

Chapter 1

Introduction

Experimental systems based on plant cell and tissue culture are characterized by the use of isolated parts of plants, called explants, obtained from an intact plant body and kept on, or in a suitable nutrient medium. This nutrient medium functions as replacement for the cells, tissue, or conductive elements originally neighboring the explant. Such experimental systems are usually maintained under aseptic conditions. Otherwise, due to the fast growth of contaminating microorganisms, the cultured cell material would quickly be overgrown, making a rational evaluation of experimental results impossible.

Some exceptions to this are experiments concerned with problems of phytopathology in which the influence of microorganisms on physiological or biochemical parameters of plant cells or tissue is to be investigated. Other examples are co-cultures of cell material of higher plants with *Rhizobia* to study symbiosis, or to improve protection for micro-propagated plantlets to escape transient transplant stresses (Peiter et al. 2003; Waller et al. 2005).

Using cell and tissue cultures, at least in basic studies, aims at a better understanding of biochemical, physiological, and anatomical reactions of selected cell material to specified factors under controlled conditions, with the hope of gaining insight into the life of the intact plant also in its natural environment. Compared to the use of intact plants, the main advantage of these systems is a rather easy control of chemical and physical environmental factors to be kept constant at reasonable costs. Here, the growth and development of various plant parts can be studied without the influence of remote material in the intact plant body. In most cases, however, the original histology of the cultured material will undergo changes, and eventually may be lost. In synthetic culture media available in many formulations nowadays, the reaction of a given cell material to selected factors or components can be investigated. As an example, cell and tissue cultures are used as model systems to determine the influences of nutrients or plant hormones on development and metabolism related to tissue growth. These were among the aims of the “fathers” of tissue cultures in the first half of the 20th century. To which extent, and under which conditions this was achieved will be dealt with later in this book.

The advantages of those systems are counterbalanced by some important disadvantages. For one, in heterotrophic and mixotrophic systems high concentrations of organic ingredients are required in the nutrient medium (particularly sugar at 2% or more), associated with a high risk of microbial contamination. How, and to which extent this can be avoided will be dealt with in Chapter 3. Other disadvantages are the difficulties and limitations of extrapolating results based on tissue or cell cultures, to interpreting phenomena occurring in an intact plant during its development. It has always to be kept in mind that tissue cultures are only model systems, with all positive and negative characteristics inherent of such experimental setups. To be realistic, a direct duplication of in situ conditions in tissue culture systems is still not possible even today in the 21st century, and probably never will be. The organization of the genetic system and of basic cell structures is, however, essentially the same, and therefore tissue cultures of higher plants should be better suited as model systems than, e.g., cultures of algae, often employed as model systems in physiological or biochemical investigations.

The domain *cell and tissue culture* is rather broad, and necessarily unspecific. In terms of practical aspects, basically five areas can be distinguished (see Figs. 1.1, 1.2), which here shall be briefly surveyed before being discussed later at length. These are callus cultures, cell suspensions, protoplast cultures, anther cultures, and organ or meristem cultures.

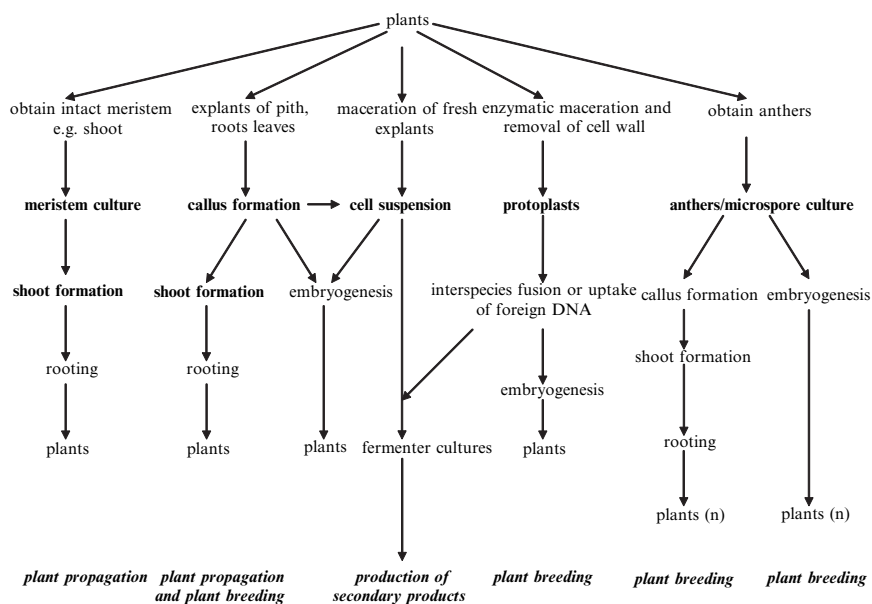


Fig. 1.1 Schematic presentation of the major areas of plant cell and tissue cultures, and some fields of application

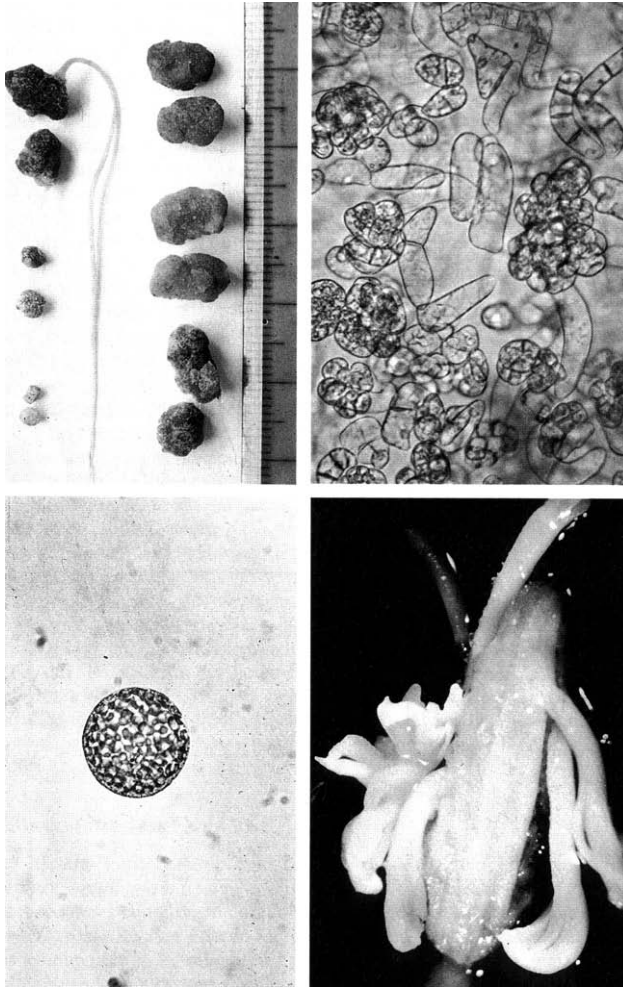


Fig. 1.2 Various techniques of plant cell and tissue cultures, some examples: *top left* callus culture, *top right* cell suspension culture, *bottom left* protoplast culture, *bottom right* anther culture

Callus cultures (see Chap. 3)

In this approach, isolated pieces of a selected tissue, so-called explants (only some mg in weight), are obtained aseptically from a plant organ and cultured on, or in a suitable nutrient medium. For a primary callus culture, most convenient are tissues with high contents of parenchyma or meristematic cells. In such explants, mostly only a limited number of cell types occur, and so a higher histological homogeneity

exists than in the entire organ. However, growth induced after transfer of the explants to the nutrient medium usually results in an unorganized mass or clump of cells—a callus—consisting largely of cells different from those in the original explant.

Cell suspensions (see Chap. 4)

Whereas in a callus culture there remain connections among adjacent cells via plasmodesmata, ideally in a cell suspension all cells are isolated. Under practical conditions, however, also in these cell populations there is usually a high percentage of cells occurring as multicellular aggregates. A supplement of enzymes is able to break down the middle lamella connecting the cells in such clumps, or a mechanical maceration will yield single cells. Often, cell suspensions are produced by mechanical shearing of callus material in a stirred liquid medium. These cell suspensions generally consist of a great variety of cell types (Fig. 1.2), and are less homogenous than callus cultures.

Protoplast cultures (see Chap. 5)

In this approach, initially the cell wall of isolated cells is enzymatically removed, i.e., “naked” cells are obtained (Fig. 1.2), and the explant is transformed into a single-cell culture. To prevent cell lysis, this has to be done under hypertonic conditions. This method has been used to study processes related to the regeneration of the cell wall, and to better understand its structure. Also, protoplast cultures have served for investigations on nutrient transport through the plasmalemma, but without the confounding influence of the cell wall. The main aim in using this approach in the past, however, has been interspecies hybridizations, not possible by sexual crossing. Nowadays, protoplasts are still essential in many protocols of gene technology. From such protoplast cultures, ideally plants can be regenerated through somatic embryogenesis to be used in breeding programs.

Anther or microspore cultures (see Chap. 6)

Culturing anthers (Fig. 1.2), or isolated microspores from anthers under suitable conditions, haploid plants can be obtained through somatic embryogenesis. Treating such plant material with, e.g., colchicines, it is possible to produce dihaploids, and if everything works out, within 1 year (this depends on the plant species) a fertile homozygous dihaploid plant can be produced from a heterozygous mother plant. This method is advantageous for hybrid breeding, by substantially reducing the time required to establish inbred lines.

Often, however, initially a callus is produced from microspores, with separate formation of roots and shoots that subsequently join, and in due time haploid plants

can be isolated. Here, the production of “ploidy chimeras” may be a problem. Another aim in using anther or microspore cultures is to provoke the expression of recessive genes in haploids to be selected for plant breeding or gene transfer purposes.

Plant propagation, meristem culture, somatic embryogenesis (see Chap. 7)

In this approach, mostly isolated primary or secondary shoot meristems (shoot apex, axillary buds) are induced to shoot under aseptic conditions. Generally, this occurs without an interfering callus phase, and after rooting, the plantlets can be isolated and transplanted into soil. Thereby, highly valuable single plants—e.g., a hybrid—can be propagated. The main application, however, is in horticulture for mass propagation of clones for the commercial market, another being the production of virus-free plants. Thus, this technique has received a broad interest in horticulture, and also in silviculture as a major means of propagation.

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