Novel Applications of Plant Tissue Culture©

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INTRODUCTION

In 1902 Gottleib Haberlandt proposed the idea of growing individual plant cells on artificial medium. While Haberlandt never realized his idea, the 105 years since has seen the concept evolve into a powerful tool utilized throughout the plant sciences. Plant tissue culture broadly refers to growing plant cells, tissues, organs, seeds, or other plant parts in a sterile environment on a nutrient medium. Tissue culture is being used for an increasing variety of purposes. Originally used largely for fundamental research to study cell division, plant growth, and biochemistry, the technology has grown and is being widely implemented on a more applied scale. In many cases, protocols have been developed and refined so that they have become a standard and commercially viable practice for propagating many important horticultural crops.

The key to the successful application of tissue culture is the manipulation of media compositions to achieve desired outcomes (Benson, 2000; Gamborg, 2002). By altering media components, tissue can be induced to produce shoots, roots, callus, or somatic embryos or inhibit growth for long-term storage. The most common application of tissue culture is micropropagation, which usually involves growing plants in an agar solidified nutrient media. Micropropagation can facilitate the rapid production and propagation of plant species.

Somatic embryogenesis also has become an important technique for plant regeneration and multiplication. Somatic embryogenesis refers to the initiation of embryos from previously differentiated somatic cells. This process usually involves two distinct steps. The first step, induction of embryonic cells or proembryoids, is required to initiate cell division and establish a new polarity in the somatic cells. The second step involves the development of the proembryoid cells into embryos (McKersie and Brown, 1996; Jiménez, 2005). As plants are often regenerated from a single or a small number of cells, this approach may provide a powerful mechanism to induce polyploidy, recover mutations, or stabilize chimeras.

Several other specialized tissue culture techniques have been developed to overcome barriers associated with conventional breeding. For example, embryo rescue and ovule culture techniques may be used to alleviate problems associated with post fertilization barriers such as triploid blocks (Mohapatra and Rout, 2005; Olsen, et al., 2006). At the North Carolina State University, Mountain Horticultural Crops Research and Extension Center, in Mills River, North Carolina, tissue culture has become an integral part of the nursery crops breeding program. Here we present an overview of some of the novel approaches used in our program.

APPLICATIONS

Micropropagation. Micropropagation, also known as in vitro multiplication of plants, is the foremost application of plant tissue culture. For many species, micro-

propagation provides a means for rapid propagation and commercial production of plants compared to traditional propagation techniques.. For example, we have developed a micropropagation protocol for the rapid multiplication of *Hydrangea arborescens*. Shoots were maintained on a multiplication media comprising of MS basal salts supplemented with 2 μM 6-benzylamino purine (BAP). Six shoots were placed in a 180 ml jar and transferred to fresh media every 5 weeks. Average shoot proliferation rate was 21.0±0.7 per jar (3.5 shoots per plantlet) every subculture period. Based on this data, we could produce over 1.5 million plants yearly starting with only 50 plantlets. In addition we have developed micropropagation procedures for a wide range of genera including *Rhododendron* (azalea), *Hypericum*, *Miscanthus*, and *Rudbeckia*.

Developing Sterile Cultivars. One focus of our research is to develop improved, non-invasive, seedless nursery crops. One of the most effective means for developing seedless plants is to create triploids (plants with three sets of chromosomes) (Ranney, 2004). While triploids grow normally, they are unable to divide equally during meiosis and typically fail to produce viable gametes. Triploids have been developed for many food crops including watermelon, bananas and grapes. Triploids can be bred by hybridizing tetraploids with diploids.

In North Carolina, miscanthus has escaped cultivation and naturalized in many areas. We have recently utilized tissue culture to develop a series of triploid miscanthus. Somatic embryogenesis was induced from callus derived from seed or shoot apices. Callus was maintained in the dark and subcultured to fresh media every 4 weeks. To induce polyploidy, callus was treated with the mitotic inhibitor oryzalin that resulted in approximately 60% of the regenerated plants being tetraploid. Tetraploids were transferred to the glass house and hybridized with diploids to create triploids.

A common problem associated with the successful development of sterile triploids is a phenomenon referred to as "triploid block," generally thought to result from imbalances in the ploidy levels between the embryo and endosperm. Triploid blocks are common in interploid crosses and typically results in embryo abortion. For miscanthus, embryo rescue techniques were required to overcome this phenomenon. Immature embryos were rescued from aborting seed using a microscope and germinated in vitro. Recovery rates remained low with approximately 600 plantlets being obtained from over 10,000 seed to date.

Wide Crosses. Wide crosses refer to hybridizations between species of the same or different genera. Wide crosses are useful in crop improvement as they allow for recombination of diverse genes and traits. However, post fertilization barriers resulting from genome incompatibility and ploidy levels often make hybridizations difficult. Similar to interploid crosses, seed from wide crosses often fail to develop and abort. Embryo rescue techniques (i.e., rescuing an embryo from an aborting seed) and ovule culture can be used to recover progeny from wide crosses. We have used this technique in developing wide crosses between *Derivilla* and *Weigela* (Touchell et al., 2006).

Progeny from wide crosses may also be sterile due to uneven chromosome paring leading to meiotic failure. Chromosome doubling can help in restoring fertility to wide hybrids by providing an exact homologous duplicate of each chromosome that can pair together during mitosis. Tissue culture procedures, particularly so-

matic embryogenesis, provide a mechanism to induce and recover polyploids. For example, *Rhododendron* 'Fragrantissimum Improved' is a sterile wide hybrid. We have developed a protocol to induce embryogenesis from leaves using a combination of the cytokinin thidiazuron (TDZ) and an auxin indole acetic acid (IAA). Embryogenic cultures were treated with oryzalin at different concentration and durations and will be assessed for ploidy.

Polyploid Induction. The development of new polyploids, through chromosome doubling, may increase ornamental characteristics, expand breeding opportunities, and restore fertility in sterile hybrids — ultimately leading to the development of improved cultivars (Contreras, et al., 2007; Olsen et al., 2006a,b; Ranney, 2006). Polyploid induction has been employed to improve ornamental characteristics and facilitate breeding programs for a wide range of plant taxa (Allum et al., 2007; Dunn and Lindstrom, 2007).

Rudbeckia is one genus in which we are exploring the effect of polyploidy on ornamental characteristics. Embryogenic systems were used to establish clonal lines in tissue culture. In-vitro treatments, ranging from 15 to 60 μM oryzalin over 3 to 5 days, were effective at inducing polyploidy, depending on taxa. New tetraploids of *R. maxima, R. subtomentosa*, and a novel interspecific hybrid were successfully developed and will be evaluated for ornamental characteristics (Palmer et al., 2008).

Stabilizing Chimeras. Chimera's occur when a plant or part of a plant is composed of genetically different layers. Chimeras often result in leaf variegations. As somatic embryogenesis offers a unique capability of regenerating a plant from a single cell, it is possible to regenerate plantlets with different genetic compositions from different layers of a single leaf or flower.

Rhododendron 'Little John' has a leaf variegation resulting from an apparent mutation causing the L1 layer (outer layer) to produce red pigments. Embryogenic procedures have allowed for the development of both red and green plants to be initiated from the same leaf.

Mutation Breeding. Induced mutations using irradiation or chemical mutagens is another advance in biotechnology that may have potential benefits for the production of sterile plants and novel forms (dwarfs) and foliage types (variegation). Mutation breeding is also beneficial to increase variability in species with low genetic diversity such as *Hypericum frondosum*. Gamma irradiation has been used for several decades for whole plants and seed; however, more recently the procedures have been used to induce mutations in tissue cultures (Ahloowalia and Maluszynski, 2001; Charbaji and Nabulsi, 1999). It is particularly desirable to treat callus cultures and to regenerate plants from single cell lines to eliminate chimeral tissue. In our laboratory we have developed embryogenic systems for *Hypericum* and investigated the effects of gamma irradiation and chemical mutagens.

Problems. While plant tissue culture provides powerful mechanisms to assist in breeding programs, there are several problems to overcome. Regeneration systems have proven difficult for many woody plants and only a small percentage of ornamental species have successfully been introduced into tissue culture. Comprehensive research needs to be conducted on a species-by-species manner to optimize regeneration protocols that can be applied to a breeding program. Further, some species are recalcitrant to tissue culture and may be better suited to conventional propagation protocols.

Future. Perhaps some of the greatest impacts of plant tissue culture are yet to come. The use of the technology for genetic modification of plant cells provides a powerful tool for both fundamental and applied research. The application of gene transfer technology has already been successfully applied to many crops species to increase resistance to herbicides and pests and to increase yields. Research on genetic modification on ornamentals may also lead to improved characteristics and to induce sterility.

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