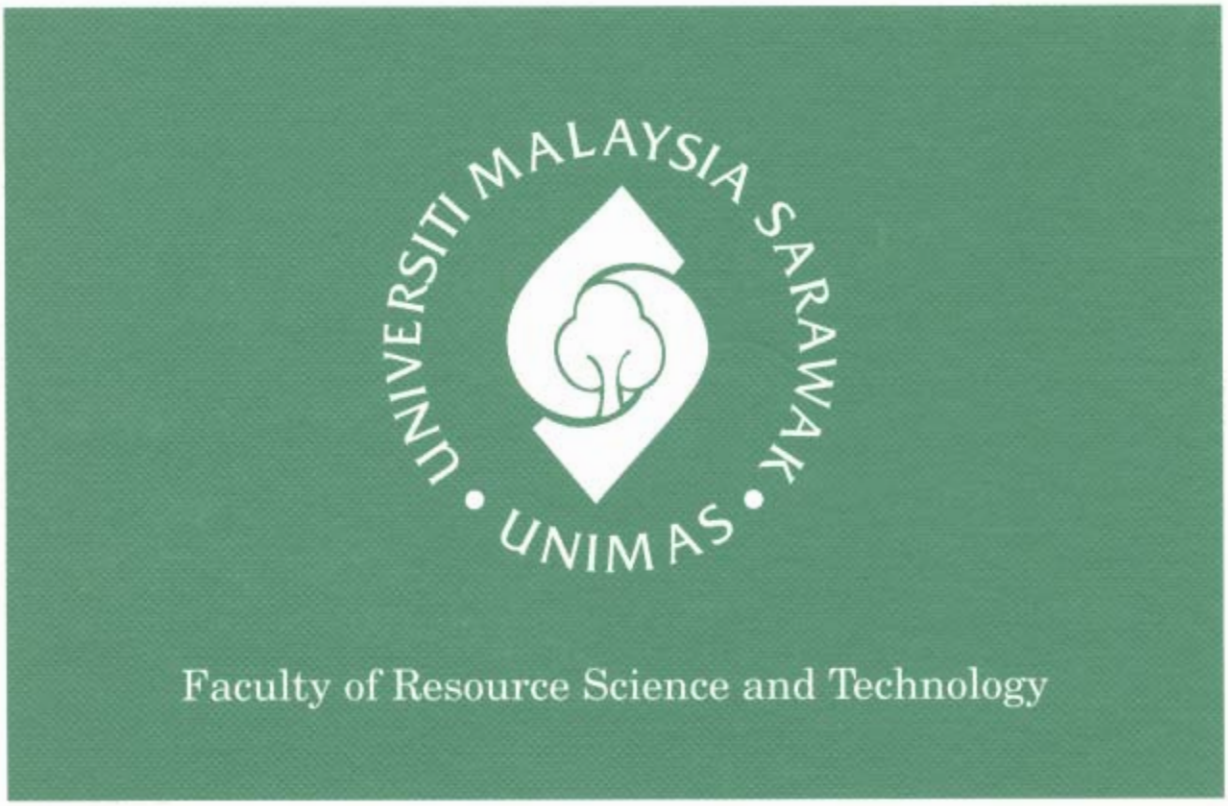

CRYOPRESERVATION OF *CUCUMIS MELO* [L.]. SEEDS USING DEHYDRATION TECHNIQUE

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Bachelor of Science With Honours
(Resource Biotechnology)
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ABSTRACT

A study was conducted to evaluate suitable dehydration techniques in cryopreserving *C. melo* seeds. The initial seed moisture content was 13.07% and germination was 92.50%. Viability determined using 1.0% solution of 2, 3, 5-triphenyl-tetrazolium chloride gave 84% viability. Three dehydration techniques used and compared were: silica gel, laminar flow and sucrose solution. The highest values of germination percentage obtained were from dehydration for 24 hours gave 80%, using laminar flow for a period of 6 hours produced 96% germination while using 0.6 M sucrose solutions for 60 minutes gave 98% germination. The two techniques, dehydration using sucrose and laminar flow, which gave the highest results were selected and compared whereby the seeds were stored for 8 weeks after treatment. Dehydrated and non-dehydrated seeds were placed in environment of different temperature: ambient room temperature (28-30°C), and liquid nitrogen (-196°C). Regression analysis showed that *C. melo* seeds after treated with sucrose and laminar flow then stored in liquid nitrogen were viable up to 8 years 24 days and 8 years 1 month, respectively.

Key words: *Cucumis melo*, cryopreservation, dehydration, regression

ABSTRAK

Kajian ini dijalankan untuk menentu teknik dehidrasi yang sesuai penyimpanan secara krioawetan biji benih Cucumis melo. Kandungan kelembapan permulaan biji benih adalah 13.07% dengan 92.50% percambahan. Pernilaian kebolehidupan dijalankan menggunakan 1.0% larutan 2, 3, 5-triphenyl tetrazolium klorida yang memberikan kebolehidupan 84%. Tiga teknik dehidrasi diguna dan dibanding: silika gel, 'laminar flow' dan larutan sukrosa. Dehidrasi menggunakan silika gel selama 24 jam memberikan keputusan 80% percambahan, menggunakan 'laminar flow' selama 6 jam memberikan keputusan 96%, sementara dehidrasi menggunakan 0.6 M larutan sukrosa selama 60 minit menghasilkan percambahan 98%. Hanya dua teknik dehidrasi yang memberikan keputusan yang terbaik, iaitu dehidrasi menggunakan sukrosa dan 'laminar flow' dipilih, dibanding dan setelah didehidrasi, disimpan di dalam cecair nitrogen selama 8 minggu. Biji benih yang telah didehidrasi dan tidak didehidrasi juga disimpan dalam persekitaran penyimpanan yang berbeza suhunya: suhu bilik (28-30°C), dan cecair nitrogen (-196°C). Analisis regresi menunjukkan bahawa biji C.melo yang telah dehidrasi menggunakan larutan sukrosa dan 'laminar flow' yang disimpan dalam cecair nitrogen dapat disimpan selama 8 tahun 24 haridan 8 tahun 1 bulan, masing-masing.

Kata kunci: Cucumis melo, krioawetan, dehidrasi, regresi

INTRODUCTION

Rock Melon *(Cucumis melo L.)* is known locally as Melon Wangi and is believed to originate from Africa. Three varieties of F1 hybrids developed from seeds imported from Taiwan are grown in Malaysia. These hybrids are Sky Rocket, Milky Way and Red Queen. The three hybrids are different in their morphological characters such as size, shape, color, texture, and composition.

The *C. melo* fruit has firm crisp flesh and contain a few hundred seeds. It can be eaten fresh as sweet melon or cooked and eaten as vegetables (Sahadevan *et al.*, 1987). Rock melon has high content of Vitamin C and A. *C. melo* is grown from seeds, and hence quality seeds are important. Conservation of *C. melo* is difficult owing to the recalcitrant nature of its seeds. Recalcitrant is used to describe the category of seeds, which cannot be stored under classical storage condition without rapid deterioration (Roberts *et al.*, 1973).

Cryopreservation is a technique used for treatment of the seeds prior to the storage of seeds because all chemical reactions in the cell will be halted due to the inability to proceed at the very low temperature of -196°C (Hauptmann *et al.*, 1982). It causes the specimen to dehydrate and have a low molecular energy. Low storage costs, combined with the ease of seed distribution and regeneration of whole plants offer distinct advantages for the cryostorage of seeds (Sakai *et al.*, 1984; 1986), and liquid nitrogen is a non-reactive reagent (Towill *et al.*, 1991).

One of the techniques used in cryopreservation of seeds is dehydration or removal of water from the specimen. This can be achieved using silica gel, laminar flow or sucrose. After dehydration, the specimen is then stored in liquid nitrogen. Seeds are withdrawn at intervals from the liquid nitrogen tank and thawed. Germination and moisture content evaluations are conducted to confirm the success of the dehydration treatments. It has been demonstrated that dry seeds of about 300 species tolerate exposure to liquid nitrogen (Styles *et al.*, 1982).

A study was carried out to assess the suitability of cryopreservation for the preservation of *C. melo* seeds and to determine the most suitable cryopreservation using dehydration technique for the storage of the *C. melo* seeds. The effectiveness of each dehydration techniques was evaluated for use in long term storage of the seeds.

MATERIALS AND METHODS

Material

Fresh and matured fruits of *C. melo* were obtained from the local farms in areas around Kuching and Kota Samarahan. Seeds were extracted from the fruits, cleaned and dried under shade. Dried seeds were dusted with fungicide and placed in an air-tight bottle to keep for use in on-going experiments.

Method

Moisture Content Test

Four replications of 15 seeds were placed in saucers made of aluminium foil. The seeds were arranged in a single layer in the aluminium foil, then were weighed using an electrical balance. The specimen is then placed in an oven at 60°C for 48 hours and reweighed again.

Seed moisture content percentage is calculated according to the formula endorsed by the International Seed Testing Association (1976), as appended below:

$$\text{Moisture Content (\%)} = \frac{b - c}{b - a} \times 100\%$$

a = weight of aluminum foil

b = weight of (a) + weight of seeds placed in oven

c = weight of (a) + weight after dried in oven

Viability (TZ) Test

Viability (Tetrazolium) test was done using solutions of 2, 3, 5-triphenyl tetrazolium chloride (Zhang, 2001), to determine the seeds' ability to survive. Seeds used were divided into four replications. 15 seeds were used in each replication, which were then reacted with tetrazolium solution at the concentrations of 0.1, 0.5 and 1.0%. The seeds were placed in an oven at 35°C for periods of 0, 30, 60, 90, and 120 minutes. Seeds which were stained carmine red were considered alive and viable.