Tissue Culture Storage of Brazilian Medicinal Plants Germplasm

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Abstact

Modern agriculture depends on a coordinated system to evaluate, introduce, distribute and maintain germplasm, since plant germplasm is the base for a productive agriculture. It is estimated that 90% of the germplasm collections are stored as seeds, of which 40% are cereals. Medicinal plants are particularly difficult to store as seeds, due to the lack of knowledge of their reproduction biology and seed behavior. Besides, there are a large number of important tropical and subtropical medicinal plants species which produce recalcitrant seeds that quickly lose viability and do not survive desiccation, hence conventional seed storage strategies are not possible. There is also a number of other import species that are sterile or do not easily produce seeds, or seeds are highly heterozygous and clonal propagation is preferred to conserve elite genotypes. Although field genebanks provide easy access to conserved material for use, they have a risk of destruction by natural calamities, pest and diseases. For this reason, safety duplicates of the living collections are established using alternate strategies of conservation and it is in this area that biotechnology contributed significantly by providing complementary in vitro conservation options through tissue culture techniques. In vitro conservation also offers other distinct advantages. For example, the material can be maintained in a pathogen-tested state, thereby facilitating safer distribution and germplasm exchange. Further, the cultures are not subject to environmental disturbance. The National Genetic Resources and Biotechnology Research Center (CENARGEN), was created by the Brazilian Organization for Agricultural Research (EMBRAPA), an institution linked to the Ministry of Agriculture, in order to coordinate and organize all activities related to genetic resources in Brazil, including genetic resources of medicinal plants. The in vitro collection of medicinal plants of CENARGEN is constituted by at least 395 accessions of five genus: *Mentha* (74), *Lippia* (47), *Pfaffia* (15), *Stevia* (16) and *Cochlospermum* (10). Experiments have shown that in vitro shoot cultures stored at temperature in the range of 18-20°C on half strength Murashige and Skoog medium nutrient with 2% sucrose reduce the plant growth and significantly extend the subculture intervals of accessions to fresh medium. We conclude that this in vitro conservation system can be greatly useful for conservation and exchange of genetic resources of these medicinal plants.

INTRODUCTION

The germplasm conservation technology has as a basic principle to preserve the maximum gene pool of a determined species, considering their actual and potential use for the future (Razdan, 2003). Conservation of threatened germplasm includes seed banks, field preservation, tissue culture and cryopreservation. Seed storage is considered the ideal method; seeds considered orthodox can be dried and are able to be preserved at sub-zero temperatures (-20°C), while recalcitrant seeds, including most tropical species, lose their seed viability when subjected to the same conditions. Maintenance of the germplasm in field collections is costly, requires large areas, and can be affected by adverse environmental conditions. Tissue culture or cryopreservation techniques can be also considered in many cases.

There are basically two approaches for germplasm conservation: ex situ or in situ. In an ex situ procedure, the germplasm is collected from fields, markets, small farms, and other sites, in form of seeds, cuttings, underground systems, and sprouts. The collected samples should represent the original population with passport data and herbarium vouchers. In a long term, mutation can take place over the years in a cold chamber or in vitro conservation. In contrast, in situ conservation maintains population in its preserved natural area, allowing the evolutionary process to continue, although genetic reserves are subject to anthropogenic action and environmental effects (Vieira, 1999).

In vitro conservation strategy is mainly used when the species produces recalcitrant seeds or do not produce viable seeds, and has a typically vegetative propagation. Besides that, there are intermediate seeds that can tolerate desiccation, but not subzero conditions (Engelmann, 2004; Panis and Lambardi, 2006).

A simple introduction of a plant material in vitro can constitute a form of germplasm conservation. However, due to the difficulty of maintaining a large number of samples, coupled to the periodic replications needed (4-6 weeks), it is almost unviable to use a single in vitro plant material for this purpose. Associated to these factors, the loss of plant material due to human mistakes or microbiological contaminations during continuous subculture protocols can be added. Besides, limitations associated to the genetic instability generated, either by replications or use of growth hormones, can be problematic (Withers et al., 1990).

As an alternative, modifications into the in vitro culture growth pattern have been used for conserving plant germplasm, considering the species and facilities available. In this context, germplasm conservation can be made using two systems: slow growth and cryopreservation system at ultra low temperatures (-196ºC) (Pérez, 1997).

The in vitro collection of medicinal and aromatic plants of Embrapa Genetic Resources and Biotechnology is constituted by accessions of five genera: *Mentha*, *Lippia*, *Pfaffia*, *Stevia* and *Cochlospermum*. This paper presents the strategies used for each medicinal and aromatic species at the in vitro germplasm collection.

MATERIALS AND METHODS

In vitro collections of *Mentha*, *Lippia*, *Pfaffia*, *Stevia* and *Cochlospermum* germplasm have been maintained at the Tissue Culture and Germplasm Conservation laboratory of Embrapa Genetic Resources and Biotechnology, Brazil. Usually, accessions are kept under minimum growth conditions, using a specific culture medium, with salt and vitamins of MS media modified (Murashige and Skoog, 1962). Besides the use of culture media modified, the majority of materials have been conserved at 20°C temperature, although preliminary results indicated that *Mentha* can be stored at 10°C.

Accessions of *Mentha*, *Lippia*, *Pfaffia*, *Stevia* and *Cochlospermum* in vitro collections are maintained in microtubes (25 × 150 mm) with 10 ml of MS medium and preserved at 20°C in a growth chamber, with light intensity of 30 $\mu mol.m^{-2}\ s^{-1}$ and 12h of photoperiod.

Each accession is maintained in six replications, formed by a single bud. Regeneration every six month is performed.

RESULTS AND DISCUSSION

Table 1 shows the collections of medicinal and aromatic plants maintained in vitro collection at Embrapa Genetic Resources and Biotechnology. A total of 162 accessions are maintained: *Cochlospermum regium* (10), *Stevia* (16), *Pfaffia* (15), *Lippia* (47) and *Mentha* (74). The best results for in vitro conservation of *C. regium* has been achieved with maintenance of explants at 20°C temperature and culture medium ½WPM (Camillo et al., 2009). This medium is indicated for *C. regium* conservation since it has 45% of total ionic concentration of MS medium and lower concentrations of nitrate (MS 40 μM; WPM 9.7 μM) and ammonia (MS 20 μM; WPM 4.9 μM). Also, this medium presented a lower concentration of total nitrogen (14.7 μM) when compared to MS (60.00 μM), promoting a slower growth of explants.

For *Stevia* and *Lippia*, all 63 accessions of these species are kept under 20°C temperature on a culture medium composed by salts and vitamins of MS modified media, reduced to half of the salt concentration.

The *Pfaffia glomerata* collection showed two forms of conservation: a) at MS modified medium under 20°C temperature and b) under MS modified plus mannitol. The addition of mannitol into the culture medium (14.6 and 29.2 mM) has showed to be a good protocol for this species' in vitro conservation, increasing time between sub-culture procedures.

In *Mentha* species, although this collection has been maintained under 20ºC, previous work has demonstrated that this species can be kept in vitro at 10ºC, without any problem. This temperature can avoid more frequent replications when compared to 20ºC or 25ºC.

CONCLUSIONS

The major challenge in these in vitro collections is to promote the chemical, agronomical, and genetic characterization of all these accessions in order to identify materials for use in breeding programs, including nematodes and fungi resistance, and high content of active substances. The improvement of this species cultivation will reduce the exploitation from wild population. Also, in vitro conservation can safely increase the germplasm interchange among research groups.

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Tables

Table 1. Germplasm collection of *Mentha*, *Lippia*, *Pfaffia*, *Stevia* and *Cochlospermum* maintained at Embrapa Genetic Resources and Biotechnology on in vitro conditions.

Product	Genus	Species	Number of accessions	Conservation temperature (°C)	Tissue culture medium
Algodão do Cerrado	*Cachlospermum*	*regium*	10	20	WPM*modified
Estévia	*Stevia*	*rebaudiana*	16	20	½MS modified
Ginseng brasileiro	*Pfaffia*	*glomerata*	15	20	MS** modified; MS modified + manitol
Erva Cidreira	*Lippia*	*alba*	44	20	MS modified
		rotundifolia	1		
		lacunosa	1		
		filifolia	1		
Menta	*Mentha*	*aquatica*	4	10-20	½ MS modified
		suaveolens	6		
		piperita	11		
		spicata	15		
		longiflora	4		
		arvensis	5		
		silvestris	1		
		pulegium	1		
		canadensis	3		
		gracilis	3		
		rotundifolia	1		
		sp.	16		

* Wood Plant Medium (1980); ** Murashige & Skoog medium (1962).