Meetings

Crop Improvement through Plant Cell and Tissue Culture

Three aspects of cell and tissue culture are of great potential significance for crop improvement: (i) meristem culture which allows rapid clonal multiplication; (ii) culture of haploids from anthers which allows fixation and analysis of genetic combination and speeds up the achievement of homozygosity; and (iii) cell hybridization by protoplasmic fusion which will overcome existing barriers to achieving hybrids between species or genera of important food or fiber plants. The first two approaches are immediately feasible and should be applied widely to crops; the third approach is still experimental, but it shows great promise.

The Rockefeller Foundation sponsored a conference on Crop Improvement through Plant Cell and Tissue Culture from 14 to 16 May at the Villa Serbelloni on Lake Como, Italy. This session, built around major topics suggested by the chairman, J. G. Torrey (Harvard University), stressed two aims: (i) to determine which facets of the field of plant cell and tissue culture have not received enough emphasis and to develop such areas, and (ii) to determine how best to utilize the techniques of plant cell and tissue culture in crop improvement and thus help conquer hunger.

Regeneration of plants from callus and cell cultures has now been accomplished with enough species to consider that it can be done with all plants—that is, only technical details remain to be worked out for each individual case. Therefore, the use of mutagens (both ionizing radiations and chemicals) is being reevaluated as a means of increasing variation which would be of potential benefit to breeding programs. Among markers already in use for genetic analyses are biochemical characters such as isoenzyme patterns, albinism, nucleic acid patterns, and blocks in biosynthetic pathways, as well as morphological characters such as chromosome number and karyotype, cell morphology, and the phenotypic expression of organized structures.

Since the somatic tissues in some plants have cells with varying chromosome numbers, some of the regenerated plants from cell cultures of such "mosaic" plants should be different from the "parent" plant. This approach is being used successfully with sugar cane, where plants thus obtained are now growing in the field for comparison with standard varieties.

Since it is theoreticaly possible to culture any tissue from any plant, the question arose as to why so little work has been done in cell and tissue culture on certain major crop plants—particularly corn, the crop plant about which the most is known genetically. Little success has been reported in culturing certain other crop plants—especially the sugar beet. Succulents, in general, have been difficult to work with except for the cactus *Opuntia*. No reports are known of successful callus culture of sorghum or manihot.

Although the isolation of protoplasts was reported as early as 1892, and fusion of isolated protoplasts from the same plant or from different plants was observed in 1937, current interest in this subject is high because of the potential of this technique for fusion of somatic cells from distantly related plants to produce new plants. Recent progress in manipulating protoplasts suggests that asexual fusion might become a major method for "crossing" unrelated plants which are not easily crossed using standard sexual methods. At the conference, special attention was given to the latest methods for producing protoplasts by removal of the cell walls through enzyme treatment (by cellulases, hemicellulases, pectinases, or combinations of these enzymes). Useful and effective commercial enzymes are now available, best results having been obtained with purified cellulases obtained from the fungi *Myrothecium verrucaria* or *Trichoderma viride*. Regeneration of new cell walls around isolated protoplasts and fusion of protoplasts in sterile culture were also reported.

Apparent genetic instability of tissue and cell cultures was much discussed. One question kept occurring: "Is this so-called instability of plant cells and tissues in culture simply the result of the removal of the pressure of being part of a multicellular environment?" Frequent subculture of diploid cells was reported to assure greater stability in culture. The auxins indoleacetic acid and naphthaleneacetic acid were both reported to be better than 2,4-dichlorophenoxyacetic acid in the culture media for maintaining stability of chromosome number.

Synchrony of cell division in plant systems was discussed at length. In rapidly growing cell-suspension cultures about 10 percent of the cells routinely divide synchronously. This can be increased to approximately 40 percent with hydroxyurea. Second and subsequent divisions show lowered percentages, and the culture soon reverts to normal. Suggestions for increasing the number of cells dividing in synchrony included use of the following agents: (i) osmotic pressure shock with polyethylene glycols and subsequent release of the pressure; (ii) lowered temperature; (iii) removal of a required compound; (iv) removal of water; (v) treatment with blue light followed by red light; and (vi) limitation of available carbon or nitrogen in the medium.

The potential for meristem culture of clonal material (as well as for obtaining virus-free young shoot tips), used so effectively in propagating orchid, carnation, and asparagus, was discussed in detail. Application of the method to other species was explored in relation to crop species and to other plants of economic importance, including trees.

Considerable attention was given also to the recently reported successes in producing haploid plants from cultured anthers, studied already in *Nicotiana, Datura*, and *Oryza*. Reports of failures in other genera suggest that intensive experimentation is needed to make the technique generally applicable. Implications to geneticists and plant breeders of rapid production of haploid plants by this method were elaborated.

There was a consensus among the conferees that recent successes in the culture of tissues of monocotyledons were not the result of a scientific breakthrough but were instead the result of the concentrated effort made during the last decade to culture a number of important crop plants, particularly sugar cane and rice and other cereals. Even

though the inclusion of coconut milk in the medium first made it possible to grow callus tissues of monocots successfully, it is believed that much of the recent success with these plants can be attributed to the development of effective synthetic media. Such media usually have higher amounts of auxin than those used for dicots, as well as raised concentrations of inorganic constituents.

In the final session, in addition to discussing such topics as research support, publications, more effective channels of communications, and problems of shipping cultures from one country to another, the conferees agreed upon those qualities which would make a particular plant ideal for research. Desirable characteristics would include absence of endopolyploidy, stable low chromosome number, stable concentrations of deoxyribonucleic acid, large chromosomes, good growth rate, good growth on defined medium, easy separation into suspension, easy organ regeneration, and a well-defined genetic background. Regrettably, no known plant has all of these attributes.

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Note

1. Conferees from eight countries included ten plant physiologists who have specialized in plant cell and tissue culture, two geneticists, and two scientific observers. Participants were: A. C. Braun, United States; E. C. Cocking, United Kingdom; F. D'Amato, Italy; T. Eriksson, Sweden; O. L. Gamborg, Canada; A. C. Hildebrandt, United States; G. Morel, France; L. G. Nickell, United States; J. Reinert, Germany; R. Riley, United Kingdom; J. G. Torrey, United States; Y. Yamada, Japan; L. M. Roberts, Rockefeller Foundation; and H. J. Carlson, National Science Foundation.

Forthcoming Events

December

7–9. American Soc. of **Hematology**, Cleveland, Ohio. (F. H. Gardner, Presbyterian–Univ. of Pennsylvania Medical Center, Philadelphia 19104)

7–12. American Soc. for **Testing and Materials**, Cincinnati, Ohio. (T. A. Marshall, Jr., ASTM, 1916 Race St., Philadelphia, Pa. 19103)

8–10. **Applications of Simulation**, 3rd conf., Los Angeles, Calif. (P. J. Kiviat, Simulation Associates, Inc., 1263 Westwood Blvd., Los Angeles 90024)

8–10. **Circuit Theory**, intern. symp., San Francisco, Calif. (B. J. Leon, School of