Applications of Plant Tissue Culture

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The history and, in turn, the applications of plant tissue culture have differed from those of animal tissue cultures as a consequence of three fundamental differences in origin and organization between the tissues of higher plants and higher animals. Of these, the first—and most important in comprehending what is to follow—is that plant organs develop from meristems, i.e., from organized clusters of dividing cells. These may be terminal, situated at the extremities of developing organs, or lateral, in the form of cylindrical sheets of dividing tissue enclosing a central core of derivatives and in turn enclosed by a hollow cylinder of outward derivatives. Cell divisions in other parts of the plant are rare and usually occur only in response to injury.

The second important difference lies in the encasement of plant cells in rigid cellulose walls. These walls are plastic and can be changed in shape and size by growth processes, but effectively prevent any motility. Consequently plant tissues in culture do not exhibit the appearance of a spreading colony, but produce instead either complete organs or mounded growths. Each cell is cemented in the position in which it formed, except as it is displaced by division or enlargement of adjacent cells.

Finally, plant cells are not typically bathed in any tissue fluid like the animal lymph. In fact, most plant juices are definitely detrimental to tissues in culture, and while it has been possible to obtain useful fractions from some of these extracts, it has not been possible to culture plant parts on the vegetable counterparts of plasma clots and embryo extracts. Consequently it was necessary with plant tissue cultures to develop synthetic media, and this had to wait for further development of our knowledge of compounds involved in growth.

So, although the first real attempts at plant tissue culture date back to Haberlandt in 1902 (8), the period that followed was marked by little real progress. With few exceptions, investigators would publish one or two papers describing negative results, then turn to more productive researches. There did develop during this period, however, the gradual realization that mature or differentiated tissues offered little promise, whereas the organized meristems would develop for a short time at least. This realization, together with the unfolding of the bios story in the '30's, made it possible for several investigators almost simultaneously in 1934 to publish reports of successful culture of plant tissue. P. R. White (16) in this country was first to obtain unlimited growth of tomato roots while Gautheret (3) in France obtained extensive development of fragments of cambium.

This development led naturally to the first of the applications of plant tissue cultures of which I wish to speak—to the study of the heterotrophic nutrition of autotrophic plants. Green plants, of course, are nutritionally independent of other living organisms. Since they can synthesize all their requirements from inorganic materials and the sun's energy, they are the fundamental source of food for all living organisms.

Yet even within the plant, the manufacture of food is confined to limited regions, usually the leaves. The rest of the plant—the stem tissues, the roots, and all of the meristems—are incapable of manufacturing food and are just as dependent on photosynthesizing tissues as are the animals. With the plant intact, there is no way of determining the essentiality of the materials exchanged between the leaf and the non-green parts. The success of the first tissue culture opened the way for the study of the nutrition of separate plant organs—a study that is by no means completed.

In general, the isolated parts require an energy source and certain growth factors as organic supplements to the media. Rather surprisingly, sucrose has proved superior to other sugars for the majority of tissues. In tomato roots, however, only one-fourth of the sucrose provided is actually utilized in growth, the remainder appearing in the medium as hexoses. Phosphate must be available for sucrose utilization and the process is inhibited by phloridzin (15). This and other evidence indicates that sugar is absorbed as hexose-phosphate formed at the cell surface from sucrose by a phosphorylase. This is quite different from the absorption mediated by hexokinase in mammalian kidney and intestine and raises the question of how and where the abundant free glucose and fructose of plant tissues is utilized.

Among the growth substances, thiamine is quite generally required. Pyridoxine and niacin are beneficial for some roots but not for others. Biotin and pantothenic acid must be supplied for willow cambium (7), but carrot cambium succeeds without either of these. Coconut milk provides for certain embryos and callus cultures factors which have not yet been identified. Beyond this there are numerous tissues and organs which cannot be cultured on present media and which must require other nutrients. It is of particular interest that no one has yet demonstrated in plants a requirement for any of the fat-soluble compounds required for growth or full development of mammals and birds.

From all of this there emerges *no* clear evidence that there exists a special nutrition of roots that differs consistently from the nutrition of stems, or of the cambium, or of any other part. The various requirements of the heterotrophic portions of the plant seem to be rather random losses of synthetic abilities which are unnecessary and extravagant in view of the greater synthetic capacities of the photosynthetic tissues. The heterotrophic portions of plants depend on their normal environment in much the same fashion that the ubiquitous saprophytic bacteria and molds depend on theirs. This does not mean that the relative growth of different parts of the plant cannot be regulated by materials exchanged between the parts. The exchange, however, seems to control the amount of growth, rather than the nature of the growth.

Synthetic abilities are often more precisely allocated to specific organs or tissues. One example of this, which was demonstrated with the help of tissue culture, is the restriction of nicotine synthesis to the roots of the tobacco plant. Cultured roots continue to form nicotine and excrete it into the culture fluid (2).

While there are no specific requirements for the *maintenance* of particular patterns of development, there are rather precise requirements

for the *initiation* of new patterns. This takes us into the area of morpho-
genesis—to which plant tissue culture has perhaps made its greatest
contribution. The first—and a major—contribution was already at hand
with the achievement of successful root cultures. The demonstration that
a small portion, 1mm. or less, of the root tip could be separated from the
remainder of the plant and would continue to produce normal root (12)
served to establish that the whole of the organizational requirement for
root was contained in this small region. No morphogenetic field extends
from other parts of the plant or even from older portions of the same root.
The developmental pattern is inherent in the organization of the root
meristem itself. This restriction of the morphogenetic field occurs also in
stem meristems (10) and in very young fruits (11).

Cambium cultures may be an exception. These meristems, when
placed in culture, demonstrate their original polarity by producing cells
characteristic of wood on one side of the dividing layer and quite differ-
ently specialized cells on the other. Some morphogenetic influence is
lacking, however, for the specialized derivatives lack their original orien-
tation. With continued culture the plate-like structure of the meristem
disappears and is replaced by isolated clusters of dividing cells which
produce unspecialized derivatives (6). Here it seems apparent that cam-
bium is dependent for its continued development as cambium on physical
or chemical influences from the tissues which normally surround it. The
morphogenetic field must be more diffuse than in the case of the root
meristem, hence essential components of the field can be, and are, left
behind when the tissue is extirpated.

Some small progress has been made toward an elucidation of the
components of such morphogenetic fields in a few cases. White in 1939
(17) made the important observation that cultures of tobacco callus,
which display no differentiation of organized tissues when cultivated on
the surface of agar media, would produce well-organized stem tips if
submerged in liquid. Subsequently Skoog and Tsui (14) demonstrated
that the frequency of stem meristem formation could be markedly in-
creased by incorporation into the medium of critical concentrations of
adenine. If we add to this the assumption, which is probably justified by
analogy with other stems, that tobacco stem tips do not require added
adenine for their continued growth, we have here two components of a
field capable of organizing a complex meristem from a relatively undiffer-
entiated, but proliferating, tissue mass. The first component is probably
physical-chemical in nature, perhaps a gradient in oxygen availability;
the other is a specific chemical compound.

In contrast to the effect of adenine, auxin, the plant growth hormone,
will induce the formation of root initials by undifferentiated callus cul-
tures of various sorts (4). Roots of the same species are capable of
unlimited growth in the absence of auxin. It is the initiation of the
meristem that requires specific conditions.

This chemical regulator, auxin, deserves further attention here. It
is probably the most versatile hormone known. It is produced, or at least
activated, in plant meristems, thence it is transported to other portions of
the plant where it induces morphogenetic responses. To mention a few of
these, it has been associated with the elongation of cells, the inhibition of

bud growth, the initiation of cambial activity, the initiation of root primordia, the suppression of root growth, and the induction or suppression of flowering. The particular nature of the response induced depends on the concentration of the hormone at the site of response and on the competence of the exposed tissue.

In plant tissue cultures, auxin has been of special importance because certain tissues which fail to proliferate in usual nutrients will proliferate rapidly if critical concentrations of auxin are included in the medium. This observation was made first by Gautheret with cultures of carrot cambium (4). At first, auxin was considered to have a nutrient role, although it was recognized that the concentration was extremely critical and that excesses led to formative effects in the tissue. Later, it was established that tissues which had been grown in media containing auxin could be transferred to auxin-free media where they would continue their proliferation at an undiminished rate (5). This phenomenon has been called "habituation." Subsequent analyses have shown that the habituated tissues produce their own auxin. Hence it seems that auxin induces its own formation in at least a portion of the tissues exposed to it.

Auxin induces another self-sustaining response in cultures of sunflower roots. Roots grown in liquid media containing auxin are shorter, more transparent, and more irregular in outline than roots of the same clone grown without auxin. Such auxin-treated roots will continue the modified pattern when subsequently cultured in auxin-free media (9).

Now I wish to turn to the field of plant pathology where there are many instances in which a parasite by means of its activities and its secretions induces a divergence from the typical growth pattern of its host. The growths which develop upon such stimulation have been called galls, but some have been likened, quite justifiably, to cancers. One in particular, crown gall caused by a bacterium, *Phytomonas tumefaciens*, has lent itself to investigation by the tissue culture technique. Infection of a susceptible host results in irregular tumorous outgrowths of the stem accompanied by secondary outgrowths of the stem or leaves above the primary infection. Braun in 1941 (1) demonstrated that secondary tumors in sunflowers were frequently free of bacteria and he and White in the same year (18) found that tissues of these secondary galls would proliferate rapidly in culture whereas normal stem tissues would not. When cultured tissue was grafted into healthy plants, tumors resulted. Quite obviously the activities of the bacteria had produced a sustained alteration in the growth proclivities of the tissue. Subsequently it has been demonstrated that the bacteria-free gall tissue can itself induce normal stem tissue to proliferate, provided it is united to the normal tissue by a completed graft union (13). Extracts of bacteria or of galls do not have this ability.

Apparently the bacteria exerts on the host tissues, and gall tissues on normal tissues, an influence that is similar to the habituation to auxin that was observed by Gautheret. Auxin itself, however, fails to induce proliferation of certain stem tissues that respond readily to the bacteria. In cases where habituated and crown gall tissues from the same plant could be compared, the cultures differ in growth rate, in morphological characters, and in auxin content.

From these various experiments, performed with different original objectives, there are two conclusions to be derived which seem to me of general importance in comprehending plant development. In the first place, while the nutritional requirements of individual meristems may vary, there does not seem to be a nutrition characteristic of particular classes of meristems such as roots, stems, or cambia. Secondly, there are many cases in which a particular pattern of development requires special conditions for its initiation which are not required for its maintenance.

It appears, therefore, that the property of inducing a direction of development which is subsequently self-sustaining is not a restricted phenomenon in plants. In fact, meristems, once organized, are essentially in steady states of varying degrees of stability.[1] The critical points in plant development seem to lie in the organization of new patterns compatible with the genetical and physiological capacities of the cells.

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[1] It is necessary, however, to recognize also the existence of progressive states which lead through a sequence of stages of the production of determinate structures. Meristems of this sort are represented by the leaf initial and the flower primordium.

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