Novel approaches to plant propagation and development of new crops
Camborg, Oluf L.

Publisher's version / Version de l'éditeur:

Report of proceedings of the Western Canadian Society for Horticulture, 1972

Novel Approaches to Plant Propagation and Development of New Crops

Oluf L. Gamborg

Prairie Regional Laboratory,

National Research Council,

Saskatoon, Saskatchewan

Presented February 26, 1972 at the

Annual Meeting

of the Western Canadian Society for

Horticulture

NRCC No. __________

Introduction

The seedless grapes and the tangello are well known examples of novel horticultural achievement. These achievements are significant from a scientific as well as from a practical viewpoint. We can think of a great many other plants for which specific modifications would be desirable. The development of hardy fruit crops and ornamentals for western Canada are an example. Developing plants resistant to diseases such as Fireblight or Dutch Elm disease is another important objective. These changes require genetic modifications. The usual procedures of plant breeding involves sexual crosses. These methods have been invaluable and will always be required. However, plant breeders recognize the need for making broad crosses in order to expand the gene pool and gain access to new genes representing desirable characteristics. Such a task is difficult because of incompatibility barriers in the pollination process, and interspecific crosses are very rare.

Pollination involves fusion of sex cells. In the last 3-4 years a new idea has been proposed and is being tested. Rather than fusing sex cells it may be possible to fuse somatic or asexual plant cells. On division the fused cells would give rise to hybrids which would contain chromosomes from separate species.

Such hybrids are available from animal cells. Since 1960 it has been possible to fuse cultured cells of man-mouse,

mouse-rat etc. and obtain hybrids. However, these cells will always remain hybrid cells and organs are not produced. On the other hand, somatic plant cells can be cultured, and also be induced to differentiate and develop into complete plants. If it was possible to obtain hybrid plant cells, it may also be possible to develop hybrid plants by the cell fusion procedure.

Plant Cell Culture

In order to study and obtain such hybrids it is necessary to use a series of techniques involving the culture of sterile plant cells. Such techniques are now available, and it is possible to grow plant cells indefinitely on sterile, defined media on a small as well as on a large industrial scale. We began the plant cell culture program at the NRC Prairie Regional Laboratory in 1963. Since then we have cultured cells of about 60 different species and cultivars, bean, pea, carrot, cereals, apple and several ornamental plants.

The cell culture is initiated from a piece of sterile tissue placed on agar medium containing salts, sucrose, the B-vitamins and a growth regulator (generally 2,4-D). The mass of cells produced is referred to as a callus. The cells can be cultured either on solid agar medium or in liquid, shake culture.

Differentiation and Morphogenesis

As mentioned previously, the cultured cells of some species have been induced to differentiate and grow into complete plants. This property was first demonstrated in the late 1950's. Plant development may take place by embryogenesis or by organogenesis. Organogenesis occurs as a result of chemical treatment. When the cells are grown in the presence of specific hormones shoot development takes place. Root formation can then be achieved by changing the culture medium and the hormone. The first successful experiments were performed with tobacco cells by Skoog et al. in Wisconsin. When the tobacco cells are grown in the nutrient medium in the presence of an auxin (indoleacetic acid) and a cytokinin (kinetin) at the proper concentrations bud formation occurs.

The tobacco cells readily differentiate and form buds. Other species have been more difficult. In our laboratory we have determined the conditions required for bud and shoot development from pea cells.

Plant development may also occur by embryogenesis. This process is rare and cannot be induced. It was first discovered in cultured cells of carrot. Any cell culture of carrot produces embryos, which resemble in every respect an embryo derived from the zygote. We have discovered the same phenomenon in cells of brome grass. This type of morphogenesis is quite rare but a very desirable property, because it takes place without mediation of exogenous hormones and occurs more rapidly and in far greater proportion of the cells than hormone-induced morphogenesis.

Application of the Morphogenetic Property

The morphogenetic property of cultured plant cells has many uses in research, but has also several practical applications.

1. Plant Propagation

The orchid producers in France had a serious problem in propagating orchids from seeds. It was discovered by Dr. Morel, that shoot meristems of the orchids could be cultured. The meristem produced protocorms (the juvenile stage of the orchid), which could be subdivided into many sections. Each section can give rise to a plant, or to more protocorms, depending upon culture conditions. The procedure is now the commercial process, and has reduced the cost of production 50 fold. It is estimated that more than 4 million protocorms can be produced in one year from a single meristem. The shoot meristem procedure has been adapted for practical use in asparagus and citrus fruits propagation by Dr. Murashige, University of California, Riverside.

2. Virus-free Stocks

Viral diseases are a serious problem in many horticultural crops. It is well known that the virus infect all cells except those in the shoot tip. In several cases it has been possible to remove these cells and grow them in tissue culture to obtain a mass of cells. These cells are then grown on media which induce the cells to differentiate and produce plants. Most of the plants are virus-free, and can be used for stock plants. This procedure is used commercially for potatoes, gladiolus, geranium, carnation and chrysanthemum. There is no report that the procedure has been developed for example for raspberry, although viral diseases are still an important problem in the culture of these plants.

3. Hybrid Plants

The morphogenetic capability also is an important step in the proposed development of hybrid plants referred to earlier. The procedure involves the production of genetically modified cells. This may be brought about by cell fusion or possibly by transformation with DNA.

Cell Hybridization

Plant cells cannot be fused until the rigid walls have been removed. This can be done by enzymatic degradation and naked cells or protoplasts are produced. These protoplasts are fragile and sensitive to chemical and physical factors, and are easily destroyed. Methods have been developed in our laboratory for preparing and culturing large numbers of protoplasts of several plant species. The protoplasts reform the cell wall and undergo division.

The fusion of the protoplasts from the same as well as from different species have been demonstrated. Multinucleated protoplasts of soybean produced by fusion have been shown to enter mitosis. For the most part the nuclei divide synchronously. Fusion products of soybean and wheat as well as wheat and rice have been obtained, but lack of suitable markers has made it impossible to follow the progress of the heterokaryons.

Protoplasts of carrot and brome grass cells have been cultured, and plants have been obtained by tissue culture procedures already discussed.

It is apparent that many of the basic procedures have been developed and the progress is quite rapid. One current difficulty is the detection and isolation of hybrid cells from amongst a population of parent cells.

Cell fusion is one procedure for obtaining genetically modified cells. The other method which is being studied is cell transformation. For this purpose one can use either cultured cells or whole plants. The procedure involves the uptake of DNA from plant A by plant B. The DNA from A should become integrated with the genome of plant B and cause changes in the phenotype. Recent results by Dr. Ledoux in Belgium and Dr. Hess in Germany suggest the procedure may be feasible.

Future Prospects

The techniques of cell culture and cell hybridization are important in the search for more productive crops. The techniques are looked upon with particular interest and anticipation by people such as Dr. Borlaug, (International Wheat and Maize Improvement Center, Mexico) who are responsible for the development of new, high-yielding cereal crops. Such crops have a high fertilizer demand. Consequently research is being persued to introduce the nitrogen-fixing ability of the legumes into cereals and other plants.

The techniques being developed allow experiments on the transfer of any characteristic from one plant to another, and widens the scope tremendously for what can be investigated.

At present the cell culture methods are mainly research tools. However, I hope the examples discussed have illustrated some of the immediate practical possibilities as well as the potentials of the techniques.