Using Traditional and Biotechnological Breeding for New Plant Development©

Mark Bridgen
Cornell University, 3059 Sound Ave., Riverhead, New York 11901 U.S.A.
Email: mpb27@cornell.edu

INTRODUCTION

The constant search for new forms and colors of plants for the horticultural industry is making the development of new cultivars a permanent endeavor. There exists a tremendous potential to introduce noncultivated species from nature and to breed new ornamentals from these native species (Bridgen, 2001). There are several native Chilean geophytes that have potential as commercial and ornamental plants. Species such as *Leucocoryne, Conanthera, Rhodophiala, Alstroemeria,* and *Zephyra* could be bred and used as cut flower crops, potted plant crops, and garden flowers.

The Chilean territory is an "ecological island" with geographical barriers that have isolated the biological communities from the rest of the continent and produced a high percentage of endemism. The Atacama Desert to the north, the Pacific Ocean to the west and south, and the Andes Mountains to the east have made Chile one of the world's few biodiversity hotspots. Continental Chile is home to some 5,100 species, of which about 2,630 are endemic (Marticorena and Rodriguez, 1995). Such a proportion of endemism is one of the highest found in any region on Earth.

Traditional breeding is still the most important source for releasing new cultivars to the market. However, there are several in vitro techniques that can be used to make traditional breeding quicker and more successful.

IN VITRO BREEDING TECHNIQUES

Plant tissue culture is the art and science of aseptically growing plant cells, tissues, organs, and plants on a nutrient medium under controlled environmental conditions. Biotechnological techniques such as embryo culture, somaclonal variation, in vitro mutation techniques, and micropropagation are incorporated into breeding programs to circumvent incompatibilities and to elicit novel changes.

Embryo Culture. Embryo culture is the sterile isolation and growth of an immature or mature zygotic embryo under aseptic conditions on a nutrient medium with the goal of obtaining a viable plant. It is most often used to rescue embryos from interspecific and intergeneric crosses that do not fully develop naturally or where the embryo aborts. This method can also be used to rescue seedless triploid embryos, produce haploids, or overcome seed dormancy. The embryo culture technique depends upon the isolation of the embryo without injury, formulation of a suitable nutrient medium, and the induction of continued embryogenic growth and seedling formation. The basic premise for this technique is that the integrity of the hybrid genome is retained in a stalled or abortive embryo and that its potential to resume normal growth may be realized if it is supplied with nutrient substances in vitro.

Plant embryos are located in the sterile environment of the ovule, and thus surface sterilization of embryos is not necessary. Instead, entire ovules or ovaries are surface sterilized, and subsequently embryos are removed aseptically from the sur-

rounding tissues. The procedure makes it relatively easy to obtain pathogen-free embryos because harsh surface disinfection procedures can be used to the ovaries or ovules.

Large embryos are not very difficult to excise. However, small embryos are easily damaged and require the use of microdissecting tools and a dissecting microscope to excise them without injury. It is important that the excised embryo does not become desiccated during dissection. Embryos from the Chilean geophytes that we are breeding are removed aseptically at different time periods (7, 10, 14 days) post-fertilization. They are then usually cultured on a Murashige and Skoog medium with dilute (50%) nutrient concentrations (Murashige and Skoog, 1962). These geophyte cultures are often placed in dark conditions and cultured under a cool temperature (18 °C).

Somaclonal Variation and In Vitro Mutation. Somaclonal variation is genetic alteration generated by the use of a tissue culture cycle and is a tool that is used for crop improvement. Somaclonal variation can be a heritable variation that is sexually transmitted to the progeny. Some in vitro techniques such as adventitious shoot formation, callus culture, cell suspension culture, and protoplast culture increase the chance that genetic mutations may occur. Somaclonal variation can result from two sources. It can arise from preexisting genetic variation that is expressed in regenerated plants and it can be induced by the culture process (Lu and Bridgen, 1997).

Genotype is an important variable to consider when trying to induce somaclonal variation and can influence the frequency of regeneration. With the large germplasm source that is available from Chile, several genotypes have been tested for their ability to mutate in vitro. Different explant sources from *Leucocoryne, Conanthera, Rhodophiala, Alstroemeria,* and *Zephyra* have been tested because genotype is a critical variable for success. Meristems and other organized growing points produce fewer mutations than unorganized growing points. Indirect organogenesis (via callus), somatic embryos, and shoots via direct organogenesis have been compared for their ability to produce mutations. Long-term cultures have been shown to induce more mutations than short-term cultures.

It is known that the growth regulator composition of the medium can influence the frequency of somaclonal variation. Different auxins and cytokinins can be used at high concentrations to induce mutations. Some herbicides and chemicals such as colchicines can also be used to mutate plants in vitro. Gross chromosomal changes such as polyploidy and aneuploidy can be induced. If tetraploids form, they can be crossed with the normal diploids to produce sterile triploids.

Micropropagation. Micropropagation is the aseptic propagation of plants in vitro. This is a clonal technique that is often used once a new plant is developed because the procedure can hasten the introduction of new plants. When new plants are developed by using the previous protocols, the following five stages of micropropagation are used for each of the new plants.

Stage 0 involves the preparation of the stock or mother plant from which the primary explant is to be excised. Careful selection is made at this stage to be certain that these plants are disease-free and healthy. The time of year in which the explant is removed from the mother plant may affect the results of the micropropagation program. Changes in temperature, water availability, light intensity, and photoperiod will affect the levels of carbohydrates, proteins, and phytohormones in the stock

plant and may affect the response of the explant in vitro. Best results in vitro are generally achieved when the explant is taken during the active phase of growth.

Stage I is the establishment phase in which the explant is disinfected and cultured aseptically on a nutrient medium. The main objective of Stage I is to obtain explants free from surface pathogens such as bacteria and fungi. The selection of a suitable explant source is essential at this stage. Almost any plant tissue or organ can be used, but the degree of success will depend upon the species, the removal of surface contaminants from the explant, and the culture system that is used. Disinfecting the surface of an explant generally involves washing the tissue, followed by sterilization with one or more disinfectants. Washing the explant under running tap water greatly reduces the amount of contamination. Washing the explant with soapy water before placing it under running tap water may further reduce the number of pathogens present on the explant or make them more accessible to sterilants. After washing, the explant is immersed in an antiseptic solution to kill contaminants present on the surface. A 0.5%–5.25% solution of sodium hypochlorite is the most commonly used sterilant with plant tissue cultures. Ethanol, calcium hypochlorite, and hydrogen peroxide are other common disinfectants. Various sterilants may be used alone, in sequence or in combination to obtain the most effective sterilization procedure. The explant must then be rinsed one to several times in sterile distilled water to remove any remaining traces of the disinfectant. Damaged tissues of the explant are then removed and the explant is subdivided into appropriate sizes for culture. The explant is then placed on a nutrient medium designed for maximum growth of that particular species. If contaminants are present after disinfection, they will become apparent within 3 to 5 days after culturing.

Stage II is the multiplication phase and is used to rapidly increase the number of propagules. The clean plant material from Stage I is repeatedly subcultured in Stage II until the desired number of propagules are obtained. Propagules may be from terminal buds, axillary branching, or from adventitious bud formation. As the new shoots develop, they in turn produce buds along their axis. Through repeated subculture, this process can be repeated indefinitely. Cytokinin concentrations of 1–20 $mg \cdot L^{-1}$ are used to enhance axillary bud proliferation. Optimum concentrations are determined for each species. Adventitious shoots originate in tissues located in areas other than leaf axils or shoot tips. Adventitious shoots, roots, bulblets, and other specialized structures may originate from stems, leaves, tubers, corms, bulbs or rhizomes. The number of propagules is increased by subdividing and reculturing the in vitro-derived organs.

Stage III is the rooting stage. In vitro-derived plants may be induced during this phase to produce roots in vitro or in vivo. If rooting occurs in vivo, Stage IV, the acclimation phase, is combined with Stage III. In the past, the majority of all shoots were rooted in vitro. Although this procedure is still used, it is quicker and more economical to combine rooting and in vivo acclimation. In some species, in vitro rooting techniques are the only practical methods of rooting plantlets. A separate root-inducing medium is used during Stage III because the presence of cytokinins in Stage II inhibits root formation. Other factors that may influence rooting include other growth regulators, support substances, nutrients, organic substances, light, and temperature. With some species, all that is required to induce rooting is to transfer shoots to a cytokinin-free medium. However, in many species, the initia-

tion of roots occurs only in the presence of auxin. The response of shoots to auxins is dependent upon the species, the type of auxin, and the concentration of auxin.

Stage IV is the acclimation or "hardening-off" phase. This is the process by which the tissue cultured plant adapts to the new environment. Acclimation is necessary for in vitro-derived plants because they are produced under very high relative humidity, very low air movements, optimum nutrition, and ideal light and temperature conditions. A suitable environment can be created by placing plantlets in a clear plastic bag or box or under intermittent water mist/fogging. Plants are acclimated by gradually reducing the relative humidity in their surroundings. The application of antitranspirants and the treatment with fungicides are often beneficial and may increase the survival of plants outside of the culture vessel.

The diverse and colorful genera of Chilean geophytes create a tremendous potential for the breeding of new hybrids. Our breeding work since 1985 has demonstrated that interspecific hybridization of these plants can be successful. In 2007, the winter-hardy *Alstroemeria* cultivar, 'Mauve Majesty' is being released.

LITERATURE CITED

Bridgen, M. 2001. A nondestructive harvesting technique for the collection of native geophyte plant species. Herbertia 56:51–60.

Bridgen, M.P. 1997. Alstroemeria, pp. 341-348. In: V. Ball, ed., Ball Red Book, 16th Ed., Geo. J. Ball Publ. Co., W. Chicago, Illinois.

Bridgen, M., and **E. Olate.** 2004. Alstroemeria of Chile. Bulbs 6(2): 9–14.

Lu, C., and **M.P. Bridgen.** 1997. Chromosome doubling and fertility study of *Alstroemeria aurea* × *A. caryophyllaea*. Euphytica 94:75–81.

Marticorena, C., and **R. Rodriguez.** 1995. Flora de Chile, Vol. 1: Pteridophyta-Gymnosperma. Concepción, Chile.

Murashige, T., and **F. Skoog.** 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plant. 15: 473–497.

Schiappacasse, F., P. Peñailillo, P. Yáñez, and **M. Bridgen.** 2005. Propagation studies on Chilean geophytes. Acta Hort. 673:121–126.