Journal of Nematology 18(1):18–22. 1986.

Germplasm Modification and Its Potential for Finding New Sources of Resistance to Diseases[1]

RICHARD E. LITZ[2]

Abstract: In vitro procedures are playing a major role in plant breeding. Embryo rescue, either through the culture of excised embryos derived from incompatible crosses or by means of ovule culture, has been a standard procedure for the introgression of genes conferring disease resistance into economically important plants. Somatic hybridization (i.e., protoplast fusion) has also been demonstrated to have some potential in obtaining hybrids that result from very wide interspecific and intergeneric crosses. Wide crosses have also been achieved by means of in vitro pollination of excised ovaries or ovules. Tissue culture-induced variability in regenerated plant (i.e., somaclonal variation) appears to be an effective way of obtaining undirected genetic change that can enhance disease resistance and yield and alter the growth habit of crops that are normally propagated vegetatively (e.g., potato) or by seed (e.g., tomato). In the near future, the isolation and sequencing of genes that confer resistance to specific plant pathogens will be possible, and transfer of this information between species will become a reality.

Key words: plant tissue culture, somatic cell genetics, disease resistance.

Current interest in the application of biotechnology to plant improvement generally has been focused on the potential for somatic cell and molecular genetic approaches to crop improvement, although other plant tissue culture procedures involving embryo rescue, cloning, and haploid plant production have been used quite effectively for several years to complement conventional approaches to plant breeding. This review is concerned with the application of in vitro systems to plant improvement, with particular emphasis on enhancing disease resistance in crops.

Embryo rescue: Perhaps the oldest area of research within plant tissue culture has been the study of the growth requirements of excised, immature zygotic embryos representing specific developmental stages. Application of these studies to plant breeding involves excision and culture of hybrid zygotic embryos following hybridization between economic plant species and wild but sexually incompatible species. This procedure, first reported by Laibach (20,21), has been used most effectively for rescuing hybrid embryos that would normally abort due to failure of endosperm development. Embryo rescue is now a standard procedure for the transfer of genes or gene complexes that confer disease resistance to economic species. The tomato (*Lycopersicon esculentum*) has been hybridized with the sexually incompatible *L. peruvianum*. As a result of this cross, endosperm does not develop; consequently, embryo abortion would normally occur. By carefully isolating the hybrid embryo on tissue culture medium, normal development can occur (28). In this manner, resistance to tobacco mosaic virus, tomato spotted wilt virus, root-knot nematode, and several fungal pathogens has been transferred to the cultivated species. Other examples of successful crosses involving the interspecific transfer of disease resistance facilitated by embryo rescue or culture have included hybridizations among *Hordeum* species (16). Wild barley (*H. bulbosum*) is resistant to mildew, whereas winter barley (*H. vulgare* and *H. sativum*) is susceptible to infection. The interspecific hybrids are resistant to mildew. Resistance to papaya ringspot virus has been transferred to papaya from the incompatible, resistant *Carica cauliflora* by embryo rescue (24). By making successive back crosses, the undesirable wild characteristics can be removed from the breeding population.

In vitro pollination and fertilization: Sexual incompatibility among species has also been overcome by in vitro pollination and fertilization. This procedure involves the fertilization of excised ovule masses by germinated pollen under sterile conditions (26). It has been successfully demonstrated

Received for publication 11 July 1985.

[1] Florida Agricultural Experiment Station Journal Series No. 6570.

Symposium paper presented at the annual meeting of the Society of Nematologists, 24–28 June 1985, Atlantic City, New Jersey.

[2] Tropical Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, Homestead, FL 33031.

for interspecific and intergeneric crosses within the Caryophyllaceae. Although in vitro pollination and fertilization have not been used to resolve difficult plant breeding problems, the technique has considerable potential, particularly because of its simplicity.

Protoplast fusion: Recovery of somatic hybrids that would otherwise be impossible to obtain using conventional sexual hybridization has been made possible by the development of effective methods for large-scale production of wall-less plant cells or protoplasts. Controlled fusion of protoplasts from different species is possible using biochemical complementation of protoplasts in a selection medium or co-culturing protoplasts having distinct cellular differences, such as pigmentation or size of plastids. Somatic hybrid plants have been produced in the following genera: *Brassica, Datura, Daucus, Nicotiana, Petunia,* and *Solanum.* In some cases these hybrids represent new genetic combinations that can be utilized in conventional plant breeding programs. Somatic hybrids have been recovered from fused protoplasts of eggplant (*Solanum melongena*) and *S. sisymbriifolium,* a wild weed resistant to *Meloidogyne incognita.* Resistance to the nematode appears to have been transferred to the hybrid since no reproduction was found in infectivity tests (13).

Intergeneric protoplast fusions have also resulted in the formation of unique somatic hybrids that would have been unobtainable using conventional sexual hybridization. Somatic hybrids have been recovered following protoplast fusion between *Solanum* and *Lycopersicon* (23), *Atropa* and *Datura* (19), *Daucus* and *Aegopodium* (5), and between *Nicotiana* and *Atropa* (12). These somatic hybrids are normally aberrant and sterile, although some evidence suggests that stable plants can be recovered (5) from which gene transfer to economic plant species would be possible.

Clonal propagation: Use of tissue culture procedures such as shoot tip culture, somatic embryogenesis, and organogenesis for the rapid clonal propagation of plants has been reported for many species. In breeding programs involved with the production of F_1 hybrid seed, tissue culture propagation has been used to produce large numbers of parental plants, particularly male sterile plants, in order to standardize the production of seed as much as possible. Generally, plants derived from seed produced by F_1 plants are inferior to the parent (F_1) plants. Hence, there is a need to continuously monitor hybrid seed production. Increasingly, there is interest in the production of artificial hybrid seed, either through encapsulation of somatic embryos or by direct planting of somatic embryos, insuring that certain combinations of valuable characteristics, such as disease resistance, in hybrid seed propagated plants can be maintained indefinitely.

Somaclonal variation: Passage of plant tissue through an in vitro cycle, particularly through a callus phase, has long been known to produce genetic alterations in cells that may become apparent in the regenerated plants. For many years, this apparent instability of the genome in tissue cultures was considered to be a major problem for clonal propagation because clonal fidelity was altered. It is now recognized, however, that these tissue culture-induced changes represent a unique source of genetic diversity in crops normally propagated vegetatively (e.g., potato, fruit trees, etc.) and for developing new breeding lines in seed-propagated crops. This approach is particularly useful for improving crop species for which little genetic information is available and for unmasking useful genes or gene complexes that can be incorporated into a conventional plant breeding program. Somaclonal variation has been reported in many plant species, including sugarcane (15), potato (27), and tomato (6).

Plants regenerated from sugarcane cell cultures exhibit considerable variation, in part because the plant itself is a chromosomal mosaic but also because of variability induced in culture (17). Regenerated plants have demonstrated greater sucrose yield and enhanced resistance to Fiji virus disease (18), downy mildew (14), eyespot disease (14), and culmicolous smut (22). These traits have remained stable for several years under field conditions. Somaclonal variation in regenerated populations of Russet Burbank potato plant included several horticultural features such as growth habit, tuber color, and uniformity (27). In addition, several regenerated plants demonstrated greater resistance to both late blight (*Phytophthora infestans*) and early blight (*Al-*

ternaria solanii) (27). Resistance to all five races of *Phytophthora infestans* was observed. Variable results, however, have been obtained in retention of root-knot nematode resistance in plants reproduced through tissue culture (1,8–10).

At one time it was believed that somaclonal variability in potato could be obtained only in plants derived from protoplast cultures. Bright (3), however, has reported similar somaclonal variability in potato plants regenerated from petiole, leaf, and rachis explants. Most of the variability in plants derived from potato callus is probably caused by cultural conditions (2). This has been corroborated by studies with sugarcane and by recent evidence from Taiwan that banana plants derived from shoot tip cultures also exhibit somaclonal variation and demonstrate resistance to *Fusarium* (Panama Disease) infection.

The genetic basis for somaclonal variation is not well understood, particularly in plants such as potato and sugarcane that are polyploids. However, changes in chromosome number or structure, single gene mutations, and in cytoplasmic DNA have been observed in seed propagated plants (7). Aneuploidy and polyploidy have been observed in regenerated plants. Moreover, rearrangement of genes on the chromosomes can also occur as a result of translocations, deletions, and inversions. Cytoplasmic genetic changes have been observed in regenerated plants (11), and single gene mutations also have been reported in tomato plants regenerated from tissue cultures (6). Although there is substantial evidence that most somaclonal variation is associated with chromosomal alterations, there is speculation that transposable elements may be responsible for some somaclonal variation (4).

Although somaclonal variation in regenerated plants can represent considerable savings in time, compared with conventional approaches, it is still essential that plants be grown under field conditions in order to determine their response to selection pressure. In some instances, it has been possible to correlate responses to certain chemicals at the cellular level with responses at the whole plant level. For example, it has been possible to select for resistance to pathotoxins at the cellular level. Gengenbach et al. (11) selected *Zea mays* cells for resistance to the toxin of *Drechslera maydis* race T, the causative agent of southern corn leaf blight. Plants regenerated from those toxin-resistant cells were also resistant to infection by the fungal pathogen. This research has interesting implications, because toxins are known to be involved in many host–pathogen associations. Unfortunately, at this time, very few toxins have been purified and postively identified.

Gametoclonal variation: When in vitro conditions stimulate variability in cultures of male gametes (microspores), this type of variability has been referred to as gametoclonal. Both dominant and recessive mutant genes induced by cultured conditions in haploid plants can be expressed because only a single copy of each gene is present. Anther culture has been widely used in wheat, tobacco, and rice breeding, particularly in China (29). Usually, anthers are cultured from an F_1 hybrid. After recovering haploid plants from these cultures, the chromosome complement is restored to the diploid number by treatment with colchicine. Because single gene recessive mutations can be visible in plants restored to the diploid number, this can be a useful approach in plant breeding. Oono (25) has used this method to recover homozygous diploid rice plants that demonstrated variation in plant height, morphology, fertility, and other characters. Obviously, gametoclonal variability would be useful also for unmasking resistance to certain diseases and pests.

Recombinant DNA: Early attempts to introduce isolated DNA into higher plants yielded controversial results; it was usually impossible to verify the fate or the expression of the foreign nucleic acid. With the improvement of cell culture techniques (e.g., protoplast isolation and culture, high frequency somatic embryogenesis from cell cultures, etc.) and with greater understanding of the natural transformation of plant cells by *Agrobacterium tumefaciens*, a bacterium causing tumor formation in many different plants, attention has been redirected to the genetic modification or transformation of plants. Various different vectors have been proposed for facilitating uptake of foreign DNA fragments into plant cells or protoplasts, including the Ti plasmid of *Agrobacterium tumefaciens*. The

tumor-inducing genes are found on the Ti plasmid. Part of the Ti plasmid becomes integrated directly into the nucleus of the infected host cell, thus suggesting a probable method for introducing foreign DNA into plant cells. Other probable vectors include cauliflower mosaic virus, the plasmid-like DNA in the mitochondria of plants, and the plasmid of the bacterium *Pseudomonas savastanoi.*

Transformation of plant cells also has been attempted in other ways, including microinjection of foreign DNA into pollen, ovaries, or protoplasts. In some of these studies, the pollen tube itself would serve as a vector. Much of this work appears to be promising. Inevitably, however, these remain shotgun approaches for achieving transformation, with little control over the recombination and transformation event.

Plant cell and tissue culture has already had a major impact on plant breeding and crop improvement. Wide hybridizations have been achieved by means of embryo rescue. Selected breeding lines and unique plant selections can be clonally propagated. New, improved cultivars have emerged from programs making use of somaclonal and gametoclonal variation in regenerated populations. The next decade will almost certainly witness cultivar development involving the use of recombinant DNA technology.

Literature Cited

1. Ammati, M., T. Murashige, and I. J. Thomason. 1984. Retention of resistance to the root-knot nematode, *Meloidogyne incognita,* by *Lycopersicon* plants reproduced through tissue culture. Plant Science Letters 35:247–250.

2. Austin, S., and A. C. Cassels. 1983. Variation between plants regenerated from individual calli produced from separated potato stem callus cells. Plant Science Letters 31:107–114.

3. Bright, S., V. Jarrett, R. Nelson, G. Creissen, A. Karp, J. Franklin, P. Norbury, J. Kueh, S. Rognes, and B. Miflin. 1983. Modification of agronomic traits using in vitro technology. Pp. 251–265 *in* S. H. Mantell and H. Smith, eds. Plant biotechnology. Cambridge, England: Cambridge University Press.

4. Chourey, P. S., and R. J. Kemble. 1982. Transposition event in tissue cultured cells of S-cms genotype of maize. Maize Genetics Crop Newsletter 56: 70.

5. Dudits, D., O. Fejer, G. Hadlaczky, C. Koncz, G. B. Lazar, and G. Horvath. 1980. Intergeneric gene transfer mediated by plant protoplast fusion. Molecular General Genetics 179:283–288.

6. Evans, D. A., and W. R. Sharp. 1983. Single gene mutations in tomato plants regenerated from tissue culture. Science 221:949–951.

7. Evans, D. A., W. R. Sharp, and H. P. Medina-Filho. 1984. Somaclonal and gametoclonal variation. American Journal of Botany 71:759–774.

8. Fassuliotis, G. 1980. Effect of cloning on resistance of *Solanum sisymbriifolium* to *Meloidogyne incognita* and *M. javanica.* Nematropica 10:66 (Abstr.).

9. Fassuliotis, G. 1981. The effect of cloning on the resistance of 'Patriot' tomato to *Meloidogyne incognita* Race 1. Nematropica 11:81.

10. Fassuliotis, G. 1984. Reversal of resistance to *Meloidogyne incognita* of NC 95 tobacco plants regenerated from leaf discs. 1st International Congress of Nematology, 5–10 August 1984, Guelph, Ontario, p. 78 (Abstr.).

11. Gengenbach, B. G., C. E. Green, and C. M. Donovan. 1977. Inheritance of selected pathotoxin resistance in maize plants regenerated from cell cultures. Proceedings of the National Academy of Science 74:5113–5117.

12. Gleba, Y. Y. 1979. Nonchromosomal inheritance in higher plants as studied by somatic cell hybridization. Pp. 775–788 *in* W. R. Sharp, P. O. Larsen, E. F. Paddock, and V. Raghavan, eds. Plant cell and tissue culture: Principles and applications. Columbus: Ohio State University Press.

13. Gleddie, S., G. Fassuliotis, W. A. Keller, and G. Setterfield. 1985. Somatic hybridization as a potential method of transferring nematode and mite resistance into eggplant. Zeitschrift für Pflanzenzuchtung 94:348–351.

14. Heinz, D. J., M. Krishnamurthi, L. G. Nickell, and A. Maretzki. 1977. Cell, tissue and organ culture in sugarcane improvement. Pp. 3–17 *in* J. Reinert and Y. P. S. Bajaj, eds. Applied and fundamental aspects of plant, cell, tissue and organ culture. Berlin: Springer-Verlag.

15. Heinz, D. J., and G. W. P. Mee. 1971. Morphologic, cytogenetic and enzymatic variation in *Saccharum* species hybrid clones derived from callus tissue. American Journal of Botany 58:257–262.

16. Konzak, C. F., L. F. Reynolds, and N. F. Jensen. 1951. Embryo culture of barley species hybrids. Cytological studies of *Hordeum sativum* × *Hordeum bulbosum.* Journal of Heredity 42:125–134.

17. Krishnamurthi, M. 1981. Sugarcane improvement through tissue culture and review of progress. Pp. 70–77 *in* A. N. Rao, ed. Tissue culture of economically important plants. Singapore: Asian Network for Biological Sciences.

18. Krishnamurthi, M., and J. Tlaskal. 1974. Fiji disease resistant *Saccharum officinarum* var. Pindar subclones from tissue cultures. Proceedings of the International Society of Sugarcane Technologists 15:130–137.

19. Krumbiegel, G., and O. Schieder. 1979. Selection of somatic hybrids after fusion of protoplasts from *Datura innoxia* Mill. and *Atropa belladonna* L. Planta 145:371–375.

20. Laibach, F. 1925. Das Taubwerden von Bastardsamen und die kunstliche Aufzucht fruh absterbender Bastardembryonen. Zeitschrift für Botanische 17:417–459.

21. Laibach, F. 1929. Ectogenesis in plants. Journal of Heredity 20:201–208.

22. Liu, M. C. 1981. In vitro methods applied to sugarcane improvement. Pp. 299–323 *in* T. A.

Thorpe, ed. Plant tissue culture. New York: Academic Press.

23. Melchers, G., M. D. Sacristan, and A. A. Holder. 1978. Somatic hybrid plants of potato and tomato regenerated from fused protoplasts. Carlsburg Research Communications 43:203–218.

24. Moore, G. A., and R. E. Litz. 1984. Biochemical markers for *Carica papaya, C. cauliflora* and plants from somatic embryos of their hybrid. Journal of the American Society for Horticultural Science 109:213–218.

25. Oono, K. 1981. In vitro methods applied to rice. Pp. 273–298 *in* T. A. Thorpe, ed. Plant tissue culture. New York: Academic Press.

26. Rangaswamy, N. S. 1977. Applications of in vitro pollination and in vitro fertilization. Pp. 412–425 *in* J. Reinert and Y. P. S. Bajaj, eds. Applied and fundamental aspects of plant cell, tissue and organ culture. Berlin: Springer-Verlag.

27. Shepard, J. F., D. Bidney, and E. Shahin. 1980. Potato protoplasts in crop improvement. Science 208: 17–24.

28. Smith, P. G. 1944. Embryo culture of a tomato species hybrid. Proceedings of American Horticultural Science 44:413–416.

29. Zeng, J. Z. 1982. Application of anther culture technique to crop improvement in China. Pp. 351–363 *in* S. K. Sen and K. L. Giles, eds. Plant cell culture in crop improvement. New York: Plenum Press.