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A review on *in-vitro* micropropagation of agave and other plants†

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ABSTRACT

This article gives a brief review on research progress on the use of tissue culture in the micropropagation of agaves and a few economic plants. Fragments of leaf meristem, rhizomes, meristematic apex, root and excised embryo are used as explants in MS medium. Techniques used in the micropropagation of several species of Agave gave promising results. *In vitro* propagation is extensively used for propagation of Agave spp. and native plant species in some established centers in some countries. *In vitro* somatic embryogenesis is considered as an important pre-requisite for genetic improvement, as well as for mass propagation. The asceptic mass production of callus is efficiently used in the extraction of secondary metabolites of medicinal use. The technique is recommended specially for the propagation of perennial native economic plant species like Agave which takes more than eight years to reach reproductive stages and produces seeds. The massive production of micropropagated native plants could be effectively used in the reforestation of the species in their natural habitats.

Key words : Agave, economic plants, *in vitro* micropropagation, mass propagation, somatic embgryogenesis

INTRODUCTION

Since remote times before the advent of agriculture, human beings depend on plants for food, medicine, wood and other necessary domestic goods. Agaves are extensively exploited in Mexico for the extraction of wine from crushed culm and also for fibres and some medicinal uses. Owing to over-exploitation, the population of agaves is decreasing day by day which may lead to the danger of exinction. Very little attempts are made to plant agaves owing to the difficulties in propagation by vegetative method and seeds. Agave plant takes 8-10 years to reach reproductive stage and produces seeds. No efficient technique is available for inducing germination.

In this respect, owing to the problems of seed availability, henequen produces flowers only once towards the end of its long life cycle and then dies. Owing to its high levels of ploidy (5x) and sterility, it produces seeds with very low viability in the laboratory. Therefore, henequen plantations are multiplied only through vegetative propagation using rhizomes. It has been observed that the vegetative propagated plants showed large variability in morphological characters of the individuals obtained from the same mother plant. Analysis with AFLP indicated that differences also existed at the genomic level. After micropropagation through somatic embryogenesis of the elite lines and the cluster analysis indicated that each mother plant and its somatic embryogenesis derived daughter plants clustered, that indicated the conservation of molecular marker patterns in the micropropagated daughter plants (Gonzalez *et al.*, 2003).

In the context of above facts, tissue culture is considered as a valuable and viable instrument for the massive production of perennial plants. Tissue culture from leaf tissue, stem segments, root tissue or fruit and seed fragments can be used for vegetative propagation of a plant, production of haploid plants and somatic cell hybridization. In the following is given a brief review of literature on the vegetative and micropropagation of Agave species and some native plants of economic importance.

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CALLUS AND MICROPROPAGATION IN AGAVE

In vitro propagation is extensively used for propagation of Agave spp. *In vitro* somatic embryogenesis is considered as an important pre-requisite for the use of many biotechnological tools for genetic improvement, as well as for mass propagation (Santacruz-Ruvalcaba *et al.,* 1998). Robert *et al.* (1987) obtained *in vitro* plant regeneration of *Avave fourcroydes* Lem. (Agavaceae). It is suggested that the balance in the culture medium is a key factor controlling callus growth and organogenesis in rhizome cultures. Although stem callus had a limited organogenic capacity, but high cytokinin concentrations induced adventitious shoot formation on stem explants. The shoots from stem explants and rhizome callus formed extensive root systems *in vitro* and were transferred to pot culture with a 90% survival rate.

Sisal (*Agave sisalana* P.) induced *in vitro :* expression of variability of spineless of spiny character of the leaf margins. Sisals (*A. sisalana* P.) induits *in vitro :* expression de la variabilite du caractere inerme ou epineux des marges foliaires (Robert *et al.,* 1991). Tapati (1992) was not successful to obtain micropropagation of *A. sisalana* from sections of rhizome and immature leaves in M. S. medium, but when excised buds with a leaf at least 2 cm long were transferred to basal SH medium with 1% sucrose and no growth regulators they rooted (80-90%) within 30 days. Rodriguez-Garay *et al.* (2003) reported somatic embryogenesis of *Agave victoria-reginae* Moore. Somatic embryogenesis was evident in a 6-week period on agar-solidified MS medium supplemented with L2 vitamins and 2, 4-dichlorophenoxyacetic acid (1, 4 mu-M), and germination of somatic embryos was achieved after eight weeks on half-strength MS medium and four weeks on half-strength SH medium, without adding growth regulators. Vargas and Garcia *et al.* (1996) developed protocol for clonal mass propagation of *A. sisalana* (Sisal) using leaf segments. Leaf segments produced a big amount of calluses only in Dustand and Short medium, but failed to regenerate shoot. But axillary buds produced new buds and shoots in both Dustand and Short medium and MS, when media were supplemented with 2 mg 6-benzylaminopurine (benzyladenine) and 0.1 mg NAA per litre. Multiplication was more efficient in MS medium.

Vargas and Garcia (1996) developed protocol for clonal mass propagation of *A. sisalana* (Sisal) from leaf segments and axillary buds. Leaf segments produced a huge mass of calluses when they were cultured on Dustand and Short medium, but no shoot regeneration was observed. Axillary buds produced new buds and shoots in both Dustand and Short medium and MS, when media were supplemented with 2 mg, 6-benzylaminopurine (benzyladenine) and 0.1 mg NAA per litre. Garcia-Suarez *et al.* (1996) were successful to obtain *in vitro* propagation of *Agave marmorata* Roezi. (Agavaceae) and *Beaucamea gracilis* Lem using a Murashige-Skoog medium, supplemented with 0.25, 0.5 and 1.0 mg/1 benzyladenine (BA) and 1-3 mg/1 2, 4-D for callus formation, and 0.9, 1.0 and 1.2 mg/1 BA with a constant 0.2 mg/1 2, 4-D for organogenesis. All media were supplemented with 3% (w/v) sucrose.

Moreno-Salazar and Martinez-Heredia (1996) were successful to obtain *in vitro* propagation of *Agave pacifica* (bacanora maguey) for its conservation, repopulation from adventitious buds, using 2 culture media (Gamborg or MS) and various auxin (2, 4-D) and cytokinin (benzyladenine (BA) concentrations, as well as different explant types, are reported. It was observed that shoot and root cultures in MS medium containing 1.13 and 4.44 micro M 2, 4-D and BA, respectively, gave the best results in terms of speed, biomass production and organogenesis from callus. Successful rooting has been achieved with medium free from cytokinins but containing 0.025 mg 2, 4-D/ litre.

Groenewald *et al.* (1977) reported the development of calli from seed fragments of an Agave sp. and subsequent regeneration of shoots and roots from callus tissue. Garcia-Suarez *et al.* (1997) reported somatic embryogenesis from callus cultures of *Agave marmorata* Roezl. Callus formation was induced using a basal medium supplemented with 1.0 mg/1 benzyladenine and 0.25 mg/1 2, 4-D. Somatic embryogenesis induction was obtained on a basal medium plus 0.25 mg/1 2, 4-D and 1.0 mg/1 kinetin, pH was adjusted to 5.8. The cultures were incubated under a 16-h light photoperiod at 29°C.

Callus of *A. sisalana* was induced on LS (Linsmaier and Skoog) medium supplemented with 2, 4-D at 3 mg/litre, NAA at 0.4 mg/litre, benzyladenine at 2.5 mg/litre and various concentrations of different nitrogen forms. Callus growth rate was highest with addition of ammonium and nitrate to the culture medium, while organic N at 1-10 g/litre was most effective for enhancing the activity of agavain-SH. Callus growth rate was negatively correlated with agavain-SH activity (Li *et al.,* 1998).

Malda *et al.* (1999) considered *in vitro* culture as a potential method for the conservation of endangered plants possessing crassulacean acid metabolism. *In vitro* somatic embryogenesis is an important pre-requisite for the use of many biotechnological tools for genetic improvement, as well as for mass propagation (Santacruz-Ruvalcaba *et al.,* 1998). Callus of *A. sisalana* was induced on LS (Linsmaier and Skoog) medium supplemented with 2, 4-D at 3 mg/litre, NAA at 0.4 mg/litre, benzyladenine at 2.5 mg/litre and various concentrations of different nitrogen forms. Callus growth rate was highest with addition of ammonium and nitrate to the culture medium, while organic N at 1-10 g/litre was most effective for enhancing the activity of agavain-SH. Callus growth rate was negatively correlated with agavain-SH activity (Li *et al.,* 1998).

An efficient method for the *in vitro* propagation of *Agave parrasana* Berger, a native to the state of Coahuila, Mexico, was developed with the proliferation of good quality shoots in agar-solidified basal MS medium supplemented with L2 vitamins and 13.3 nuM enzyladenine. Rooting was successful in the basal medium without growth regulators; under a light intensity of 100 µmol m-2 s-1 (Santacruz-Ruvalcaba *et al.,* 1999).

Nikam (1997) reported that callus of *A. sisalana* was initiated from rhizome, and stem explants on MS, SH, Gamborg and White's medium supplemented with different concentrations of BA, kinetin, NAA, IAA and 2, 4-D either in combination or singly. In a later study, Hazra *et al.* (2001) successfully obtained *in vitro* multiple shoot induction from rhizome buds os sisal (*A. sisalana* Perr ex. Engelm) for onbtaining quick planting material by using the basal media of MS, White, BS, SH and Litvay. PGRs benzyladenine (BA), 2iP (isopentenyladenine), kinetin, adenine sulfate and n-benzyl-9 (2-tetrahydro-pyranyl) adenine (BPA) at 1, 3, 5, 8 or 10 mg/litre supplied to the media alone or in combination with NAA at 0.5 mg/litre. SH medium was the most effective media, followed by MS, in inducing multiple shoots, in the presence of 1 or 8 mg/litre kinetin. In trials SH basal medium supplied with the PGRs, kinetin was the most effective in inducing multiple shoots at all concentrations followed by BA and BPA.

In another study, Hazra *et al.* (2002) obtained sisal plant regeneration via organogenesis. Callus was initiated from *in vitro* grown immature leaf and *ex vitro* grown mature leaf and rhizome explants of *A. sisalana* Perr. ex. Engelm, on MS medium containing 2, 4-D (9.05 micro M) and kinetin (4.6 micro M) or 2, 4-D (9.05 micro M), kinetin (4.6 micro M) and CH (1000 mg 1^{-1}) or mod. MS (NH_4NO_3, 1500 mg 1^{-1}) containing 2, 4-D (9.05 micro M) and kinetin (4.6 micro M). Light was necessary for callus formation. While increasing NH_4^+ had a negative impact, addition of CH had a positive impact on callus formation.

A protocol has been developed for somatic embryogenesis and plant regeneration of sisal (*A. sisalana* Perr. ex. Engelm). Embryogenic callus cultures were initiated from young shoots raised *in vitro* from the stem portion of the bulbil on medium supplemented with 1-2 mg 1 SUP-SUP 1 kinetin (KN) and 0.2-0.5 mg 1 SUP-SUP 1 alpha-naphthaleneacetic acid plus KN or 1-1.5 mg 1 SUP-SUP 1 benzylaminopurine (BAP) or 0.25-0.5 mg 1 SUP-SUP 1 2, 4-dichlorophenoxyacetic acid plus BAP or 0.5-1.0 mg 1 SUP-SUP 1 KN. Plantlets regenerated from embryos were transferred to the field where their survival rate was 100% (Nikam *et al.,* 2003). Lima *et al.* (2000) determined the optimum size of basic experimental unit for determination of micropropagation technology for sisal (*A. fourcroydes* L.). Increase in the basic unit resulted in an increase in heterogeneity. The use of 8-10 tubes gave the best results for evaluation of the multiplication index, while 12-16 tubes gave the best results for the other variables.

Somatic embryogenesis and plant regeneration of henequen (*Agave fourcroydes*) was performed using an established protocol at four different stages. Embryogenic calluses were developed from immature leaf and stem explants of *in vitro* propagated plants. It was assessed that the addition of BAP during the second stage and culture in photoperiodic conditions during the third stage promoted the development of cotyledons in somatic embryos and induced plant recovery during the germination stage (Piven *et al.,* 2002). In view of excellent results, Vazquez-Flota and Loyola-Vargas (2003) used *in vitro* plant cell culture as the basis for the development of a research institute in Mexico with a great exit. Martinez-Palacios *et al.* (2003) induced somatic embryogenesis and organogenesis of *Agave victoriae reginae*. The optimal treatment was MS medium with 2.26 muM 2, 4-D. Multiple shoot regeneration was induced from axillary buds from stem segments cultured on MS medium with 2.2-4.4 muM BA.

Production of Secondary Metabolites

Tissue culture with massive production of callus under asceptic condition is used as an efficient intrument for the extraction of metabolites of high medicinal and economic importance. Ackerman *et al.* (1973) detected the presence of free lysopine in tissue culture of *Agave toumeyana*. Sharma and Khanna (1980) obtained from steroidal sapogenins from tissue cultures of *Agave wightii*. Eighteen-month-old unorganized callus tissue of *A. wightii* raised from seedlings on RT medium (Murashige and Skoog's medium+1 ppm 2, 4-D+1% agar) was maintained as a static culture. The analysis of the cultures for saponin content produced gitogenin, hecogenin and tigogenin. The addition of cholesterol to the submerged culture medium increased considerably sapogenin content. Hall (1981) studied the factors influencing the production of saponins and other steroidal compounds from *A. sisalana* tissue culture. Castro-Concha *et al.* (1990) studied glutamate dehydrogenase activity in normal and vitrified plants of *Agave tequilana* Weber propagated *in vitro*. Glutamate dehydrogenase activity was high in non-vitrified tissues and decreased significantly in the vitrified ones.

Indrayanto *et al.* (1993) observed that calcium, strontium and magnesium ions had effects on the formation of phytosteroids in callus cultures of *Agave amaniensis*. Andrijany (1998) studied simultaneous effect of calcium, magnesium, copper and cobalt ions on sapogenin steroids content in callus cultures of *A. amaniensis.*

Vinsencia *et al.* (1998) studied the simultaneous effect of calcium, cobalt, copper and magnesium ions and their interactions on growth and sapogenin steroids accumulation in callus cultures of *A. amaniensis.* The absence of calcium ions in media increased the sapogenin steroid content, while relatively high concentration of magnesium, cobalt and copper ions inhibited the sapogenin steroid formation.

Kartosentono *et al.* (2002) studied the uptake of copper ions by cell suspension cultures of *A. amaniensis,* and its effect on the growth, amino acids and hecogenin content. Cell suspension cultures of *A. amaniensis* were able to grow in media containing 10-240 micro M copper ions. Certain amino acids were released in high concentration into the media. The hecogenin content in the biomass increased upto 157.9% at 20 micro M copper ions.

Few studies reported the physiology of the control and micropropagated plants. Santamaria *et al.* (1995) reported that detached leaves of micropropagated *A. tequilana* plants lost water at similar rates as did field-grown plantlets when dehydrated in air. It was concluded that *in vitro* culture did not affect the capacity of leaves to control water loss nor did it alter the nocturnal stomatal opening of this CAM plant. Wen *et al.* (1997) studied growth and nocturnal acid accumulation during early ontogeny of *Agave attenuata* grown in nutrient solution and *in vitro* culture. In the earliest ontogenetic phases of development (cotyledon and primary leaf stage), the plants were able to carry out considerable nocturnal organic acid accumulation. *In vitro* cultivated plants, from the beginning of their development, were also capable of diurnal acid fluctuation, though of distinctly weaker activity than the pot plants.

Tissue Culture Used in the Propagation of Some Plants of Economic Importance

Research on the use of tissue culture in the micropropagation of some plants of economic importance started in early seventies. Few examples are mentioned here about the use of tissue culture in the propagation of different plants of economic importance showing reasonably good results. The potential of *in vitro* propagation of Coffea and Agave in Mexico-coffee and henequen propagation was discussed. *In vitro* (20, 3, Pt. 2, 244) 1984.

Leaf disks from peppermint, spearmint, orange mint, lavender mint and Scotch spearmint cultured on various Murashige-Skoog-based media regenerated shoots on basal medium containing 44.4 muM benzyladenine (BA) and 250 ml 1^{-1} coconut water (CW). Shoots regenerated from peppermint leaf disks cultured on basal medium containing 44.4 muM BA and 250 ml or 450 ml 1^{-1} CW (Vaneck and Kitto, 1992). In an earlier study on Yuca, casein added to the nutrient medium favourably affected tissue growth in *Yucca elata, Y. aloifolia, Agave americana, A. attenuata* and *A. regia.* The presence of antioxidants in the medium reduced phenolic compound accumulation in the tissues. Glutathione was the most suitable antioxidant. Callus tissues from the roots had a higher sapogenin content than those from the leaves. AttaAlla and VanStaden (1997) were successful to obtain micropropagation and establishment of *Yucca aloifolia* from shoot tips cultured on half strength. Murashige and Skoog medium supplemented with 3% sucrose and 0.2% gelrite produced roots. The proliferated shoots readily rooted *in vitro* on 1/2 or 1/4 strength MS medium with and without 2.5 or 4.9 muM IBA and 1% charcoal and the rooted plantlets were successfully acclimatized in soil. Bouza *et al.* (1992) obtained *in vitro* propagation of *Prunus tenella* showing increase of the multiplication and growth of the micropropagated plant by chilling.

Bergmann and Stomp (1992) reported that embryos of *Pinus echinata* Mill., *Pinus taeda* L., *Pinus serotina* Michx., *Pinus eldarica* Medwed., *Pinus caribaea* Morelet., *Pinus oocarpa* Scheide, *Pinus tecunumanii* (Schwd.) Equiluz & Perry, *Pinus strobus* L. and *Pinus radiata* D. Don cultured indicating half-strength modified LePoivre medium containing 2.5 mg/l benzyladenine but no auxin gave the best results with most species. Significant differences in shoot production were found among *Pinus oocarpa* provenances. Sita and Vaidyanathan (1980) used tissue culture methods for the production of some medicinal plants such as Sandalwood, Eucalyptus, Mucuna and Agave.

Kartosentono *et al.* (2002) studied the uptake of copper ions by cell suspension cultures of *Agave amaniensis*, and its effect on the growth, amino acids and hecogenin content callus culture, suspension cell culture, propagation and heavy metal effect for drug manufacture. The hecogenin content in the biomass increased upto 157.9% at 20 muM copperions. Micropropagation of desert Mexican endangered plant species (Cactaceae and Agavaceae) which were cultured in Murashige and Skoog (MS) medium containing 1-2 mg/1 benzyladenine and 0-0.01 mg/1 naphthaleneacetic acid was induced. Species studied included : *Agave victoriae reginae, Echinocactus grusonii, Opuntia ficus indica, Optunia microdasys, Coryphanta elephant-idens, Mammillaria duwei, Mammillaria* Sanchez-Mejoradae, *Mammil-laria pectinifera, Mammallaria carmenae, Mammillaria ochoterenae* and *Ariocarpus kotschoubeyanus*. Grownewald *et al.* (1979) reported the use of tissue cultures in the propagation and possible hybridization of aloes and related plants. There was little success in production of haploid aloe species using tissue culture.

The potential for advanced plant biotechnology (APB; manipulation of plants by tissue culture and genetic engineering) to deal with specific socio-economic and technical problems is discussed for different plants including henequen (*Agave fourcroydes*). It is concluded that owing to the fact, APB is mostly applied to certain high-value crops and its potential to help poor consumers will not be exploited in Mexico. Malda *et al.* (1999) suggested that *in vitro* micropropagation was a potential method for the conservation of endangered plants possessing crasulacean metabolism like mebbers of the Cactaceae, Agavaceae. *In vitro* micropropagation produces virus free plants of superior quality (Ammirato *et al.*, 1990; Koizumi and Kitaura, 1997).

CONCLUSIONS

In vitro micropropagation is effectively used in the mass propagation of agaves and some economic plants. The asceptic mass production of callus is efficiently used in the extraction of secondary metabolites of medicinal use. The technique is recommended specially for the propagation of perennial native economic plant species like Agave which takes more than eight years to reach reproductive stages and produces seeds. The massive production of micropropagated native plants could be effectively used in the reforestation of the species in their natural habitats. Therefore, a schematic protocol should be planned for utilising the techniques of *in vitro* micropropagation of the native plant species of economic plants.

REFERENCES

Ackerman, L., Teller, G. and Hirth, L. (1973). Presence of free lysopine in tissue culture of different origins [*Agave toumeyana*, tobacco]. C. R. Hebd Seances. *Acad Sci. Ser. D. Sci. Nat.* **277** : 573-76.

Ammirato, P. V., Evans, D. A., Sharp, W. R. and Bajaj, Y. P. S. (1990). Handbook of plant cell culture. Ornamental species. Barnard College, Columbia University, New York, USA **XIII** (5) 833. Publisher : McGraw-Hill, Inc. New York, USA.

Andrijany, V. S. (1998). Simultaneous effect of calcium, magnesium, copper and cobalt ions on sapogenin steroids content in callus cultures of *Agave amaniensis. Plant Cell, Tissue and Organ Culture* **55** : 103-08.

Koizumi, T. and Kitaura, T. (1997). Establishment of virus-free and micropropagation techniques of superior variety. Influence of phytohormone on growth of 2 kinds genus Cordyline fruticosa. Kanaga-waken Nogyo Sogo Kenkyujo Shiken Kenkyu Seisekisho, Kaki, Kanshoju, 1997. pp. 31-32.

AttaAlla, H. and VanStaden, J. (1997). Micropropagation and establishment of *Yucca aloifolia. Plant Cell*

Tissue and Organ Culture **48** : 3-4.

Bergmann, B. A. and Stomp, A. M. (1992). Influence of taxonomic relatedness and medium composition of meristematic nodule and adventitious shoot formation in nine pine species. *Canadian J. Forest Res.* **22** : 750-55.

Bouza, L., Jacques, M., Maziere, Y. and Arnaud, Y. (1992). *In vitro* propagation of *Prunus tenella* Batsch cv. Firehill-Control of vitrification-increase of the multi-plication rate and growth by chilling. *Scientia Horticulturae* **52** : 143-55.

Castro-Concha, L., Loyola-Vargas, V. M., Chan, J. L. and Robert, M. L. (1990). Glutamate dehydrogenase activity in normal and vitrified plants of *Agave tequilana* Weber propagated *in vitro. Plant Cell, Tissue and Organ Culture* **22** : 147-51.

Garcia-Suarez, M. D., Osorio, R., Lechuga, J. A., Cruz, F. and Serrano, H. (1996). *In vitro* propagation of *Agave marmorata* Roezl. (Agavaceae) and *Beaucamea gracilis* Lem. (Nolinaceae) from a Mexican semi-arid environment *in vitro* seed germination and propagation from foliar bud culture and apical meristem culture. *In Vitro* **32** : 108-09.

Garcia-Suarez, M. D., Osorio, R., Lechuga, J. A., Cruz, F. and Serrano, H. (1997). Somatic embryogenesis from callus cultures of *Agave marmorata* Roezl. (Agavaceae) callus culture and propagation. *In Vitro* **33** : 2-3.

Gonzalez, G., Aleman, S. and Infante, D. (2003). Asexual genetic variability in *Agave fourcroydes*. II. Selection among individuals in a clonally propagated population. *Plant Sci.* **165** : 595-601.

Groenewald, E. G., Koeleman, A. and Wessels, D. C. J. (1979). The use of tissue cultures in the propagation and possible hybridization of aloes and related plants. *Aloe* **17** : 37-40.

Groenewald, E. G., Wessels, D. C. J. and Koeleman (1977). Callus formation and subsequent plant generation from seed tissue of an *Agave sp*. Agavaceae. *Zeitschrift fuer Pflanzenphysiologie* **81** : 369-73.

Hall, Andrea Cecelia (1981). Factors influencing the production of sapogenins and other steroidal components of *Agave sisalana* tissue culture. Degree : Ph. D. Volume 4210B of Dissertations Abstracts International. p. 3917.395.

Hazra, S. K., Sudripta, D. and Das, A. K. (2001). *In vitro* multiple shoot induction from rhizome buds of sisal (*Agave sisalana* Perr. ex. Engelm) for obtaining quick planting material. *J. Plant Biol.* **28** : 165-71.

Hazra, S. K., Sudripta, D. and Das, A. K. (2002). Sisal plant regeneration via organogenesis. *Plant Cell, Tissue and Organ Culture* **77** : 235-40.

Indrayanto, G., Rahayu, L., Rahman, A. and Noeraeni, P. E. (1993). Effect of calcium, strontium, and magnesium ions on the formation of phytosteroids in callus cultures of *Agave amaniensis. Planta Medica* **59** : 97-98.

Kartosentono, S., Indrayanto, G. and Zaini, N. C. (2002). The uptake of copper ions by cell suspension cultures of *Agave amaniensis,* and its effect on the growth, amino acids and hecogenin content. *Plant Cell, Tissue and Organ Culture* **68** : 287-92.

Li, B., Peng, Z. and He, Q. (1998). Effects of nitrogen on tissue culture and agavain-SH inducement of *Agave sisalana. J. South China Agric. Univ.* **19** : 45-49.

Lima, D. S., Gonzalez, O. G., Liviano, G. R. and Sanchez, L. P. (2000). Study of optimum size of basic experimental unit for determination of micropropagation technology for sisal (*Agave fourcroydes* L.). Universidad "Camilo Cienfuegos", Matanzas, Cuba. *Centro Agricola* **27** : 79-86.

Malda, G., Suzan, H. and Backhaus, R. (1999). *In vitro* culture as a potential method for the conservation of endangered plants possessing crassulacean acid metabolism. *Scientia Horticulturae* **81** :71-87.

Martinez-Palacios, A., Ortega-Larrocea, M. P., Chavez, V. M. and Bye, R. (2003). Somatic embryogenesis and organogenesis of *Agave victoriae reginae* : Considerations for its conservation. *Plant Cell, Tissue and Organ Culture* **74** : 135-42.

Moreno-Salazar, S. F. and Martinez-Heredia, D. (1996). *In vitro* propagation of *Agave pacifica* (bacanora maguey) for its conservation, repopulation and commercial exploitation. Proc. Ninth International Conference on Jojoba and its Uses and of the Third International Conference on New Industrial Crops and Products, Catamarca, Argentina, 25-30 September, 1994. Princen, L. H. and Rossi, C. (eds.). pp. 385-88.

Nikam, T. D. (1997). High frequency shoot regeneration in *Agave sisalana. Plant Cell, Tissue and Organ Culture* **51** : 225-28.

Nikam, T. D., Bansude, G. M. and Aneesh-Kumar, K. C. (2003). Somatic embryogenesis in sisal (*Agave sisalana* Perr. ex. Engelm). *J. Plant Cell Reports* **22** : 188-94.

Piven, N. M., Barredo-Pool, F. A., Borges-Argaez, I. C. and Robert, M. L. (2002). Key events in the regulation of somatic embryogenesis in monocots : agaves. *Byulleten Gosudarstvennogo Nikit-skogo Botanicheskogo Sada* **86** : 12-16.

Robert, M. L., Herrera, J. L., Chan, J. L., Contreras, F. and Berlin, W. (1991). Micropropgation of *Agave* spp. *Biotechnology in Agriculture and Forestry* **19** : 306-29.

Robert, M. L., Herrera, J. L., Contreras, F. and Scorer, K. N. (1987). *In vitro* propagation of *Agave fourcroydes* LEM. Henequen. *Plant Cell, Tissue and Organ Culture* **8** : 37-48.

Rodriguez-Garay, B., Santacruz-Ruvalcaba, F. and Portillo,

M. L. (2003). *In vitro* regeneration of *Agave tequilana* plants by indirect somatic embryogenesis, from a single cell, inclues embryogenesis then germination of embryos plant propagation by somatic embryogenesis has potential for transgenic plant generation. Patent Assignee : Centr. Investigacion Asist Tech Queretaro 2003. Patent Number : WO 200339244, Patent Date : 20030515 WPI Accession No. : 2003-441432 (200341).

Santacruz-Ruvalcaba, F., Gutierrez-Mora, A. and Rodriguez-Garay, B. (1998). Somatic embryogenesis in some cactus and agave species. *J. Professional Assoc. for Cactus Dev.* **3** : 15-26.

Santacruz-Ruvalcaba, F., Gutierrez-Pulido, H. and Rodriguez-Garay, B. (1999). Efficient *in vitro* propagation of *Agave parrasana* Berger. *Plant Cell, Tissue and Organ Culture* **56** : 163-67.

Santamaria, J. M., Herrera, J. L. and Robert, M. L. (1995). Stomatal physiology of a micropropagated CAM plant, *Agave tequilana* (Weber). *Plant Growth Regulation* **16** : 211-14.

Sharma, O. P. and Khanna, P. (1980). Studies on steroidal sapogenins from tissue cultures of *Agave wightii*. *J. Natural Products* **43** : 459-62.

Sita, G. L. and Vaidyananthan, C. S. (1980). Tissue culture of medicinal plants : Sandalwood, Eucalyptus, Mucuna and Agave. 4th Asian Symposium on Medicinal Plants and Spices : ts Mahidol Univ., Bangkok (Thailand). Faculty of Science. Dept. of Chemistry. Publisher : Bangkok (Thailand). p. 106.

Tapati, D. (1992) Micropropagation of *Agave sisalana. Plant Cell, Tissue and Organ Culture* **31** : 253-55.

Vaneck, J. M. and Kitto, S. L. (1992). Regeneration of pippermint and orangemint from leaf disks. *Plant Cell, Tissue and Organ Culture* **30** : 41-49.

Vargas, T. E. and Garcia, E. (1996). Clonal mass propagation of *Agave sisalana* (Sisal). Propagacion clonal masiva de *Agave sisalana* (Sisal). *Acta Biologica Venezuelica* **16** : 39-44.

Vazquez-Flota, F. A. and Loyola-Vargas, V. M. (2003). *In vitro* plant cell culture as the basis for the development of a research institute in Mexico : Centro de Investigacion Cientifica de Yucatan. *In Vitro Cellular and Developmental Biology Plant* **39** : 250-58.

Vinsencia, S. A., Iindrayanto, G. and Loekito, A. S. (1998). Simultaneous effect of calcium, magnesium, copper and cobalt ions on sapogenin steroids content in callus cultures of *Agave amaniensis. Plant Cell, Tissue and Organ Culture* **55** : 103-08.

Wen, H., Wagner, J. and Larcher, W. (1997). Growth and nocturnal acid accumulation during early ontogeny of *Agave attenuata* grown in nutrient solution and *in vitro* culture. *Biologia Plant.* **39** : 1-11.