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FOREWORD

The International Seminar on Tropical Horticulture 2016 was held in IPB International Convention Center, Bogor, Indonesia 28 – 29 November 2016. This seminar was organized by Center of Excellence for Tropical Horticulture Studies (PKHT), Center of Excellence in University (PUI-PT), Bogor Agricultural University (IPB), and supported by an excellent collaboration with International Tropical Fruits Network (TF Net).

We're very glad to know the fact that the seminar displayed a very wide discussion about tropical horticulture with delegates from 5 countries (Taiwan, Thailand, Malaysia, Japan and Indonesia) as keynote speech and participants. 24 papers were selected to be included in this proceeding from 28 oral and 31 poster presentation.

This proceeding is contained of three sub chapter, that is fruits, vegetables and miscellaneous. There are 9 papers of fruits chapter, 12 papers of vegetables chapter and 3 papers of miscellaneous chapter. We wish to thank Sanjeet Kumar, Ph.D, Prof. Sobir, Prof Masayoshi Shigyo, Dr. Mohd Desa Haji Hassim, Parson Saradhuldhat, Ph.D for being keynote speech at this international seminar and all participants for very lively atmosphere during and after the seminar.

Bogor, May 2017

Editor

Dr. Darda Efendi
Dr. Awang Maharijaya

SYMPOSIUM PROGRAM

28 November 2016

07.30 – 09.00	*Registration desk open and morning coffee*
09.00 – 09.30	Welcome addresses **Dr. Darda Efendi**, Director of Center for Tropical Horticulture Studies, Indonesia **Prof. Herry Suhardiyanto,** Rector of Bogor Agricultural University, Indonesia
09.30 – 12.00 (20 minutes presentation + 10 minutes discussion)	**Session 1: Introductory Topics** **Dr. Sanjeet Kumar,** World Vegetable Center, Taiwan *"Science and Art of Tropical Horticulture: Stories, Impacts and Prospects"* **Prof. Sobir,** Indonesian Center of Excellence for Tropical Horticulture *"Tropical Horticulture: Past, Present and Future"* **Gregori Hambali, MSc,** Mekarsari, Indonesia *"Managing Tropical Fruit Collection"*
12.00 – 13.00	*Lunch*
13.00 – 14.30 (20 minutes presentation + 10 minutes discussion)	**Session 2: Opportunity in Tropical Horticulture Industry** **Prof. Muhammad Firdaus,** Bogor Agricultural University *"Enhancing the Competitiveness of Tropical Horticulture Products"* **Dr. Mohd Desa Haji Hassim,** International Tropical Fruit Network, Selangor, Malaysia *"Issues and Challenges in The Global Tropical Fruit Market"* **Parson Saradhuldat, Ph.D,** Department of Horticulture, Kasestsart University, Thailand *"Tropical Horticulture Business in Thailand"*
14.30 – 16.00 (20 minutes presentation + 10 minutes discussion)	**Session 3: Quality of Horticultural Products** **Dr. Darda Efendi,** Center for Tropical Horticulture Studies, Indonesia *"Quality Issues in Tropical Horticultural Products"*

	Tatas H. P. Brotosudarmo, PhD, Ma Chung University *"*Non-optical and optical spectroscopy as metabolomics platforms for determining the quality of horticultural products*"* **Dr. Irmanida Batubara,** Tropical Biopharmaca Research Center *" Quality Control on Herbal Medicine"*

29 November 2016

07.30 – 08.30	*Registration desk open*	
08.30 – 10.15	**Parallel session 1**	**Parallel session 2**
10.15 – 10.30	*Coffee Break and Poster Session*	
10.30 – 12.15	**Parallel session 3**	**Parallel session 4**
12.15 – 13.00	*Lunch*	
13.00 – 15.00 (@20 minutes presentation + 10 minutes discussion)	**Session 4 : Technology Needs for Improving Horticulture in The Tropics** **Prof. Masayoshi Shigyo,** Yamaguchi University, Japan *"Proposal for a forwarding model in order to encourage social interaction among HRs and/or PGRs via platform operation based on research collaboration in Indonesian vegetable crops"* **Prof. Sri Hendrastuti Hidayat,** Department of Plant Protection. Faculty of Agriculture. Bogor Agricultural University *"Integrated Disease Management for Vegetable Crops: Concepts and Practices"* **Dr. Catur Hermanto,** Indonesian Vegetables Research Institute (IVEGRI) *"Pest And Disease Threats and Challenges For Future Vegetable In The Tropic*"	
15.00 – 16.00	*Concluding and Remarks*	
16.00 – 18.00	*Farewell Drink*	

ORAL PRESENTATION SCHEDULE

Tuesday, November 29th 2016

Paralel 1

TIME	PRESENTER NAME	CODE	TITLE
08.30 – 08.45	Slamet Susanto	OP 1	Prolong Shelflife of Seedless Pummelo (*Citrus maxima* (L.) Osbeck) Fruit During Storage
08.45 – 09.00	Dini Hervani	OP 2	Cryopreservation of Long-term Plant Germplasm Storage
09.00 – 09.15	Sulassih	OP 3	Variability of Jackfruit Based on Morphology and Molecular ISSR
09.15 – 09.30	Ahmad Solikin	OP 4	Characterization of Local Durian Varieties In Central Java Using Molecular Markers Inter Simple Sequence Repeats (ISSR)
09.30 – 09.45	Nelinda	OP 5	Packaging Design and Postharvest Treatment to Maintain the Quality of Rambutan (*Nephelium Lappaceum* L.) in Distribution System
09.45 – 10.00	Maxmilyand Leiwakabessy	OP 6	Disease Incidence and Molecular Analysis of Banana Bunchy Top Virus in Bogor, West Java
10.00 – 10.15	Ajmir Akmal	OP 7	Transpiration rate of relationship fruit with Gamboge of Mangosteen (*Garcinia mangostana* L.)

Paralel 2

TIME	PRESENTER NAME	CODE	TITLE
08.30 – 08.45	Juang Gema Kartika	OP 8	Growth and Production of Some *Moringa oleifera* Lam. Accession at Several Harvesting Interval
08.45 – 09.00	Lutfi Izhar	OP 9	Conservation Agriculture with Soil Health: Optimal Fosfor Fertilizer Rate for Tomato (*Lycopersicon esculentum* Mill. L) on Inceptisols
09.00 – 09.15	Adhitya Mahendra K	OP 10	Stakeholders Analysis in Seed Provision System Development Originated from True Seed of Shallot
09.15 – 09.30	Endro Gunawan	OP 11	Policy Analysis on Shallot Stock Seed Program Though The Botanical Seed (*True Shallot Seed*) TSS
09.30 – 09.45	Ali Asgar	OP 12	Integrating Understanding of Indigenous Vegetable Nutrients and Benefits
09.45 – 10.00	Marlin	OP 13	Metabolite Changes in Shallot (*Allium cepa* var *aggregatum*) during Vernalization
10.00 – 10.15	Suhesti Kusuma Dewi	OP 14	The Effects of Vernalization and Photoperiod on Flowering of Shallot (*Allium cepa* var. *ascalonicum* Baker) in Lowland Area

Paralel 3

TIME	PRESENTER NAME	CODE	TITLE
10.30 – 10.45	Satriyas Ilyas	OP 15	Study of Phenology and Determination of Seed Physiological Maturity of Long Bean (*Vigna sinensis* L.) Based on Heat Unit
10.45 – 11.00	Endah Retno Palupi	OP 16	Chromosome Number Estimation of Diploid, Autotetraploid and Triploid Hybrid 'Rejang' Banana Using Protoplast from Male Flower (anther)
11.00 – 11.15	Yudiwanti Wahyu	OP 17	Performance of Some First Generation Corn Populations derived from Selfing and Sibbing for Developing Baby Corn Varieties
11.15 – 11.30	Ady Daryanto	OP 18	Inheritance of Chili Pepper Resistance Against Infestation of *Aphis gossypii* Glover (Hemiptera: Aphididae)
11.30 – 11.45	Edi Santosa	OP 19	Variation in Floral Morphology of Agamosporous *Amorphophallus Muelleri* Blume of Natural and Gibberellins Treatment
11.45 – 12.00	Kusuma Darma	OP 20	The Eco-Friendly Technology to Control Pests and Diseases of Shallot
12.00 – 12.15	Filemon Yusuf	OP 21	Phylogenetic Study of Indigenous Pulses Based on Morphological and Inter Simple Sequence Repeat (ISSR) Markers

Paralel 4

TIME	PRESENTER NAME	CODE	TITLE
10.30 – 10.45	Ririh Sekar Mardisiwi	OP 22	Growth and Production of Black Cumin (*Nigela sativa* L.) at Several Composition Media and Watering Interval
10.45 – 11.00	Evi Setiawati	OP 23	Growth and Production of Black Cumin (*Nigela sativa* L.) at Shade Levels and Nitrogen Doses
11.00 – 11.15	Tatik Raisawati	OP 24	The Nutritional Value and Total Flavonoid Content of *Sonchus arvensis* L. Leave
11.15 – 11.30	Dewi Sukma	OP 25	Diversity Analysis of Phalaenopsis by Using SNAP Marker
11.30 – 11.45	Widya Sari	OP 26	Morphological, Molecular Charactheristics and Pathogenicity of *Fusarium* spp. from Some Cultivars of Banana
11.45 – 12.00	Juwartina Ida Royani	OP 27	In Vitro Shoots Multiplication of Sapodilla (*Manilkara zapotta* Van Royen) with Modified MS Media
12.00 – 12.15	Willy B. Suwarno	OP 28	Melon Breeding: Past Experience and Future Challenge

Table of Content

Fruits

Evaluation of Morphological and Cytological Character of F1 Diploid Hybrid Banana Sapon and *Musa acuminata* var. *tomentosa* (K.Sch) Nasution
Diyah Martanti, Tri Handayani and Yuyu Suryasari Poerba 1

Fruit Plants of Kalimantan : Results of Field Exploration and Conservation
Sudarmono 9

Melon Breeding: Past Experiences and Future Challenges
Willy B. Suwarno, Sobir, and Endang Gunawan 16

In vitro shoots multiplication of Sapodilla (*Manilkara zapotta* Van Royen) with modified MS media
Juwartina Ida Royani 24

Confirmation Number of Chromosome Diploid, Autotetraploid and Triploid Hybrid 'Rejang' Banana Using Digested Anther
Tri Handayani, Diyah Martanti, Yuyu S. Poerba, Witjaksono 31

Disease Incidence and Molecular Analysis of Banana Bunchy Top Virus in Bogor, West Java
Maxmilyand Leiwakabessy, Sari Nurulita, Sri Hendrastuti Hidayat 37

The Potential of Liquid Smoke Coconut Shell in Extending The Shelf Life of Tropical Fruits
Ira Mulyawanti, Sari Intan Kailaku and Andi Nur Alamsyah 46

The Effects of The Application of Edible Coating, Antimicrobial Agent, Packaging and Absorber on Snake Fruit (*Salacca edualis* REINW)
Sari Intan Kailaku, Ira Mulyawanti, Asep W Permana and Evi Savitri Iriani 50

Packaging Design and Postharvest Treatment to Maintain the Quality of Rambutan (*Nephelium Lappaceum* L.) in Distribution System
Nelinda, Emmy Darmawati, Ridwan Rachmat, Lilik Pujantoro Eko Nugroho 57

Characterization of Local Durian Varieties in Central Java Using Molecular Markers Inter Simple Sequence Repeats (ISSR)
Ahmad Solikin, Amin Retnoningsih, and Enni S. Rahayu 65

Vegetables

Shallot Varieties Adaptation in Napu Highlands, Central Sulawesi
Saidah, Abdi Negara and Yogi P Rahardjo 77

Collection and Characterization of Shallot Germplasm in Effort to Support National Food Security
Ita Aprilia, Erviana Eka Pratiwi, Awang Maharijaya, Sobir, Heri Harti 81

Optimum Fertilizer of Shallot on Andisol and Latosol Soils
Gina Aliya Sopha, Suwandi 86

Effect of Organic Fertilization on The Growth and Yields of New Onion Varieties in Limited Land
I Ketut Suwitra dan Yogi P. Raharjo 94

Interaction Between Varieties and Plastic Mulch on Shallot Growth in Dryland South Kalimantan
Lelya Pramudyani 98

Effect of *Trichoderma* and *Penicillium* Application (Isolated From Pine Rhizosphere) to The Shallot Growth
Shinta Hartanto dan Eti Heni Krestini 107

The Effects of Vernalization and Photoperiod on Flowering of Shallot (*Allium cepa* var. *ascalonicum* Baker) in Lowland Area
Suhesti Kusumadewi, Hamim, Sobir 112

Metabolite Changes in Shallot (*Allium cepa* var *aggregatum*) during Vernalization
Marlin, Awang Maharijaya, Sobir, Agus Purwito 118

Stakeholders Analysis in the Development of Seed Provision System Originating from True Seed of Shallot
Adhitya Marendra Kiloes, Puspitasari, and Turyono 124

Policy Analysis on Shallot Stock Seed Program through The Botanical Seed (True Shallot Seed/TSS)
Endro Gunawan and Rima Setiani 131

The Dynamic of Shallot Production, Supply and Price after the Implementation of Horticulture Import Regulations
Puspitasari and Adhitya Marendra Kiloes 136

Characterization and Resistance to Bacterial Wilt Diseases (*Ralstonia solanacearum*) of 20 Eggplant (*Solanum melongena* L.) Genotypes
Heri Harti, Teni Widia, Pritha, Awang Maharijaya 143

Miscellaneous

Cryopreservation for Long-term Plant Germplasm Storage
Dini Hervani, Darda Efendi, M. Rahmad Suhartanto, Bambang S. Purwoko 149

Good Manufacturing Practices (GMP) for Fresh-Cut Fruits and Vegetables
Sari Intan Kailaku, Ira Mulyawanti and Andi Nur Alam Syah 154

Breeding of Anthurium (*Anthurium andreanum*) : A strategy to produce new clones as tropical ornamental plants
Ridho Kurniati, Kurnia Yunianto, Suskandari Kartikaningrum 161

Cryopreservation for Long-term Plant Germplasm Storage

Dini Hervani [1)], Darda Efendi[2)], M. Rahmad Suhartanto[2)], Bambang S. Purwoko[2)]

[1)] *Postgraduate student of Plant Breeding and Biotechnology study program, Department of Agronomy and Horticulture, Graduate School of IPB.*

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Abstract

Germplasm is a source of genetic diversity that is essential for sustained through the long term. Seeds germplasms storage that are not resistant to desiccation can be carried out by cryopreservation. Cryopreservation is a method of storing the plant material in liquid nitrogen (-196oC) so the metabolic processes in cells, tissues or organs that are stored can be stopped so the plant material can be stored in a very long time without a genetic change or somaclonal variation. Cryoprotectants in cryopreservation storage is needed for plant material protection to adapt to the very low temperature. Some examples of successful treated-plant for cryopreservation are cocoa and papaya. In cocoa, proembryo somatic derived from cryopreservation cocoa zygotic embryo can creating embryos survived and were able to grow a callus back as much as 46.67% is dimethylsulfoxide (DMSO) and glycerol with a concentration of 15% each in MS0 solution, at a temperature of thawing (melting) 35oC by using culture media such as media restorer MS + picloram 0.1 g L-1 + thiamin 0.2 g L-1 + IAA 1 g L-1 + kinetin 0.1 g L-1. In Sukma papaya seed varieties treatment with cryoprotectants of PVS2 submersion for 30 minutes and the rate of 11-13% water content provides the best germination value (DB), the maximum growth potential (PTM), and the better rate of growth (KCT), 38.39%, 38.39%, and 2:24%, respectively.

Keywords: cryopreservation, cryoprotectant, germplasm

1. Introduction

Indonesia has a big biodiversity, including genetic diversity of plants, of which approximately 11% of the world's plant species are tucked away in the woods as a wild species and other agricultural lands spread over thousands of islands in Indonesia (Leunufna 2007). Sources of genetic diversity of plant crop as germplasm is very important to be utilized in the future, for the science development and understand in depth the plant's benefits. Germplasm is a term of living organism or groups that determine their characteristics (Mugnozza and Perrino 2002).

Along with land exploration due to population race, uncontrolled forest exploitation, as well as the growing number of high-yielding varieties of plant breeding, it can create the wealth of plants available plasmanutfah shrinking very rapidly. Wattimena and Ansori (1992) states that the collection and preservation of plasmanutfah needed both to improve the quality and quantity of plants and is also required to facilitate the testing of other experiments with certain techniques. Thus the storage and preservation seed plants are not only intended to supply planting the following season, but can also be intended to supply the medium term and long term.

Germplasm preservation in plants can be carried out either in situ or ex situ outside the habitat. In situ plant preservation is an attempt to maintain the plants growth in the natural habitat. This method is benefit for plants because plants will not adapt to stress condition on the new environment, but also has some risks against the possibility of germplasm loss in open field and operating costs (Leunufna 2007), in addition to biotic and abiotic stress also affects the level of genetic diversity of plants its self (Roostika 2013). Ex situ plant preservation is a such plant conservation by moving the plants from the growth point of origin. By using ex situ preservation, the collection can be observed. Ex situ preservation includes botanical gardens, collection, storage of seeds, in vitro preservation, pollen storage and genebanks (Engelmann 2000).

The germplasm storage system are not the same from one plant to another. This is due to the diversity of the natural characteristic and condition of the crop. The diversity of the seeds character will make the way of germplasm plants storage are also not the same from one plant to another. Most plants produce orthodox seeds, where the seeds can be dried up by the resilience of the water content (KA) <7% and stored at low temperature, so the seeds do not have problems when stored for a long period of time (Engelmann 2000). Most other plants produce seeds are recalcitrant, where the seed is less resistant or have constraints to be saved in the long term because it is not resistant to low temperatures and low water content, where resistance moisture content> 20%. Seeds that have condition of orthodox seed but resistant to moisture (KA) 10-12% referred to intermediate seeds (Uyoh *et al.* 2003). Approximately 70% of tropical plant species produce recalcitrant seeds, including forest plants, crops and fruit trees (Normah 2000).

Germplasm storage solutions for plants with intermediate and recalcitrant seeds usually through in vitro culture. In connection with the storage length of in vitro techniques can be divided into two general categories: (1) storage of short or medium term with the aim of simply growth suppressing for a while, using a low temperature, the addition of inhibitors, the addition of mineral oil and impoverishment of the culture media, and (2) with the objective of long-term storage, where the metabolic activity actually stopped but the cells keep live. In this case usually used liquid nitrogen (N2) with a very low temperature (-196°C). This technique is known as cryopreservation.

In general if the plant material is stored at very low temperatures can cause damage to the plant cell. The plant cell membrane will act as a barrier to the formation of ice crystals from the water content within cell so the cell can not survive if a temperature of liquid nitrogen are very low. During freezing and melting, plant cells can be damaged as a result of being forced to adapt with very low temperature, the formation of ice crystals, and dehydrated (Reinhoud *et al.* 2000).

Cell damage that occurs when plant material facing very low temperatures can be overcome by dehydration or drying stages to avoid the formation of ice crystals using cryoprotectants, such as glycerol, dimethylsulfoxide (DMSO), and sucrose. When the cryopreservation process in plants has been completed and the plant will be continously activated then cryoprotectants should be immediately removed from the planting material through washing stages with liquid culture such as cryoprotectants but it can be toxic to the plant cells (Watanabe *et al.* 1999). In general, the stages of cryopreservation techniques is including pre-growth, pre-culture, loading, dehydration, freezing, melting, replacement charge (deloading or unloading), recovery, and regeneration (Roostika 2013).

2. Methodology

In general, the cryopreservation technique is often done by classical and new cryopreservation techniques. Classical cryopreservation techniques (slow cooling cryopreservation) which cover or soaking the planting material in a solution of cryoprotectants and stages to reach the temperature that can freeze, followed by storage in liquid nitrogen. Techniques of new cryopreservation (fast freezing cryopreservation / vitrification), based on the phase transition from liquid form into the shape of non-crystalline or amorphous glassy (invisibility) for the planting material are placed on media cryoprotectants that highly concentrate then immediately placed in liquid nitrogen (Engelmann 2000). Solution in vitrification often used PVS2 [*Plant Vitrification Solution* 2 by Sakai *et al.* (1991)], which contains 30% gliserol, 15% dimethylsulfoxide (DMSO), and 15% etilen glicol (EG)].

Cryopreservation in plant cell culture and in the culture of others, includes several steps: providing sterile tissue, the addition of cryoprotectants and providing early treatment, freezing, storage, melting, viability testing and plants growth and regeneration. Increasing the viability of planting material either morphologicaly and physiologicaly must be improved because it will adapt to the freezing conditions at temperatures of -196°C.

Some networks or institution often manage the planting material to be stored in the cryopreservation in form of apical meristem and lateral organs of plants (embryo, endosperm, ovule, anthers / pollen), seeds, cell culture, somatic proembryo, protoplast, callus, etc. In general, the smaller planting material is rich in cytoplasmic and meristem cells will be able to last longer in this cryopreservation technique.

Permeability of cells during cryopreservation needs to be maintained by adding protective material in the form of a solution called cryoprotectants. The application of cryoprotectants will keep the form of the cells during the freezing process occurs by increasing the concentration of the solution and prevent the formation of ice crystals by dehydration or drying stage (Simione 1998). Cryoprotectants are frequently used and give the best effectiveness are dimethylsulfoxide (DMSO) and glycerol. Cryoprotectants should be used in the appropriate composition and soaking time.

3. Discussion

Some plants, especially plants that have recalcitrant or intermediate seed properties has been successfully stored in cryopreservation. Examples of successful treated-plant for cryopreservation are cocoa and papaya. In cocoa, proembryo somatic derived from cryopreservation cocoa zygotic embryo used as an plant material of slow cooling technique, because proembryo somatic cell more compact, has denser cytoplasm and still in the globular phase. The combination of cryoprotectants that creating embryos survived and were able to grow a callus back as much as 46.67% is dimethylsulfoxide (DMSO) and glycerol with a concentration of 15% each in MS0 solution, at a temperature of thawing (melting) 35°C by using culture media such as media restorer MS + picloram 0.1 g L-1 + thiamin 0.2 g L-1 + IAA 1 g L-1 + kinetin 0.1 g L-1, as shown in Figure 1 (Hervani 2006).

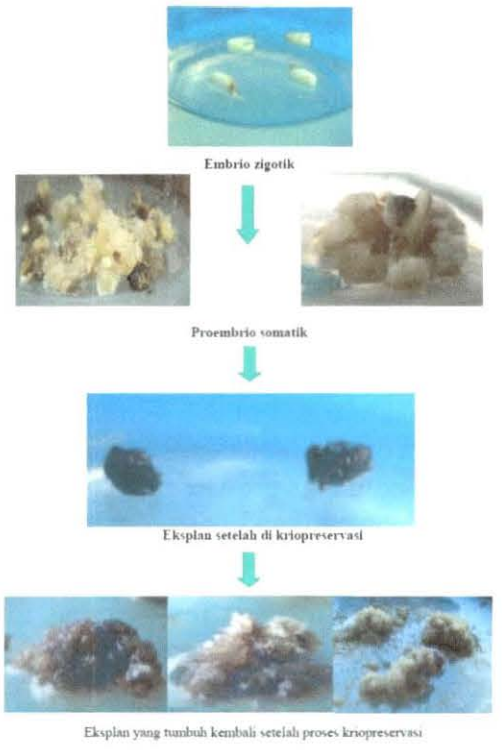

Figure 1. Development of proembryo somatic explants after cryopreservation using cryopreservation method of slow cooling at the age of 24 weeks after planting

In the next stage of the study, the researchers work with Sukma papaya seed varieties whose seeds are intermediates seed to be preserve in the long term as cryopreservation. Treatment with cryoprotectants of PVS2 submersion for 30 minutes and the rate of 11-13% water content provides the best germination value (DB), the maximum growth potential (PTM), and the better rate of growth (KCT), 38.39%, 38.39%, and 2:24%, respectively. Seed without cryoprotectants is not able to grow back and the seeds are soaked too long in PVS2 deliver the low value of DB and KCT (Hervani *et al.* 2016).

Seeds that are not protected or immersed in cryoprotectant prior to entry of liquid nitrogen is not able to grow at all. This is because the cells contain a lot of water so it would not survive if a temperature of liquid nitrogen are very low. During freezing and melting process, plant cells can be damaged due to: (1) force adaptation a very low temperature, (2) the formation of ice crystals, (3) dehydrated, and (4) the formation of free radicals. During the cooling process, the lipids in the membrane will undergo a transition phase from the liquid crystal into a gel phase. This process results in leakage of the cell so the cell becomes damaged, since not all the lipid transition phase at the same temperature. At low temperatures, some proteins in plant cells will be inactive due to the nature of the protein that is sensitive to low temperatures (Reinhoud *et al.* 2000).

4. Conclusion

Cryopreservation currently offers the only safe and cost effective option for the long-term conservation of genetic resources. An important key to the activities of cryopreservation is appropriate cryoprotectant concentration, soaking period time, the selection of planting material to be stored in cryopreservation, thawing process and reactivation cell growth after cryopreservation.

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