Cryopreservation by Encapsulation-Dehydration of *Aerides multiflora* Roxb. Protocorms

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ABSTRACT

This study was undertaken to establish an effective protocol for storage of *Aerides multiflora* Roxb. protocorms using encapsulation-dehydration method. To establish an efficient protocol for cryopreservation using encapsulation-dehydration method, protocorms were encapsulated in Ca-alginate, followed by preculture with sucrose. Prior to storage in liquid nitrogen (LN), encapsulated protocorms were dehydrated by air-drying. The efficient protocol for cryopreservation was achieved by the pretreatment of encapsulated protocorms in 0.7 M sucrose for 24 hrs, followed by 5 hrs of dehydration (16.21 % MC fresh weight basis) before storage in LN. The encapsulated protocorms cooled to -196 °C produced a high level of survival (93.33%). Flow cytometry showed that no genetic alterations occurred after cryostorage in LN. This method is promising for cryogenic storage of *A. multiflora* protocorms.

Keywords: *Aerides multiflora*; cryopreservation; encapsulation-dehydration; flow cytometry

INTRODUCTION

Low moisture content and soil nutrient defciency are primary causes for low crop productivity under arid and semi-arid conditions in Jordan. Agriculture is the major consumer of water in Jordan, where about 72% of Jordanian annual water demand goes for agricultural uses (Ministry of Water and Irrigation, 2008). Under high temperature environment such as Jordan, irrigation is a very wasteful practice, where huge quantities of water are evaporated during irrigation (Hudson, 1994; Montemurro, 2004; Gholizadeh et al., 2006). Jordanian soils are suffering from nutrient deficiency, which is mainly due to high soil calcium carbonate content (15-35%), alkaline condition and low soil organic matter (Al-Rawashdeh and Abdel-Ghani, 2008). Therefore, under these low input conditions (drought and low soil fertility), water-saving agriculture practices and improving nutrient use efficiency are essential to enhance the economic yield and give the opportunity for small scaled farmers to reduce their input cost per unit area.

One possible solution to minimize the effect of drought and to conserve and enhance soil fertility is using soil amendments (Reganold, 1995; Baikova and Semekhina, 1996; Liu et al., 1996; Conacher and Conacher, 1998; Loboda, 1999). A soil amendment is any organic or inorganic material could be added to a soil to improve its physical properties with a goal to provide a better environment for roots growth and development (Hudson, 1994; Montemurro, 2004; Gholizadeh et al., 2006).Natural zeolite is among the

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Received on 1/7/2013 and Accepted for Publication on 31/12/2013.

minerals often used in attempts to develop new substrates for plant growing: for seedling production, rooting of cuttings, potting of ornamental plants etc. Natural zeolite' strong sorption properties, high cation exchange capacity (CEC) and high macro- and micro-nutrients content make them an attractive alternative to peatmoss and other natural products used in agricultural applications (e.g., Ming and Dixon, 1986; Ibrahim et al., 2001; Mohammad et al., 2004; Gul et al., 2005; Noor et al., 2006). Jordan has rich mineable deposits of zeolites with attractive physical and chemical properties for agriculture; it has been estimated that zeolite reserves in various areas in Jordan is about 2037.2 million ton (Natural Resources Authority NRA; 2010). In several literatures, zeolite was reported to be useful in various agricultural crops as a soil conditioner in order to improve drainage and aeration, reduce leaching of pesticides and fertilizers from the soil and save water during irrigation (eg., Ming et al., 1995; Baikova and Semekhina, 1996; Loboda, 1999). While many studies investigated the ability of natural zeolites and other amendments such as lime and red mud to reduce heavy metal availability in contaminated soils (Garau et al. 2007; Bertocchi et al. 2006; Gray et al. 2006), the influence of fresh and weathered volcanic tuff (VT) on growth, irrigation water saving and nutrient uptake by plants remains poorly investigated. Therefore, the present study was carried out to: (i) assess the effects of Jordanian VT as a soil amendment on plant growth and yield of salvia (*Salvia officinalis*), (ii) estimate the amount of irrigation water that could be saved from using Jordanian VTand (iii) investigate the influence Jordanian VT on leaves mineral content.

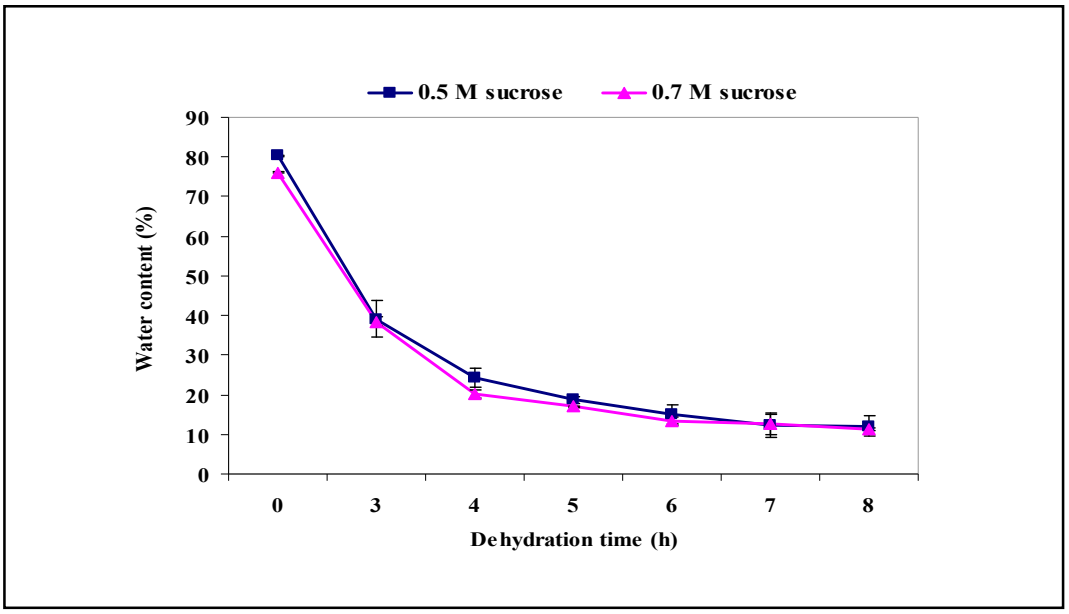

Figure 1: Effect of sucrose concentrations (0.5 and 0.7 M) in preculture mediums and dehydration time on changes in water content of encapsulated protocorms.

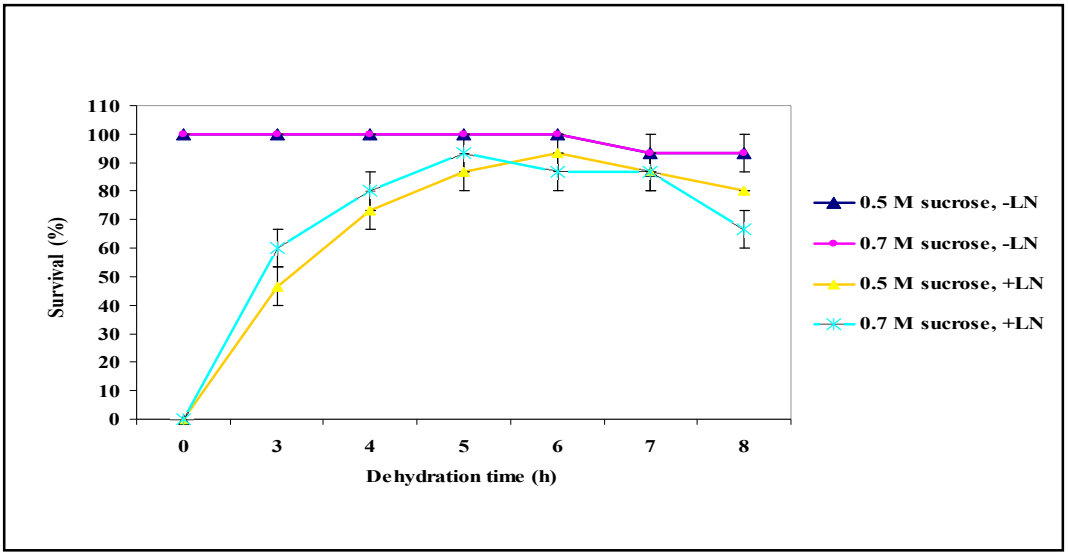

Figure 2: Effect of sucrose concentrations (0.5 and 0.7 M) in preculture mediums and dehydration time on survival percentage of non-cryopreserved (-LN) and cryopreserved protocorms (+LN).

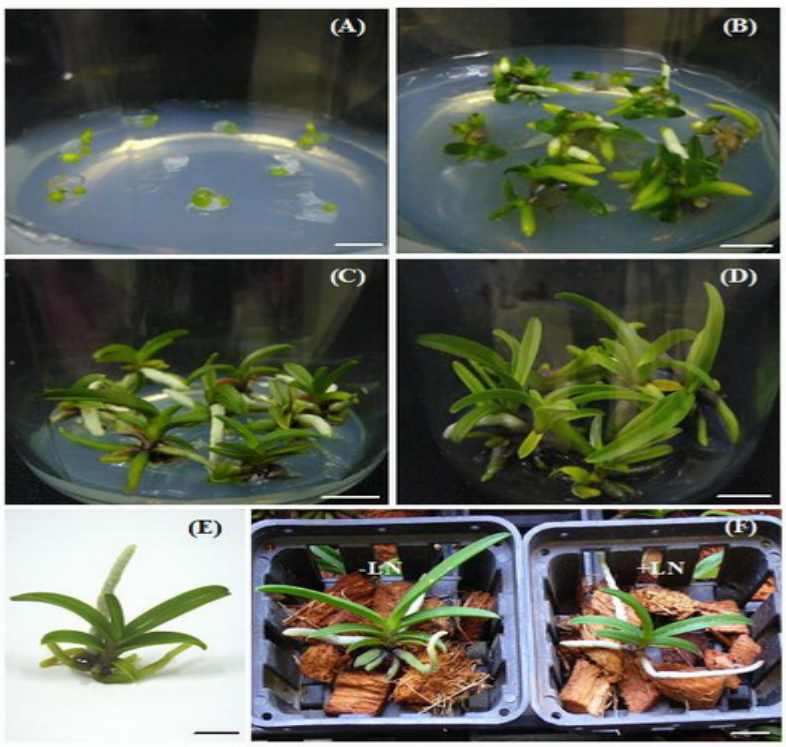

Figure 3: Re-growth of cryopreserved; A: Cryopreserved protocorms after recovery for 15 days; B: Shoot and root formation within 45 days on ND medium containing 2 mg/l BA; C: 3-month-old cryopreserved plantlets; D-E: 6-month-old plantlets; F: 9-month-old plantlets developed from non-cryopreserved (-LN) and cryopreserved plantlets (+LN). Scale bar; A = 5 mm, B-F = 1 cm.

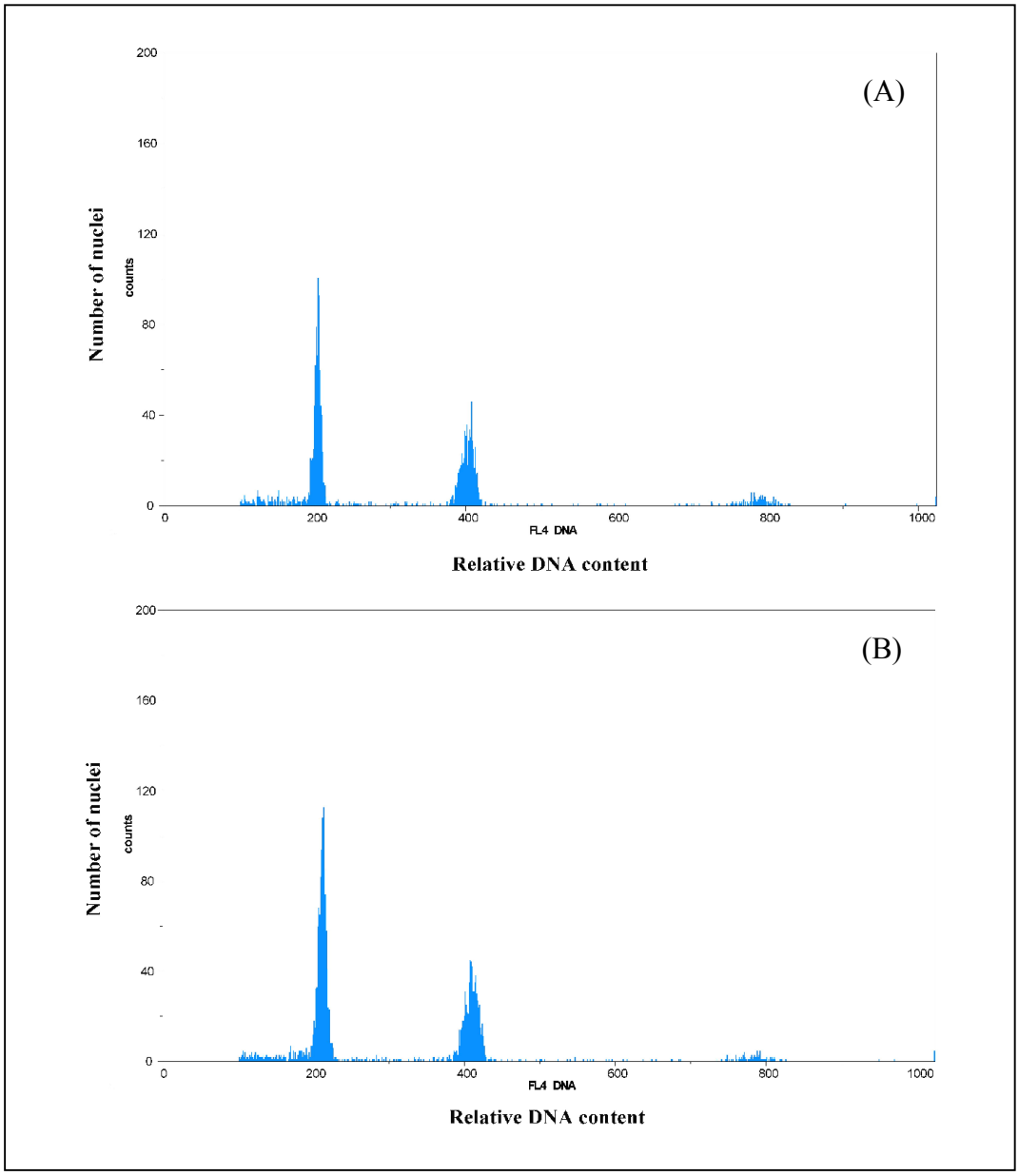

Figure 4: Flow cytometric DNA histograms of plants recovered from the cryopreservation and cultured for 6 month; A: -LN, B: +LN.

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الحفظ طويل الأمد بالتجفيف بالكبسلة لنبات *Aerides multiflora Roxb.* باستخدام الكورمات الأولية(Protocorms)

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ملخـــص

تم القيام بعمل هذه الدراسة لتأسيس بروتوكول فاعل لحفظ بادئات الكورمات في نباتAerides multiflora Roxb وذلك باستخدام طريقة الكبسلة بالتجفيف. ومن أجل تأسيس بروتوكول فعال للحفظ طويل الامد بالكبسلة بالتجفيف لبادئات الكورمات، فقد تم عمل كبسلة لبادئات الكورمات باستخدام ألجينات الكالسيوم ومن ثم الزراعة على وسط غذائي مزود بالسكورز. و قبل وضع الكورمات بالنتروجين السائل تم تجفيف بادئات الكورمات المكبسلة عن طريق التجفيف الهوائي. و قد تم تحقيق البروتوكول الفاعل للحفظ طويل الأمد بالنتروجين السائل عن طريق المعاملة القبلية لبادئات البروتوكورم المكبسلة في 0.7 مولار من السكروز لمدة 24 ساعه، متبوعة بتجفيف لمدة 5 ساعات من التجفيف (على أساس 16.21% من الرطوبة النسبية) قبل الحفظ في النتروجين السائل. تم تجميد البروتوكورمات المكبسلة إلى درجة حرارة −196 سليسيوسية و قد انتجت مستوى عال من الحياة بعد التجميد في النترجين السائل (93.33%). وقد أظهر التدفق الخلوي بأنه لا يوجد هناك تغيرات وراثية حدثت بعد الحفظ بالتجميد في النتروجين السائل. تعد هذه الطريقه من الطرق الواعدة للحفظ بالتجميد لكورمات *A. multiflora*.

الكلمات الدالة: الحفظ طويل الأمد، الكبسلة بالتجفيف، التدفق الخلوي *Aerides multiflora*.

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تاريخ استلام البحث 2013/7/1 وتاريخ قبوله 2013/12/31.