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CLONING PROTOCOL OF Aloe vera AS A STUDY-CASE FOR "TAILOR-MADE" BIOTECHNOLOGY TO SMALL FARMERS

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Abstract

Aloe vera has been used worldwide both for pharmaceutical, food, and cosmetic industries due to the plethora of biological activities of some of its metabolites. A study case is reported focusing on the development of a cloning protocol of A. vera to provide propagation material with superior quality to the private sector in southern Brazil, i.e., A. vera juice industry. Such biotechnological approach afforded ca. 4,300 plantlets from 20 explants, over a 6-month period, overcoming the drawback of the lack of propagation material. Typically, the results have led to the increase of the cultured area and juice production of that species coming true the goal of the partnership between the public and private sectors herein involved. The transference of the resulting technology was successfully performed to the company and a patent covering the biotechnological process has been recently requested to official organisms on behalf of the partners.

Keywords:
Biotechnological process innovation, Aloe vera, technology transference, university-private sector cooperation.

Introduction

Historical evidences point to Africa as origin center of Aloe vera Müller, a species already cultured by Egyptian and Mediterranean people thousands of years B.C. Phytochemical studies have shown the presence of several bioactive compounds originated from Aloe vera's primary and secondary metabolism pathways, which have been widely used in formulations (gels and juices, e.g.) in commercial scale all over the world. As example of those compounds is worth mentioning enzymes (lipases, bradykinases and proteases), mono e polysaccharides (glucomannans), amino acids, vitamins (A, B12, C and D), anthraquinones (aloine and emodine), saponins, salicilic acid, lignin and steroids (lupeol, campesterol, and sitosterol). A number of biological activities have been claimed to those substances, such as antiseptic [saponins and anthraquinones], anti-tumoral [mucopolysaccharides], anti-inflammatory [steroids and salicilic acid], antioxidant [vitamins], and immune-regulator [glucomannans] effects (Weiner & Weiner, 1994). Due to the wide spectrum of application in human health, the products containing A. vera's compounds have showed a strong demand in both national and international markets. Accordingly, an increasing search for A. vera biomass of high quality can be envisages in local markets. However, since the biomass availability in Brazil is restricted, the expansion of this activity finds its "bottleneck". In order to overcome this constraint, high yields should be a goal to be pursued in conventional cultures systems of Aloe vera, as well as the enlargement of the cultivated area. For that, a basic question should be addressed, i.e., the utilization of propagation material of high genetic and sanitary quality and its commercial availability.

Aloe vera is usually propagated through lateral shoots which the donor-plant produces mostly over the growth season. The number of lateral shoots/donor plant is low and also variable over time, becoming difficult to plan in a rational basis a production system in commercial scale for obtainment of plant propagation material. In general, 3 to 4 lateral shoots/donor plant/year are found in conventional production systems, what is a time-consuming and tedious task, as well as an obviously meaningful drawback for purpose of large scale production of plant propagation material. In fact, this can be more easily visualized as one aims at obtaining propagation material to, for instance, 1 hectare, having a plant density of 12,000 to 16,000 plants/ha, as usually found in commercial production systems. Additionally, a higher incidence of diseases is expected to happen due to lesions caused to donor-plant after withdrawing of the lateral shoots.

This picture points to a significant constraint concerning the availability of propagation material from A. vera with superior genetic and sanity qualities, so that the enlargement of the cultured area is a difficult task. Accordingly, the production of cosmetics, foods and pharmaceuticals containing A. vera has experienced a slow increase due to limited offering of raw material with high quality.

As a feasible solution for the production of higher quality propagation material of A. vera in large scale, biotechnological techniques have been thought of. Such approach has allowed the in vitro cloning and multiplication (by means of development of apical meristems as explants cultured in appropriate basal medium, for instance) in commercial scale of several plant species, as well as of superior germoplasms, being especially important for that recalcitrant species to conventional propagation techniques (cuttings and grafting, e.g.).

Over the last years, a micropropagation protocol for genotypes of A. vera was developed by Plant Morphogenesis and Biochemistry Laboratory (University Federal of Santa Catarina PMBL/UFSC) upon request and on behalf of small farmers, hereafter named Naturama Sucos Integrais do Brasil Ltd. Naturama is a small scale industry of A. vera juice in southern Brazil (Paulo Lopes county, Santa Catarina State), with an increasing participation in the local market. This partnership found financial support with the Brazilian Service for Support to Micro and Small Enterprises (SEBRAE/SC), a private institution that aims to promote the development of small producers in both rural and urban areas. Based in such a study case, some technical subjects are presented and discussed afterwards, which allowed in a consistent manner to increase the availability of A. vera's propagation material to the partner company, with the consequent enlargement of the cultured area of that medicinal plant. Furthermore, it was possible to realize a significant decrease in the occurrence of diseases in the A. vera population at field conditions just by replacing of the plant propagation material obtained through conventional methods (lateral shoots) for the micropropagated one. The transference of the developed technology was successfully performed to the company and a patent for purpose of registration of the intellectual property has been recently requested to official organisms. To the best of our knowledge, no previous report has been published on the transference of a biotechnological product to the private sector, i.e., A. vera juice industry, as herein described in Brazil.

Method

The protocol for micropropagation of Aloe vera was previously reported (Araújo et al. 2002). Briefly, the protocol was developed using apical meristems as explants cultured in basal medium of Murashige and Skoog (1962) – MS medium, supplemented with sucrose (3g%), agar (0.7%) and the growth regulators naphthalene acetic acid (4.03μM) and 6-benzylaminopurine (6.67μM). The in vitro multiplication rate (number of lateral shoots/explant) of sixteen A. vera genotypes furnished by small farmers and originated from Florianopolis and Araquari counties (Santa Catarina State), Pato Branco, Rolandia, and Colombo counties (Parana State) and also from Paraguay was assayed, over a 8 months period. During the experimental time the cultures were kept in growth chamber at 24±1°C, 85% relative humidity of the air, 14-hour-photoperiod, and 100 ± 5μM photons.m-2.s- (as active photosynthetic radiation), with a subculture interval of 35 days.

After the in vitro growth stage, plantlets taller than 3cm were transferred to trays containing commercial substrate (Plantmax®) and kept at growth chamber under intermittent mist, for seven days. Further, the plantlets were transferred to plastic containers (~ 200mL internal volume) containing a mixture of soil and commercial substrate (Plantmax®, 1:1) and grown in greenhouse with periodic irrigation (3 days) and leaf manuring (15 and 21 day intervals, respectively for summer and winter seasons). After ca. two months growing under greenhouse conditions, the plantlets were directly transferred to field conditions, at Paulo Lopes county, and monitored over a 18-month period as to their agronomic treats (survival rate, phenological state evolution, resistance to pest and disease, and yield) and somaclonal variation occurrence (visual analysis of the phenotypes).

Results

The data obtained for in vitro multiplication of the lateral shoots showed that experimental conditions induced a mean rate of new lateral shoots/explant of ca. 1:2. However, this value was strongly dependent of the genotype, i.e., the donor-plant, since a multiplication rate of 1:8 was obtained as well. These findings suggest the existence of distinct morphogenetic potentials among the genotypes in study. More specifically the genotype originated from Paraguay showed the highest multiplication rate, with values ranging from 1:6 to 1:8 and was further selected to massal multiplication. Similar results have been reported for the micropropagation of other medicinal species with high commercial value as Coleus forskohlii, Camptotheca acuminate, and Valeriana edulis sp. procera, for instance (Pletsch, 1998).

The protocol used for multiplication of lateral shoots was also able in inducing the rhizogenesis, but in a time-dependent way. Thus, in all the cultures older than 35 days were found platelets in different rooting stages, so that the subculture interval was changed to forty-five days. The data suggest that rhizogenesis induction is correlated, in any extension, to depletion of nutrients in culture medium. In fact, this methodology has allowed the obtainment of whole plants, i.e., aerial and rooting systems, without the need of transferring of the plantlets to rooting culture medium. Furthermore, in vitro rooting strongly influenced the survival rate of the plantlets over the acclimatization step. Notably, the rooted plantlets reached survival values of about 80% to 95%, as lower value was found to non-rooted plantlets (about 30% of survival). Interestingly, just after the transplant to commercial substrate, all the A. vera plantlets presented pronounced root degeneration, followed by the emission of new primary and secondary roots. In average, in vitro growth stage of the plantlets lasted for about 45-50 days, followed by an acclimatization step no longer than 2 months (spring and summer seasons) or 3 months (autumn and winter seasons) without temperature control in the greenhouse.

The occurrence of individuals evidencing any phenotypic variation both in vitro or ex vitro was not detected over 18 months after transplantation to the field, except for two young plants which showed albinism and variegation in leaf tissue during the acclimatization step. Such findings probably results from somaclonal variation, a common phenomenon with aleatory occurrence in plant cell and tissue cultures that leads to the arising of variant phenotypes with genetic or epigenetic origin. Up to date, the results indicate that somaclonal variation is not a meaningful phenomenon as the protocol herein reported is used for the micropropagation of A. vera genotypes.

Discussion

The described protocol seems to be technologically suitable for clonal multiplication of A. vera in large scale, affording propagation material with high genetic and sanitary quality. Over a 6-month-period, about 4,300 plantlets were obtained in vitro from 20 initial explants, clearly revealing the potential of such a technological strategy to overcome the lack of propagation material of superior quality. Preliminary estimation point to a cost of ca. US$ 1, 00/plantlet (December - 2006), but it is assumed that the methodology herein described might be optimized in any extension, leading to a reduction in that value. However, comparatively to the conventional propagation system used to A. vera the results herein shown are significantly superior. In fact, it was possible to produce a large number of propagation materials, which are presently

in cultivation by the partner company, in a less expensive and time-consuming way. Additionally, this biotechnological approach has allowed the increase of the economic activity of Naturama Sucos Integrais do Brasil Ltd. as technological improvements in its A. vera juice production system are claimed and are matter of currently concerns. In a near future, it is possible to envisage the complete autonomy of that company property regarding both the production of A. vera raw material and its transformation to juices and other derivative products (i.e., nutraceuticals).

In a more wide sense, the adoption of this biotechnological strategy could, in any extension and taking into account the specific conditions of each enterprise, be applied as a model ("tailor made") to increase the incoming of Aloe-related companies, as a short-term goal, since it seems to be both technological and economical feasible.

References

Weiner, M., & Weiner, J. A. (1994). Herbs that heal. Mill Valley: Quantum Books.

Araújo, P. S., Silva, J. M. O., Neckel, C., Ianssen, C., Oltramari, A. C., Passos, R., Tiepo, E., Bach, D. B., & Maraschin, M. (2002). Micropropagação de babosa (Aloe vera) – Biotecnologia de Plantas Medicinais. Biotecnologia Ciência e Desenvolvimento, 25, 54-57. Available at www.biotecnologia.com.br

Murashige, T., & Skoog, D. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiology Plantarum, 15, 473-497.

Pletsch, M. (1998). Compostos naturais biologicamente ativos: a aplicação da biotecnologia à produção de compostos naturais biologicamente ativos. Biotecnologia Ciência e Desenvolvimento, 4, 12-15.