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Plant tissue culture methods in somatic hybridization by protoplast fusion and transformation.

Oluf L. Gamborg

A contribution to the Symposium on"Control Mechanisms in Development: Activation, Differentiation and Modulation in Biological Systems."

Presented October 14, 1974 at the School of Life Sciences, University of Nebraska, Lincoln, Nebraska.

Current concepts of gene structure and regulation were formulated largely on the basis of biochemical and molecular genetical analyses of prokaryote cells. Their rapid growth makes it possible to obtain large quantities of single cells and measure biochemical and physiological events relatively quickly. It is not surprising that microorganisms became the material of choice for investigations on the metabolic and molecular genetic regulation of growth and differentiation in living cells.

Parallel with the expanding knowledge of molecular genetics and cell regulation came discoveries of transfer of genetic material in microorganisms. This process can occur through uptake of DNA (transformation), by transduction involving viral carriers, or by conjugation (Merrill and Stanbro, 1974).

By using phages and plasmids, it is feasible to construct and transfer specific genes from one organism to another, where the genetic material may integrate and become functional (Cohen et al, 1973).

Similar genetic manipulations are now being envisaged with plant cells. The procedures focus on DNA-mediated transformation and hybridization by cell fusion and involve methods of plant cell culture (Nickell and Heinz, 1973; Gamborg and Miller, 1973; Gamborg et al, 1975).

Plant Cell Culture and Morphogenesis

Plant cells can be grown indefinitely as masses on agar (callus) or in liquid suspension (Fig. 1,A), and produced in any desired quantity. Generally the cells require only mineral salts, sucrose, and certain

vitamins and growth hormones (Street, 1973). The latter are needed to induce cell division.

Cells grown in suspension culture appear to be undifferentiated and generally do not show any structural or biochemical evidence for functions other than continued division. However, totipotency may be expressed. When produced and cultured under suitable conditions, the cells can be induced to differentiate, and regernerate complete plants (Street, 1973; Murashige, 1974)(Fig. 2).

The process of morphogenesis may occur by embryogenesis in the absence of exogenous hormones or by organogenesis induced by cytokinins. The cytokinins are adenine-derivatives, which stimulate shoot formation in most plants except cereals and grasses. Shoots may be readily obtained from stem sections, and can be produced from callus grown in the presence of cytokinins (Kartha et al, 1974a; Gamborg et al, 1974a)(Fig. 1, B, C). When shoots are excised and transferred to other media, rooting can be achieved (Fig. 1, D).

There are numerous examples of plant regeneration from callus, and in some cases the techniques can be utilized as a routine method for vegetative propagation (Earle and Langhans, 1974; Kartha et al, 1974b; Murashige, 1974)(Fig. 3).

Apart from hormones and tissue origin, the culture medium and light and temperature play a significant role in the success of plant regeneration (Murashige, 1974). The specific conditions must be determined for each plant species.

Plant Protoplast isolation and culture

Significant advances have been made in the technology for isolating plant protoplasts (Cocking, 1973; Tempè, 1972; Gamborg et al, 1973). They are released by incubating the tissues with hydrolytic enzymes (cellulases, hemicellulases, pectinases) in solutions containing osmotic stabilizers such as hexitols or glucose (Fig. 4, A). Protoplasts can be prepared in high yields within 6-12 hours depending upon the tissue (Kao et al, 1971; Tempe, 1973; Constabel, 1975). The most common materials are leaf tissues and cells from suspension cultures, but they can be obtained from fruits, root and shoot materials (Nickell and Heinz, 1973).

Isolated protoplasts can be cultured and in some cases be induced to divide (Table I)(Fig. 4, B). Growing protoplasts in droplets has been very successful (Kao et al, 1971). The nutritional requirement of protoplasts is similar to those of plant cells, but initially they often have special needs for sugars, calcium, glutamine or hormones. The first divisions may occur within 24-72 hours and a callus would subsequently be produced.

Cultured protoplasts reform a cell wall which in composition and structure resembles the original wall. (Albersheim, private communication; Fowke et al, 1974).

Mitosis and division on the reconstituted cells resembles in every respect that of cells grown in suspension culture (Fowke et al, 1974). Although sustained division has been possible in protoplasts of some species, the lack of success in many others remains a serious handicap

and warrants continued study (Eriksson et al, 1975).

Genetic modifications in plants

Various methods are being explored to develop procedures which permit gene transfer and modification in somatic cells of plants (Fig. 5). Apart from their potential value in genetic analyses, these methods may provide a means for achieving wide crosses in plants. The natural barriers which prevent sexual crosses between plant genera normally do not exist when somatic cells are hybridized. Two methods have been considered for gene transfer: DNA-mediated transformation and cell fusion. Protoplasts are required for cell fusion, while transformation can be investigated also with other plant materials. Transformation or fusion with protoplasts involves four steps (Fig. 5):

1. Protoplast isolation and culture

   Develop procedures for isolating viable protoplasts, and establish methods for culturing the protoplast and regenerating dividing cells.

2. Fusion/DNA-uptake

   Carry out fusion/DNA-uptake.

3. Cell selection and culture

   Reconstitute the cells and select the hybrid/transformed cells.

4. Plant production

   Culture the genetically modified cells, under conditions appropriate for differentiation and plant regeneration.

Protoplast fusion

Fusion can occur spontaneously between adjacent protoplasts during isolation. The process appears to commence with dilation of plasma-desmata connecting plant cells. Fusion of protoplasts from different sources must be induced. The isolated protoplasts are spherical, and an agglutinating agent is required to increase the area of membrane contact to facilitate fusion.

Although several compounds such as nitrate, lysozyme and treatments involving sudden osmotic changes and centrifugal forces were reported to facilitate fusion, the rates were very low (Eriksson et al, 1975). Hartmann et al (1973) prepared antisera and achieved effective agglutination of protoplasts from different plant genera, but fusion did not occur. Treatment at alkaline pH in the presence of calcium induced fusion of protoplasts from two tobacco mutants (Keller and Melcher, 1973) and interspecies hybrid tobacco plants were produced (Melcher et al, 1974).

An effective method for protoplast fusion employing polyethylene glycol (PEG) has been introduced recently (Kao and Michayluk, 1974; Eriksson et al, 1975). The polymer facilitates fusion of protoplasts of different plant genera. Protoplasts aggregate immediately when exposed to high concentrations of PEG (Fig. 6, A, B). Fusion occurs when the PEG is eluted out and heterokaryons are formed (Fig. 4, C) (Kao et al, 1975; Fowke et al, 1975). The fusion rate depends upon protoplast quality, molecular size of the PEG, and is also influenced by compounds and treatments which modify the plasma membrane (Kao et al, 1975; Constabel and Kao, 1974).

Intergeneric fusion can be achieved consistantly at rates between

10 and 40% of the surviving protoplasts. There are no apparent difficulties in fusing protoplasts of widely different plant genera and families, and the tissue origin of the protoplasts does not seem to be critical for heterokaryon formation and division (Table II). Present evidence indicates that if at least one of the parental protoplasts normally can be induced to divide, there is a greater chance for division and hybrid cell formation after fusion. It is therefore essential that conditions for the culture and division of at least one of the parent protoplasts be developed before any attempts are made to obtain cell progeny from fused protoplasts. Leaf mesophyll protoplasts exhibit relatively low frequencies of division. However, after fusion with protoplasts from the PRL soybean cell culture line, the resulting heterokaryocytes divide. The stimulus for division seems to reside in the soybean protoplasts (Fig. 6, D).

When heterokaryocytes produced by fusion divide, most of the leaf protoplasts deteriorate and, initially, the hybrids are the only cells with green chloroplasts (Table II). The hybrid cells contain a single nucleus, and it is apparant that nuclear fusion occurs prior to or during mitosis. The dividing cells contain chromosomes from both parental species (Fig. 6, E).

Recognition of the hybrid cells based on the presence of green chloroplasts has been adequate as an aid in demonstrating the formation of intergeneric hybrids. Procedures for eliminating both parental cell species and the isolation of hybrids is required to obtain pure cultures of the hybrid lines. Selective culture media or mechanical isolation

by micromanipulation may be feasible methods. Other systems include the use of metabolic mutants, toxins, antimetabolites or other selective chemicals (Chaleff and Carlson, 1974).

Transformation

Transformation may provide an alternate approach for achieving exchange of genetic information in plants. The process involves donor-DNA uptake, integration, replication and expression of new genetic information in the recipient organism or cell (Fig. 7, A) (Ledoux, 1971). The desirable result is the stable phenotypic expression of the genetic information coded for by the donor DNA. While cell fusion necessitates the use of protoplasts, investigations on transformation also have been pursued with plant organs. Using seeds or plant organs has the advantage that complete plant formation is ensured after introducing the DNA. Ledoux and Colleagues have investigated extensively the uptake and fate of bacterial DNA in plants (Ledoux, 1971). They reported successful correction of biochemical mutants of _Arabidopsis thaliana_ (L. Heyhn) requiring thiamine, thiazole or pyrimidine, after treatment of the seeds with DNA from microorganisms. The radioactive DNA fed to imbibing seeds was taken up and a portion stored in the cotyledons. The DNA was later translocated to the flower, and biochemical evidence was presented for a hybrid DNA in the F_1 progeny. Some of the transformed plants responded to the addition of thiamine to the growing medium, indicating incomplete correction (Ledoux, 1974).

Hess (19) observed a genetically stable change in flower color

in progeny of *Petunia* after feeding DNA from a red-flowering (wild type) to plants of a white-flowering anthocyanin-mutant. Several molecular genetic models have been proposed to account for these observations (Holl et al, 1975; Ledoux, 1971).

Cultured cells and particularly protoplasts may provide a more effective material. Protoplasts from cell suspension cultures of soybean, carrot and *Ammi* take up bacterial DNA (Ohyama et al, 1972). Up to 1% of the radioactive DNA was incorporated into TCA-insoluble fractions during a 4-hour incubation period. The bacterial DNA after uptake appears inside the protoplasts as the double-stranded form (Ohyama, 1975).

The protoplasts reform a cell wall and divide after the DNA treatment. Plant DNA supplied to Petunia protoplasts has been observed to enter the nucleus (Hoffmann and Hess, 1973). Although protoplasts may be highly desirable as materials for transformation experiments, their usefulness is restricted at present because cell regeneration and division at high frequency is limited to a few species. Moreover, biological marker systems for selection of transformed cells are not generally available.

The capability of plant cells to utilize new carbon sources should have potential as a selection system. Plant cells rarely grow on mannitol or lactose although these compounds are separated from the glycolytic pathway by only one or two enzymes. DNA from bacteria which utilize these compounds has been fed to soybean protoplasts, and cells were obtained which grew on lactose-mannitol media. The growth rates were extremely low and biochemical analyses have not been possible.

An alternative approach to feeding isolated DNA involves the use of transducing phages as carriers (Doy et al, 1973). Specific genes may be carried by phages which then serve as vectors to facilitate the introduction of DNA into the plant cells (Merrill and Stanbro, 1974).

Although controversies exist about classifying the present observations as genetic transformation, there is substantial evidence for DNA uptake and integration into the plant genome. However, considerable uncertainty still exists about the mode of integration and the stability of the donor DNA in the host cells.

Mutant cells

The plant cell and protoplast systems can be adapted and utilized for the purpose of mutant cell production. Cells from suspension cultures can be exposed to chemical or physical mutagens followed by selection for desirable mutants (Chaleff and Carlson, 1974).

The isolation of metabolic mutants of plant cells from the 'wild' type is not as readily achieved as with bacteria. The procedure of 5-bromodeoxyuridine BUdR) treatment followed by light exposure can be employed. Actively growing cells on minimal media incorporate BUdR and are destroyed while slow-growing mutants survive (Kao and Puck, 1968; Chaleff and Carlson, 1974).

Mutants with nucleic-acid-base analogs or antibiotic resistance may be effective, because a positive selection system for eliminating the normal, sensitive cells can be employed. The same principle applies

to the isolation of cells resistant to amino acid analogs and certain pathogenic toxins.

Ohyama has employed protoplasts from soybean cell cultures to obtain BUdR - resistant mutants (Fig. 7, B). The mutants grow rapidly in liquid suspension in the presence of BUdR, which is incorporated into the DNA (Ohyama, 1974).

At present the most readily available plant material consists of diploid cells. For dominant or organelle residing characteristics such cells may be adequate, but haploid cells would have wide application in mutqgenic studies. Haploid cells are becoming readily available through anther and pollen culture (Smith, 1974). In some plant species, anthers (pollen) can be cultured on defined media, where they form embryos and complete (haploid) plants (Sunderland, 1971). Cells obtained from anthers may not remain haploid but undergo chromosome changes to diploid and aneuploids.

Since haploid plants can be obtained, leaf mesophyll protoplasts from such plants could be used as materials for mutagenesis.

Hybrid plants

Cells obtained after fusion or transformation can be subjected to conditions for inducing plant regeneration. It is imperative, however, to determine the specific requirements for inducing organogenesis in cells regenerated from the particular protoplasts employed in the genetic experiment. Plants have been obtained from protoplasts of several species including carrot (Grambow et al, 1972), rapeseed (Fig 4, C)

(Kartha et al, 1974) and from cells produced by fusion of protoplasts from two tobacco species (Carlson et al, 1972; Melcher et al, 1974). The latter constituted interspecies hybrid plants. Similar plant regeneration may be anticipated from intergeneric somatic hybrid cells. In view of the ease with which hybrid cells from widely different plants can be produced and induced to divide, the process of hybrid plant formation may be realized when suitable culture conditions have been developed.

Concluding Discussion and Applications

Many advances in the technology of tissue culture and the handling of protoplasts have now made it possible to investigate genetic manipulation of somatic plant cells. The development of a new and efficient technique has resulted in fusion of protoplasts and the production of intergeneric hybrids between cells of various cultivated plants. The results show that the natural barriers which prevent sexual crosses between plant genera are circumvented in the fusion and growth of somatic hybrid cells.

Hybrid cells can be recognized by the presence of green chloroplasts from the parental leaf protoplasts. Such a detection system is made possible by designing conditions in which the unfused parental leaf protoplasts fail to survive. Appropriate selection systems which permit isolation of hybrids and elimination of all parental cells are being developed. It will then be possible to obtain clones and determine the stability of the karyotypes, which consist of a combination of the

parental chromosomes, and utilize the materials for biochemical and genetic analyses.

The evidence for transformation in plants is restricted largely to correction of biochemical lesions with bacterial DNA. The practical value of the technique, however, may be limited unless plant DNA can be used. Progress in the testing and development of gene transfer procedures depends upon the careful choice of suitable plant material such as the use of isogenic lines with single gene differences expressed as a clear cut phenotypic variation.

There is no information on the role of competence for DNA-mediated transformation in plants. The relative importance of molecular size, purity and degree of DNA denaturation has not been ascertained. An assessment of these problems becomes feasible with genetically characterised systems in which the encoded information is expressed in a relatively short time.

Present techniques also permit investigation of the uptake of nuclei, plastids and other organelles by protoplasts (see review by Eriksson et al, 1975). Organelle transfer to protoplasts and subsequent reconstitution of the cells may provide an elegant approach for genetic analyses and for investigations on gene control of biochemical and developmental processes in higher plants. Such systems may be helpful in elucidating the control of morphogenesis, a phenomenon which is very readily achieved in cells of such plants as tobacco or carrot, but not in cells of most cultivated plants (e.g. soybean, corn).

As hybrids and genetically modified cells become available, it is

expected that methods can be designed for inducing differentiation and plant regeneration. Although the capability for totipotency in somatic cells has been adequately documented, attempts to elicit the manifestation of the process is frequently unsuccessful. It is imperative to determine the necessary conditions and requirements for plant regeneration in one of the parental lines before attempting hybrid plant production.

Present progress in tissue culture and genetic manipulation procedures makes it possible to direct their application to problems of practical importance (Nickell and Heinz, 1973). Methods for producing wide crosses are required for plant breeding and crop improvement (Borland, 1971; Wittwer, 1974). Existing methods which depend upon sexual crosses are limited to closely related species. The somatic cell fusion and transformation method would make it possible to obtain an infinite variety of crosses. The products of these combinations may then be employed in conventional plant breeding systems. Somatic cell genetics, thus, provide the means to transfer desirable characteristics between plants of different families.

The objectives of more effective disease resistance, greater tolerance to extreme environments and plants with improved protein quality and quantity depend on such techniques (Wittwer, 1974).

The need for novel genetic procedures is suggested by more distant goals of significant agronomic potential. Current objectives include plans to introduce greater photosynthetic efficiency into crop plants. The product of photosynthesis of some plants are C-3 compounds, in others it is a C-4 compound. In the latter type the photo-

synthetic process is more efficient because the photorespiration is lower. The sites of genetic control and regulation of these properties have not been established.

Another objective of great importance is directed towards equipping non-leguminous plants with the ability to fix nitrogen from the air. Nitrogen fixation in legumes such as soybean and peanuts is a symbiotic process involving the plant and a *Rhizobium* species. Fixation takes place in nodules formed on the roots. The process is determined by bacterial and plant genes.

Although skepticism exists about the feasibility of manipulating the complex biochemical processes of photosynthesis and nitrogen fixation, somatic cell genetics may provide a tool to investigate these objectives. The design of less complicated systems to ascertain the genetics of separate steps of these processes should be the primary consideration (Holl et al, 1975).

The predictions of an imminent shortage of food may be alleviated by raising the efficiency of production and improving the yields of existing crop types. However, in view of continued population increases the urgency for new crops with superior productivity, growth efficiency and product quality remains a vital and compelling objective, which can be realized only through methods of somatic cell hybridization.

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References

Borlaug, N.E. 1971. The Green Revolution, Peace and Humanity. Cereal Science Today 16: 401-411.

Carlson, P.S., Smith, H.H. and Dearing, R.D. 1972. Parasexual interspecific plant hybridization. Proc. Nat. Acad. Sci. (USA) 69: 2292-2294.

Chaleff, R.S. and Carlson, P.S. 1974. Somatic cell genetics of higher plants. Am. Rev. Gen. (In Press)

Cocking, E.C. 1972. Plant cell protoplasts, isolation and development. Ann. Rev. Plant Physiol. 23: 29-50.

Cohen, S.N., Chang, A.C.Y., Boyer, H.W. and Helling R.B. 1973. Construction of biologically functional bacterial plasmids *in vitro*. Proc. Nat. Acad. Sci. (USA) 70: 3240-3244.

Constabel, F. 1975. Protoplast isolation and culture. *In* O.L. Gamborg and L.R. Wetter [ed.]. Plant Tissue Culture Methods. National Research Council of Canada, Saskatoon.

Constabel, F. and Kao, K.N. 1974. Agglutination and fusion of plant protoplasts by polyethylene glycol. Can. J. Bot. 52: 1603-1606.

Doy, C.H., Gresshoff, P.M. and Rolfe, B. 1973. Transgenosis of bacterial genes from *E. coli* to cultures of haploid *Lycopersicon esculentum* and haploid *Arabidopsis*. *In* J. Pollak and J. Wilson Lee [ed]. The biochemistry of gene expression in higher organisms. Australia and New Zealand Book Co. 21-37.

Earle, E.D. and Langhans, R.W. 1974. Propagation of chrysanthemum-in-vitro. I. Multiple plantlets from shoot tips and establishment of tissue cultures. J. Amer. Soc. Hort. Sci. 99: 128-131.

Eriksson, T., Bonnett, H., Glimelius, K. and Wallin, A. 1975. Technical advances in protoplast isolation culture and fusion. In E.H. Street and E.C. Cocking [ed.]. Advances in plant science through tissue culture.

Fowke, L.C., Bech-Hansen, C.W., Constabel, F., and Gamborg, O.L. 1974b. Comparative study on ultrastructure of cultured cells and protoplasts of soybean during cell division. Protoplasma 81: 189-203.

Fowke, L.C., Rennie, P.J., Kirkpatrick, J.W., and Constabel, F. 1975. Ultrastructural characteristics of intergeneric protoplast fusion. Can. J. Bot. (In Press).

Gamborg, O.L., and Miller, R.A. 1973. Isolation, culture and uses of plant protoplasts. Can. J. Bot. 51: 1795-1799.

Gamborg, O.L., Kao, K.N., Miller, R.A., Fowke, L.C. and Constabel, F. 1973. Cell regeneration division and plant development from protoplasts. Coll. Int. Cent. Nat. Rech. Sci. 212: 155-173.

Gamborg, O.L., Constabel, F., and Shyluk, J.P. 1974a. Organogenesis in callus from shoot apices of Pisum sativum. Physiol. Plant. 30: 125-128.

Gamborg, O.L., Constabel, F., Fowke, L., Kao, K.N., and Ohyama, K. 1975. Protoplasts and cell culture methods in somatic hybridization in higher plants. Can. J. Gen. Cytol. (In Press).

Grambow, H.J., Kao, K.N., Miller, R.A. and Gamborg, O.L. 1972. Cell division and plant development from protoplasts of carrot cell suspension cultures. Planta (Berlin) 103: 348-355.

Hartmann, J.X., Kao, K.N., Gamborg, O.L. and Miller, R.A. 1973. Immunological methods for the agglutination of protoplasts from cell suspension cultures of different genera. Planta 112: 45-56.

Hess, D. 1972. Transformationen an hoheren organismen. Naturwissenschaften 59: 348-355.

Hoffmann, F. and Hess, D. 1973. Uptake of radioactively labelled DNA into isolated protoplasts of Petunia hybrida. Z. Pflanzenphysiol. 69: 81-83.

Holl, F.B., Gamborg, O.L., Ohyama, K. and Pelcher, L. 1974. Genetic transformation in plants. In E.H. Street and E.C. Cocking [ed.]. Advances in plant science through tissue culture.

Johnson, C.B. and Grierson, D. 1974. The uptake and expression of DNA by plants. In H. Smith [ed.]. Current Advances in Plant Science. Vol. 2, No. 9, Commentaries. pp. 1-12. Maxwell, Oxford.

Kao, F.T. and Puck, T.T. 1968. Genetics of somatic mammalian cells. VII. Induction and isolation of nutritional mutants in Chinese Hamster cells. Proc. Nat. Acad. Sci. 60: 1275-1281.

Kao, K.N., Gamborg, O.L., Miller, R.A. and Keller, W.A. 1971. Cell divisions in cells regenerated from protoplasts of soybean and Haplopappus gracilis. Nature (London) New Biology 232: 124.

Kao, K.N. and Michayluk, M.R. 1974. Method for high-frequency intergeneric fusion of plant protoplasts. Planta 115: 355-367.

Kao, K.N., Constabel, F., Michayluk, M.R. and Gamborg, O.L. 1975. Plant protoplast fusion and growth of intergeneric hybrid cells. Planta (Berlin). (In Press).

Kartha, K.K., Gamborg, O.L., Constabel, F. and Kao, K.N. 1974a. Fusion of rapeseed and soybean protoplasts and subsequent division of Heterokaryocytes. Can. J. Bot. (In Press).

Kartha, K.K., Gamborg, O.L., Constabel, F. and Shyluk, J.P. 1974b. Regeneration of cassava plants from apical meristems. Plant Sci. Lett. 2: 107-113.

Kartha, K.K., Michayluk, M.R., Kao, K.N., Gamborg, O.L. and Constabel, F. 1974. Callus formation and plant regeneration from mesophyll protoplasts of rape plants (Brassica napus L. Cr. Zephyr.). Plant Sci. Lett. 3: 265-271.

Keller, W.A. and Melchers, G. 1973. Effect of high pH and calcium on tobacco leaf protoplast fusion. Z. Naturforschung 28: 737-741.

Ledoux, L. [ed]. 1971. Informative molecules in biological systems. North-Holland Publ. Co., Amsterdam.

Ledoux, L., Huart, R. and Jacobs, M. 1971. Fate of exogenous DNA in Arabidopsis thaliana. Eur. J. Biochem. 23: 96-108.

Ledoux, L., Huart, R., and Jacobs, M. 1974. DNA-mediated genetic correction of thiamineless Arabidopsis thaliana. Nature London 249: 17-21.

Melchers, G., Keller, W. and Labib, G. 1974. The consequences of the fusion of mesophyll protoplasts. Proc. Cong. Plant Tissue and Cell Culture. (Abstracts). p. 147. Leicester.

Merrill, C.R. and Stanbro, H. 1974. Intercellular gene transfer. Z. Pflanzenphysiol. (In Press).

Murashige, T. 1974. Plant propagation through tissue cultures. Ann. Rev. Plant Physiol. 25: 135-166.

Nickell, L.G. and Heinz, D.J. 1973. Tissue culture techniques as aids in economic plant improvement. In A.M. Srb [ed.]. Genes enzymes and populations. Plenum, New York. pp. 109-128.

Ohyama, K., Gamborg, O.L. and Miller, R.A. 1972. Uptake of exogenous DNA by plant protoplasts. Can. J. Bot. 50: 2077-2080.

Ohyama, K. 1974. Properties of 5-Bromodeoxyuridine-resistant lines of higher plant cells in liquid culture. Expt. Cell. Res. (In Press).

Ohyama, K. 1975. Introduction of genetic information into plant protoplasts. In O.L. Gamborg and L.R. Wetter [ed.]. Plant Tissue Culture Methods. National Research Council of Canada, Saskatoon.

Smith, H.H. 1974. Model systems for somatic cell plant genetics. BioScience 24 (5): 269-276.

Street, H.E. 1973. Plant tissue and cell culture. In Botanical Monographs, Vol. II. Blackwell Scientific Publications, London.

Sunderland, N. 1971. Anther Culture, A Progress Report. Sci. Prog. 59: 527-549.

Tempe, J. [ed.]. 1973. Protoplastes et fusion de cellules somatique vegetales. Coll. Int. Cent. Nat. Rech. Sci. 212, Paris.

Wittwer, S.H. 1974. Maximum production of capacity food crops. BioScience 24 (4): 216-224.

Table I. Examples of protoplasts in which cell regeneration and Division has been observed.

Systematic	Common Name	Cell Origin
Ammi visnaga		culture
Bromus inermis	Brome grass	culture
Cicer arietinum	Chick pea	leaf
Brassica napus	Rapeseed	culture, leaf
Daucus carota	Carrot	culture, leaf
Glycine max	Soybean	culture
Linum usitatissimum	Flax	leaf
Medicago sativa	Alfalfa	leaf, culture
Melilotus alba	Sweet clover	leaf
Phaseolus vulgaris	Bean	leaf, culture
Pisum sativum	Pea	leaf
Pisum sativum	Pea	culture, shoot tip
Vicia hajastana		culture
Vigna sinensis	Cow pea	leaf

Table II. Intergeneric plant protoplast fusion and heterokaryocyte division.

SOURCE OF PROTOPLASTS		
Leaf mesophyll		Cell Culture
Barley (Hordeum vulgare)	x	soybean (Glycine max)
Pea (Pisum sativum)	x	Vicia hajastana
Corn (Zea mays)	x	soybean (Glycine max)
Pea (Pisum sativum)	x	soybean (Glycine max)
Rapeseed (Brassica napus)	x	soybean (Glycine max)
Alfalfa (Medicago sativa)	x	soybean (Glycine max)
Sweet clover (Melilotus alba)	x	soybean (Glycine max)
Chick pea (Cicer arietinum)	x	soybean (Glycine max)
Angelica archangelica	x	carrot (Daucus carota)

* References

Constabel and Kao, 1974; Kao and Michayluk, 1974; Kao et al., 1975; Kartha et al., 1974; Fowke et al., 1975; Dudits, unpublished.

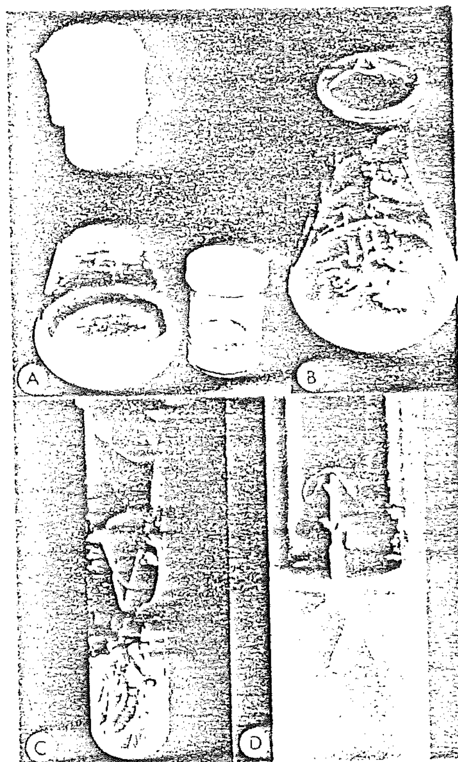

Fig. 1. Plant tissue culture and morphogenesis.

A. Cell suspension and callus culture of pea (Pisum sativum).

B. Plant regeneration from pea shoot tip callus.

C. Cassava plantlet produced by meristem culture.

D. Pea plantlet grown from shoot tip.

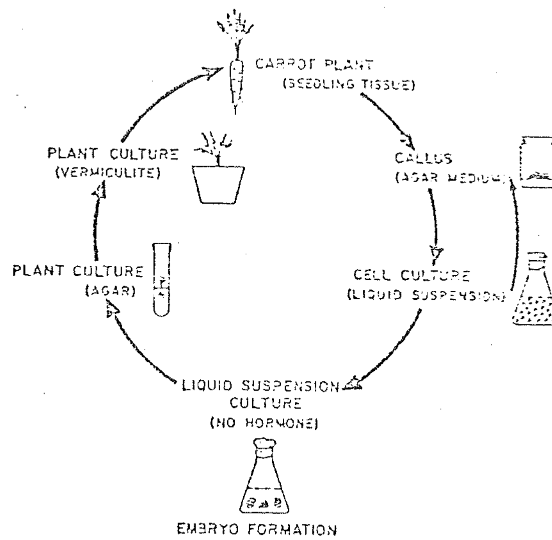

Fig. 2. Outline of tissue culture and embryogenesis in carrot cells.

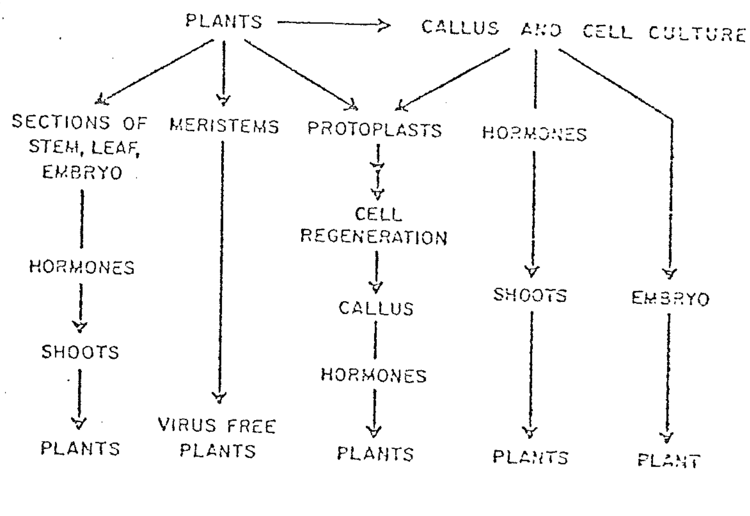

Fig. 3. Outline of protoplast and cell culture, and methods of morphogenesis.

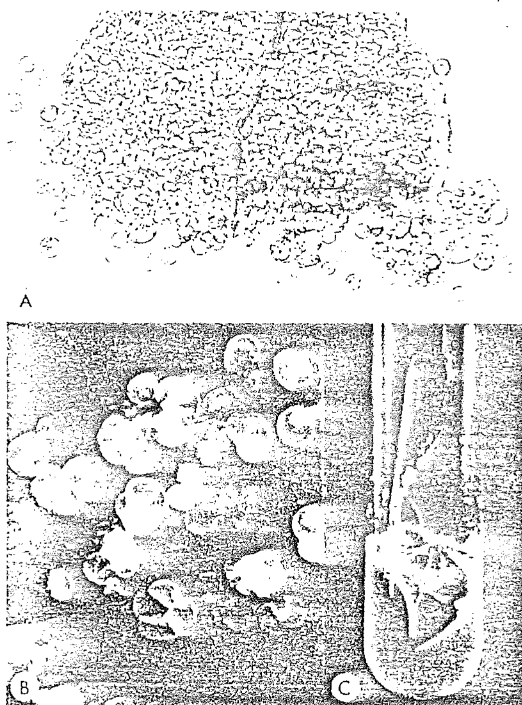

Fig. 4. Protoplasts.

A. Isolation from shoot tips of pea seedling.

B. Division in regenerated cells from rapeseed leaf protoplasts.

C. Plantlet grown from callus derived from rapeseed leaf protoplasts.

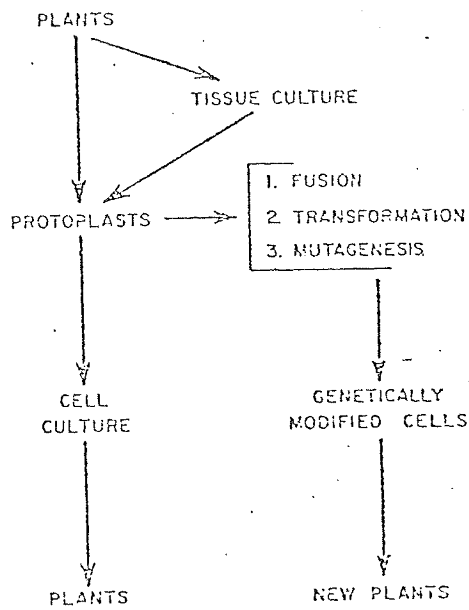

Fig. 5. Outline of methods for genetic modification in somatic plant cells.

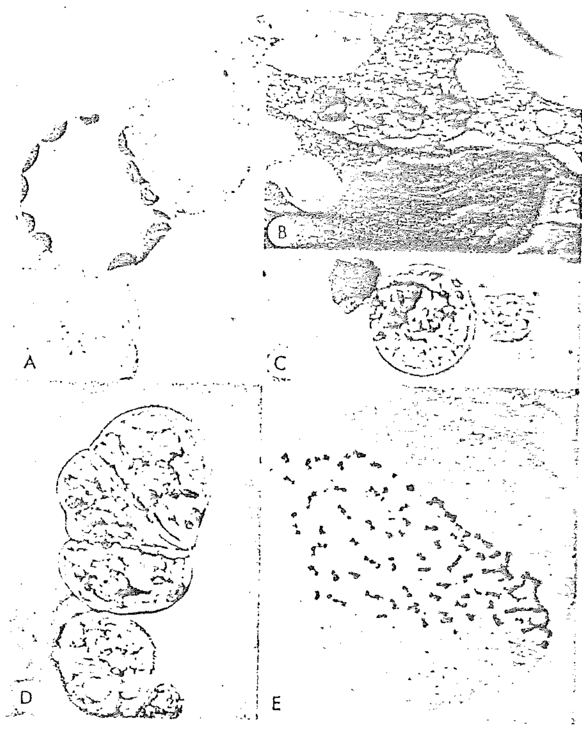

Fig. 6. Protoplast fusion.

A. Aggregate of protoplasts from pea leaf and *Vicia hajastana* cell culture produced in polyethylene glycol.

B. Ultrastructural section of A.

C. Fusion product (heterokaryocyte) of barley + soybean protoplasts.

D. Cells from barley-soybean heterokaryocyte.

E. Heterokaryon of corn-soybean in mitosis.

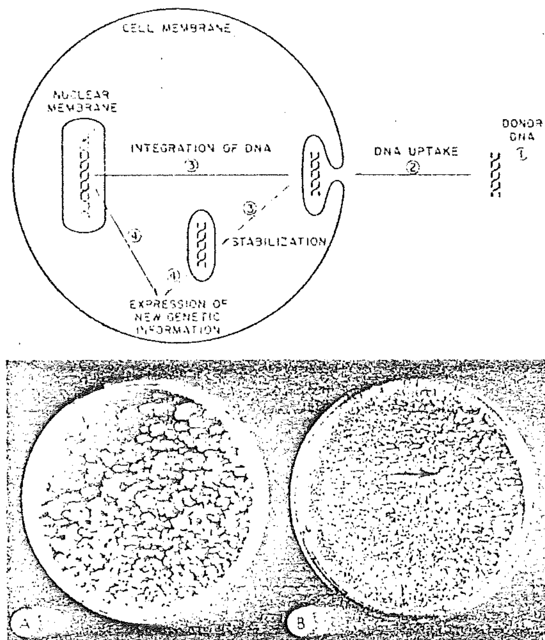

Fig. 7. Model of DNA-mediated transformation in plants.

Fig. 8. Selection for BUdR-resistant soybean cells regenerated from protoplasts treated with mutagens (Ohyama, 1975).

A. Control. Growth in absence of BUdR.

B. Colonies of BUdR-resistant cells.