

## Education Posters

### E-2000

A Classroom Exercise in Hand Pollination and *In Vitro* Asymbiotic Orchid Seed Germination. P. J. KAUTH<sup>1</sup>, T. R. Johnson<sup>1</sup>, S. L. Stewart<sup>2</sup>, and M. E. Kane<sup>1</sup>. <sup>1</sup>Environmental Horticulture Department, University of Florida, PO Box 110675, Gainesville, FL 32611, USA and <sup>2</sup>PhytoTechnology Laboratories, 14335 West 97th Terrace, Lenexa, KS 66215. Email: pkauth@ufl.edu

Many scientific reports on orchid seed germination provide germination protocols, but few provide concise descriptions of plant selection, hand pollination, and asymbiotic seed culture for use in science classroom exercises. A major limitation for conducting orchid seed germination exercises are seed and flower availability. Flower availability must be consistent throughout the year so instructors can hand pollinate flowers to produce seed on demand for laboratory exercises. However, many orchids do not flower reliably throughout the year. Seed capsule development is often a lengthy process with maturation often taking more than 100 d. An efficient and reliable classroom exercise using the orchid *Spathoglottis* to demonstrate hand pollination and subsequent asymbiotic seed germination is described. *Spathoglottis* species are excellent candidates for this exercise because they flower throughout the year, their floral parts are large and distinct, seed capsules mature quickly, and seeds germinate readily. In this exercise, *Spathoglottis* flowers are hand pollinated and subsequent seed capsule development is carefully monitored. Capsules are harvested prior to dehiscence approximately 30 d after pollination. Seed capsules are surface sterilized, seeds excised, and then sown on P723 Orchid Seed Sowing Medium. Germination begins 2 wk after initial sowing, and mature flowering plants can be observed 1 y after seed inoculation. Hand pollination of orchid flowers provides an opportunity to discuss floral morphology and associated reproductive biology. This exercise introduces students from high school to college levels to basic skills commonly employed in plant tissue culture, plant biotechnology, and orchid seed culture.

### E-2001

Interrelations of Polyploidy and Development of Contractile System during Normal and Hypertrophic Growth of Heart Cells In Vitro. PAVEL A. BORISOV. Greenhills School, Ann Arbor, MI 48105. Email: pborisov@umich.edu, gryphon\_green@yahoo.com

Myocytes in the mammalian heart become tetraploid and octaploid during the postnatal period. The functional significance of this process is poorly understood. The goal of this study was to test the hypothesis that polyploid cells provided more effective support of contractile function and response to hypertrophy than did diploid cells. Primary cell cultures of neonatal rat cardiac myocytes undergoing intense polyploidization during differentiation in vitro were used as an experimental model. We comparatively analyzed the capability of diploid and polyploid cells to assemble new contractile structures during terminal differentiation and under conditions of pharmacologically induced hypertrophic growth in culture. Nuclear DNA content was determined using DNA staining with propidium iodide. Formation of new contractile structures was evaluated using phase contrast microscopy and immunofluorescent labeling of sarcomeric proteins. Image J software was used for morphometric analysis. Myocytes isolated from 1-d-old neonatal rats were predominantly mononucleated and diploid during the G1 phase of the mitotic cycle. We found that intense assembly of contractile structures rapidly activated and progressed during 24–72 h immediately following polyploidization of muscle cells. Tetraploid myocytes developed significantly higher (up to 60–80%) number of new myofibrils during terminal differentiation than diploid cells. Stimulation of hypertrophic growth with phenylephrine resulted in considerably more intense formation of new contractile structures in polyploid cells compared to diploid cells. The results of this study show that polyploid myocytes possess an enhanced capability to form new contractile structures both during differentiation and under conditions of hypertrophic growth. These findings suggest that polyploidization increases the adaptive potential of cardiac myocytes and is essential for

successful compensatory response of the overloaded heart to increasing functional demand.

### E-2002

Effect of Homocysteine on Insulin Resistance in Cultured Hep G2 Cell Line. V. KADIYALA<sup>1</sup>, M. Patil<sup>2</sup>, S. J.Barve<sup>2</sup>, and S. Barve<sup>2</sup>. <sup>1</sup>DuPont Manual and <sup>2</sup>Department of Internal Medicine, University of Louisville Medical Center, Louisville, KY 40292. Email: vishnukadiyala@gmail.com

Inability to use insulin (insulin resistance) and high plasma levels of homocysteine (hyper-homocysteinemia) are important clinical features of non-insulin dependent diabetes mellitus (NIDDM) or type II diabetes. Although association between elevated plasma homocysteine and insulin resistance in diabetes has been observed, it is still not clear whether hyperhomocysteinemia induces insulin resistance. The liver is the main organ that is involved in carbohydrate and fat metabolism. Accordingly, insulin resistance in the liver is an important clinical feature in diabetic patients. This experiment was based on the hypothesis that homocysteine induces insulin resistance in liver cells (hepatocytes).

To test the hypothesis, the researchers evaluated the effects of homocysteine on key features of insulin signaling by examining (1) the phosphorylation of insulin receptor substrate (IRS)-1 and (2) the glucose uptake/consumption by hepatocytes that have been exposed to insulin in the presence or absence of homocysteine. The experiments were carried out by using the human hepatocyte cell line, HepG2, which is a well-characterized, commonly used model system for liver studies. The data obtained from these studies demonstrated that phosphorylation of IRS-1 in response to insulin (that leads to its degradation) is greatly increased when the hepatocytes are subject to elevated homocysteine levels between the concentrations of 0.5 and 1.5 mM. Increased degradation of IRS-1 can lead to decreased insulin signaling and consequent insulin resistance in the liver. Accordingly, the data also showed that the pretreatment of hepatocytes with homocysteine significantly reduced their ability to utilize and consume glucose. These results support the hypothesis that high levels of homocysteine could play a causal role in the development of insulin resistance in the liver, and hence play an important clinical role in the development of type II diabetes.