between others, are propagated using bioreactors to certified seeds production (Escalona *et al*., 1999, 2003; Ibaraki and Kurata, 2001; Etienne and Berthouly, 2002; Chakrabarty *et al*., 2003; Arencibia *et al*., 2008).

Priming: One of the important plant adaptations to complex environment challenges is priming behavior (Conrath *et al*., 2006; Beckers and Conrath, 2007; Frost *et al*., 2008). In principle, priming means that plants that previously experienced abiotic or biotic stress have altered and most often enhanced their ability to resist and survive recurring stress conditions. In the current terminology, 'priming' is usually associated with biotic stresses while 'hardening' is used for response adaptations of plants to abiotic factors (Bruce *et al*., 2007). Priming effects span trophic levels: plants can be primed by herbivore attack, pathogen infection and colonization with micro-organisms, exposure to the metabolites these organisms produce and even synthetic compounds (Conrath *et al*., 2001).

Conrath *et al*. (2001) proposed that the accumulation of signaling proteins in their inactive form and their rapid activation in new stress situations can contribute to the formation of short-term stress imprints. Protein phosphorylation and dephosphorylation is one of the most important reversible post-translational modifications that causes inactive proteins to become active and vice versa (Van Bentem and Hirt, 2007). Members of a diverse class of Mitogen Activated Protein Kinases (MAPK) are known to play important roles in mediating pathogen resistance as well as in JA-dependent signal transduction cascades (Seo *et al*., 2007; Takahashi *et al*., 2007; Wu *et al*., 2007; Iriti *et al*., 2007). A concrete example in *Arabidopsis* shows that the priming competence induced by treatment with salicylic acid analog benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH), sold under trade name BionTM (Syngenta AG, Basel, Switzerland) and used commercially for priming and protecting crops against plant pathogens (Iriti *et al*., 2007), can be attributed to the accumulation of inactive MPK3 proteins (Conrath *et al*., 2006); these are activated in response to pathogen infection, thereby enhancing the expression of defense genes and the accumulation of antifungal metabolites.

In addition, more permanent but still reversible changes in gene expression-namely those involved in protection from stress-have been described in plants. In particular, DNA methylation

(Mathieu *et al*., 2007), histone modifications and changes in small-RNA (smRNAs) populations play a role in plant defense-related memory mechanisms. Pandey and Baldwin (2008) can lead to heritable or transgenerational alterations in plant behavior, some of which cannot be explained by Mendelian genetics. Readers are directed to examples of several current reviews that analyze the broad areas of DNA and histone modifications in epigenetic control of development and stress responses (Bronner *et al*., 2007; Boyko and Kovalchuk, 2008).

Phenolic metabolites as priming molecules: Sugarcane, a major source of world sugar and bioenergy production (Waclawovsky *et al*., 2010), is growing throughout the tropics and subtropics. In this sense, as all major plant-based industries require extensive breeding programs to produce/introduce improved genotypes (e.g., sugar content) that are matched to specific selection sites, accounting for biotic (e.g., pest and disease resistance) and abiotic factors in each part of the world (Snyman *et al*., 2011).

The use of temporary immersion systems in sugarcane promoted both phenolic excretion and shoots formation (Lorenzo *et al*., 2001). In this way, a genomic characterization of plants has been achieved by suppressing key genes of the phenylpropanoid pathway; as a result, a new function of phenolic metabolites as priming molecules has been characterized during sugarcane micropropagation in TIBs. Genes related to cell metabolism and development (10), plant defenses (9), phenylpropanoids (7), methyl jasmonate response (5), ethylene (5), oxidative burst (3) and auxins (3) pathways, among others (8) were found to be induced in sugarcane plants micropropagating in TIBs with phenolic metabolites, supporting that phenylpropanoids might act as elicitor molecules of others biochemical pathways (Arencibia *et al*., 2008). As conclusion phenolics related to the brown color in the culture medium display a beneficial role for the induction/expression of genes during sugarcane propagated in TIBs.

The priming approach has been integrated into the sugarcane micropropagation technology by TIBs. Sugarcane micropropagation in CO_2-rich TIBs induces a mixotrophic condition adequate for the production of natural phenolic metabolites (Fig. 2a). Scaling up has been conducted in several commercial genotypes. While phenolics demonstrate to act as priming molecules during the *in vitro* culture, vitroplantlets growing and shooting in the presence of phenolic metabolites display an enhanced vigor measured as plant size (Fig. 2b,c), emitted functional roots and increase adaptability to the natural environment. Additionally, when combined with the inoculation of the endophytic *Gluconacetobacter diazotrophicus*, a significant improvement of the percentage of survival has been attached through this critical step (Arencibia *et al*., 2008; Bernal *et al*., 2008).

Sugarcane phenolic metabolites as elicitors of resistance to tomato bacterial wilt in the *Solanum lycopersicum and Ralstonia solanacearum* pathosystem has also been identified (Yang *et al*., 2010). The culture media was collected and the phenolics were sprayed onto tomato plants infected with *R. solanacearum*, eliciting and/or maintaining an early defense signaling mechanism that resulted in the protection of the plant against the tomato bacterial wilt disease. RT-PCR analyses confirmed that selected genes from defense-related pathways were differentially expressed between plants treated with sugarcane metabolites, non-treated pathogen-free plants and non-treated plants inoculated with *R. solanacearum* (Yang *et al*., 2010). Results indicate a promising potential for diversification of the sugarcane micropropagation industry by the production of useful phenolic metabolites as byproducts.

The phenylpropanoid pathway is an important pathway in secondary plant metabolism, yielding a variety of phenolics with structural and defense related functions. These phenolic

compounds include lignins, phenolic acids, flavonoids and stilbenes. In addition, enzymes such as phenylalanine ammonialyase (PAL; EC 4.3.1.5), cinnamate-4-hydroxylase (C4H; EC 1.14.13.11) and 4-coumarate:coenzyme A ligase (4CL, EC 6.2.1.12) are considered to be crucial to phenylpropanoid metabolism. A number of reports have shown that phenylpropanoid derivatives are capable of protecting plants against various biotic (infection by viruses, bacteria, fungi) and abiotic (low and high temperatures, UV-B light, wounding) stresses (Sgarbi *et al*., 2003; Solecka and Kacperska, 2003). Stilbene synthase (STS) (EC 2.3.1.95) catalyses the last step of the phenylpropanoid biosynthesis pathway leading to the formation of stilbene phytoalexins. Expression of STS genes is often induced in response to biotic and abiotic stresses (Jeandet *et al*., 2002).

Beside, BABA (beta aminobutyric acid) a non-protein amino acid, was used to induce resistance in grapevine against downy mildew (Slaughter *et al*., 2008). BABA-induced resistance was observed in the susceptible cv. Chasselas as well as in the resistant cv. Solaris. Following BABA treatment, sporulation of *Plasmopara viticola* was strongly reduced and the accumulation of stilbenes increased with time following infection. Furthermore, BABA-treatment of Solaris led to a rapid increase in transcript levels of three genes involved in the phenylpropanoid pathway: phenylalanine ammonialyase, cinnamate-4-hydroxylase and stilbene synthase. BABA-primed Chasselas showed increased transcript levels for cinnamate-4-hydroxylase and stilbene synthase. As a result, the susceptible cultivar became more resistant to downy mildew. In parallel, in grapevine, it has been shown that callose deposition as well as defense mechanisms depending on the phenylpropanoid and the Jasmonic Acid (JA) pathways all contributed to BABA-IR (Hamiduzzaman *et al*., 2005).

Biopriming: Many symbiotic organisms contribute to the vigor and ability of the tissue-cultured plants to perform well in the field. In the course of tissue culture, plants are made free from such beneficial microbes, e.g., symbiotic nitrogen fixing endophytic bacteria and mycorrhizae. There is no practical way to retain the beneficial microorganisms during tissue culture. The deliberate re-infection of propagules with selected strains can be a valid way of retrieving the benefits of such microbes as a biopriming approach.

Endophytic microorganisms can promote plant growth, as well as suppress diseases. Plant growth promotion is taken to result from improved nutrient acquisition or hormonal stimulation (Suman *et al*., 2005). Disease suppression can occur through induction of resistance in the plant (Arencibia *et al*., 2006).

Several bacterial strains have been shown to act as plant growth-promoting bacteria (PGPR) through both stimulation of growth and Induced Systemic Resistance (ISR) but it is not clear in how far both mechanisms are connected. Induced resistance is manifested as a reduction of the number of diseased plants or in disease severity upon subsequent infection by a pathogen. Such reduced disease susceptibility can be local or systemic, result from developmental or environmental factors and depend on multiple mechanisms (Van Loon, 2007).

The spectrum of diseases to which PGPR elicited ISR confers enhanced resistance overlaps partly with that of pathogen-induced Systemic Acquired Resistance (SAR). Although ISR-eliciting bacteria can induce typical early defense-related responses in cell suspensions, in plants they do not necessarily activate defense-related gene expression. Instead, they appear to act through priming of effective resistance mechanisms, as reflected by earlier and stronger defense reactions once infection occurs (Van Loon, 2007).

While, these bacteria utilize the nutrients that are released from the host for their growth, they also secrete metabolites. Several of these metabolites can act as signaling compounds that are perceived by neighboring cells within the same micro-colony, by cells of other bacteria that are present or by cells of the host plant (Van Loon and Bakker, 2003; Bais *et al*., 2004; Gray and Smith, 2005; Kiely *et al*., 2006).

The specificity in the reactions of different plant species to individual strains indicates that the reactions of plants to resistance-inducing PGPR must be the outcome of a dynamic interplay between the production and the perception of ISR eliciting signals. Whereas, some PGPR activate defence-related gene expression, others appear to act solely through priming of effective resistance mechanisms, as reflected by earlier and stronger defence reactions once infection occurs (Gray and Smith, 2005; Kiely *et al*., 2006).

Gluconacetobacter diazotrophicus as biopriming: *Gluconacetobacter diazotrophicus*, *Herbaspirillum* sp., *Azospirillum amazonense*, *Burkholderia* spp., capable of fixing nitrogen have been reported to colonize the epidermis of sugarcane stem and roots, of which *Gluconacetobacter* (earlier *Acetobacter diazotrophicus*) seems to contribute substantially to nitrogen nutrition of the plant (Dobereiner *et al*., 1993; James *et al*., 2001). *Gluconacetobacter diazotrophicus*, a nitrogen fixing endophyte, is found in high number in all part of sugarcane and its better colonization in sugarcane is probably due to its capability to grow in the presence of high sugar and low pH (Dobereiner *et al*., 1993).

Production of plant growth hormones is the other beneficial trait associated with *Gluconacetobacter diazotrophicus* (Bastin *et al*., 1998; James *et al*., 2001). The exact role of such endophytic colonization individually or in a complex endophytic community is not yet very clear but inoculation experiments involving micropropagated plants suggest the positive colonization and its contribution to plant growth and development in terms of improved plant height, nitrogenase activity, leaf nitrogen, biomass and yield (Dobereiner *et al*., 1993; Sevilla *et al*., 2001; Muthukumarasamy *et al*., 2002).

More recently, a new role for the plant growth-promoting nitrogen-fixing endophytic bacteria *Gluconacetobacter diazotrophicus* has been identified and characterized while it is involved in the sugarcane-*Xanthomonas albilineans* pathogenic interactions. Living *G. diazotrophicus* possess and/or produce elicitor molecules which activate the sugarcane defense response resulting in the plant resistance to *X. albilineans*, in this particular case controlling the pathogen transmission to emerging agamic shoots. A total of 47 differentially expressed transcript derived fragments (TDFs) were identified by cDNA-AFLP. Transcripts showed significant homologies to genes of the ethylene signaling pathway (26%), proteins regulates by auxins (9%), β-1,3 Glucanase proteins (6%) and ubiquitin genes (4%), all major signaling mechanisms. Results point toward a form of induction of systemic resistance in sugarcane, *G. diazotrophicus* interactions which protect the plant against *X. albilineans* attack (Arencibia *et al*., 2006).

Biopriming approach has also been integrated into the sugarcane micropropagation technology by Temporary Immersion Bioreactors (TIBs). While phenolics demonstrate to enhance plant capability to be colonized by the endophytic *Gluconacetobacter diazotrophicus*, a simple procedure for *G. diazotrophicus* inoculation has been developed using sugarcane vitroplants during transplanting (Fig. 2d, e). As result a significant improvement of the percentage of adaptability and plant growth rate have been demonstrated in high scale plants micropropagation (Bernal *et al*., 2008).

Fig. 2(a-f): Large-scale sugarcane micropropagation in (a) CO_2-rich TIBs, (b) induction of a functional rooting during the last *in vitro* step as an evidence of the photomixotrophic stage, (c-d) sugarcane vitroplantlets obtained in a TIB using 5 L of the culture medium, (e) inoculation of vitroplantlets with a suspension of *G. diazotrophicus* and planting in a soil-compost-zeolite mixture and (f) twenty-days olds sugarcane plants adapted to the natural environment

Besides sugarcane, *Gluconacetobacter diazotrophicus* colonizes many other sugar and non-sugar plants like *Pennisetum purpureum*, *Ipomea batatas* Döbereiner, *Coffea arabica* (Jimenez-Salgado *et al*., 1997), *Eleusine coracana* (Loganathan *et al*., 1999) and *Ananas comosus* (Tapia-Hernandez *et al*., 2000). Present strategy could be applied to other plants from which recently *Gluconacetobacter diazotrophicus* has also been isolated (Cocking *et al*., 2006), *i.e.*, *Arabidopsis thaliana* and the crop plants maize (*Zea mays*), rice (*Oryza sativa*), wheat (*Triticum aestivum*), oilseed rape (*Brassica napus*), tomato (*Lycopersicon esculentum*) and white clover (*Trifolium repens*).

For a bioinoculant to benefit the plant, it should establish and compete with the native heterotrophic bacterial population and also should acclimatize the local conditions. Under such conditions the indigenous bioinoculant strains may perform better than introduced alien ones for promoting plant growth due to their superior adaptability to the environment. Therefore, selection of superior strains of *Gluconacetobacter diazotrophicus* is essential for its exploitation as bioinoculant for improving growth of *in vitro* sugarcane plants (Oliveira *et al*., 2002).

As summary views of the sugarcane micropropagation process is show in Fig. 2.

An assessment between the conventional micropropagation technology (agar base) and the new one including CO_2-rich TIBs producing phenolics and a further inoculation with *G. diazotrophicus* is show in Table 1. The crucial steps influencing the main parameters related to productive efficiency are under layer considering the conventional micropropagation as the comparison base.

Table 1: Comparing high-scale plant micropropagation technologies in sugarcane as model plant

	Conventional	TIBs
Multiplication rates	1: 3-5	1: 3-5
	1: 10-15	1: 80-120
Steps (handwork)	Initiation	Initation
	10 multiplication subcultures	3 multiplication subcultures
	rooting	TIBs multiplication
Complete cycle	12 months including 10	5 months including 3
	multiplication subcultures	multiplication subcultures
Expected production in 5 months starting from 10 explants	3000 units	20000 units
In vitro consumables and cultures medium to produce 1 million plants	100%	30%
Time of laminar flow operations to produce 1 million plants (handwork)	100%	30%
Total laboratory area to produce 1 million units	100%	40%
Cost per unit	100%	25-30%

Remarks:

- The optimization for photomixotrophic conditions is required in a case by case manner using the TIBs as a plant multiplication platform. A permanent goal must be reach the whole autotrophic stage at least in the final *in vitro* stage
- Wherever possible, the priming and biopriming strategies should be standardized according to local conditions, as well as considering the specific plant-microbe interactions
- The integration of new approaches for plant micropropagation should result in a more efficient and competitive technology, where both basic and applied researches must be primarily focused to take profit of the natural plant physiology during the *in vitro* stage

ACKNOWLEDGMENTS

To Sugar Ministry and CITMA, Cuba, for supports this work. Thanks to researchers and technicians staffs at Genetic and Phytopathology Program, National Institute for Sugarcane Research, Cuba.

REFERENCES

Adelberg, J. and E.P. Simpson, 2002. Intermittent Immersion Vessel Apparatus and Process for Plant Propagation. In: Automation and Environmental Control in Plant Tissue Culture, Aitken-Christie, J., T. Kozai and M.A.L. Smith (Eds.). Kluwer Academic Publishers, The Netherlands, pp: 19-26.

Ahloowalia, B.S., J. Prakash, V.A. Savangikar and C. Savangikar, 2004. Plant Tissue Culture, Low Cost Options for Tissue Culture Technology in Developing Countries. International Atomic Energy Agency, Austria, pp: 3-10.

Arencibia, A.D., F. Vinagre, Y. Estevez, A. Bernal and J. Perez *et al.*, 2006. *Gluconoacetobacter diazotrophicus* elicitate a sugarcane defense response against a patogenic bacteria *Xanthomonas albilineans*. Plant Signaling Behav., 1: 265-273.

Arencibia, A.D., A. Bernal, L. Yang, L. Cortegaza and E.R. Carmona *et al.*, 2008. New role of phenylpropanoid compounds during sugarcane micropropagation in temporary immersion bioreactors (TIBs). Plant Sci., 175: 487-496.

Baginsky, S., L. Hennig, P. Zimmermann and W. Gruissem, 2010. Gene expression analysis, proteomics and network discovery. Plant Physiol., 152: 402-410.

Bais, H.P., S.W. Park, T.L. Weir, R.M. Callaway and J.M. Vivanco, 2004. How plants communicate using the underground information superhighway. Trends Plant Sci., 9: 26-32.

Bastin, F., A. Cohen, P. Piccoli, Y. Luna, R. Bottini, R. Baraldi and R. Bottini, 1998. Production of indol-3-acetic acid and gibberellins A1 and A3 by *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae* in chemically-defined culture media. Plant Growth Regul., 24: 7-11.

Beckers, G.J.M. and U. Conrath, 2007. Priming for stress resistance: From the lab to the field. Curr. Opin. Plant Biol., 10: 425-431.

Bernal, A., P. Machado, L. Cortegaza, E.R. Carmona and O. Rivero *et al*., 2008. Priming and biopriming integrated into the sugarcane micropropagation technology by temporary immersion bioreactors (TIBs). Biomed. Life Sci., 10: 42-47.

Boyko, A. and I. Kovalchuk, 2008. Epigenetic control of plant stress response. Environ. Mol. Mutagen., 49: 61-72.

Bronner, C., T. Chataigneau, V.B. Schini-Kerth and Y. Landry, 2007. The "epigenetic code replication machinery", ECREM: A promising druggable target of the epigenetic cell memory. Curr. Med. Chem., 14: 2629-2641.

Bruce, T.J.A., M.C. Matthes, J.A. Napier and J.A. Pickett, 2007. Stressful memories of plants: Evidence and possible mechanisms. Plant Sci., 173: 603-608.

Chakrabarty, D., E.J. Hahn, Y.S. Yoon and K.Y. Paek, 2003. Micropropagation of apple root stock 'M9 EMLA' using bioreactor. J. Hortic. Sci. Biotech., 78: 605-609.

Cocking E.C., P.J. Stone and M.R. Davey, 2006. Intracellular colonization of roots of *Arabidopsis* and crop plants by *Gluconacetobacter diazotrophicus*. *In vitro* Cell. Dev. Biol. Plant, 42: 74-82.

Conrath, U., G.J.M. Beckers, V. Flors, P. Garcia-Agustin and G. Jakab *et al*., 2006. Priming: Getting ready for battle. Mol. Plant Microbe Interact., 19: 1062-1071.

Conrath, U., O. Thulke, V. Katz, S. Schwindling and A. Kohler, 2001. Priming as a mechanism in induced systemic resistance of plants. Eur. J. Plant Pathol., 107: 113-119.

Debnath, S., 2011. Bioreactors and molecular analysis in berry crop micropropagation: A review. Can. J. Plant Sci., 91: 147-157.

Dobereiner, J., R.M. Reis, M.A. Paula and F. Olivares, 1993. Endophytic Diazotrophs in Sugarcane, Cereals and Tuber Plants. In: New Horizons in Nitrogen Fixation, Palacios, R., J. Mora and W.E. Newton (Eds.). Kluwer Academic Publishers, USA., pp: 671-676.

Escalona, M., J.C. Lorenzo, B. Gonzalez, M. Daquinta, J.L. Gonzalez, Y. Desjardins and C.G. Borroto, 1999. Pineapple (*Ananas comosus* L. Merr.) micropropagation in temporary immersion systems. Plant Cell Rep., 18: 743-748.

Escalona, M., G. Samson, C. Borroto and Y. Desjardins, 2003. Physiology of effects of temporary immersion bioreactors on micropropagated pineapple plantlets. *In Vitro* Cell. Dev. Biol. Plant, 39: 651-656.

Etienne, H. and M. Berthouly, 2002. Temporary immersion systems in plant micropropagation. Plant Cell Tiss. Org. Cult., 69: 215-231.

Frost, C.J., M.C. Mescher, J.E. Carlson and C.M. de Moraes, 2008. Plant defense priming against herbivores: Getting ready for a different battle. Plant Physiol., 146: 818-824.

Gray, E.J. and D.L. Smith, 2005. Intracellular and extracellular PGPR: Commonalities and distinctions in the plant-bacterium signaling processes. Soil Biol. Biochem., 37: 395-412.

Grieneisen, V.A., J. Xu, A.F. Maree, P. Hogeweg and B. Scheres, 2007. Auxin transport is sufficient to generate a maximum and gradient guiding root growth. Nature, 449: 1008-1013.

Hamiduzzaman, M.M., G. Jakab, L. Barnavon, J.M. Neuhaus and B. Mauch-Mani, 2005. β-aminobutyric acid-induced resistance against downy mildew in grapevine acts through the potentiation of callose formation and jasmonic acid signaling. Mol. Plant Microbe Interact., 18: 819-829.

Ibaraki, Y. and K. Kurata, 2001. Automation of somatic embryo production. Plant Cell. Tissue Org. Cult., 65: 179-199.

Iriti, M., S. Mapelli and F. Faoro, 2007. Chemical-induced resistance against post-harvest infection enhances tomato nutritional traits. Food Chem., 105: 1040-1046.

James, E.K., F.L. Olivares, A.L.M. de Oliveira, F.B. Jr. dos Reis, L.G. da Silva and V.M. Reis, 2001. Further observations on the interaction between sugarcane and *Gluconacaetobacter diazothrophicus* under Laboratory and greenhouse conditions. J. Exp. Bot., 52: 747-760.

Jeandet, P., A.C. Douillet-Breuil, R. Bessis, S. Debord, M. Sbaghi and M. Adrian, 2002. Phytoalexins from the Vitaceae: Biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity and metabolism. J. Agric. Food Chem., 50: 2731-2741.

Jiao, Y., O.S. Lau and X.W. Deng, 2007. Light-regulated transcriptional networks in higher plants. Nat. Rev. Genet., 8: 217-230.

Jimenez-Salgado, T., L.E. Fuentez-Ramirez, A. Tapia-Hernandez, M.A. Mascarua-Esparza, E. Martinez-Romero and J. Caballero-Mellado, 1997. *Coffea Arabic* L., a new host plant for *Acetobacter diazotrophicus* and isolation of other nitrogen-fixing acetobacteria. Appl. Environ. Microbiol., 63: 3676-3683.

Kiely, P.D., J.M. Haynes, C.H. Higgins, A. Franks, G.L. Mark, J.P. Morrissey and F. O'Gara, 2006. Exploiting new systems-based strategies to elucidate plant-bacterial interactions in the rhizosphere. Microbial. Ecol., 51: 257-266.

Leifert, C., 2000. Quality assurance systems for plant cell and tissue culture: The problem of latent persistance of bacterial pathogens and *Agrobacterium*-based transformed vector systems. Proceedings of the International Symposium Methods and Markers For Quality Assurance in Micropropagation (MMQAM'00), Acta Horticulturae, Kenya, pp: 87-91.

Levin, R. and G. Tanny, 2004. Bioreactors as Low Cost Option for Tissue Culture. In: Low Cost Options for Tissue Culture Technology in Developing Countries, IAEA (Ed.). IAEA, Vienna, pp: 47-54.

Loganathan, P., R. Sunitha, A.K. Prida and S. Nair, 1999. Isolation and characterization of two genetically distant group of *Acetobacter diazotrophicus* from a new host plant (*Eleusine coracona* L.). J. Appl. Microbiol., 87: 167-172.

Lorenzo, J.C., M.A. Blanco, O. Pelaez, A. Gonzalez and M. Cid *et al.*, 2001. Sugarcane micropropagation and phenolic excretion. Plant Cell Tissue Organ Culture, 65: 1-8.

Mathieu, O., J. Reinders, M. Caikovski, C. Smathajitt and J. Paszkowski, 2007. Transgenerational stability of the *Arabidopsis* epigenome is coordinated by CG methylation. Cell, 130: 851-862.

Morini, S. and A. Melai, 2004. CO_2 dynamics and growth in photoautotrophic and photomixotrophic apple cultures. Biol. Plant., 47: 167-172.

Muthukumarasamy, R., G. Revathi and P. Loganathan, 2002. Effect of inorganic N on the population, *in vitro* colonization and morphology of *Acetobacter diazotrophicus* (syn. *Gluconacetobacter diazotrophicus*). Plant Soil, 243: 91-102.

Nemhauser, J.L., 2008. Dawning of a new era: Photomorphogenesis as an integrated molecular network. Curr. Opin. Plant Biol., 11: 4-8.

Oliveira, A.L.M., S. Urquiaga, J. Dobereiner and J.I. Baldani, 2002. The effect of inoculating endophytic N_2-fixing bacteria on micropropagated sugarcane plants. Plant Soil, 242: 205-215.

Paek, K.Y., E.J. Hahn and S.H. Son, 2001. Application of bioreactors of large-scale micropropagation systems of plants. *In Vitro* Cell. Dev. Biol. Plant, 37: 149-157.

Paek, K.Y., D. Chakrabarty and E.J. Hahn, 2005. Application of bioreactor systems for large scale production of horticultural and medicinal plants. Plant Cell Tissue Organ Cult., 81: 287-300.

Pandey, S.P. and I.T. Baldwin, 2008. Silencing RNA-directed RNA polymerase 2 increases the susceptibility of *Nicotiana attenuate* to UV in the field and in the glasshouse. Plant J., 54: 845-862.

Seo, S., S. Katou, H. Seto, K. Gomi and Y. Ohashi, 2007. The mitogen-activated protein kinases WIPK and SIPK regulate the levels of jasmonic and salicylic acids in wounded tobacco plants. Plant J., 49: 899-909.

Sevilla, M., R.H. Burris, N. Gunapala and C. Kennedy, 2001. Comparison of benefit to sugarcane plant growth and 15 N_2 incorporation following inoculation of sterile plants with *Acetobacter diazotrophicus* wild type and Nif-mutants strains. Mol. Plant Microbe. Interact., 14: 358-366.

Sgarbi, E., R.B. Fornassiero, A.P. Lins and P.M. Bonatti, 2003. Phenol metabolism is differentially affected by ozone in two cell lines from grape (*Vitis vinifera* L.) leaf. Plant Sci., 165: 951-957.

Sivakumar, G., 2006. Bioreactor technology: A novel industrial tool for high-tech production of bioactive molecules and biopharmaceuticals from plant roots. Biotechnol. J., 1: 1419-1427.

Slaughter, A.R., M. Hamiduzzaman, K. Gindro, J.M. Neuhaus and B. Mauch-Mani, 2008. β-aminobutyric acid-induced resistance in grapevine against downy mildew: Involvement of pterostilbene. Eur. J. Plant Pathol., 122: 185-195.

Snyman, S.J., G.M. Meyer, A.C. Koch, M. Banasiak and M.P. Watt, 2011. Applications of *in vitro* culture systems for commercial sugarcane production and improvement. *In Vitro* Cell. Dev. Biol. Plant, 47: 234-249.

Solecka, D. and A. Kacperska, 2003. Phenylpropanoid deficiency affects the course of plant acclimation to cold. Physiol. Plant., 119: 253-262.

Suman, A., A. Gaur, A.K. Shrivastava and R.L. Yadav, 2005. Improving sugarcane growth and nutrient uptake by inoculating *Gluconacetobacter diazotrophicus*. Plant Growth Regul., 47: 155-162.

Takahashi, F., R. Yoshida, K. Ichimura, T. Mizoguchi and S. Seo *et al*., 2007. The mitogen-activated protein kinase cascade MKK_3- MPK_6 is an important part of the jasmonate signal transduction pathway in *Arabidopsis*. Plant Cell, 19: 805-818.

Tapia-Hernandez, A., M.R. Bustillo-Cristales, T. Jimenez-Salgado, J. Caballero-Mellado and L.E. Fuentes-Ramirez, 2000. Natural endophytic occurrence of *Acetobacter diazotrophicus* in pineapple plants. Microbiol. Ecol., 39: 49-55.

Van Loon, L.C. and P.A. Bakker, 2003. Signalling in Rhizobacteria-Plant Interactions. In: Root Ecology, Ecological Studies, De Kroon, H. and E.J.W. Visser (Eds.). Vol. 168, Springer-Verlag, Berlin, pp: 297-330.

Van Bentem, S.D. and H. Hirt, 2007. Using phosphoproteomics to reveal signalling dynamics in plants. Trends Plant Sci., 12: 404-411.

Van Loon, L.C., 2007. Plant responses to plant growth-promoting rhizobacteria. Eur. J. Plant Pathol., 119: 243-254.

Waclawovsky, A.J., P.M. Sato, C.G. Lembke, P.H. Moore and G.M. Souza, 2010. Sugarcane for bioenergy production: An assessment of yield and regulation of sucrose content. Plant Biotechnol. J., 8: 263-276.

Weitz, J.S., P.N. Benfey and N.S. Wingreen, 2007. Evolution, interactions and biological networks. PLoS Biol., 5: e11-e11.

Wu, J.Q., C. Hettenhausen, S. Meldau and I.T. Baldwin, 2007. Herbivory rapidly activates MAPK signaling in attacked and unattacked leaf regions but not between leaves of *Nicotiana attenuate*. Plant Cell, 19: 1096-1122.

Yang, L., Y. Zambrano, C.J. Hu, E.R. Carmona and A. Bernal *et al*., 2010. Sugarcane metabolites produced in CO_2-rich temporary immersion bioreactors (TIBs) induce tomato (*Solanum lycopersicum*) resistance against bacterial wilt (*Ralstonia solanacearum*). *In vitro* Cell. Dev. Biol. Plant, 46: 558-568.

Zeilinger, M.N., E.M. Farre, S.R. Taylor, S.A. Kay and F.J. Doyle, 2006. A novel computational model of the circadian clock in *Arabidopsis* that incorporates PRR7 and PRR9. Mol. Syst. Biol., 2: 58-58.