Fruit, Vegetable and Cereal Science and Biotechnology ©2007 Global Science Books

Minimal Growth *in Vitro* Culture for Preservation of Plant Species

Suriyan Cha-um* • **Chalermpol Kirdmanee**

National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Thailand Science Park, Paholyothin Rd, Klong Luang, Pathumthani 12120, Thailand

Corresponding author: * **suriyanc@biotec.or.th**

ABSTRACT

Plant genetic resources for sustainable usage have been investigated as a basis for breeding programs for crop improvement and the advance of target gene(s) in genetic engineering. *Ex situ* conservation is used to preserve plant species manually in addition to *in situ* techniques. Orthodox seeds of temperate and subtropical species are mainly conserved as a genebank. However, methods of conservation of seedless and heterozygous orthodox tropical plant species and those with recalcitrant seeds are still limited. *In vitro* or live vegetative preservation is the preferred way to conserve elite species. There are many approaches to this, including short-, medium- and long-term preservation. Minimal growth *in vitro* culture or medium-term preservation has been widely exploited as a source of disease-free plants, which are promptly available for international material exchange. This technique is well established and is applied to a wide range of genetic conservation measures with high recovery growth and maintenance of genetic stability when compared to cryopreservation or long-term storage, identified by RAPD and AFLP genetic variation assay. Recently developed techniques and the application of minimal growth preservation will be described in this review. There are many techniques, which can be used to control the *in vitro* physical and chemical environments, including low temperature, low light intensity, short-photoperiod, high osmotic adjustment, low nutrient concentration and plant growth retardant supplementation for *in vitro* germplasm. In addition, the combination of both physical and chemical factors is a progressive channel, which can be used to develop general practices for medium-term preservation of tropical plant species.

Keywords: *ex situ* germplasm, genetic stability, low ambient temperature, medium-term preservation, plant growth retardants, slow growth

Abbreviations: 2,4-D, 2,4-dichlorophenoxy acetic acid; **ABA**, abscisic acid; **AFLP**, amplified fragment length polymorphism; **BAP**, N^6-benzyl aminopurine; **Boxus,** Boxus and Terzi media (1987); **DCR**, Gupta and Durzan media (1985); **GD**, Gresshoff and Doy media (1972); **LT**, low temperature; **MSAP**, methylation sensitive amplified polymorphism; **MS**, Murashige and Skoog medium (1962); **MT**, Murashige and Tucker medium (1969); **MW**, Morel and Wetmore medium (1951); **NAA**, α-naphthalene acetic acid; **OM**, Rugini medium (1984); **PPF**, photosynthetic photon flux; **RAPD**, random-amplified polymorphic DNA; **RFLP**, restriction fragment length polymorphism; **ROS**, reactive oxygen species; **SH**, Schenk and Hildebrandt medium (1972); **SM**, Smith and Murashige medium (1970); **STS**, silver thiosulfate; **VNTR**, variable number of tandem repeats; **WP**, Woody plant media (1981)

CONTENTS

INTRODUCTION

Plant conservation is one of the most attractive topics to concern and intensively manage on plant genetic resource for sustainable usages. Plant genetic resource for human life is a critical topic because the world's population is estimated to rapidly increase from 6 billion in 2000 to 8 billion in 2025 (Rao 2004; Khush 2005). An increasing population, modern agriculture and industry worldwide not only damage plant habitats but also directly reduce plant genetic diversity (Heywood and Iriondo 2003; Olorode 2004). To date, plant genetic resources have been continuously destroyed at an unpredicted rate through deforestation, human activity, modern agricultural practices, and modern variety introduction (Rao and Hodgkin 2002; Uyoh *et al*. 2003). It is an urgent priority to conserve native natural resources, protect-

Received: 8 December, 2006. Accepted: 4 February, 2007.

Invited Review

ted areas, forest reserves and national parks, namely through *ex situ* conservation and to establish artificial habitat preservation, botanic gardens, and field genebanks, and to use *in vitro* methods and cryopreservation for seed storage and *in situ* conservation (Rao and Hodgkin 2002; Uyoh *et al*. 2003; Rao 2004; Schroth *et al*. 2004). However, the main barrier of *in situ* conservation is the requirement of a large amount of area, high cost of operation, complicated management and risks to damage by biotic and abiotic environments. An *ex situ* method is thus an efficient conservation of plant genetic resources over *in situ* or natural habitats. The most convenient technique for long-term *ex situ* conservation is seed storage or seed genebanks. Normally, plants with orthodox seed plants, desiccation and low-temperature (LT) storage tolerance, are successfully preserved in both medium- and long-term conservation. Alternatively, tropical and subtropical plant species are mostly defined as seedless and recalcitrant seeds, which are sensitive to desication and LT and quickly lose viability. *Ex situ* live-gene banks or *in vitro* preservation is an effective method to slow growth storage (Engelmann and Engels 2002; Theilade and Petri 2003; Uyoh *et al*. 2003; Rao 2004). *In vitro* culture or plant tissue culture has been reported as an effective method to conserve many recalcitrant species, especially tropical plants. *In vitro* conservation is a good way to store disease-free elite species, to control the growth and development, and to exchange genetic resources easily and to simply manage storage space area, labor and maintenance costs (Withers 1991; Crouch *et al*. 1998; Engelmann 1998; Theilade and Petri 2003). There are three categories of *in vitro* conservation: short-term (*in vitro* culture), medium-term (slow growth or minimal growth) and long-term (cryopreservation). In the short and medium terms, various techniques have been investigated for slow growth and reduced interval-time subculturing, leading to genetic stability. Normally, somaclonal variation or genetic instability of *in vitro* plants depends on the plant species, culture media composition and subculturing times (Modgil *et al*. 2005; Bairu *et al*. 2006; Guo *et al*. 2006; Peredo *et al*. 2006; Thomas *et al*. 2006). Alternatively, cryopreservation has been developed as a long-term genetic conservation at a very low temperature (–196°C) with safe and cost-effective storage. Tropical plant species are still, however limited because these species are very sensitive to LT (<10°C) or to chilling injury (Angeli *et al*. 2003; Li *et al*. 2004a; Gusta *et al*. 2005), resulting in a low survival percentage or death after storage. Additionally, the genetic instability of cryopreserved plants is one of the greatest obstacles to be aware of (Theilade and Petri 2003; Rao 2004; Shibli *et al*. 2006). In this review, the critical factors affecting medium-term plant conservation of genetic resources are intensively described and the genetic stability of *in vitro* storage is mentioned.

SLOW OR MINIMAL GROWTH PRESERVATION

Disease-free production using *in vitro* meristem culture is a progressive channel to eliminate bacteria, fungi and viruses from host plants. There are many plants species that have escaped from diseases by meristem culture such as garlic, shallot (Walkey *et al*. 1987), lily turf (Strandberg 1993), ginger (Sharma and Singh 1997), sweet potato (Kuo *et al*. 1985), sugarcane (Homhual *et al*. 1999; Cha-um *et al*. 2006b) and banana (Ko *et al*. 1991; Cha-um *et al*. 2006a). The disease-free plants – especially those are virus-free and are derived from meristem culture – have been widely utilized as genetic resources. Minimal growth preservation is one of the most common practices for disease-free living genebanks in terms of small spaced areas and required labor input for subculturing and viability testing. A number of subculture is the main obstacle to the genetic stability inducing somaclonal variation in aseptic conditions. The growth reduction of *in vitro* plants for slow-growing, sequential subcultures has been developed using both physical and chemical factors. It has been successfully applied to preserve many recalcitrant plants, especially tropical species (Uyoh *et al*. 2003; Rao 2004; Keller *et al*. 2006; Shibli *et al*. 2006).

Physical factors

Physiological environments, including temperature, light and culture vessel are the most important factors to strongly delay plant growth and develop *in vitro* storage. The first factor is LT, which is a fruitful environmental condition for medium-term preservation in many plant species. A LT (0-20°C) affects higher plants allowing them to establish well a broad spectrum of chilling injuries or ice crystal damage (Brush *et al*. 1994; Concellon *et al*. 2007) and chilling-induced water deficit (Guy 1990; Buitink and Leprince 2004; Sato *et al*. 2004), especially warm ecotype species (Xin and Browse 2000; Smallwood and Bowles 2002). The plant plasma membrane, ultrastructure macromolecules and intracellular pH are sensitively damaged by cold-induced injuries (Shattuck *et al*. 1991; Nantes *et al*. 1999; Yoshida *et al*. 1999; Kratsch and Wise 2000; Neefs *et al*. 2000; Chen and Li 2002; Lee *et al*. 2002; Aroca *et al*. 2005), leading to electrolyte leakage, pigment degradation, diminished photosynthetic activity and a reduction in photosynthesis (Nilsen and Orcutt 1996; Du *et al*. 1999; de Oliveira *et al*. 2002; Campos *et al*. 2003; Nicotra *et al*. 2003; Mahajan and Tuteja 2005; Pietrini *et al*. 2005; Renaut *et al*. 2005; Kalberer *et al*. 2006; Renaut *et al*. 2006; Uemura *et al*. 2006; Wang *et al*. 2006). Alternatively, reactive oxygen species (ROS) generated by cold stress have been reported as secondary injury, damaging both membranes (causing leakage) and photosynthetic systems (Prasad 1996; Gechev *et al*. 2003; Janda *et al*. 2003; Kornyeyev *et al*. 2003; Tambussi *et al*. 2004; Nayyar *et al*. 2005b; Posmyk *et al*. 2005; Chen *et al*. 2006; Kalberer *et al*. 2006; Hola *et al*. 2007). The biochemical and physiological changes of cold-stressed plants are the major causes of toxic symptoms in germination, vegetative and reproductive stages prior to cell death (Lavee *et al*. 1985; Ranwala and Miller 2000; Allen and Ort 2001; Birch *et al*. 2003; Loik and Redar 2003; Clarke and Siddique 2004; Ercoli *et al*. 2004; Nayyar *et al*. 2005a; Bois *et al*. 2006; Farrell *et al*. 2006; Niimi *et al*. 2006; Ensminger *et al*. 2006). Nevertheless, there are many investigations that have been able to harden tropical plant species by a temperature drop or ABA treatment before exposure to chilling temperature, namely cold acclimation (Sysoeva *et al*. 1999; Xin and Browse 2000; Lindqvist 2001; Sysoeva *et al*. 2005; Zhou *et al*. 2005). It is an effective approach to improve the plant defense mechanisms in cold stress conditions, i.e. the production of LT-defensive proteins (dehydrin, antifreeze and cryoprotectin proteins) (Rinne *et al*. 1998; Pearce 1999; Ohno *et al*. 2003; Sror *et al*. 2003; Buitink and Leprince 2004; Griffith and Yaish 2004; Dhanaraj *et al*. 2004, 2005), a membrane system (Murai and Yoshida 1998; Aroca *et al*. 2003; Uemura and Steponkus 2003; Lee *et al*. 2005; Bakht *et al*. 2006; Bohn *et al*. 2007), improved water relations (Aroca *et al*. 2003; Bloom *et al*. 2004; Kalberer *et al*. 2006), compatible solute accumulation [carbohydrates (Vandenbussche *et al*. 1999; Tabaei-Aghdaei *et al*. 2003; Kerepesi *et al*. 2004; Zuther *et al*. 2004; Livingston *et al*. 2005; Nagao *et al*. 2005; Hekneby *et al*. 2006; Livingston *et al*. 2006; Malone *et al*. 2006; Priscila *et al*. 2006; Miao *et al*. 2007), proline (Nayyar *et al*. 2005a; Chen *et al*. 2006; Hekneby *et al*. 2006), polyamines (Hummel *et al*. 2004; Imai *et al*. 2004; Sahin-Cavik and Moore 2006; Akiyama and Jin 2007) and betaine (Alia *et al*. 1998; Allard *et al*. 1998; Sakamoto and Murata 2002; Park *et al*. 2004; Quan *et al*. 2004)] and an antioxidant system (Gechev *et al*. 2003; Kornyeyev *et al*. 2003; Pennycooke *et al*. 2005; Posmyk *et al*. 2005; Chen *et al*. 2006; Kalberer *et al*. 2006; Tommasi *et al*. 2006). The LT-acclimated plants are highly adapted to chilling temperature in terms of CO_2-assimilation, photochemistry and photosynthesis limitation (Boese *et al*. 1997; Savitch *et al*. 2002; van Heerden *et al*. 2004; Syros *et al*. 2005; Hu *et al*. 2006; Zhou *et al*. 2006), resulting in slow leaf appearance, leaf

area decline and overall growth reduction (Birch *et al*. 2003; Ercoli *et al*. 2004). There are many genes in both upstream (transcription factors) and downstream (cold-responsive genes) expressions associated with those plant metabolites by cold temperature regulation (Ndong *et al*. 1997; Thomashow 1999; Browse and Xin 2001; Sung *et al*. 2003; Gusta *et al*. 2005; Chinnusamy *et al*. 2006).

This is an excellent tool to preserve living plants at a LT for sustainable use. Secondly, light – both intensity and photoperiod – is an alternative factor to control plant growth and development. A higher pigment concentration, thylakoid frequency and grana stacks in the chloroplast with low CO_2 assimilation on plants grown under a low light and short-photoperiod flux have been reported in pumpkin, big-leafed periwinkle (Logan *et al*. 1998), mahogany, tonka bean (Gonçalves *et al*. 2001) and soybean (Lichtenthaler and Burkart 1999), leading to a 7-16 fold decrease in photochemistry of photosystem II (Logan *et al*. 1998), 2-3 fold lower net-photosynthetic CO_2 assimilation rate (Lichtenthaler and Burkart 1999) and 1.5-2 fold shorter stems when compared to those grown under high light condition (Grace and Logan 1996; Nilsen and Orcutt 1996; de la Vina *et al*. 2001). In addition to this, the storage atmosphere such as ethylene, oxygen (O_2), carbon dioxide (CO_2) and relative humidity (%RH) is a state topic (Dorion *et al*. 1994; Legnani *et al*. 2004). Ethylene gas, an endogenous phytohormone plays a central role in physiological processses i.e. chlorophyll degradation (Gong and Mattheis 2003), stomatal closure (Tanaka *et al*. 2005; Desikan *et al*. 2006) and net-photosynthesis reduction (Khan 2005) and plant morphogenesis (Gonzalez *et al*. 1997; Fal *et al*. 1999; Arigita *et al*. 2003; Kepczynski *et al*. 2006) as well as in signal transduction in response to environmental stimuli (Alonso and Stepanova 2004; Chang and Bleecker 2004; Guo and Ecker 2004; Klee 2004; Chen *et al*. 2005; Pierik *et al*. 2006). On the other hand, without aeration ethylene accumulated in *Lagerstroemia thorelli* (1.2 $\mu L.L^{-1}$) (Zobayed 2000) and papaya (0.15 ppm) (Lai *et al*. 1998) cultures in closed vessels and had a negative effect on plant growth and development while also inducing senescence. Low ethylene concentration (0.05-0.1 $\mu L.L^{-1}$) in the culture vessels is an alternative way to retard leaf emergence and plant height (Lai *et al*. 1998; Zobayed 2000). There are several approaches to reduce ethylene concentration in culture vessels i.e. air ventilation (Lai *et al*. 1998; Zobayed 2000) and the application of ethylene inhibitors, leading to typical, normal growth and development in cauliflower (Zobayed *et al*. 1999), potato (Sarkar *et al*. 2002), *Hacncornia speciosa* (Pereira-Netto 2001) and cucumber (Mohiuddin *et al*. 1997). Furthermore, a small culture vessel limits both the root zone and the aeration part of *in vitro* culture. *In vitro* elongation, proliferation and growth of plantlets develop slowly in the small closed vessel (Jackson 2003; Martin and Pradeep 2003; Islam *et al*. 2005; Keller *et al*. 2006).

Low temperature storage

Plant evolution in a cool climate has been identified to geographical zones, and is related to adaptive mechanisms i.e. alpine plant species (sub-zero temperatures), temperate species (non-freezing temperatures), subtropical- and tropical-species (low temperature sensitive). Subtropical and tropical plant species are very sensitive to cool environments, leading to ice crystal damage or nucleation and water deficit stress prior to cell death (Nilsen and Orcutt 1996; Breton *et al*. 2000; Li *et al*. 2004a). As described above, cold acclimation is one of the most effective ways to harden plants before expose to chilling temperature. In addition, the main physical factor of *in vitro* minimal growth storage is a low temperature as the medium-term preservation tool (**Fig. 1**). It can be classified into three classes: very low (<10°C), low (10-20°C) and room temperature (>20°C). In **Table 1**, several plant species have been shown to survive in temperatures below 10°C over a 3-96 month storage period, depending on the plant species,

Fig. 1 Minimal growth preservation of living plant cells in a Plant Growth Incubator at 10°C and 10-15 µmol m^{-2} s^{-1} PPF by fluorescent lamps with 12 h photoperiod.

explants and storage organs. Coco yam and poplar *in vitro* shoots have a high potential for medium-term storage for 5-8 years at a very low temperature (Son *et al*. 1991; Bessembinder *et al*. 1993). Likewise, cork oak (shoots) and lily (bulblets) plants can be preserved *in vitro* at 2-5°C for 2 years (Bonnier and van Tuyl 1997; Romano and Martins-Loução 1999). The short-term storage (<2 years) at a very low temperature has been accomplished in many plant species i.e. apple (Negri *et al*. 2000; Hao and Deng 2003), cedar (Renau-Morata *et al*. 2006), chokecherry (Pruski *et al*. 2000), date palm (Bekheet *et al*. 2002), mint (Islam *et al*. 2003), olive (Rugini and Pesce 2006), paradise tree (Scocchi and Mroginski 2004) and potato (Lopez-Delgado *et al*. 1998; Sarkar *et al*. 1999; Pruski *et al*. 2000; Sarkar *et al*. 2001). In addition, an increase in storage temperature (10-20°C) has also been applied for *in vitro* preservation (**Table 2**). Banana (wild species), elephant's ear and miscanthus plants have been successfully preserved under aseptic conditions with 13-17°C air-temperature for 2 years (Ko *et al*., 1991; Zandvoort *et al*. 1994; Hansen and Kristiansen 1997). In contrast, banana (commercial species), cordyline, enset, mint and sugarcane *in vitro* plants cultured under low temperatures could be conserved in shorter periods than these other species (Hvoslef-Eide 1992; Taylor and Dukic 1993; de Oliveira *et al*. 2000; Negash *et al*. 2001; Islam *et al*. 2005; Cha-um *et al*. 2006a). In **Table 3**, there are several plant species that have been stored at room temperature for more than two years, including coffee (Dussert *et al*. 1997; Naidu and Sreenath 1999), lily (Renau-Morata *et al*. 2006), orchid (Martin and Pradeep 2003) and sword fern (Hvoslef-Eide 1992). Coconut, wild coffee, dierama, kokum, pineapple, sweet potato and vanilla plant species on the other hand have been preserved at room temperature for a year or less (Bertrand-Desbrunais *et al*. 1991; Jarret and Gawel 1991; Assy-Bah and Engelmann 1993; Canto *et al*. 2004; Malik *et al*. 2005; Divakaran *et al*. 2006; Madubanya *et al*. 2006). This shows that a very low temperature is suitable to exploit medium-term minimal growth preservation. Incidentally, the successful preservation at low temperatures depends on the plant species, initial explants, plant growth regulators and the osmotic agents applied.

Table 1 *In vitro* storage of plant species using very low temperature (<10°C) as a major factor in combination with other factors.

Common name	Scientific name	Explants/ Storage organ	*In vitro* storage	Size and age	Storage period (month)	Survival (%)	Ref.
Apple	*Malus pumila* cv. Moscatella	Apical/ node	1. ½ MS basal media, 3% sucrose, darkness 2. 4°C air temperature	10 mm	12	100	1
	M. pumila cv. Starkspur Red	Apical/ node	1. ½ MS basal media, 3% sucrose, darkness 2. 4°C air temperature	10 mm	18	90	1
	M. pumila cv. *Gala*	Shoot tip	1. MT basal media, 0.5 mg.L^{-1} BAP, 0.05 mg.L^{-1} NAA, 2% sucrose, 2% mannitol, 11 µmol m^{-2} s^{-1} PPF, 12 h photoperiod 2. 4°C air temperature	5 mm	12	100	2
Cedar	*Cedrus atlantica* *C. libani*	Shoot	1. ½ MS basal media with or without 10 mg.L^{-1} ABA, 70 µmol m^{-2} s^{-1} PPF, 16 h photoperiod 2. 4°C air temperature	20 mm	>6	40-50	3
Chokecherry	*Prunus virginiana*	Shoot	1. MS minimal organic media, 3% sucrose, 3 µmol m^{-2} s^{-1} PPF, 16 h photoperiod 2. 4°C air temperature	10 mm	3	-	4
Cock oak	*Quercus suber*	Shoot	1. GD basal media, 58 mM sucrose, 30 µmol m^{-2} s^{-1} PPF, 16 h photoperiod 2. 5°C air temperature	10-20 mm	24	50	5
Coco yam	*Colocasia esculenta*	Single shoot	1. MS basal media, 10 µM BAP, 1-2% mannitol, 80 µmol m^{-2} s^{-1} PPF, 16 h photoperiod 2. 9°C air temperature	5 mm	96	90	6
Date palm	*Phoenix dactylifera*	Shoot bud	1. MS basal media and darkness 2. 5°C air temperature	20 mm	12	70	7
Lily	*L. hybrid*	Scale bulblet	1. ¼ MS basal media, 9% sucrose, 10-20 µmol m^{-2} s^{-1} PPF, 16 h photoperiod 2. 2°C air temperature	2 months	28	73-90	8
Mint	*Mentha* spp.	Apical explants	1. MS basal media, 3% sucrose, 35-55 µmol m^{-2} s^{-1} PPF, 16 h photoperiod 2. 2°C air temperature	10 mm	6	71.5	9
Olive	*Olea europaea*	Shoot	1. OM media without hormones, darkness or 20 µmol m^{-2} s^{-1} PPF, 8 h photoperiod 2. 4°C air temperature	-	8	-	10
Paradise tree	*Melia azedarach*	Apical meristem	1. ¼ MS basal media, 0.5 µM BAP, darkness 2. 4°C air temperature	0.2-0.3 mm	12	67	11
Poplar	*Populus alba* × *P. grandidentata*	Shoot	1. MS basal media and 1.33 µM BAP 2. 4°C air temperature	-	60	25	12
Potato	*Solanum tuberosum*	Single node	1. MS basal media, 2% mannitol, 6-9 mgL^{-1} STS, 20 µmol m^{-2} s^{-1} PPF, 16 h photoperiod 2. 6°C air temperature	-	16	88-100	13
	S. tuberosum	Shoot	1. MS minimal organic media, 3 µmol m^{-2} s^{-1} PPF, 16 h photoperiod 2. 4°C air temperature	40 mm	3	-	4
	S. tuberosum	Single node	1. MS basal media, 4% mannitol or 100 µM acetylsalicylic acid, 11 µmol m^{-2} s^{-1} PPF, 16 h photoperiod 2. 8°C air temperature	-	8-12	>90	14
	S. tuberosum	Single node	1. MS media, 10 mM ancymidol, 6% sucrose 2. 6°C air temperature	-	16	79	15

Abbreviations: MS = Murashige and Skoog (1962); MT = Murashige and Tucker media (1969); BAP = N^6-benzyl amino purine; NAA = naphthalene acetic acid; PPF = photosynthetic photon flux; ABA = abscisic acid; GD = Gresshoff and Doy media (1972); OM = Rugini media (1984); STS = silver thiosulfate

Ref. represents the following references: 1 = Negri *et al*. (2000); 2 = Hao and Deng (2003); 3 = Renau-Morata *et al*. (2006); 4 = Pruski *et al*. (2000); 5 = Romano and Martins-Loução (1999); 6 = Bessembinder *et al*. (1993); 7 = Bekheet *et al*. (2002); 8 = Bonnier and van Tuyl (1997); 9 = Islam *et al*. (2003); 10 = Rugini and Pesce (2006); 11 = Scocchi and Mroginski (2004); 12 = Son *et al*. (1991); 13 = Sarkar *et al*. (1999); 14 = Lopez-Delgado *et al*. (1998); 15 = Sarkar *et al*. (2001).

Low light intensity and short photoperiod

There are many publications that mention how the quality and quantity of light are factors for *in vitro* slow growth conservation. Generally, *in vitro* plantlets are incubated in light conditions at 60-100 µmol m^{-2} s^{-1} PPF and 16 h photoperiod. Low light intensity and a short photoperiod flux have been elevated to delay growth and development of *in vitro* plantlets. In most case, low light intensities (3-50 µmol m^{-2} s^{-1} PPF) with 8-12 h photoperiod have been applied to slow growth storage of coffee (Bertrand-Desbrunais *et al*. 1991; Dussert *et al*. 1997), coconut (Assy-Bah and Engelmann 1993), miscanthus (Hansen and Kristiansen 1997), potato (Lopez-Delgado *et al*. 1998), chokecherry (Pruski *et al*. 2000), apple (Hao and Deng 2003), orchid (Martin and Pradeep 2003), pineapple (Canto *et al*. 2004), vanilla (Divakaran *et al*. 2006), cedar (Renau-Morata *et al*. 2006), strawberry (Nishizawa *et al*. 1997) and olive (Rugini and Pesce 2006) (**Table 1-3**). A major cost of *in vitro* cassava preservation is that of preservation space with facilities (58-60%), intensive skill labors (20-25%), somaclonal variation assay (8-10%), and management (8-10%) (Blakesley *et al*. 1996; Epperson *et al*. 1997; Virchow 1999). The reduction in maintenance facilities involving light intensity and photoperiod is an excellent channel to reduce either plant growth or light energy costs. The electric and labor costs of preservation in developing countries (0.47 US$/accession/year) is lower than those in developed countries (1.32 US$/accession/year) by 2.85-fold and is positively related to the genebank management (Koo *et al*. 2003).

Table 2 *In vitro* storage of plant species using low temperature (10-20°C) as a major factor in combination with other factors.

Common name	Scientific name	Explants/ Storage organ	*In vitro* storage	Size and age	Storage period (months)	Survival (%)	Ref.
Banana	*Musa* spp.	Shoot	1. MS basal media, 2% sucrose, 40 µmol m^{-2} s^{-1} PPF, 16 h photoperiod 2. 17°C air temperature	12 mm	15	66	1
	M. acuminata	Meristem tip	1. SM basal media, 3% ribose, 47 µmol m^{-2} s^{-1} PPF, 24 h photoperiod 2. 17°C air temperature	-	24	67	2
	Musa spp.	Shoot	1. ½ MS basal media, 136 µM paclobutrazol, 30 µmol m^{-2} s^{-1} PPF, 16 h photoperiod 2. 10°C air temperature	10 mm	12	100	3
Cordyline	*Cordyline fruticosa*	Shoot	1. Pre-storage in 9°C air temperature, 3-5 µmol m^{-2} s^{-1} PPF 2. 18°C air temperature with 15 µmol m^{-2} s^{-1} PPF	20 mm	18	-	4
Elephant's ear	*Xanthosoma* spp.	Single shoot	1. MS basal media, 3% mannitol 2. 13°C air temperature	-	24	-	5
Enset	*Ensete ventricosum*	Shoot	1. MS basal media, 3% sucrose, darkness 2. 15°C air temperature	-	15	35-56	6
Mint	*Mentha* spp.	Apical/nodal explants	1. MS basal media, 53 mL culture vessel, 35-55 µmol m^{-2} s^{-1} PPF, 16 h photoperiod 2. 20°C air temperature	10 mm	6	100	7
Miscanthus	*Miscanthus* × *ogiformis*	Single shoot	1. ½ SH basal media, 2% sucrose, 0.1 µM NAA, 20 µmol m^{-2} s^{-1} PPF, 16 h photoperiod 2. 8-16°C air temperature	3 days	6	95-100	8
Sugarcane	*Saccharum* spp.	Apical meristem	1. ½ MS basal media, 1% sucrose, 12 h photoperiod 2. 18°C air temperature	5-10 mm	12	-	9

Abbreviations: SM = Smith and Murashige medium (1970); PPF = photosynthetic photon flux; SH = Schenk and Hildebrandt medium (1972); NAA = naphthalene acetic acid; MS = Murashige and Skoog medium (1962)
Ref. represents the following reference list: 1 = de Oliveira *et al*. (2000); 2 = Ko *et al*. (1991); 3 = Cha-um *et al*. (2006a); 4 = Hvoslef-Eide (1992); 5 = Zandvoort *et al*. (1994); 6 = Negash *et al*. (2001); 7 = Islam *et al*. (2005); 8 = Hansen and Kristiansen (1997); 9 = Taylor and Dukic (1993)

Table 3 *In vitro* storage of plant species using room temperature (>20°C) as a major factor in combination with other factors.

Common name	Scientific name	Explants/ Storage organ	*In vitro* storage	Size and age	Storage period (months)	Survival (%)	Ref.
Coconut	*Cocos nucifera*	Zygotic embryo	1. MS basal media, 100 $mg.L^{-1}$ sodium ascorbate, 1.5% sucrose, 35 µmol m^{-2} s^{-1} PPF, 12 h photoperiod 2. 27°C air temperature	10 mm	12	51	1
Coffee	*Coffea liberica* *C. congensis*	Shoot tip	1. MS salts with MW vitamins, 1.3 µM BAP, 50 µmol m^{-2} s^{-1} PPF, 12 h photoperiod 2. 25°C air temperature	10-15 mm	-	85	2
	C. canephora *C. racemosa*	Shoot tip	1. MS salts with MW vitamins, 50 µmol m^{-2} s^{-1} PPF, 12 h photoperiod 2. 25°C air temperature	10-15 mm	-	85	2
	Coffea spp.	Shoot tip	1. MS salts with MW vitamins, 1.3 µM BAP, 30 µmol m^{-2} s^{-1} PPF, 12 h photoperiod 2. 27°C air temperature	10-20 mm	36	-	3
	C. arabica	Zygotic embryo	1. MS basal media and 18.9 or 37.8 µM ABA 2. 25°C air temperature	1.5-2.0 mm	24	74.2	4
Dierama	*Dierama luteoalbidum*	Corm	1. MS basal media, 5-10 $mg.L^{-1}$ paclobutrazol, 71 µmol m^{-2} s^{-1} PPF, 16 h photoperiod 2. 25°C air temperature	6 months	3	100	5
Lily	*Lilium* spp.	Bulblet	1. ¼ MS basal media, 9% sucrose, 10-20 µmol m^{-2} s^{-1} PPF, 16 h photoperiod 2. 25°C air temperature	2 months	28	71-100	6
Kokum	*Garcinia indica*	Shoot	1. ½ MS basal media, 5 µM BAP, 3% sucrose 2. 25°C air temperature	15-25 mm	10-11	95	7
Orchid	*Ipsea malabarica*	Shoot	1. ½ MS basal media without sugar, 25 µmol m^{-2} s^{-1} PPF, 16 h photoperiod 2. 25°C air temperature	-	27	100	8
Pineapple	*Ananus comosus*	Single shoot	1. MS basal media, 3% sucrose, 0.5 $mg.L^{-1}$ paclobutrazol, 22 µmol m^{-2} s^{-1} PPF, 16 h photoperiod 2. 27°C air temperature	-	3	100	9
Sweet potato	*Ipomoea batatas*	Axillary buds	1. MS basal media, 10 $mg.L^{-1}$ ABA, 50-60 µmol m^{-2} s^{-1} PPF, 16 h photoperiod 2. 28°C air temperature	5 mm	12	20-80	10
Sword fern	*Nephrolepis exaltata*	Shoot	1. Pre-storage in 9°C air temperature, darkness 2. 24°C air temperature, 30 µmol m^{-2} s^{-1} PPF	15-20 mm	36	-	11
Vanilla	*Vanilla* spp.	Shoot buds	1. ½ MS basal media, 1.5% sucrose, 1.5% mannitol, 12 h photoperiod, 40 µmol m^{-2} s^{-1} PPF 2. 25°C air temperature	3-5 mm	12	90	12

Abbreviations: MW = Morel and Wetmore medium (1951); BAP = N^6-benzyl amino purine; MS = Murashige and Skoog medium (1962); ABA = abscisic acid; PPF = photosynthetic photon flux
Ref. represents the following reference list: 1 = Assy-Bah and Engelmann (1993); 2 = Bertrand-Desbrunais *et al*. (1991); 3 = Dussert *et al*. (1997); 4 = Naidu and Sreenath (1999); 5 = Madubanya *et al*. (2006); 6 = Bonnier and van Tuyl (1997); 7 = Malik *et al*. (2005); 8 = Martin and Pradeep (2003); 9 = Canto *et al*. (2004); 10 = Jarret and Gawel (1991); 11 = Hvoslef-Eide (1992); 12 = Divakaran *et al*. (2006).

Application of ethylene inhibitors

An atmospheric aerial zone of an *in vitro* culture is one of the best and easiest ways to modify the culture for slow growth preservation. As explained above, ethylene is a plant growth regulator, which, in a gas form plays a key role in *in vitro* plant growth and development. Normally, plants grown in a closed vessel of *in vitro* culture for a long period release ethylene gas which accumulates in the culture vessel (>1.2 $\mu L.L^{-1}$) and negatively affects the plants' physiological and morphological characters (Zobayed 2000). A low ethylene concentration (0.05-0.1 $\mu L.L^{-1}$) controlled by ethylene inhibitors i.e. silver nitrate (Goh *et al*. 1997; Mohiuddin *et al*. 1997; Zobayed 1999; Naik and Chand 2003), silver thiosulfate (Zobayed 1999; Pereira-Netto 2001), α-amino isobutaric acid (Pereira-Netto 2001), cobalt chloride (Zobayed 1999; Pereira-Netto 2001), (aminooxy) acetic acid (Pereira-Netto 2001), aminoethoxyvinyl glycine (Goh *et al*. 1997; Pereira-Netto 2001; Naik and Chand 2003), 1-amino-cyclopropane-1-carboxylic acid (Goh *et al*. 1997; Zobayed 1999; Pereira-Netto 2001) and 1-methylcyclopropene (Zobayed 1999; Pereira-Netto 2001) has been applied with the objective of improving *in vitro* morphogenesis. In addition to this, the reduction of ethylene using silver thiosulfate has been applied for potato slow-growth preservation (Sarkar *et al*. 1999, 2002; **Table 1**).

Small size of culture vessel

A small culture vessel is an alternative goal to minimize the atmospheric space for plant growth and development. Small glass vessels, of 53 mL and 35 mL, were applied to preserve four mint accessions (Islam *et al*. 2005) and banana (Cha-um *et al*. 2006a), respectively. The leaf number of mint plantlets grown in small vessels is 1.3-fold less than that in a control vessel (380 mL). Small culture vessels can be effectively stored with a high density of plants, i.e. a large number of accessions or *in vitro* genebanks. In addition, the interval of subcultures of slow growth in a small vessel are extended, leading to a decrease in somaclonal variation as well as a reduced labor cost. The cost of *in vitro* cassava preservation (11.98 US$/accession/year) is 7.46-fold lower than that of cryopreservation technology (89.35 US$/accession/year) (Koo *et al*. 2003). Liquid nitrogen (-196°C) is a major cost of cryopreservation, which was spent at an estimated 400 US$/accession/year (Benford 1992).

Chemical factors

Chemical composition i.e. media strength, plant growth retardants and osmosis in the culture media are the most popular topics to reduce the growth of various plant species. A half and quarter strength of culture media directly decrease nutrient absorption, translocation and utilization, resulting in growth reduction (Bonnier and van Tuyl 1997; Garcia-Jimenez *et al*. 2006). MS medium (Murashige and Skoog 1962) is well known for its enriched nutrient composition. In some cases, the appropriate media might be developed or modified for specific plant species, especially woody and endemic species (Hansen and Kristiansen 1997; Lucchesini and Mensuali-Sodi 2004; Scocchi and Mroginski 2004). The plant growth retardants including abscisic acid (ABA) and the anti-GA group are well applied as growth inhibitors or for dwarfing plants. These agents function to slow the growth rate of plants and are normally applied in various fruit species for flowering and setting fruit out of season. The anti-GA group is the most adequate agent to play a role in dwarfism in orchid (Torres and Mogollon 2002) and cauliflower (Rodrigues-Otubo *et al*. 1998), without reducing leaf number and resulting in the absence of phytotoxicity symptoms. Moreover, the shoot number of *in vitro* plantlets increased slightly with an increase in anti-GA concentration in the culture media (Daquinta *et al*. 2001). Moreover, limitations in available water or a drought condition using osmoticum treatment are alternatively techniques to slow the growth rate of *in vitro* plantlets. There are several osmotic agents i.e. mannitol (Li *et al*. 2004b), sorbitol (Perales *et al*. 2005), and polyethylene glycol (Morsy *et al*. 2007) to retard the growth of *Centaurea ragusina* (Radic *et al*. 2006), maize (Li *et al*. 1998; Valentovic *et al*. 2006), rice (Cabuslay *et al*. 2002; Hsu and Kao 2003; Pandey *et al*. 2004; Zhang *et al*. 2006), poplar (Watanabe *et al*. 2000), *Brassica juncea* (Gangopadhyay *et al*. 1997), tepary bean (Mohamed and Tawfik 2006), cotton (Nepomuceno *et al*. 1998), common bean (Turkan *et al*. 2005), grass (van den Berg and Zeng 2006) and Mexican marigold (Mohamed *et al*. 2000). The major function of osmoticum application in the culture media is to reduce water deficit or drought, causing on low root relative water content and decrease in leaf water potential (Lawlor and Cornic 2002; Tambussi *et al*. 2005), reduction in photosynthesis (Reddy *et al*. 2004) through stomatal closure (CO_2-assimilated limitation) (Cornic 2000; Bhargava and Paranjpe 2004; Rouhi *et al*. 2007) and through chlorophyll degradation (water oxidation restriction) (Chaves *et al*. 2002; Sircelj *et al*. 2005; Lizana *et al*. 2006) as well as overall growth reduction (Sanchez *et al*. 2004; Kumar *et al*. 2006; Mohsenzadeh *et al*. 2006). A mild water-deficit condition using osmotic agents is an alternative tool for *in vitro* slow growth. Consequently, the basic knowledge on growth reduction by chemical factors would apply for medium-term preservation of *in vitro* plant storage.

Minimal media and plant growth regulators

Strength reduction of the culture medium is the first aspect to result in fruitful minimal growth preservation. A half and one-forth strengths of media are generally modified in many species i.e. apple (Negri *et al*. 2000), banana (Cha-um *et al*. 2006a), cedar (Renau-Morata *et al*. 2006), kokum (Malik *et al*. 2005), lily (Bonnier and van Tuyl 1997), miscanthus (Hansen and Kristainsen 1997), orchid (Martin and Pradeep 2003), paradise tree (Scocchi and Mroginski 2004), sugarcane (Taylor and Dukic 1993) and vanilla (Divakaran *et al*. 2006). Moreover, the growth rate of *in vitro* plantlets is retarded by the application of certain plant growth regulators. The application of ABA to the culture media was effectively applied to inhibit the growth of cedar (Renau-Morata *et al*. 2006), arabica coffee (Naidu and Sreenath 1999) and sweet potato (Jarret and Gawel 1991). Paclobutrazol and ancymidol, chemical agents which are plant growth retardants, delayed the growth and development of banana (**Fig. 2**) (Cha-um *et al*. 2006a), dierama (Madubanya *et al*. 2006), pineapple (Canto *et al*. 2004) and potato (Sarkar *et al*. 2001). Acetyl salicylic acid, activated charcoal and sodium ascorbate have been provided in the culture media to delay senescence, functioning as an antioxidant for *in vitro* preservation of potato (Lopez-Delgado *et al.* 1998; Rugini and Pesce 2006) and coconut (Assy-Bah and Engelmann 1993).

Osmotic potential preservation

Osmotic potential adjustment is an attractive topic for minimal growth of *in vitro* plants by water use limitation. There are many osmotic agents, including sugars and sugar alcohols, to apply in the media for slow growth of apple (20 $g.L^{-1}$ sucrose and 20 $g.L^{-1}$ mannitol) (Hao and Deng 2003), banana (20 $g.L^{-1}$ sucrose) (de Oliveira *et al*. 2000), coco yam (30 $g.L^{-1}$ mannitol) (Bessembinder *et al*. 1993), elephant's ear (30 $g.L^{-1}$ mannitol) (Zandvoort *et al*. 1994), lily (90 $g.L^{-1}$ sucrose) (Bonnier and van Tuyl 1997; Renau-Morata *et al*. 2006), potato (40-60 $g.L^{-1}$ sucrose and/or 20-40 $g.L^{-1}$ mannitol) (Lopez-Delgado *et al*. 1998; Sarkar *et al*. 1999, 2001) and vanilla (15 $g.L^{-1}$ sucrose and 15 $g.L^{-1}$ mannitol) (Divakaran *et al*. 2006). Normally, sugar supplementation in the media plays a central role as carbon source. A high concentration of sugars (6-9%) should function as low water potential or osmotic stress similar to sugar alcohols,

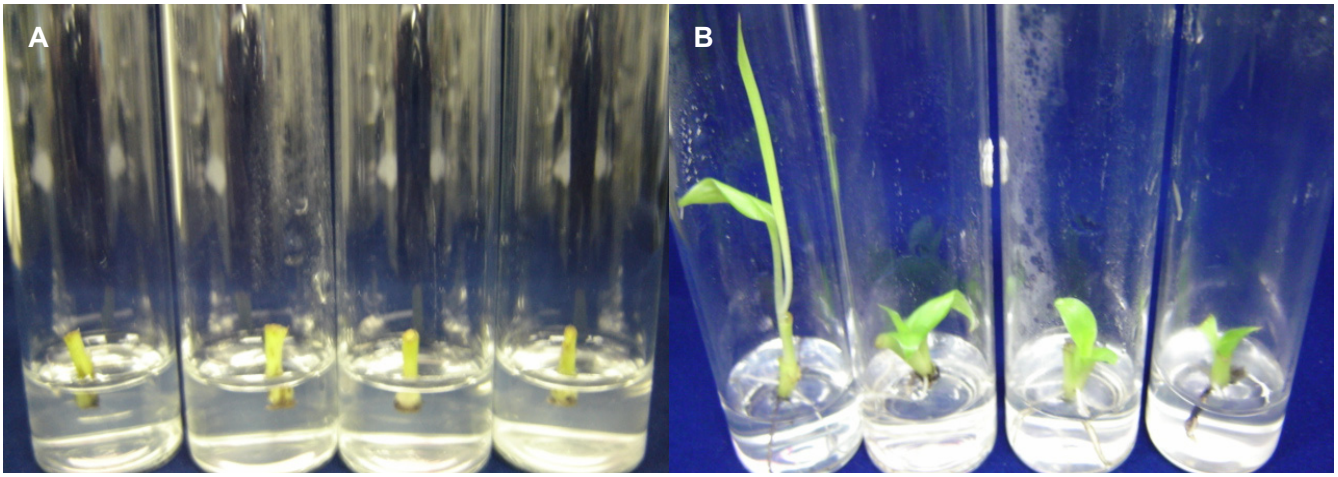

Fig. 2 Slow growth preservation of disease-free banana plantlets in media supplemented with various concentrations of paclobutrazol (PBZ) and incubated at 10°C in a Plant Growth Incubator (A) or at 25°C in the culture room (B). Reprinted from Cha-um S, Kirdmanee C, Huyen PX, Vathany T An effective procedure for disease-free production and minimal-growth preservation of *in vitro* banana (*Musa* spp.). *Acta Horticulturae* (in press), with kind permission, International Society for Horticultural Science.

resulting in a slow growth rate or minimal growth (Bonnier and van Tuyl 1997; Renau-Morata *et al*. 2006; Shibli *et al*. 2006). Therefore, the most effective way for medium-term preservation has been to modify both physical and chemical factors to slow the rate of *in vitro* growth and development.

PRESERVATION OF *IN VITRO* STORAGE ORGANS

On the other hand, *in vitro* storage organs, including protocorm-like bodies (Orchidaceae), somatic embryos, microrhizome (Zingiberaceae), microtubers (Solanaceae), and bulblets (Liliaceae), and artificial seeds have also played a role as alternative sources for medium-term preservation. There are many reports to successfully produce storage organs in rhizomes of ginger (Sharma and Singh 1995; Nalawade *et al*. 2003; Tyagi *et al*. 2006), orchid (Roy and Banerjee 2002), bamboo (Kapoor and Rao 2006), turmeric (Nayak 2000; Shirgurkar *et al*. 2001) and microtubers of yam (Alizadeh *et al*. 1998) and potato (Gopal *et al*. 1998) as well as bulblets of onion (Kastner *et al*. 2001), *Lachenalia* (Slabbert and Niederwieser 1999). As explained above, carbohydrate accumulation i.e. glucose (Vandenbussche *et al*. 1999; Tabaei-Aghdaei *et al*. 2003), fructose (Vandenbussche *et al*. 1999; Tabaei-Aghdaei *et al*. 2003), sucrose (Vandenbussche *et al*. 1999; Kerepesi *et al*. 2004; Miao *et al*. 2007), galactinol (Zuther *et al*. 2004; Miao *et al*. 2007), raffinose (Zuther *et al*. 2004), fructan (Kerepesi *et al*. 2004; Livingston *et al*. 2006), stachyose (Miao *et al*. 2007) and enzyme activities associated with sugar biosynthesis (Sturm and Tang 1999; Strand *et al*. 2003; Zuther *et al*. 2004; Hekneby *et al*. 2006) in cold-acclimated plants play an important role in reducing the nucleation or chilling injury (Priscila *et al*. 2006). Furthermore, the storage organs of both potato tubers (Malone *et al*. 2006) and lily bulbs (Xu *et al*. 2006) have been reported as carbohydrate-enriched tissues, which are tolerant to low temperature storage (du Toit *et al*. 2002; Kawakami *et al*. 2002; Goulas *et al*. 2003). Storage organs have more potential to be preserved at low temperatures as well as rapid recovering growth, micropropagation, and acclimatization in the pro-

Table 4 *In vitro* storage of plant species using sodium alginate encapsulation method.

Common name	Scientific name	*In vitro* storage	Storage period (months)	Survival (%)	Ref.
Cypress	*Chamaecyparis pisifera*	1. WP basal media and 17 μM BAP 2. 4°C air temperature	-	60-100	1
Patula pine	*Pinus patula*	1. DCR media, 100 μmol m^{-2} s^{-1} PPF 2. 2°C air temperature	4	73	2
Pomegranate	*Punica granatum*	1. MS media, 4.44 μM BAP, 0.54 μM NAA, 30-50 μmol m^{-2} s^{-1} PPF 2. 25°C air temperature	1	15-20	3
Potato	*Solanum tuberosum*	1. MS media, 2% mannitol, 4% sucrose, 0.5-1.0 mM STS, 20 μmol m^{-2} s^{-1} PPF, 16 h photoperiod 2. 6°C air temperature	16	83-92	4
Raspberry	*Rubus idaeus*	1. MS media, 4% sucrose 1% mannitol or 3.4 μM paclobutrazol, darkness 1. 4°C air temperature	9	25	5
Sissoo	*Dalbergia sissoo*	1. Water agar media, darkness 2. 4°C air temperature	2	30	6
Strawberry	*Fragaria* x *ananassa*	1. Boxus media, 4% glucose, 1% mannitol or 1.7 μM paclobutrazol, darkness 2. 4°C air temperature	9	50	5
Vanilla	*Vanilla* spp.	1. Sterile water 2. 22°C air temperature	12	10	7
White cedar	*Cedrela fissilis*	1. Water-solidified 0.4% agar 2. 25°C air temperature	6	44	8

Abbreviations: WP = Woody plant media (McCown and Lloyd 1981); DCR = Gupta and Durzan media (1985); MS = Murashige and Skoog media (1962); STS = Silver thiosulfate; Boxus = Boxus and Terzi media (1987)
Ref. represents the following references: 1 = Maruyama *et al*. (2003); 2 = Malabadi and van Staden (2005); 3 = Naik and Chand (2006); 4 = Sarkar *et al*. (2002); 5 = Lisek and Orlikowska (2004); 6 = Chand and Singh (2004); 7 = Divakaran *et al*. (2006); 8 = Nunes *et al*. (2003).

cess of *in vitro* post-storage (Wannakrairoj 1998; Tyagi *et al*. 2006). Alternatively, low temperature storage or vernalization of bulb and tuber species may enhance the flowering stage after cultivated in the field (Wurr *et al*. 2000; Roh 2005). Artificial seeds and vegetative organs i.e. nodal segments (Sarkar *et al*. 2002; Nunes *et al*. 2003; Chand and Singh 2004; Naik and Chand 2006), shoot tips (Nunes *et al*. 2003; Lisek and Orlikowska 2004; Divakaran *et al*. 2006), cotyledon (Nunes *et al*. 2003), somatic embryos (Anthony *et al*. 1996; Maruyama *et al*. 2003; Malabadi and van Staden 2005), protocorm-like bodies (Divakaran *et al*. 2006), pollen (Marcellan and Camadro 1996), embryogenic cell suspension and protoplasts (Anthony *et al*. 1996) have been successfully preserved as medium-term storage organs (**Table 4**). In addition to this, seed storage is manually preserved as large gene banks (Koo *et al*. 2003) i.e. rice (IRRI 2004), maize (Pardey *et al*. 2001), wheat (Pardey *et al*. 2001), *Avena* (Pita *et al*. 1998), *Trichilia emetica* (Kioko *et al*. 2006) and forest tree species (Phartyal *et al*. 2002).

GENETIC STABILITY

Genetic stability is an important requirement for genetic resource conservation. In the case of *in vitro* storage, there are several techniques to assay genetic stability as well as DNA methylation generated from environmental effects (**Table 5**). In one-year storage of apple (4°C) and citrus (10°C), methylation sensitive amplified polymorphism (MSAP) was displayed in the different bands between control and stored shoot-tip in both *Msp*I-*Eco*RI and *Hpa*II-*Eco*RI restriction enzyme digestions (Hao and Deng 2003; Hao *et al*. 2004). DNA methylation may be generated from low temperature in citrus (Hao *et al*. 2004), apple (Hao and Deng 2003), snapdragon (Kitamura *et al*. 2001; Hashida *et al*. 2005), wheat (Horvath *et al*. 2003) and chicory (Demeulemeester *et al*. 1999) as well as plant growth regulators application in wheat (Vlasova *et al*. 1995), barrel medic (Santos and Fevereiro 2002), true myrtle (Parra *et al*. 2001), tobacco (Toldi *et al*. 2005), Siberian ginseng (Chakrabarty *et al*. 2003), banana (Peraza-Echeverria *et al*. 2001) and potato (Joyce and Cassells 2002). Conversely, DNA stability is still maintained, and can be defined by either RAPD or AFLP, implying that DNA repair systems may automatically restore DNA methylation in short-term preservation. Moreover, RAPD, RFLP and AFLP techniques have been assayed for genetic maintenance in slow growth preservation of cassava (Angel *et al*. 1996), cedar (Renau-Morata *et al*. 2006), kangaroo paw (Turner *et al*. 2001), potato (Harding 1991) and silver birch (Ryynanen and Aronen 2005). In ten-year cassava preservation, the genetic variation is doubly checked using RAPD and RFLP-homologous with RAPD to confirm genetic stability (Angel *et al*. 1996). It should be noted that slow growth preservation is an effective approach to maintain genetic resources as gene banks.

CONCLUSION

In vitro slow-growth preservation is an alternative way to conserve the genetic resources of higher plants for sustainable uses, especially tropical plant species or recalcitrant plants. The physical and chemical factors of *in vitro* storage have been applied to reduce living plant growth and development for medium-term preservation with genetic stability. Genetic resources of medium-term preservation are more stable than cryopreservation or long-term preservation. In general, *in vitro* slow-growth storage has been widely investigated together with cryopreservation at –196°C as genebanks in many plant species. Moreover, freezing damage in cryopreservation technology may induce the genetic instability of preserved plant materials. It is a large barrier to overcome in order to develop in the near future long-term preservation goals.

Table 5 Genetic stability assay in *in vitro* slow-growth preservation of several plant species.

Plant species	Assay techniques	Results	References
Apple	1. DNA methylation	+	Hao and Deng (2003)
	2. AFLP	O	
Cassava	1. RAPD	O	Angel *et al*. (1996)
	2. RFLP homolog with RAPD	O	
Cedar	1. DNA methylation	O	Renau-Morata *et al*. (2006)
	2. RAPD	O	
Citrus	1. DNA methylation	+	Hao *et al*. (2004)
	2. RAPD	O	
Kangaroopaw	1. AFLP	O	Turner *et al*. (2001)
Potato	1. RFLP	O	Harding (1991)
Silver birch	1. RAPD	O	Ryynanen and Aronen (2005)

Different and similar patterns between controlled and preserved materials are represented by + and O, respectively.

REFERENCES

Akiyama T, Jin S (2007) Molecular cloning and characterization of an arginine decarboxylase gene up-regulated by chilling stress in rice seedlings. *Journal of Plant Physiology* **164**, 645-654

Alia, Hayashi H, Chen THH, Murata N (1998) Transformation with a gene for choline oxidase enhances the cold tolerance of *Arabidopsis* during germination and early growth. *Plant Cell and Environment* **21**, 232-239

Alizadeh S, Mantell SH, Viana AM (1998) *In vitro* shoot culture and microtuber induction in steroid yam *Dioscorea composita* Hemsl. *Plant Cell, Tissue and Organ Culture* **53**, 107-112

Allard F, Houde M, Krol M, Ivanov A, Huner NPA, Sarhan F (1998) Betaine improves freezing tolerance in wheat. *Plant and Cell Physiology* **39**, 1194-1202

Allen DJ, Ort DR (2001) Impacts of chilling temperatures on photosynthesis in warm-climate plants. *Trends in Plant Science* **6**, 36-42

Alonso JM, Stepanova AN (2004) The ethylene signaling pathway. *Science* **306**, 1513-1515

Angel F, Barney VE, Tohme J, Roca WM (1996) Stability of cassava plants at the DNA level after retrieval from 10 years of *in vitro* storage. *Euphytica* **90**, 307-313

Angeli SD, Malho R, Altamura M (2003) Low-temperature sensing in olive tree: calcium signaling and cold acclimation. *Plant Science* **165**, 1303-1313

Anthony P, Ausseil J, Bechler B, Benguria A, Blackhall N, Briarty LG, Cogoli A, Davey MR, Garesse R, Hager R, Loddenkemper R, Marchant R, Marco R, Marthy HJ, Perry M, Power JB, Schiller P, Ugalde C, Volkmann D, Wardrop J (1996) Preservation of viable biological samples for experiments in space laboratories. *Journal of Biotechnology* **47**, 377-393

Arigita L, Tames RS, Gonzalez A (2003) 1-Methylcyclopropene and ethylene as regulators of *in vitro* organogenesis in kiwi explants. *Plant Growth Regulation* **40**, 59-64

Aroca R, Amodeo G, Fernandez-Illescas S, Herman EM, Chaumont F, Chrispeels MJ (2005) The role of aquaporins and membrane damage in chilling and hydrogen peroxide induced changes in the hydraulic conductance of maize roots. *Plant Physiology* **137**, 341-353

Aroca R, Vernieri P, Irigoyen JJ, Sanchez-Diaz M, Tognoni F, Pardossi A (2003) Involvement of abscisic acid in leaf and root of maize (*Zea may* L.) in avoiding chilling-induced water stress. *Plant Science* **165**, 671-679

Assy-Bah B, Engelmann F (1993) Medium-term conservation of mature embryos of coconut. *Plant Cell, Tissue and Organ Culture* **33**, 19-24

Bairu MW, Fennell CW, van Staden J (2006) The effect of plant growth regulators on somaclonal variation in Cavendish banana (*Musa* AAA cv. 'Zelig'). *Scientia Horticulturae* **108**, 347-351

Bakht J, Bano A, Dominy P (2006) The role of abscisic and low temperature in chickpea (*Cicer arietinum*) cold tolerance. II Effects on plasma membrane structure and function. *Journal of Experimental Botany* **57**, 3707-3715

Bekheet SA, Taha HS, Saker MM (2002) *In vitro* long-term storage of date palm. *Biologia Plantarum* **45**, 121-124

Benford G (1992) Saving the "library of life". *Proceedings of the National Academy of Science USA* **89**, 11098-11101

Bertrand-Desbrunais A, Noirot M, Charrier A (1991) Minimal growth *in vitro* conservation of coffee (*Coffea* spp.). *Plant Cell, Tissue and Organ Culture* **27**, 333-339

Bessembinder JJE, Staritsky G, Zandvoort EA (1993) Long-term *in vitro* storage of *Colocasia esculenta* under minimal growth conditions. *Plant Cell, Tissue and Organ Culture* **33**, 121-127

Bhargava S, Paranjpe S (2004) Genotypic variation in the photosynthetic competence of *Sorghum bicolor* seedlings subjected to polyethylene glycol-mediated drought stress. *Journal of Plant Physiology* **161**, 125-129

Birch CJ, Vos J, van der Putten PEL (2003) Plant development and leaf area

production in contrasting cultivars of maize grown in a cool temperature environment in the field. *European Journal of Agronomy* **19**, 173-188

Blakesley D, Pask N, Henshaw GG, Fay MF (1996) Biotechnology and the conservation of forest genetic resources: *in vitro* strategies and cryopreservation. *Plant Growth Regulation* **20**, 11-16

Bloom AJ, Zwieniecki MA, Passioura JB, Randall LB, Holbrook NM, St. Clair DA (2004) Water relations under root chilling in a sensitive and tolerant tomato species. *Plant Cell and Environment* **27**, 971-979

Boese SR, Wolfe DW, Melkonian JJ (1997) Elevated CO_2 mitigates chilling-induced water stress and photosynthetic reduction during chilling. *Plant and Cell Physiology* **20**, 625-632

Bohn M, Luthje S, Sperling P, Heinz E, Dorffling K (2007) Plasma membrane lipid alterations induced by cold acclimation and abscisic treatment of winter wheat seedlings differing in frost resistance. *Journal of Plant Physiology* **164**, 146-156

Bois JF, Winkel T, Lhomme JP, Raffaillac JP, Rocheteau A (2006) Response of some Andean cultivars of quinoa (*Chenopodium quinoa* Willd.) to temperature: Effects on germination, phenology, growth and freezing. *European Journal of Agronomy* **25**, 299-308

Bonnier FJM, van Tuyl JM (1997) Long term *in vitro* storage of lily: effects of temperature and concentration of nutrients and sucrose. *Plant Cell, Tissue and Organ Culture* **49**, 81-87

Boxus PH, Terzi JMV (1987) Big losses due to bacterial contamination can be avoided in mass propagation schemes. *Acta Horticulturae* **212**, 91-93

Breton G, Danyluk J, Ouellet F, Sarhan F (2000) Biotechnological applications of plant freezing associated proteins. *Biotechnology Annual Review* **6**, 57-100

Browse J, Xin Z (2001) Temperature sensing and cold acclimation. *Current Opinion in Plant Biology* **4**, 241-246

Brush RA, Griffith M, Mlynarz A (1994) Characterization and quantification of intrinsic ice nucleators in winter rye (*Secale cereale*) leaves. *Plant Physiology* **104**, 725-735

Buitink J, Leprince O (2004) Glass formation in plant anhydrobiotes: survival in the dry state. *Cryobiology* **48**, 215-228

Cabuslay GS, Ito O, Alejar AA (2002) Physiological evaluation of responses of rice (*Oryza sativa* L.) to water deficit. *Plant Science* **163**, 815-827

Campos PS, Quartin V, Ramalho JC, Nunes MA (2003) Electrolyte leakage and lipid degradation account for cold sensitivity in leaves of *Coffea* sp. plants. *Journal of Plant Physiology* **160**, 509-515

Canto AMME, Souza FVD, Costa MAC, Souza AS, Ledo CAS, Cabral JRS (2004) *In vitro* conservation of pineapple germplasm treated with paclobutrazol. *Pesquisa Agropecuaria Brasileira* **36**, 717-720

Chakrabarty D, Yu KW, Paek KY (2003) Detection of DNA methylation changes during somatic embryogenesis of Siberian ginseng (*Eleuterococcus senticosus*). *Plant Science* **165**, 61-68

Chand S, Singh AK (2004) Plant regeneration from encapsulated nodal segments of *Dalbergia sissoo* Roxb., a timber-yielding leguminous tree species. *Journal of Plant Physiology* **161**, 237-243

Chang C, Bleecker AB (2004) Ethylene biology. More than a gas. *Plant Physiology* **136**, 2895-2899

Cha-um S, Hien NT, Kirdmanee C (2006b) Disease-free production of sugarcane varieties (*Saccharum officinarum* L.) using *in vitro* meristem culture. *Biotechnology* **5**, 443-448

Cha-um S, Kirdmanee C, Huyen PX, Vathany T (2006a) An effective procedure for disease-free production and minimal-growth preservation of *in vitro* banana (*Musa* spp.). *Acta Horticulturae* in press

Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CPP, Osorio ML, Carvalho I, Faria T, Pinheiro C (2002) How plants cope with water stress in the field photosynthesis and growth. *Annals of Botany* **89**, 907-916

Chen WP, Li PH (2002) Membrane stabilization by abscisic acid under cold aids proline in alleviating chilling injury in maize (*Zea mays* L.) cultured cells. *Plant Cell and Environment* **25**, 955-962

Chen Y, Zhang M, Chen T, Zhang Y, An L (2006) The relationship between seasonal changes in anti-oxidative system and freezing tolerance in the leaves of evergreen woody plants of *Sabina*. *South African Journal of Botany* **72**, 272-279

Chen YF, Etheridge N, Schaller GE (2005) Ethylene signaling transduction. *Annals of Botany* **95**, 901-915

Chinnusamy V, Zhu J, Zhu JK (2006) Gene regulation during cold acclimation in plants. *Physiologia Plantarum* **126**, 52-61

Clarke HJ, Siddique KHM (2004) Response of chickpea genotypes to low temperature stress during reproductive development. *Field Crop Research* **90**, 323-334

Concellon A, Anon MC, Chaves AR (2007) Effect of low temperature storage on physical and physiological characteristics of eggplant fruit (*Solanum melongena* L.). *LWT - Food Science and Technology* **40**, 389-396

Cornic G (2000) Drought stress inhibits photosynthesis by decreasing stomatal aperture- not by affecting ATP synthesis. *Trends in Plant Science* **5**, 187-188

Crouch JH, Vuylsteke D, Ortiz R (1998) Perspective on the application of biotechnology to assist the genetic enhancement of plantain and banana (*Musa* spp.). *Electronic Journal of Biotechnology* **1**, 1-12

Daquinta M, Lezcano Y, Escalona, Santos R (2001) *In vitro* multiplication of FHIA-18 plantain in the presence of paclobutrazol. *INFOMUSA* **10**, 22-24

de la Vina G, Barcelo-Muñoz A, Pliego-Alfaro F (2001) Effect of culture media and irradiance level on growth and morphology of *Persea americana* Mill microcutting. *Plant Cell, Tissue and Organ Culture* **65**, 229-237

de Oliveira RP, Silva SO, da Silva KM, Silveira DG (2000) *In vitro* conservation of diploid banana accessions. *Scientia Agricola* **57**, 245-249

de Oliveira JG, Alves PLCA, Magalhães AC (2002) The effect of chilling on the photosynthetic activity in coffee (*Coffea arabica* L.) seedlings. The protective action of chloroplastid pigments. *Brazilian Journal of Plant Physiology* **14**, 95-104

Demeulemeester MAC, van Stallen N, de Proft MP (1999) Degree of DNA methylation in chicory (*Cichorium intybus* L.): influence of plant age and vernalization. *Plant Science* **142**, 101-108

Desikan R, Last K, Harrett-Williams R, Tagliavia C, Harter K, Hooley R, Hancock JT (2006) Ethylene-induced stomatal closure in *Arabidopsis* occurs *via* AtrbohF-mediated hydrogen peroxide synthesis. *The Plant Journal* **47**, 907-916

Dhanaraj AL, Slovin JP, Rowland LJ (2004) Analysis of gene expression associated with cold acclimation in blueberry floral buds using expressed sequence tags. *Plant Science* **166**, 863-872

Dhanaraj AL, Slovin JP, Rowland LJ (2005) Isolation of a cDNA clone and characterization of expression of the highly abundant, cold acclimation-associated 14 kDa dehydrin of blueberry. *Plant Science* **168**, 949-957

Divakaran M, Babu KN, Peter KV (2006) Conservation of *Vanilla* species, *in vitro*. *Scientia Horticulturae* **110**, 175-180

Dorion N, Regnard JL, Serpette I, Bigot C (1994) Effects of temperature and hypoxic atmosphere on preservation and further development of *in vitro* shoots of peach ('Armking') and peach × almond hybrid ('GF 677'). *Scientia Horticulturae* **57**, 201-213

du Toit ES, Robbertse PJ, Niederwieser JG (2002) Effects of growth and storage temperature on *Lachenalia* cv. Ronina bulb morphology. *Scientia Horticulturae* **94**, 117-123

Du YC, Nose A, Wasano K (1999) Effects of chilling temperature on photosynthetic rates, photosynthetic enzyme activities and metabolite levels in leaves of three sugarcane species. *Plant Cell and Environment* **22**, 317-324

Dussert S, Chabrillange N, Anthony F, Engelmann F, Recalt C (1997) Variability in storage response with in a coffee (*Coffea* spp.) core collection under slow growth conditions. *Plant Cell Reports* **16**, 344-348

Engelmann F (1998) *In vitro* conservation of horticultural genetic resources: review of the state of the art. World Conference on Horticultural Research, 17-20 June 1998, ISHS, Rome, Italy

Engelmann F, Engels JMM (2002) Technologies and strategies for *ex situ* conservation. In: Engels JMM, Rao VR, Brown AHD, Jackson MT (Eds) *Managing Plant Genetic Diversity*, International Plant Genetic Resources Institute (IPGRI), Rome, Italy, pp 89-103

Ensminger I, Busch F, Huner NP (2006) Photostasis and cold acclimation: sensing low temperature through photosynthesis. *Physiologia Plantarum* **126**, 28-44

Epperson JE, Pachico DH, Guevara CL (1997) A cost analysis of maintaining cassava plant genetic resources. *Crop Science* **37**, 1641-1649

Ercoli L, Mariotti M, Masoni A, Arduini I (2004) Growth responses of sorghum plants to chilling temperature and duration of exposure. *European Journal of Agronomy* **21**, 93-103

Fal MA, Majada JP, Gonzalez A, Tames RS (1999) Differences between *Dianthus caryophyllus* L. cultivar in *in vitro* growth and morphogenesis are related to their ethylene production. *Plant Growth Regulation* **27**, 131-136

Farrell TC, Fox KM, Williams RL, Fukai S (2006) Genotypic variation for cold tolerance during reproductive development in rice: Screening with cold air and cold water. *Field Crop Research* **98**, 178-194

Gangopadhyay G, Basu S, Gupta S (1997) *In vitro* selection and physiological characterization of NaCl- and mannitol-adapted callus in *Brassica juncea*. *Plant Cell, Tissue and Organ Culture* **50**, 161-169

Garcia-Jimenez P, Navarro EP, Santana CH, Luque A, Robaina RR (2006) Anatomical and nutritional requirements for induction and sustained growth *in vitro* of *Cymodocea nodosa* (Ucria) Ascherson. *Aquatic Botany* **84**, 79-84

Gechev T, Willekens H, van Montagu M, van Camp W, Toneva V, Minkov I (2003) Different responses of tobacco antioxidant enzymes to light and chilling stress. *Journal of Plant Physiology* **160**, 509-515

Goh CJ, Ng SK, Lakshmanan P, Loh CS (1997) The role of ethylene on direct shoot bud regeneration from mangosteen (*Garcinia mangostana* L.) leaves cultured *in vitro*. *Plant Science* **124**, 193-202

Gonçalves JFC, Marenco RA, Vieira G (2001) Concentration of photosynthetic pigments and chlorophyll fluorescence of Mahogany and Tonka bean under two light environments. *Revista Brasileira de Fisiologia Vegecutura* **13**, 149-157

Gong Y, Mattheis JP (2003) Effect of ethylene and 1-methylcyclopropene on chlorophyll catabolism of broccoli florets. *Plant Growth Regulation* **40**, 33-38

Gonzalez A, Arigita L, Majada JP, Tames RS (1997) Ethylene involvement in *in vitro* organogenesis and plant growth of *Populus tremula* L. *Plant Growth Regulation* **22**, 1-6

Gopal J, Minocha JL, Dhaliwal HS (1998) Microtuberization in potato (*Solanum tuberosum* L.) *Plant Cell Reports* **17**, 794-798

Goulas E, Dily FL, Ozouf J, Ourry A (2003) Effects of a cold treatment of the root system on white clover (*Trifolium repens* L.) morphogenesis and nitro-

gen reserve accumulation. *Journal of Plant Physiology* **160**, 893-902
Grace SC, Logan BA (1996) Acclimation of foliar antioxidant systems to growth irradiance in three broad-leaved evergreen species. *Plant Physiology* **122**, 1631-1640
Gresshoff PM, Doy CH (1972) Development and differentiation of haploid *Lycopersicon esculentum* (tomato). *Planta* **170**, 161-170
Griffith M, Yaish MWF (2004) Antifreze proteins in overwintering plants: a tale of two activities. *Trends in Plant Science* **9**, 399-405
Guo H, Ecker JR (2004) The ethylene signaling pathway: new insights. *Current Opinion in Plant Biology* **7**, 40-49
Guo W, Li Y, Gong L, Li F, Dong Y, Liu B (2006) Efficient micropropagation of *Robinia ambigua* var. *idahoensis* (Idaho Locust) and detection of genomic variation by ISSR markers. *Plant Cell, Tissue and Organ Culture* **84**, 343-351
Gupta PK, Durzan DJ (1985) Shoot multiplication from mature trees of Douglas fir and sugar pine. *Plant Cell Reports* **4**, 177-179
Gusta LV, Trischuk R, Weiser CJ (2005) Plant cold acclimatization: The role of abscisic acid. *Journal of Plant Growth Regulation* **24**, 308-318
Guy CL (1990) Cold acclimation and freezing stress tolerance: role of protein metabolism. *Annual Review of Plant Physiology and Plant Molecular Biology* **41**, 187-223
Hansen J, Kristiansen K (1997) Short-term *in vitro* storage of *Miscanthus* × *ogiformis* Honda "Giganteus" as affected by medium composition, temperature and photon flux density. *Plant Cell, Tissue and Organ Culture* **49**, 161-169
Hao YJ, Deng XX (2003) Genetically stable regeneration of apple plants from slow growth. *Plant Cell, Tissue and Organ Culture* **72**, 253-260
Hao YJ, Wen XP, Deng XX (2004) Genetic and epigenetic evaluations of citrus calluses recovered from slow-growth culture. *Journal of Plant Physiology* **161**, 479-484
Harding K (1991) Molecular stability of the ribosomal RNA genes in *Solanum tuberosum* plants recovered from slow growth and cryopreservation. *Euphytica* **55**, 141-146
Hashida S, Kishima Y, Mikami T (2005) DNA methylation is not necessary for the inactivation of the TAM3 transposon at non-permissive temperature in *Antirrhinum*. *Journal of Plant Physiology* **162**, 1292-1296
Hekneby M, Antolin MA, Sanchez-Diaz M (2006) Frost resistance and biochemical changes during cold acclimation in different annual legumes. *Environmental and Experimental Botany* **55**, 305-314
Heywood VH, Iriondo JM (2003) Plant conservation: old problems, new perspectives. *Biological Conservation* **113**, 321-335
Hola D, Kocava M, Rothova O, Wilhelmova N, Benesova M (2007) Recovery of maize (*Zea mays* L.) inbreds and hybrids from chilling stress of various duration: Photosynthesis and antioxidant enzymes. *Journal of Plant Physiology* in press
Homhual R, Chiemsombat P, Naritoom K, Surijachaijakorn M (1999) Role of growth regulator in meristem culture and production of virus-free sugarcane germplasm. *Sugar Technology* **3**, 82-88
Horvath E, Szalia G, Janda T, Paldi E, Racz I, Lasztity D (2003) Effect of vernalisation and 5-azacytidine on the methylation level of DNA in wheat (*Triticum aestivum* L. cv. Martonvasar 15). *Plant Science* **165**, 689-692
Hsu SY, Kao CH (2003) Differential effect of sorbitol and polyethylene glycol on antioxidant enzymes in rice leaves. *Plant Growth Regulation* **39**, 83-90
Hu WH, Zhou YH, Du YS, Xia XJ, Yu JQ (2006) Differential response of photosynthesis in greenhouse- and field-ecotypes of tomato to long-term chilling under low light. *Journal of Plant Physiology* **163**, 1238-1246
Hummel I, El Amrani A, Gouesbet G, Hennion F, Couee I (2004) Involvement of polyamines in the interacting effects of low temperature and mineral supply on *Pringlea antiscorbutica* (Kerguelen cabbage) seedlings. *Journal of Experimental Botany* **55**, 1125-1134
Hvoslef-Eide AK (1992) Effects of pre-storage conditions on storage of *in vitro* cultures of *Nephrolepis exaltata* (L.) Schott and *Cordyline fruticosa* (L.) A. Chev. *Plant Cell, Tissue and Organ Culture* **28**, 167-174
Imai R, Ali A, Pramanik MHR, Nakaminami K, Sentoku N, Kato H (2004) A distinctive class of spermidine synthase is involved in chilling response in rice. *Journal of Plant Physiology* **161**, 883-886
IRRI (2004) International Rice Gene Bank Manual: The conservation of rice genetic resources at IRRI. IRRI, Los Banos, The Philippines
Islam MT, Dembele DP, Keller EJR (2005) Influence of explants, temperature and different culture vessels on *in vitro* culture for germplasm maintenance of four mint accessions. *Plant Cell, Tissue and Organ Culture* **81**, 123-130
Islam MT, Leunufna S, Dembele DP, Keller ERJ (2003) *In vitro* conservation of four mint (*Mentha* spp.) accessions. *Plant Tissue Culture* **13**, 37-46
Jackson MB (2003) Aeration stress in plant tissue cultures. *Bulgaria Journal of Plant Physiology* **Special Issue**, 96-109
Janda T, Szalai G, Rios-Gonzalez K, Veisz O, Paldi E (2003) Comparative study of frost tolerance and antioxidant activity in cereals. *Plant Science* **164**, 301-306
Jarret RL, Gawel N (1991) Abscisic acid-induced growth inhibition of sweet potato (*Ipomoea batatas* L.) *in vitro*. *Plant Cell, Tissue and Organ Culture* **24**, 13-18
Joyce SM, Cassells AC (2002) Variation in potato microplant morphology *in vi-tro* and DNA methylation. *Plant Cell, Tissue and Organ Culture* **70**, 125-137
Kalberer SR, Wisniewski M, Arora R (2006) Deacclimation and reacclimation of cold-hardy plants: Current understanding and emerging concepts. *Plant Science* **171**, 3-16
Kapoor P, Rao IU (2006) *In vitro* rhizome induction and plantlet formation from multiple shoots in *Bambusa bambos* var. *gigantean* Bennet and Gaur by growth regulators and sucrose. *Plant Cell, Tissue and Organ Culture* **85**, 211-217
Kastner U, Klahr A, Keller ERJ, Kahane (2001) Formation of onion bulblets *in vitro* and viability during medium-term storage. *Plant Cell Reports* **20**, 137-142
Kawakami S, Matsumoto Y, Matsunaga A, Mayama S, Mizuno M (2002) Molecular cloning of ascorbate peroxidase in potato tubers and its response during storage at low temperature. *Plant Science* **163**, 829-836
Keller ERJ, Senula A, Leunufna S, Grube M (2006) Slow growth storage and cryopreservation-tools to facilitate germplasm maintenance of vegetative propagated crops in living plant collections. *International Journal of Refrigeration* **29**, 411-417
Kepczynski J, Nemoykina A, Kepczynska E (2006) Ethylene and *in vitro* rooting of rose shoots. *Plant Growth Regulation* **50**, 23-28
Kerepesi I, Banyai-Stefanovits E, Galiba G (2004) Cold acclimation and abscisic acid induced alterations in carbohydrate content in calli of wheat genotypes differing in frost tolerance. *Journal of Plant Physiology* **161**, 131-133
Khan NA (2005) The influence of exogenous ethylene on growth and photosynthesis of mustard (*Brassica juncea*) following defoliation. *Scientia Horticulturae* **105**, 499-505
Khush GS (2005) What it will take to feed 5.0 billion rice consumers in 2030? *Plant Molecular Biology* **59**, 1-6
Kioko JI, Berjak P, Pammenter NW (2006) Viability and ultrastructural responses of seeds and embryonic axes of *Trichilia emetica* to different dehydration and storage conditions. *South African Journal of Botany* **72**, 167-176
Kitamura K, Hashida S, Mikami T, Kishima Y (2001) Position effect of the excision frequency of the *Antirrhinum* transposon Tam3: implications for the degree of position-dependent methylation in the ends of the element. *Plant Molecular Biology* **47**, 475-490
Klee HJ (2004) Ethylene signal transduction. Moving beyond *Arabidopsis*. *Plant Physiology* **135**, 660-667
Ko W, Hwang S, Ku F (1991) A new technique of meristem-tip cultures of 'Cavendish' banana. *Plant Cell, Tissue and Organ Culture* **25**, 179-83
Koo B, Pardey P, Wright B (2003) The price of conserving agricultural biodiversity. *Nature Biotechnology* **21**, 126-128
Kornyeyev D, Logan BA, Allen RD, Holaday AS (2003) Effect of chloroplastic overproduction of ascorbate peroxidase on photosynthesis and photoprotection in cotton leaves subjected to low temperature photoinhibition. *Plant Science* **165**, 1033-1041
Kratsch HA, Wise RR (2000) The ultrastructure of chilling stress. *Plant Cell and Environment* **23**, 337-350
Kumar R, Sarawgi AK, Ramos C, Amarante ST, Ismail AM, Wade LJ (2006) Partitioning of dry matter during drought stress in rainfed lowland rice. *Field Crop Research* **98**, 1-11
Kuo CG, Chen BJ, Green K, Lee DR (1985) Virus-free sweet potato storage roots derived from meristem-tips and leaf-cutting. *HortScience* **26**, 231-240
Lai CC, Yu TA, Yeh SD, Yang JS (1998) Enhancement of *in vitro* growth of papaya multishoots by aeration. *Plant Cell, Tissue and Organ Culture* **53**, 221-225
Lavee A, Borochov A, Halevy AH (1985) Effects of growing and storage temperatures on growth and tuber yield of caladium. *Scientia Horticulturae* **26**, 175-182
Lawlor DW, Cornic G (2002) Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell and Environment* **25**, 275-294
Lee SH, Ahn SJ, Im YJ, Cho K, Chung GC, Cho BH, Han O (2005) Differential impact of low temperature on fatty acid unsaturation and lipoxygenase activity in figleaf gourd and cucumber roots. *Biochemical and Biophysical Research Communications* **330**, 1194-1198
Lee SH, Singh AP, Chung GC, Kim YS, Kong IB (2002) Chilling root temperature causes rapid ultrastructural changes in cortical cells of cucumber (*Cucumis sativus* L.) root tips. *Journal of Experimental Botany* **53**, 2225-2237
Legnani G, Watkins CB, Miller WB (2004) Light, moisture, and atmosphere interact to affect the quality of dry-sale lily bulbs. *Postharvest Biology and Technology* **34**, 93-103
Li C, Junttila O, Heino P, Palva ET (2004a) Low temperature sensing in silver birch (*Betula pendula* Roth) ecotypes. *Plant Science* **167**, 165-171
Li Z, L Zhao, Kai G, Yu S, Cao Y, Pang Y, Sun X, Tang K (2004b) Cloning and expression analysis of water stress-induced gene from *Brassica oleracea*. *Plant Physiology and Biochemistry* **42**, 789-794
Li L, van Staden J, Jager AK (1998) Effects of plant growth regulators on the antioxidant system in seedling of two maize cultivars subjected to water stress. *Plant Growth Regulation* **25**, 81-87
Lichtenthaler HK, Burkart S (1999) Photosynthesis and high light stress. *Bulgarian Journal of Plant Physiology* **25**, 3-16
Lindqvist H (2001) Effect of different lifting dates and different lengths of cold storage on plant vitality of silver birch and common oak. *Scientia Horticul-*

turae **88**, 147-161

Lisek A, Orlikowska T (2004) *In vitro* storage of strawberry and raspberry in calcium-alginate beads at 4°C. *Plant Cell, Tissue and Organ Culture* **78**, 167-172

Livingston D, Premakumar R, Tallury SP (2005) Carbohydrate concentrations in crown fractions from winter oat during hardening at sub-zero temperatures. *Annals of Botany* **96**, 331-335

Livingston D, Premakumar R, Tallury SP (2006) Carbohydrate partitioning between upper and lower regions of the crown in oat and rye during cold acclimation and freezing. *Cryobiology* **52**, 200-208

Lizana C, Wentworth M, Martinez JP, Villegas D, Meneses R, Murchie EH, Pastenes C, Lercari B, Vernieri P, Horton P, Pinto M (2006) Differential adaptation of two varieties of common bean to abiotic stress. I. Effect of drought on yield and photosynthesis. *Journal of Experimental Botany* **57**, 685-697

Logan BA, Demmig-Adams B, Adams WW, Grace SC (1998) Antioxidants and xanthophylls cycle-dependent energy dissipation in *Cucurbita pepo* L. and *Vinca major* L. acclimated to four growth PPFDs in the field. *Journal of Experimental Botany* **49**, 1869-1879

Loik ME, Redar SP (2003) Microclimate, freezing tolerance, and cold acclimation along an elevation gradient for seedlings of the Great Basin Desert shrub, *Artemisia tridentate*. *Journal of Arid Environments* **54**, 769-782

Lopez-Delgado H, Jimenz-Casas M, Scott IM (1998) Storage of potato microplants *in vitro* in the presence of acetylsalicylic acid. *Plant Cell, Tissue and Organ Culture* **54**, 145-152

Lucchesini M, Mensuali-Sodi A (2004) Influence of medium composition and vessel ventilation on *in vitro* propagation of *Phillyrea latifolia* L. *Scientia Horticulturae* **100**, 117-125

Madubanya LA, Makunga NP, Fennell CW (2006) *Dierama luteoalbidum*: liquid culture provides an efficient system for the *ex situ* conservation of an endangered and horticulturally valuable plant. *South African Journal of Botany* **72**, 584-588

Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: An overview. *Archives of Biochemistry and Biophysics* **444**, 139-158

Malabadi RB, van Staden J (2005) Storability and germination of sodium alginate encapsulated somatic embryos derived from the vegetative shoot apices of mature *Pinus patula* trees. *Plant Cell, Tissue and Organ Culture* **82**, 259-265

Malik SK, Chaudhury R, Kalia RK (2005) Rapid *in vitro* multiplication and conservation of *Garcinia indica*: A tropical medicinal tree species. *Scientia Horticulturae* **106**, 539-553

Malone JG, Mittova V, Ratcliffe RG, Kruger NJ (2006) The response of carbohydrate metabolism in potato tubers to low temperature. *Plant and Cell Physiology* **47**, 1309-1322

Marcellan ON, Camadro EL (1996) The viability of asparagus pollen after storage at low temperatures. *Scientia Horticulturae* **67**, 101-104

Martin KP, Pradeep AK (2003) Simple strategy for the *in vitro* conservation of *Ipsea malabarica* an endemic and endangered orchid of the Western Ghats of Kerala, India. *Plant Cell, Tissue and Organ Culture* **74**, 197-200

Maruyama E, Hosoi Y, Ishii K (2003) Somatic embryo culture for propagation, artificial seed production, and conservation of sawara cypress (*Chamaecyparis pisifera* Sieb. *et* Zucc.). *Journal of Forest Research* **8**, 1-8

McCown BH, Lloyd G (1981) Woody Plant Medium (WPM) – a mineral nutrient formulation for microculture for woody plant species. *HortScience* **16**, 453

Miao M, Xu X, Chen X, Xue L, Cao B (2007) Cucumber carbohydrate metabolism and translocation under chilling night temperature. *Journal of Plant Physiology* **164**, 621-628

Modgil M, Mahajan K, Chakrabarti SK, Sharma DR, Sobti RC (2005) Molecular analysis of genetic stability in micropropagated apple rootstock MM106. *Scientia Horticulturae* **104**, 151-160

Mohamed MAH, Harris PJC, Henderson J (2000) *In vitro* selection and characterisation of a drought tolerant clone of *Tagetes minuta*. *Plant Science* **159**, 213-222

Mohamed MF, Tawfik AA (2006) Dehydration-induced alterations in growth and osmotic potential of callus from six tepary bean lines varying in drought resistance. *Plant Cell, Tissue and Organ Culture* **87**, 255-262

Mohiuddin AKM, Chowdhury MKU, Abdullah ZC, Napis S (1997) Influence of silver nitrate (ethylene inhibitor) on cucumber *in vitro* shoot regeneration. *Plant Cell, Tissue and Organ Culture* **51**, 75-78

Mohsenzadeh S, Malboobi MA, Razavi K, Farrahi-Aschtiani S (2006) Physiologiacl and molecular responses of *Aeluropus lagopoides* (Poaceae) to water deficit. *Environmental and Experimental Botany* **56**, 314-322

Morel G, Wetmore RH (1951) Fern callus tissue culture. *American Journal of Botany* **38**, 141-143

Morsy MR, Jouve L, Hausman JF, Hoffmann L, Stewart JM (2007) Alteration of oxidative and carbohydrate metabolism under abiotic stress in two rice (*Oryza sativa* L.) genotypes contrasting in chilling tolerance. *Journal of Plant Physiology* **164**, 157-167

Murai M, Yoshida S (1998) Vacuolar membrane lesions induced by a freeze-thaw cycle in protoplasts isolated from deacclimated tubers of Jerusalem artichoke (*Helianthus tuberosus* L.). *Plant and Cell Physiology* **39**, 87-96

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

Murashige T, Tucker DPH (1969) Growth factor requirements of citrus tissue culture. *Proceedings 1st International Citrus Symposium* **3**, 1155-1161

Nagao M, Minami A, Arakawa K, Fujikawa S, Takezawa D (2005) Rapid degradation of starch in chloroplasts and concomitant accumulation of soluble sugars associated with ABA-induced freezing tolerance in the moss *Physcomitrella patens*. *Journal of Plant Physiology* **162**, 169-180

Naidu MM, Sreenath HL (1999) *In vitro* culture of coffee zygotic embryo for germplasm preservation. *Plant Cell, Tissue and Organ Culture* **55**, 227-230

Naik SK, Chand PK (2003) Silver nitrate and aminoethoxyvinylglycine promote *in vitro* adventitious shoot regeneration of pomegranate (*Punica granatum* L.). *Journal of Plant Physiology* **160**, 423-430

Naik SK, Chand PK (2006) Nutrient-alginate encapsulation of *in vitro* nodal segments of pomegranate (*Punica granatum* L.) for germplasm distribution and exchange. *Scientia Horticulturae* **108**, 247-252

Nalawade SM, Sagare AP, Lee CY, Kao CL, Tsay HS (2003) Studies on tissue culture of Chinese medicinal plant resources in Taiwan and their sustainable utilization. *Botanical Bulletin of Academia Sinica* **44**, 79-98

Nantes IL, Fagian MM, Catisti R, Arruda P, Maia IG, Vercesi AE (1999) Low temperature and aging-promoted expression of PUMP in potato tuber mitochondria. *FEBS Letters* **457**, 103-106

Nayak S (2000) *In vitro* multiplication and microrhizome induction in *Curcuma aromatica* Salisb. *Plant Growth Regulation* **32**, 41-47

Nayyar H, Bains T, Kumar S (2005a) Low temperature induced floral abortion in chickpea: relationship to abscisic acid and cryoprotectants in reproductive organs. *Environmental and Experimental Botany* **53**, 39-47

Nayyar H, Bains TS, Kumar S (2005b) Chilling stressed chickpea seedlings: effect of cold acclimation, calcium and abscisic acid on cryoprotective solutes and antioxidative damage. *Environmental and Experimental Botany* **54**, 275-285

Ndong C, Ouellet F, Houde M, Sarhan F (1997) Gene expression during cold acclimation in strawberry. *Plant and Cell Physiology* **38**, 863-870

Neefs V, Leuridan S, van Stallen N, de Meulemeester M, de Proft MP (2000) Frost sensitiveness of chicory roots (*Cichorium intybus* L.). *Scientia Horticulturae* **86**, 185-195

Negash A, Krens F, Schaart J, Visser B (2001) *In vitro* conservation of enset under slow-growth conditions. *Plant Cell, Tissue and Organ Culture* **66**, 107-111

Negri V, Tosti N, Standardi A (2000) Slow-growth storage of single node shoots of apple genotypes. *Plant Cell, Tissue and Organ Culture* **62**, 159-162

Nepomuceno AL, Oosterhuis DM, Stewart JM (1998) Physiological responses of cotton leaves and roots to water deficit induced by polyethylene glycol. *Environmental and Experimental Botany* **40**, 29-41

Nicotra AB, Hofmann M, Siebke K, Ball MC (2003) Spatial patterning of pigmentation in evergreen leaves in response to freezing stress. *Plant Cell and Environment* **26**, 1893-1904

Niimi Y, Han DS, Abe S (2006) Temperature affecting embryo development and seed germination of Christmas rose (*Helleborus niger*) after sowing. *Scientia Horticulturae* **107**, 292-296

Nilsen ET, Orcutt DM (1996) *The Physiology of Plants under Stress*, John Wiley & Sons, Inc. New York, USA

Nishizawa T, Shishido Y, Kudo M, Kumakura H, Hamamoto H (1997) Petiole length, chlorophyll and carbohydrate levels, and photosynthetic rates of June-bearing strawberry plants as influenced by red-light irradiation during storage under cool conditions. *Scientia Horticulturae* **72**, 25-33

Nunes EDC, Benson EE, Oltramari AC, Araújo PS (2003) *In vitro* conservation of *Cedrela fissilis* Velloza (Meliaceae), a native tree of the Brazilian Atlantic forest. *Biodiversity and Conservation* **12**, 837-848

Ohno R, Takumi S, Nakamura C (2003) Kinetics of transcript and protein accumulation of a low-molecular-weight wheat LEA D-11 dehydrin in response to low temperature. *Journal of Plant Physiology* **160**, 193-200

Olorode O (2004) Conservation of plant genetic resources. *African Journal of Traditional, Complementary and Alternative Medicines* **1**, 4-14

Pandey R, Agarwal RM, Jeevaratnam K, Sharma GL (2004) Osmotic stress-induced alterations in rice (*Oryza sativa* L.) and recovery on stress release. *Plant Growth Regulation* **42**, 79-87

Pardey PG, Koo B, Wright BD, van Dusen ME, Skovmand B, Taba S (2001) Costing the conservation of genetic resources: CIMMYT's *ex situ* maize and wheat collection. *Crop Science* **41**, 1286-1299

Park EJ, Jeknic Z, Sakamoto A, DeNoma J, Yuwansiri R, Murata N, Chen THH (2004) Genetic engineering of glycinebetaine synthesis in tomato protects seeds, plants, and flowers from chilling damage. *The Plant Journal* **40**, 474-487

Parra R, Pastor MT, Perez-Paya E, Amo-Marco JB (2001) Effect of *in vitro* shoot multiplication and somatic embryogenesis on 5-methylcytosine content in DNA of *Myrtus communis* L. *Plant Growth Regulation* **33**, 131-136

Pearce RS (1999) Molecular analysis of acclimation to cold. *Plant Growth Regulation* **29**, 47-76

Pennycooke JC, Cox S, Stushnoff C (2005) Relationship of cold acclimation, total phenolic content and antioxidant capacity with chilling tolerance in petunia (*Petunia* × *hybrida*). *Environmental and Experimental Botany* **53**, 225-232

Perales L, Arbona V, Gomez-Cadenas A, Cornejo MJ, Sanz A (2005) A rela-

tionship between tolerance to dehydration of rice cell lines and ability for ABA synthesis under stress. *Plant Physiology and Biochemistry* **43**, 786-792

Peraza-Echeverria S, Herrara-Valencia VA, James-Kay A (2001) Detection of DNA methylation changes in micropropagated banana plants using methyl-lation-sensitive amplification polymorphism (MSAP). *Plant Science* **161**, 359-367

Peredo EL, Revilla MA, Arroyo-Garcia R (2006) Assessment of genetic and epigenetic variation in hop plants regenerated from sequential subcultures of organogenic calli. *Journal of Plant Physiology* **163**, 1071-1079

Pereira-Netto AB (2001) Effect of inhibitors of ethylene biosynthesis and signal transduction pathway on the multiplication of *in vitro*-grown *Hancornia speciosa*. *Plant Cell, Tissue and Organ Culture* **66**, 1-7

Phartyal SS, Thapliyal RC, Koedam N, Godefroid S (2002) *Ex situ* conservation of rare and valuable forest tree species through seed-gene bank. *Current Science* **83**, 1351-1357

Pierik R, Tholen D, Poorter H, Visser EJW, Voesenek LACJ (2006) The Janus face of ethylene: growth inhibition and stimulation. *Trends in Plant Science* **11**, 176-183

Pietrini F, Chaudhuri D, Thapliyal AP, Massacci A (2005) Analysis of chloro-phyll fluorescence transients in mandarin leaves during a photo-oxidative cold shock and recovery. *Agriculture, Ecosystems and Environment* **106**, 189-198

Pita JM, Perez-Garcia F, Escudero A, de la Cuadra C (1998) Viability of *Avena sativa* L. seeds after 10 years of storage in base collection. *Field Crops Research* **55**, 183-187

Posmyk MM, Bailly C, Szafranska K, Janas KM, Corbineau F (2005) Antioxidant enzymes and isoflavonoids in chilled soybean (*Glycine max* (L.) Merr.) seedlings. *Journal of Plant Physiology* **162**, 403-412

Prasad T (1996) Mechanisms of chilling-induced oxidative stress injury and tolerance in developing maize seedlings: changes in antioxidant system, oxidation of proteins and lipids, and protease activities. *The Plant Journal* **10**, 1017-1026

Priscila MS, Rene E, Sofia VA (2006) Resistance to freezing in three *Eucalyptus globules* Labill subspecies. *Electronic Journal of Biotechnology* **9**, 310-314

Pruski K, Kozai T, Lewis T, Astatkie T, Nowak J (2000) Sucrose and light effects on *in vitro* cultures of potato, chokecherry and saskatoon berry during low temperature. *Plant Cell, Tissue and Organ Culture* **63**, 215-221

Quan R, Shang M, Zhang H, Zhao Y, Zhang J (2004) Improved chilling tolerance by transformation with *betA* gene for the enhancement of glycinebetaine synthesis in maize. *Plant Science* **166**, 141-149

Radic S, Radic-Stojkovic M, Pevalek-Kozlina B (2006) Influence of NaCl and mannitol on peroxidase activity and lipid peroxidation in *Centaurea ragusina* L. roots and shoots. *Journal of Plant Physiology* **163**, 1284-1292

Ranwala AP, Miller WB (2000) Preventive mechanisms of gibberellin$_{4+7}$ and light on low-temperature-induced leaf senescence in *Lilium* cv. Stargazer. *Postharvest Biology and Technology* **19**, 85-92

Rao NK (2004) Plant genetic resources: Advancing conservation and use through biotechnology. *African Journal of Biotechnology* **3**, 136-145

Rao VR, Hodgkin T (2002) Genetic diversity and conservation and utilization of plant genetic resources. *Plant Cell, Tissue and Organ Culture* **68**, 1-19

Reddy AR, Chaitanya KV, Vivekanandan M (2004) Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of Plant Physiology* **161**, 1189-1202

Renau-Morata B, Arrillaga I, Segura I (2006) *In vitro* storage of cedar shoot cultures under minimal growth conditions. *Plant Cell Reports* **25**, 636-642

Renaut J, Hausman JF, Wisniewski ME (2006) Proteomics and low-temperature studies: bridging the gap between gene expression and metabolism. *Physiologia Plantarum* **126**, 97-109

Renaut J, Hoffmann L, Hausman JF (2005) Biochemical and physiological mechanisms related to cold acclimation and enhanced freezing tolerance in poplar plants. *Physiologia Plantarum* **125**, 82-94

Rinne P, Welling A, Kaikuranta P (1998) Onset of freezing tolerance in birch (*Betula pubescens* Ehrh.) involves LEA proteins and osmoregulation and is impaired in an ABA-deficient genotype. *Plant Cell and Environment* **21**, 601-611

Rodrigues-Otubo BM, Siqueira WJ, Filho JAU, Fahl JI, Nagai V (1998) Growth retardant effects on cauliflower micropropagation. *Revista Brasileira de Fisiologia Vegetal* **10**, 179-184

Roh MS (2005) Flowering and inflorescence development of *Lachenalia aloides* 'Pearsonii' as influenced by bulb storage and forcing temperature. *Scientia Horticulturae* **104**, 305-323

Romano A, Martins-Loução MA (1999) *In vitro* storage of cork oak shoot cultures. *Plant Cell, Tissue and Organ Culture* **59**, 155-157

Rouhi V, Samson R, Lemeur R, van Damme PV (2007) Photosynthetic gas exchange characteristics in three different almond species during drought stress and subsequent recovery. *Environmental and Experimental Botany* **59**, 117-129

Roy J, Banerjee N (2002) Rhizome and shoot development during *in vitro* propagation of *Geodorum densiflorum* (Lam.) Schltr. *Scientia Horticulturae* **94**, 181-192

Rugini E, Pesce PG (2006) Genetic improvement of olive. *Pomologia Croatica* **12**, 43-74

Rugini E (1984) *In vitro* propagation of some olive (*Olea europaeasativar* L.) cultivars with different root ability, and medium development using analytical data from developing shoots and embryos. *Scientia Horticulturae* **24**, 123-134

Ryynanen L, Aronen T (2005) Genome fidelity during short- and long-term tissue culture and differentially cryostored meristems of silver birch (*Betula pendula*). *Plant Cell, Tissue and Organ Culture* **83**, 21-32

Sahin-Cevik M, Moore GA (2006) Identification and expression analysis of cold-regulated genes from the cold-hardy *Citrus* relative *Poncirus trifoliata* (L.) Raf. *Plant Molecular Biology* **62**, 83-97

Sakamoto A, Murata N (2002) The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. *Plant Cell and Environment* **25**, 163-171

Sanchez FJ, de Andres EF, Tenorio JL, Ayerbe L (2004) Growth of epicotyls, turgor maintenance and osmotic adjustment in pea plants (*Pisum sativum* L.) subjected to water stress. *Field Crops Research* **86**, 81-90

Santos D, Fevereiro P (2002) Loss of DNA methylation affects somatic embryogenesis in *Medicago truncatula*. *Plant Cell, Tissue and Organ Culture* **70**, 155-161

Sarkar D, Chakrabarti SK, Naik PS (2001) Slow growth conservation of potato microplants: efficacy of ancymidol for long-term storage *in vitro*. *Euphytica* **117**, 133-142

Sarkar D, Kaushik SK, Naik PS (1999) Minimal growth conservation of potato microplants: silver thiosulfate reduces ethylene-induced growth abnormalities during prolonged storage *in vitro*. *Plant Cell Reports* **18**, 897-903

Sarkar D, Sud KC, Chakrabarti SK, Naik PS (2002) Growing of potato microplants in the presence of alginate-silver thiosulfate capsules reduces ethylene-induced culture abnormalities during minimal growth conservation *in vitro*. *Plant Cell, Tissue and Organ Culture* **68**, 79-89

Sato F, Yoshioka H, Fujiwara T, Higashio H, Uragami A, Tokuda S (2004) Physiological responses of cabbage plug seedlings to water stress during low-temperature storage in darkness. *Scientia Horticulturae* **101**, 349-357

Savitch LV, Leonardos ED, Krol M, Jaansson S, Grodzinski B, Huner NPA, Oquist G (2002) Two different strategies for light utilization in photosynthesis in relation to growth and cold acclimation. *Plant Cell and Environment* **25**, 761-771

Schenk RU, Hildebrandt AC (1972) Medium and techniques for induction and growth monocotyledonous and dicotyledonous plant cell cultures. *Canadian Journal of Botany* **50**, 199-204

Schroth G, da Mota MSS, Lopes R, de Freitas AF (2004) Extractive use, management and *in situ* domestication of a weedy palm, *Astrocaryum tucuma*, in the central Amazon. *Forest Ecology and Management* **202**, 161-179

Scocchi AM, Mroginski LA (2004) *In vitro* conservation of apical meristem-tip of *Melia azedarach* L. (Meliaceae) under slow growth conditions. *International Journal of Experimental Botany* **53**, 137-143

Sharma TR, Singh BM (1995) *In vitro* microrhizome production in *Zingiber officinale* Rosc. *Plant Cell Reports* **15**, 274-277

Sharma TR, Singh BM (1997) High-frequency *in vitro* multiplication of disease-free *Zingiber officinale* Rosc. *Plant Cell Reports* **17**, 68-72

Shattuck VI, Kakuda Y, Shelp BJ (1991) Effect of low temperature on the sugar and glucosinolate content of rutabaga. *Scientia Horticulturae* **48**, 9-19

Shibli RA, Shatnawi MA, Subaih WS, Ajlouni MM (2006) *In vitro* conservation and cryopreservation of plant genetic resources: A review. *World Journal of Agricultural Sciences* **2**, 372-382

Shirgurkar MV, John CK, Nadgauda RS (2001) Factors affecting *in vitro* microrhizome production in turmeric. *Plant Cell, Tissue and Organ Culture* **64**, 5-11

Sircelj H, Tausz M, Grill D, Batic F (2005) Biochemical responses in leaves of two apple tree cultivars subjected to progressing drought. *Journal of Plant Physiology* **162**, 1308-1318

Slabbert MM, Niederwieser JG (1999) *In vitro* bulblet production of *Lachenalia*. *Plant Cell Reports* **8**, 620-624

Smallwood M, Bowles D (2002) Plant in a cold climate. *Philosophical Transactions of the Royal Society of London - Biological Sciences* **357**, 831-847

Smith RH, Murashige T (1970) *In vitro* development of the isolated shoot apical meristem of angiosperms. *American Journal of Botany* **57**, 562-568

Son SH, Chun YW, Hall RB (1991) Cold storage of *in vitro* cultures of hybrid poplar shoots (*Populus alba* L. × *P. grandidentata* Michx.). *Plant Cell, Tissue and Organ Culture* **27**, 161-168

Sror HAM, Tischendorf G, Sieg F, Schmitt JM, Hincha DK (2003) Cryoprotectin protects thylakoids during a freeze-thaw cycle by a mechanism involving stable membrane binding. *Cryobiology* **47**, 191-203

Strand A, Foyer CH, Gustafsson P, Gardestrom P, Hurry V (2003) Altering flux through the sucrose biosynthesis pathway in transgenic *Arabidopsis thaliana* modifies photosynthetic acclimation at low temperatures and the development of freezing tolerance. *Plant Cell and Environment* **26**, 523-535

Strandberg JO (1993) Meristem culture of *Ophiopogon japonicus* and production of embryo-like structures. *Plant Cell, Tissue and Organ Culture* **32**, 277-82

Sturm A, Tang GQ (1999) The sucrose-cleaving enzymes of plants are crucial for development, growth and carbon partitioning. *Trends in Plant Science* **4**, 401-407

Sung DY, Kaplan F, Lee KJ, Guy CL (2003) Acquired tolerance to tempera-

ture extremes. *Trends in Plant Science* **8**, 179-187

Syros T, Yupsanis T, Petkou D, Economou AS (2005) Protein, leucine aminopeptidase, esterase acid phosphatase and photosynthetic responses of oleander (*Nerium oleander* L.) during cold acclimation and freezing treatments. *Journal of Plant Physiology* **162**, 886-894

Sysoeva MI, Markovskaya EF, Kharkina TG, Sherudilo EG (1999) Temperature drop, dry matter accumulation and cold resistance of young cucumber plants. *Plant Growth Regulation* **28**, 89-94

Sysoeva MI, Sherudilo EG, Markovskaya EF, Obshatko LA, Matveyeva EM (2005) Temperature drop as a tool for cold tolerance increment in plants. *Plant Growth Regulation* **46**, 189-191

Tabaei-Aghdaei SR, Pearce RS, Harrison P (2003) Sugars regulate cold-induced gene expression and freezing-tolerance in barley cell cultures. *Journal of Experimental Botany* **54**, 1565-1575

Tambussi EA, Bartoli CG, Guiamet JJ, Beltrano J, Araus JL (2004) Oxidative stress and photodamage at low temperatures in soybean (*Glycine max* L. Merr.) leaves. *Plant Science* **167**, 19-26

Tambussi EA, Nogues S, Araus JL (2005) Ear of durum wheat under water stress: water relations and photosynthetic metabolism. *Planta* **221**, 446-458

Tanaka Y, Sano T, Tamaoki M, Nakajima N, Kondo N, Hasezawa S (2005) Ethylene inhibits abscisic acid-induced stomatal closure in *Arabidopsis*. *Plant Physiology* **138**, 2337-2343

Taylor PWJ, Dukic S (1993) Development of an *in vitro* culture technique for conservation of *Saccharum* spp. hybrid germplasm. *Plant Cell, Tissue and Organ Culture* **34**, 217-222

Theilade I, Petri L (2003) Conservation of tropical trees *ex situ* through storage and use. Guideline and Technical Notes No. 65. Danida Forest Seed Centre, Humlebaek, Denmark

Thomas J, Vijayan D, Joshi SD, Lopez SJ, Kumar RR (2006) Genetic integrity of somaclonal variants in tea (*Camellia sinensis* (L.) O Kuntze) as revealed by inter simple sequence repeats. *Journal of Biotechnology* **123**, 149-154

Thomashow MF (1999) Plant cold acclimation: Freezing tolerance genes and regulatory mechanisms. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 571-599

Toldi O, Ahanen K, Kovacs G, Sorvari S, Toth S, Dulai S, Scott P (2005) Integrated application of physiological and molecular methods to forecast determinative morphogenetic events in tissue cultured tobacco (*Nicotiana tabacum* L. cv Samsun) leaf discs. *Plant Growth Regulation* **47**, 59-64

Tommasi F, Paciolla C,de Pinto MC, de Gara L (2006) Effects of storage temperature on viability, germination and antioxidant metabolism in *Ginkgo biloba* L. seeds. *Plant Physiology and Biochemistry* **44**, 359-368

Torres J, Mogollon N (2002) Effect of PBZ on budbreak and development *in vitro* of the leaf epidermis of *Cattleya mossiae* Parker ex Hooker previous to acclimatization. *Biologia Agronomy* **14**, 25-28

Turkan I, Bor M, Ozdemir F, Koca H (2005) Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. *Plant Science* **168**, 223-231

Turner S, Krauss SL, Bunn E, Senaratna T, Dixon K, Tan B, Touchell D (2001) Genetic fidelity and viability of *Anigozanthos viridis* tissue culture, cold storage and cryopreservation. *Plant Science* **161**, 1099-1106

Tyagi RK, Agrwal A, Yusuf A (2006) Conservation of *Zingiber* germplasm through *in vitro* rhizome formation. *Scientia Horticulturae* **108**, 210-219

Uemura M, Steponkus PL (2003) Modification of intracellular sugar content alters the incidence of freeze-induced membrane lesions of protoplasts isolated from *Arabidopsis thaliana* leaves. *Plant and Cell Physiology* **26**, 1083-1096

Uemura M, Tominaga Y, Nakagawara C, Shigematsu S, Minami A, Kawamura Y (2006) Responses of the plasma membrane to low temperatures. *Physiologia Plantarum* **126**, 81-89

Uyoh EA, Nkang AE, Eneobong EE (2003) Biotechnology, genetic conservation and sustainable use of bioresources. *African Journal of Biotechnology* **2**, 704-709

Valentovic P, Laxova M, Kolarovic L, Gasparikova O (2006) Effect of osmotic stress on compatible solutes content, membrane stability and water relations in two maize cultivars. *Plant Soil Environment* **52**, 186-191

van den Berg L, Zeng YJ (2006) Response of South African indigenous grass species to drought stress induced by polyethylene glycol (PEG) 6000. *South African Journal of Botany* **72**, 284-286

van Heerden PDR, Viljoen MM, de Villiers MF, Kruger GHJ (2004) Limitation of photosynthetic carbon metabolism by dark chilling in temperate and tropical soybean genotypes. *Plant Physiology and Biochemistry* **42**, 117-124

Vandenbussche B, Leuridan S, Verdoodt V, Gysemberg M, de Proft M (1999) Changes in sugar content and fatty acid composition of *in vitro* sugar beet shoots after cold acclimation: influence on survival after cryopreservation. *Plant Growth Regulation* **28**, 157-163

Virchow D (1999) Conservation costs of PGRFA. In: Virchow D (Ed) *Conservation of Genetic Resources – Costs and Implications for Sustainable Utilisation of Plant Genetic Resources for Food and Agriculture*, Springer-Verlag, Heidelberg, pp 101-144

Vlasova TI, Demidenko ZN, Kirnos MD, Vanyushin BF (1995) *In vitro* DNA methylation by wheat nuclear cytosine DNA methyltransferase: effect of phytohormones. *Gene* **157**, 279-281

Walkey DGA, Webb JW, Bolland CJ, Miller A (1987) Production of virus-free garlic (*Allium sativum*) and shallot (*A. Ascalonicum*) by meristem-tip culture. *HortScience* **62**, 211-20

Wang X, Li W, Li M, Welti R (2006) Profiling lipid changes in plant response to low temperatures. *Physiologia Plantarum* **126**, 90-96

Wannakrairoj S (1998) *In vitro* preservation of *Vanda coerulea* Giff. Protocorm-like bodies. *Kasetsart Journal* **32**, 329-332

Watanabe S, Kojima K, Ide Y, Sasaki S (2000) Effects of saline and osmotic stress on proline and sugar accumulation in *Populus euphratica in vitro*. *Plant Cell, Tissue and Organ Culture* **63**, 199-206

Withers LA (1991) Plant tissue culture: Maintenance of plant tissue culture. In: Kirsop BE, Doyle A (Eds) *Maintenance of Microorganisms and Cultured Cells: A Manual of Laboratory Methods* (2nd Edn), Academic Press Inc., San Diego, CA, pp 243-255

Wurr DCE, Fellows JR, Andrews L (2000) The effects of temperature and daylength on flower initiation and development in *Dianthus allwoodii* and *Dianthus alpinus*. *Scientia Horticulturae* **86**, 57-70

Xin Z, Browse J (2000) Cold comfort farm: the acclimation of plants to freezing temperatures. *Plant Cell and Environment* **23**, 893-902

Xu RY, Niimi Y, Han DS (2006) Changes in endogenous abscisic acid and soluble sugars levels during dormancy-release in bulbs of *Lilium rubellum*. *Scientia Horticulturae* **111**, 68-72

Yoshida S, Hotsubo K, Kawamura Y, Murai M, Arakawa K (1999) Alterations in intracellular pH in response to low temperature stress. *Journal of Plant Research* **112**, 225-236

Zandvoort EA, Hulshof MJH, Staritsky G (1994) *In vitro* storage of *Xanthosoma* spp. under minimal growth conditions. *Plant Cell, Tissue and Organ Culture* **36**, 309-316

Zhang X, Zhou S, Fu Y, Su Z, Wang X, Sun C (2006) Identification of a drought tolerant introgression line derived from Dongxiang common wild rice (*O. rufipogon* Griff.). *Plant Molecular Biology* **62**, 247-259

Zhou B, Guo Z, Lin L (2006) Effects of abscisic acid application on photosynthesis and photochemistry of *Stylosanthe guianensis* under chilling stress. *Plant Growth Regulation* **48**, 195-199

Zhou B, Guo Z, Liu Z (2005) Effects of abscisic acid on antioxidant systems of *Stylosanthes guianensis* (Aublet) Sw. under chilling stress. *Crop Science* **45**, 599-605

Zobayed SM (2000) *In vitro* propagation of *Lagerstroemia spp.* from nodal explants and gaseous composition in the culture head space. *Environmental Control in Biology* **38**, 1-11

Zobayed SMA, Armstrong J, Armstrong W (1999) Evaluation of a closed system, diffusive and humidity-induced convective through flow ventilation on the growth and physiology of cauliflower *in vitro*. *Plant Cell, Tissue and Organ Culture* **59**, 113-123

Zuther E, Buchel K, Hundertmark M, Stitt M, Hincha DK, Heyer AG (2004) The role of raffinose in the cold acclimation response of *Arabidopsis thaliana*. *Federation of European Biochemical Societies Letters* **576**, 169-173