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Starch Utilization During In Vitro Rooting of Easy- and Difficult-to-Acclimatize Sea Oats (*Uniola paniculata*) Genotypes©

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Starch content was evaluated during microcutting in vitro rooting of an easy- (EK 16-3) and difficult-to-acclimatize (EK 11-1) genotypes of *Uniola paniculata* L. (sea oats), a native dune species of the southeastern U.S.A. Excluding Week 0, EK 11-1 plantlets exhibited greater shoot starch reserves than EK 16-3. Starch content was lower in roots than in shoots at Weeks 6 and 9 and, during root elongation, root starch content decreased in both genotypes. The difficult-to-acclimatize genotype (EK 11-1) exhibited a lower shoot to root dry weight ratio and reduced leaf development compared to the easy-to-acclimatize genotype (EK 16-3). Sugar and starch reserves are reported to be critical for successful acclimatization. However, these results indicate that, while starch content is higher in EK 11-1 plantlets, it is insufficient for successful ex vitro acclimatization. This may be the result of a higher energy requirement of the extensive root system and the absence of photosynthetically competent leaves ex vitro.

[1] 2nd Place Student Awardee for Graduate Student Research Competition

INTRODUCTION

Sea oats (*Uniola paniculata* L.), a dune species native to the southeastern U.S.A., is commonly used for beach stabilization and dune restoration in Florida. A micropropagation protocol was developed for sea oats production to facilitate the selection and rapid production of diverse genotypes with potential valuable ecological characteristics. This protocol was initially optimized using a single sea oats genotype (Philman and Kane, 1994). However, when applied to 28 different sea oats genotypes, significant variability in shoot production, rooting, and ex vitro survival was observed. Understanding the physiological basis for differences among genotypes provides a framework for developing efficient commercial micropropagation protocols for diverse sea oats genotypes. The industry must have the tools to provide and maintain a genetically diverse germplasm for ecosystem restoration.

Valero-Aracama et al. (2003) observed that increased ex vitro survival of two sea oats genotypes (EK 11-1 and EK 16-3) occurred with increasing Stage II culture duration. Furthermore, extended Stage III rooting was a requirement for successful sea oats Stage IV acclimatization. In another study using the same two genotypes (EK 11-1 and EK 16-3), Valero-Aracama et al. (2004) found that survival ex vitro increased from 85% to 95% (EK 16-3) and from 2% to 40% (EK 11-1) with increasing Stage III rooting duration (from 3 to 9 weeks).

The photosynthetic competence and carbohydrate status of plantlets during the in vitro rooting stage can be critical for successful ex vitro acclimatization (Piqueras et al., 1998). Sufficient starch accumulation in vitro has been hypothesized as the energy reserve to facilitate the transition from the heterotrophic to the photoautotrophic mode after transfer to ex vitro conditions (Van Huylenbroeck and De Riek, 1995). Consequently, it was hypothesized that low survival of some sea oats genotypes may be the result of limited carbohydrate reserves.

The objectives of the present study were to characterize in vitro shoot and root development and assess starch content of both easy-to-acclimatize (EK 16-3) and difficult-to-acclimatize (EK 11-1) sea oats genotypes at 0, 3, 6, and 9 weeks after transfer to in vitro rooting medium.

MATERIALS AND METHODS

Culture Procedures. Five sea oats shoot clusters, each consisting of three shoots, of EK 11-1 and EK 16-3 genotypes, were subcultured on 80 mL sterile Stage II medium into separate Magenta GA-7 vessels (Magenta Corp., Chicago, IL). Medium consisted of Murashige and Skoog (MS) (1962) inorganic salts, supplemented with 87.6 mM sucrose, 0.56 mM myo-inositol, 1.2 μM thiamine-HCl, 2.2 μM N_6-benzyladenine, adjusted to pH 5.7 with 0.1 N KOH, and solidified with 8 $g \cdot L^{-1}$ TC™ agar (Phyto Technology Laboratories, Shawnee Mission, KS). Cultures were maintained for 8 weeks in a growth chamber at 24 ± 1 ºC, 16-h photoperiod provided by cool-white fluorescent lamps (General Electric F20W T12CW), at 40 ± 5 μmol $m^{-2} \cdot s^{-1}$ photosynthetic photon flux (PPF), as measured at culture level. Subsequently, single-shoot microcuttings (Fig. 1A) were harvested and transferred to sterile Stage III medium, in GA-7 vessels. Medium consisted of 80 mL half-strength MS inorganic salts supplemented with 87.6 mM sucrose, 0.56 mM myo-inositol, 1.2 μM thiamine-HCL, 10 μM α-naphthalene acetic acid, adjusted to pH 5.7, and solidified with 8 $g \cdot L^{-1}$ TC™ agar. Culture vessels were inoculated with 8 microcuttings each, and were maintained in a culture room at 22 ± 1 ºC, 16-h photoperiod, provided

Figure 1. From top to bottom, plantlets 0, 3, 6 and 9 weeks after transfer to *in vitro* rooting medium of EK 11-1 (left) and EK 16-3 (right) genotypes. **Scale bar** = 1cm

by cool-white fluorescent lamps (General Electric F96T12-CW-WM) at 100 ± 5 μmol·m^{-2} s^{-1} PPF, as measured at culture level. After 0, 3, 6, and 9 weeks under Stage III conditions, shoots and roots from 10 replicate vessels per treatment were harvested, separated, frozen in liquid N_2, and freeze-dried. Dry weights of shoots and roots were determined and shoot to root dry weight ratios were calculated. To obtain sufficient tissue for starch analysis, all 8 microcutting subsamples from each vessel were pooled to generate a sample.

Starch Extraction and Analysis. Procedures for starch determination were modified as described by Haissig and Dickson (1979) and Miller and Langhans (1989). Glass Pasteur pipettes with glass wool plugs were loaded with 50 mg of each sample and 3 extractions with 1.5 mL of 12 methanol : 5 chloroform: 3 water (by volume, MCW) were carried out for soluble sugar extraction before the residue was used for starch extraction. The tissue residue was dried overnight at 60 ºC, suspended in 4 mL Na-acetate buffer (100 mM, pH 4.5) and placed in a boiling water bath overnight. After cooling to room temperature, 1.0 mL amyloglucosidase solution (from *Rhizopus* mold, Sigma-Aldrich Co., St. Louis, MO) (50 units/assay in 0.1 M pH 4.5 Na-acetate buffer) was added to each test tube. Samples were incubated for 48 h at 55 ºC with occasional agitation. Glucose determinations via the glucose oxidase and peroxidase enzymatic method were completed on 100-μL samples. Absorbance was spectrophotometrically determined at 450 nm and starch content was calculated based on the regression equation of the glucose calibration line (0.0 to 0.5 μmol).

Statistical Analysis. The experiment was a completely randomized design, consisting of 10 replicate vessels per treatment, (per genotype and sampling time). Treatment main effects were analyzed using the General Linear Model (GLM) procedure (SAS Institute, 1999). Further analysis was conducted to determine genotype effects at each time interval and time effect within each genotype using the GLM procedure.

RESULTS AND DISCUSSION

Plantlets of EK 16-3 and EK 11-1 exhibited striking differences in shoot and root development (Figs. 1A-D). The EK 11-1 plantlets produced many small shoots with

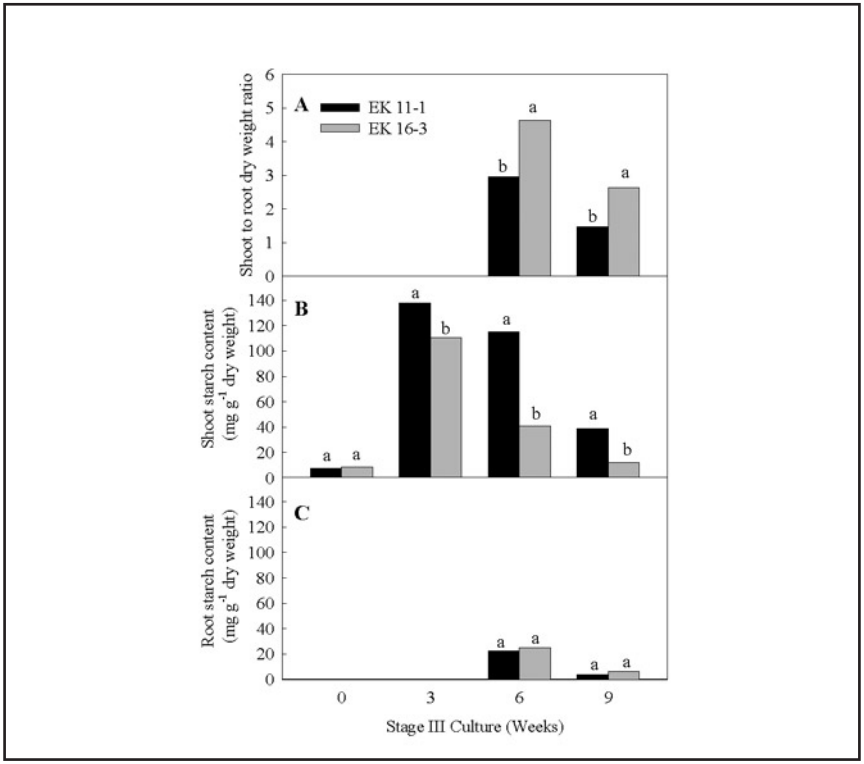

Figure 2. A) Shoot to root dry weight ratio, B) shoot starch content, and C) root starch content, of EK 11-1 and EK 16-3 genotypes on weeks 0, 3, 6 and 9 after transfer to in vitro rooting medium. Each histobar represents the mean response from 10 replicate samples. Different letters indicate significant differences between genotypes at each time interval ($P = 0.01$).

minimal leaf blade development and an extensive root system (Fig. 1D). In contrast, EK 16-3 plantlets produced few shoots with expanded leaf blades and an extensive root system (Fig. 1D). Shoot to root dry weight ratios (Fig. 2A) were 1.5 and 1.9 times higher for EK 16-3 than EK 11-1 plantlets after 6 and 9 weeks, respectively.

Starch content in shoot tissue followed similar trends in both genotypes increasing from the lowest level on Day 0 to the highest after 3 weeks, and then continuously decreasing until Week 9 (Fig. 2B). Total soluble sugars (sucrose, glucose, and fructose) in shoots followed a similar profile with time (data not shown). Furthermore, with the exception of Week 0, starch content in EK 11-1 shoots was significantly greater than in EK 16-3 shoots ($P < 0.0001$).

After 3 weeks in culture, root emergence was visible on microcuttings of both genotypes (Fig. 1B) but not a quantity sufficient for starch analysis. Root starch content was similar between genotypes and greater at Week 6 than Week 9 (Fig. 2C). Total soluble sugars were also greater at Week 6 than Week 9 (data not shown).

Numerous investigations have correlated starch accumulation and utilization in plant cells prior to the initiation and progression of developmental processes (Swarnkar et al., 1986). The low initial starch levels in both sea oats genotypes,

obtained from 8-week old Stage II cultures, indicated that the shoot clusters, from which microcuttings were derived, were starch-depleted at the end of the multiplication stage. Starch content increased rapidly after being transferred to sucrose-containing Stage III medium, prior to root emergence. Rapid accumulation of starch was also observed when *Begonia* explants were transferred to medium containing 87.6 mM sucrose (Mangat et al., 1990).

Capellades et al. (1991) established an inverse relationship between starch content in shoot chloroplasts and photosynthetic rates of in vitro *Rosa multiflora* leaves. A similar effect may be occurring during in vitro development of sea oats. Capellades et al. (1991) also concluded that during ex vitro acclimatization, leaves that exhibited low photosynthetic rates utilized starch reserves while re-establishing their photosynthetic capacity. However, in other species only leaves produced ex vitro are photosynthetically competent (Grout and Millam, 1985). In EK 11-1 plantlets, allocation of energy resources appears to be directed towards root biomass and nonleafy shoot production.

Carbon metabolism during ex vitro acclimatization evolves from heterotrophy in vitro towards photoautotrophy ex vitro (Piqueras et al., 1998). Sucrose in the medium is hydrolyzed and taken up by roots, translocated, and stored as starch in shoots and roots in vitro. In contrast, under ex vitro conditions, photosynthetically active leaves are the sources of carbon, where soluble sugars are produced and, either stored as starch reserves, or translocated into other sink organs such as roots. Given that starch levels were higher in shoots of the difficult-to-acclimatize sea oats genotype (EK 11-1) at the time of ex vitro transfer, the low ex vitro survival may actually result from limited production of photosynthetically competent leaves in vitro, presence of an extensive heterotrophic root system, and subsequent rapid depletion of energy reserves ex vitro. Further research on the effect of in vitro culture on the physiological differences between sea oats genotypes is being conducted.

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Seeking and Sharing Ideas from the Orient©

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INTRODUCTION

In the spirit of IPPS, this article shares some of our experiences as IPPS-SRNA international representatives to the Japanese International Plant Propagator's Conference, in Sept. 2004. This was a working trip to do the business of the IPPS, but it is customary for the host country to share their industry and culture with their guests. Representatives have an opportunity to get to know each other in an informal setting. They discuss opportunities and society concerns on the bus and at meals before entering into formal dialogue during the IPPS International Board meeting. It is a great format that serves the IPPS well.

ACCOMPLISHMENTS OF THE INTERNATIONAL BOARD — 2004

The IPPS International Board accomplished a great deal in 2004. Four significant measures were approved that will make seeking and sharing much easier for our members, and the IPPS more attractive for nonmembers to join:

- The secretaries of the regions will receive computer program updates that will make communications among regional members much more frequent, effective, and meaningful.
- Thanks to the IPPS Western Region, Vol. 1-10 of the Combined Proceedings of the IPPS will be placed on the web site this year; see <www.ipps.org>. The rest of the IPPS Proceedings will also be placed on the site beginning with the most recent volumes, followed by earlier editions. The two most recent volumes of the IPPS Proceedings will be excluded if password security concerns cannot be assured. This means that you do not have to be a member for 50 years to get full access to the IPPS knowledgebase.