PROSPECTS FOR CROP IMPROVEMENT IN THE FIELD OF CELL CULTURE[1]

C. E. Green[2]
Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108

Advances in plant tissue culture techniques during the past decade have fostered the hope that these techniques can be utilized for crop improvement. Simply stated, these methods allow large, relatively homogeneous populations of cells from diverse tissue sources to be grown and regenerated into plants in a defined environment. An important advantage gained by this capability is that tissue and genetic complexities associated with whole plants can be simplified by working at the cellular level. This capability has provided many new approaches for the investigation and modification of plants. The techniques being extended from model plant systems to increasing numbers of crops include reliable growth of somatic and haploid cells, protoplast isolation and culture, intra- and intergeneric hybridizations by protoplast fusion, utilization of selection systems to recover desired mutants, and the regeneration of plants in all of the above areas. Several recent reviews discuss the development and current status of these techniques and provide insight into their potential uses for crop improvement (2, 6, 7, 23, 24, 26, 27).

The prospects for successfully improving the agronomic characteristics of a given crop will be determined by several factors. First, tissue culture methods must be well developed for the species under investigation. The required capabilities will vary with the crop improvement objective but frequently will require rapidly growing diploid and/or haploid cells or protoplasts from which plants can be regenerated and grown to maturity. Second, the trait to be modified for crop improvement must be expressed in tissue culture and an effective selection system must exist which identifies the desired variant. Third, variant cell lines must be capable of regenerating plants in which the selected mutation is expressed and heritable in succeeding generations. Fourth, tissue culture methods must provide efficient or unique solutions to crop improvement problems not resolved by existing breeding or genetic methods.

Development of tissue culture methods in maize

The crucial problem facing most crops continues to be the development of tissue culture methods which allow effective plant improvement investigations. The current status of cell culture methods in maize serves to illustrate recent progress, as well as deficiencies remaining in this and other crops. Among the diverse maize tissues used to initiate somatic callus in recent years, only the scutellum of immature embryos has produced cultures capable of plant regeneration (16). This method was based on the isolation of developing embryos at an appropriate size and from appropriate genotypes, such as the inbred A188 or the single cross A188 x W22 *Rnj*. Callus initiation from the scutellum of embryos was achieved on Murashige and Skoog (MS) medium containing L-asparagine and 2,4-dichlorophenoxyacetic acid (2,4-D).

Orientation of maize embryos on the culture medium had a marked influence on callus formation (16). When the scutellum side of these embryos was oriented downward, the exposed embryonic axis germinated and produced a plant. Under these conditions no scutellar callus was formed. When the plumule-radicle axis side, however, was placed in contact with growth medium, the scutellum developed a lobed, irregular shape by 10 days and after 14 days a white or pale-yellow callus was visible. Primary cultures frequently exhibited areas of chlorophyll development, small leaves, and occasional roots after 3-4 weeks growth. These cultures were maintained by subculture and plants were readily regenerated as needed by lowering or omitting the 2,4-D from the MS medium. Callus cultures capable of plant regeneration have been isolated from a variety of maize genotypes with this method (14). The original A188 x W22 *Rnj* cultures have retained their embryogenic capacities for three years.

Regenerated plants exhibited vigorous leaf and root development in the growth medium, but a large percentage did not survive transplantation to soil in the greenhouse. This difficulty was circumvented by transferring regenerated plants to vermiculite in peat pots, watering daily with a ¼ strength MS salts solution, and permitting growth for 10-14 days in an incubator with 70-75% relative humdity, a temperature of 27-28°C, and a 16 hr photoperiod with a light intensity of 3.5 klx. Plants developed rapidly in this environment and an extensive root system was evident at the time of transplantation to soil in the greenhouse. Among the initial 108 plants transferred to these conditions 87 or 80% survived and were subsequently grown to maturity. This survival rate was compared to the 10-15% of regenerated plants which survived and were grown to maturity after transplantation directly to soil. The capability of regenerated plants to tolerate the environmental change from axenic growth in the culture medium to that found in the greenhouse varies with the species under investigation. Regenerated plants from *Avena sativa,* for example, can be transplanted from the growth medium directly to soil with a 90% success rate (10).

Understand plant characteristics

Attempts to utilize tissue culture procedures for crop improvement are especially dependent on the recovery of plants which can be utilized in conventional genetic or breeding programs. As a consequence it is important to understand the characteristics of plants derived from particular culture conditions for a given species. An essential consideration in crops which cannot be propagated asexually is the fertility of regenerated plants. In many cases the sexual cycle provides the only means to preserve desirable mutations or agronomic characteristics for transfer and potential use in plant improvement. In order to investigate the fertility of regenerated plants, various characteristics of such plants were analyzed during their maturation in the greenhouse. The cultures used for this study were initiated from 1.5-2.0 mm long A188 x W22 *Rnj* embryos on MS medium containing 1 mg 2,4-D/liter. Plants were regenerated from these cultures beginning with the 4th subculture (65 days after culture initiation) by transferring callus samples to asparagine-minus MS medium which contained 0 or 0.25 mg 2,4-D/liter. This process was continued through two additional subculture periods for a total of 60 days. A total of 108 regenerated plants were sampled, of which 87 grew to maturity in the greenhouse (Fig. 1). As these plants matured 43 partial sporocyte samples were collected for analysis of meiosis at pachytene and diakinesis stages of prophase in the laboratory of Dr. R. L. Phillips. Examination revealed that the sporocyte tissues of 2 plants appeared to contain a mosaic of normal and abnormal cells. One of these plants contained aneuploid cells monosomic for chromosome 5 and the other appeared to have tetraploid sectors in the tassel. The remaining 41 plants exhibited normal chromosome pairing and 10 bivalents. Pollen collected from 85 regenerated plants was stained with IKI (iodine) to determine fertility. The pollen from 83 of these plants was at least 97% fertile while the remaining 2 plants were 92% and 60% fertile. All regenerated plants developed ears which were sib- or self-pollinated in order to recover F_1 progeny. Kernel development and seed set was generally excellent. The F_1 progeny were evaluated by planting 10 or 20 kernel samples from each ear in the field. These plants grew vigorously, had "normal" morphology, and produced well-filled ears after self pollination. In summary, on the basis of sporocyte, pollen, and ear fertility analysis, most plants regenerated from maize callus 65-125 days after callus initiation were "normal."

Although most regenerated plants grew vigorously with alternately arranged leaves and ears, some morphological irregularities also were evident. Perhaps the most interesting was the development of 6 plants with a decussate leaf and ear arrangement (Fig. 2). This has been described previously in maize and termed the abphyl syndrome (17). This developmental irregularity resulted in plants with a typical number of nodes for regenerated plants but twice the normal number of leaves and ears. These plants grew vigorously and were fertile. Another developmental irregularity encountered in two plants was the development of twin stalks from a single node near the base.

The height and node number of mature regenerated A188 x W22 *Rnj* plants was compared to A188 x W22 *Rnj* plants produced from seed. The height of 84 regenerated plants ranged from 61-170 cm with an average of 120 cm and the node number ranged from 5-10 with an average of 8. This compared with an average height of 210 cm and 12-13 nodes for eight A188 x W22 *Rnj* plants grown from seed. Regenerated A188 x W22 *Rnj* plants were approximately 80 cm

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Fig. 1. Regenerated maize plants from A188 x W22 *Rnj* scutellar callus growing to maturity in greenhouse.

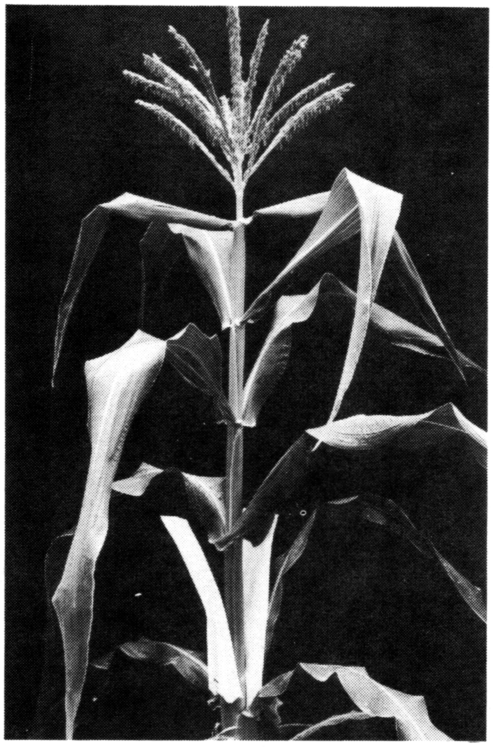

Fig. 2. Regenerated maize plant exhibiting the abphyl syndrome of decussately arranged leaves and ears.

shorter and had 5 fewer nodes on the average than the same genotype produced from seed. This reduced stature did not appear to have a genetic basis since F_1 plants exhibited a more normal height and node number.

Influence of embryo age and genotype

The original observations on plant regeneration in maize demonstrated that embryo age (size) and genotype significantly influenced the initiation of callus from the scutellum of embryos (16). The influence of embryo size and 2,4-D concn is further illustrated for inbred A188 embryos in Table 1. As embryo length increased from 0.75 mm to greater than 3 mm the initiation of scutellum callus capable of plant regeneration decreased from 74% to zero at 0.25 mg 2,4-D/liter. In addition, increasing 2,4-D concn decreased the frequency at which embryos with lengths of 0.75-3.0 mm produced callus capable of plant regeneration. High 2,4-D concn were particularly harmful. None of the embryos with lengths between 0.75 mm to greater than 3 mm produced cultures capable of plant regeneration at 4 mg 2,4-D/liter. In summary, this experiment indicated that cultures capable of plant regeneration were obtained from high percentages of A188 embryos when they were isolated at a size range of 0.75-2.5 mm and if they were cultured in the presence of 0.25 to 1.0 mg 2,4-D/liter. A sufficient number of embryos smaller than 0.75 mm have not yet been tested to determine their response to these growth conditions. Maize embryos longer than 3 mm appear to have achieved a developmental state which is no longer conducive to scutellar callus formation or plant regeneration.

The influence of genotype and 2,4-D concn on scutellar callus formation from 0.75-1.5 mm long embryos is illustrated in Table 2. The variation in response between W23, W22 *Rnj,* and A188 embryos typifies the spectrum encountered for most inbreds tested thus far. With current cultural conditions some genotypes, such as W23, produce scutellum callus capable of regenerating plants at very low frequencies, if at all. Other genotypes exhibit an intermediate response, like that shown for W22 *Rnj,* while other genotypes such as A188 produce callus capable of plant regeneration at relatively high frequencies and over a range of 2,4-D concentrations.

Perhaps the most interesting results have come from the analysis of cultures derived from the hybrids listed in Table 2. Embryos from the reciprocal crosses between A188 and W22 *Rnj* produced cultures capable of regeneration at frequencies much higher than embryos from either parent individually. Regenerating cultures of the hybrids, especially W22 *Rnj* x A188, also developed over a broader range of 2,4-D concn than from either parent. These data indicate that genotype can dramatically influence the frequency at which regenerated plants are obtained from maize tissue cultures.

The presence of considerable genetic variability for desired tissue culture characteristics is a mixed blessing. If investigations must be restricted to certain genotypes within a species for example, it would preclude working directly with many agronomically superior types. This limitation would require that the useful genetic modifications selected in culture be backcrossed into the appropriate agronomic lines. The advantage of genetic variability is that it would allow searches for genes or blocks of genetic information which determine essential tissue culture characteristics. It is easy to imagine, for example, that selection for superior tissue culture genotypes might improve our ability to manipulate the genome of crops at the cellular level as well as allow subsequent regeneration of plants for incorporation into breeding programs. The potential of this approach has recently been explored in tetraploid alfalfa (*Medicago sativa* L.) (1). Although it has been feasible to produce plants from callus cultures of alfalfa for several years, the proportion of genotypes capable of plant regeneration has been very low (0-5%) (25). This limitation was overcome by using recurrent selection to develop germplasm with a greatly increased capability to regenerate plants. Three cycles of screening and two cycles of recurrent selection increased the frequency of plant regeneration from an average of 12% for 5 parental alfalfa clones to 67% for the resulting Regen-S germplasm. It is encouraging that in alfalfa, regeneration from callus cultures appears to be highly heritable.

In the continuing development of tissue culture systems for crop

Table 1. Influence of embryo size and 2,4-D concn on the growth of scutellar callus capable of plant regeneration from inbred A188 embryos. The growth medium and culture conditions were as previously described (16). Each value represents the percent of 50 embryos from which regenerating scutellar callus was obtained.

Embryo length (mm)	2,4-D (mg/liter)		
	0.25	1.0	4.0
0.75 – 1.5	74	40	0
1.6 – 2.5	60	32	0
2.6 – 3.0	6	16	0
> 3.0	0	0	0

Table 2. Influence of genotype and 2,4-D concn on the growth of scutellar callus capable of plant regeneration from 0.75-1.5 mm long embryos. The growth medium and culture conditions were as previously described (16). Each value represents the percent of 50 embryos from which regenerating scutellar callus was obtained.

Genotype	2,4-D (mg/liter)		
	0.25	1.0	4.0
W23	6	0	0
W22 *Rnj*	36	0	0
A188	74	40	0
A188 x W22 *Rnj*	96	80	16
W22 *Rnj* x A188	100	90	76

improvement, an important objective is the successful culture of large populations of single cells. Furthermore, the totipotency of these cells must be preserved in order to obtain regenerated plants. The current status of research in this area for most crop species restricts the application of new techniques to achieve genetic modifications. The utilization of microbial-like mutant selection schemes and other available methods to engineer plant cells will have their greatest potential in crop improvement when our ability to grow and regenerate plants from single cells is vastly improved.

The development of well-dispersed suspension cell cultures is a useful intermediate step in the evolution of techniques to culture single cells. Suspension cultures frequently contain actively growing single cells but most cells in the population are usually found in aggregates containing a few to several hundred cells. In species where single cell populations have not been reliably cultured, suspension cultures provide a useful approximation of the unicellular state. Knowledge gained during the development and investigation of these cultures may provide useful insights for the growth of single cell populations. In some instances suspension cultures have been utilized directly in the development of methods to genetically modify plant cells. Soybean protoplasts, for example, have been readily isolated and cultured from cell suspensions but not from leaf mesophyll cells (21). Cell suspensions of tobacco and carrot have been utilized in the isolation of feedback insensitive mutants (28, 29, 30).

Maize cell suspensions

Although single cells of maize have not been grown reliably in culture, well-dispersed cell suspensions have recently been produced from diploid tissues (14; W. F. Sheridan, personal communication). Cell suspensions derived from triploid endosperm have been available for some time (13). Earlier attempts to grow maize tissue from several genotypes in liquid shake culture typically produced cultures with slow growth rates and very large cell clusters ranging in size from 2-10 mm in diameter (Fig. 3-A). Despite this extreme aggregation, they frequently produced significant numbers of single cells in the growth medium. Attempts to utilize these cells to establish well-dispersed suspension cultures have been unsuccessful.

The genotype 'Black Mexican Sweet', obtained from R. L. Phillips, Univ. of Minnesota, was used to establish callus cultures at the suggestion of W. F. Sheridan, Univ. of North Dakota. Kernels, which had been surface sterilized, were germinated and grown axenically on MS medium for 7 days. Cross sections, about 1 mm thick, were taken from young shoots in the region of the first node. These were placed on MS agar medium containing 4 mg/liter 2,4-D. Initially a yellow callus was produced from these tissues which was more compact than friable. At the completion of the fourth subculture cycle, regions of friable, pale-yellow callus were observed in many cultures. These regions were preferentially isolated and transferred to fresh medium where they grew rapidly as a very friable callus. Suspension cultures established from this callus grew rapidly in MS medium containing 2 mg 2,4-D/liter. These cell suspensions consisted of a mixture of single cells and cell aggregates ranging up to 1 mm in diameter (Fig. 3-B). Analysis of these cultures by phase contrast microscopy revealed a variety of cell shapes and sizes, but most cells were oval with a length of 30-50 μm. Cytoplasmic strands and active streaming were observed in most cells and the doubling time of these cultures was 72 hr at 25°C. Neither the callus nor the suspension cultures have demonstrated an ability to regenerate plants at this time.

The increasing capability to culture and regenerate plants from single cells of crops was recently demonstrated by Button and Botha (4). Highly embryogenic callus cultures of 'Shamouti' orange (*Citrus sinensis* (L.) Osbeck) were treated with a cell wall-digesting enzyme preparation to produce large populations of single cells. These cells were then cultured in or on agar basal medium where they produced pseudobulbits and heart-shaped embryos after 8-10 weeks. Plants were successfully grown from the heart-shaped embryos.

The preceding sections of this paper have attempted to emphasize the development of tissue culture technology as an essential key in the successful genetic modification of crops. The emphasis of this discussion on maize serves to illustrate 2 points. First, meaningful progress can be achieved in crops which have been difficult to work with in the past. Second, essential components of the tissue culture system remain to be developed for maize as well as most other crops. This deficiency is apparent in maize by the omission in this discussion of 2 areas where significant progress has not yet been achieved. These areas include the development of haploid cultures capable of plant regeneration and the culture and regeneration of plants from protoplasts.

Applications of tissue culture to crop improvement

Despite current limitations in tissue culture technology, significant progress towards crop improvement has already been reported. The dramatic results achieved by clonal-propagation of desired phenotypes in floriculture is described by Dr. Murashige in another paper in this symposium. He also describes the development and use of shoot tip and meristem cultures to produce virus free plants. This tecnique has been very useful for asexually propagated species.

Tissue culture methods have been applied to sugarcane improvement in several ways. Coleman (9) obtained regenerated sugarcane plants resistant to mosaic virus disease from tissue cultures of a susceptible variety. In further work with regenerated plants (24), 116 clones obtained from tissue cultures of 15 cultivars were produced which remained immune after 8 or more inoculations with the virus. Some of these "apparently" stable new genotypes also remained "immune" when grown under field conditions favorable to the spread of mosaic virus. Heinz and Mee (19) demonstrated that chromosome doubling by colchicine treatment of somatic cell suspensions resulted in the regeneration of large numbers of doubled plants from these cultures. This approach has provided sugarcane breeders with a convenient method to produce fertility in sterile progeny from interspecific crosses. The sugarcane variety used in the colchicine study also was susceptible to the pathogen *Helminthosporium sacchari.* Diploid plants regenerated from these cultures were infected with *H. sacchari* and found to range from highly susceptible to very resistant (18).

Pathotoxins also have been utilized to select resistant variants from tissue cultures derived from susceptible genotypes. The wildfire disease of tobacco is caused by *Pseudomonas tabaci,* a bacterial pathogen (3). Carlson (5) used haploid tobacco cells and methionine sulfoximine, an analog of the wildfire toxin, to select cell lines resistant to wildfire toxin. Three diploid plants were regenerated from methionine sulfoximine-resistant callus and these were less susceptible than the parent plant to the pathogenic effects of *P. tabaci* infection. The resistant phenotype of 2 of these plants was transmitted in crosses as a single semidominant gene.

Southern corn leaf blight is caused by a fungal pathogen, *Helminthosporium maydis* race T, and its pathotoxin (22). This disease preferentially attacks maize having Texas male-sterile cytoplasm (cms-T), while nonsterile (N) cytoplasm is resistant. Gengenbach and Green (12) studied the effects of *H. maydis* race T. pathotoxin on somatic tissue cultures from cms-T and N cytoplasm versions of inbred A619. Low concentrations of toxin in the growth medium inhibited cms-T callus growth while N callus grew normally, even in the presence of high toxin concentrations. The cms-T callus also was utilized in selections for pathotoxin resistance. After 4 cycles of selection in the presence of sublethal toxin concn, 14 pathotoxin-resistant lines of A619 (cms-T) callus were established. The growth rates of these resistant cultures, in the presence of toxin concn lethal to unselected A619 (cms-T) callus, ranged from 53 to 111% of their growth in the absence of toxin. This high degree of resistance remained stable during growth of these cultures in the absence of toxin for nearly 1 year.

The pathotoxin from *H. maydis* race T has been shown to disrupt membranes, oxidative phosphorylation, and electron transport in

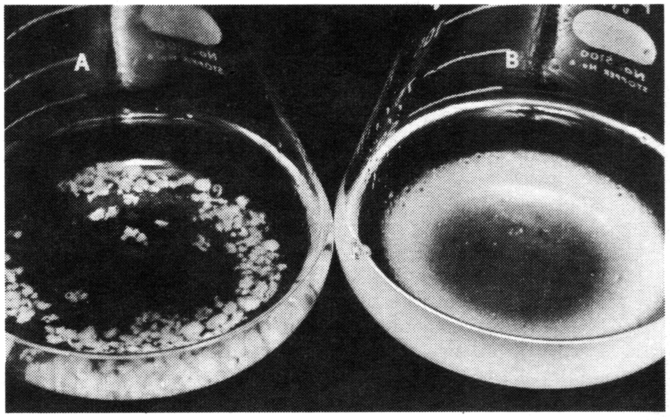

Fig. 3. Suspension cultures of maize in MS medium containing 2 mg 2.4-D/liter. Cultures were grown at 25°C and aerated by shaking at 150 rpm. A, inbred A188; B, 'Black Mexican'sweetcorn.

mitochondria isolated from cms-T but not N cytoplasm maize (11). These activity parameters of mitochondria from susceptible A619 (cms-T) callus also were disrupted by the toxin. Mitochondria isolated from A619 (N) and resistant A619 (cms-T) callus were not affected by the toxin. It was not possible to evaluate the nature of this selected pathotoxin resistance in regenerated plants because none was obtained from these cultures. Additional studies are currently in progress with new N and cms-T callus cultures that are capable of plant regeneration.

Increased synthesis of specific amino acids has been achieved in a number of studies by selecting feedback inhibition mutants resistant to toxic levels of amino acid analogs. Widholm (28, 29) selected cell lines which were resistant to 5-methyl tryptophan from carrot and tobacco cell suspensions. The resulting mutants possessed stable resistance against the analog and contained endogenous levels of free tryptophan 15 to 27 times higher than in the corresponding parental cultures. Anthranilate synthetase from these resistant cell lines was less sensitive to feedback inhibition by tryptophan and 5-methyltryptophan than the wild-type enzyme. More recently, Widholm (30) has selected for resistance to lysine, methionine, and proline analogs in tobacco and carrot cell suspensions. Resistant cell lines were isolated at low frequencies, were generally stable in the absence of the selective agent, and also accumulated the corresponding free amino acid at levels 10 to 30 times higher than normal. Carlson (5) selected tobacco cultures resistant to lethal levels of methionine sulfoximine by plating haploid cells in agar medium. Plants were regenerated from three stable resistant cell lines. Analysis of free amino acids in leaves showed that two resistant plants contained 5-6 times higher levels of free methionine than the parental genotype.

In addition to their usefulness in plant biochemistry and genetics, regulatory mutants such as feedback inhibition resistance also may have potential in crop improvement. The grain of most crops is deficient in one or more of the amino acids tryptophan, lysine, threonine, or methionine, which are essential in human or monogastric animal nutrition. Feedback resistant mutants might provide a selective mechanism to increase production of needed amino acids. Chaleff and Carlson (8) used suspension cultures of rice (*Oryza sativa*) to select cell lines resistant to the lysine analog S-(2-aminoethyl)-cysteine (AEC). Three cell lines which grew slowly in the presence of 2mM AEC were selected for further study. The free pool size of eight amino acids was 2-13 times higher in the variant cell lines as compared to unselected parental cultures. Free lysine was increased about 2 fold and protein bound lysine was increased from 13-26% in the resistant cell lines. Plants were not regenerated from these rice cultures.

An additional selection system to recover potential feedback resistant mutations in the aspartate family biosynethtic pathway of cereal crops was described by Green and Phillips (15). This method was based on a growth inhibition observed in maize callus and seedlings of several cereals when lysine and threonine were added simultaneously to the growth medium. This inhibition was competitively reversed by low concentrations of methionine and its metabolic intermediate, homoserine. None of the remaining 17 common amino acids demonstrated a capacity to reverse lysine-threonine inhibition in maize. These results were interpreted to mean that methionine production was blocked by cooperative feedback inhibition by lysine and threonine on one or more early enzymes in the aspartate family pathway. Reduced synthesis of methionine during lysine-threonine inhibition of *Mimulus cardinalis* seedlings was recently described by Henke (20). The selection system provided by lysine-threonine inhibition is the basis of an extensive cooperative program currently in progress to investigate the regulation and enzymology of the aspartate family biosynthetic pathway and to recover potential feedback inhibition mutants in maize capable of producing increased levels of lysine, threonine, and methionine. Agronomic questions concerning this approach to improved nutritional quality will be answered by recovery and analysis of feedback mutants in plants of appropriate crops.

In conclusion, enthusiasm for crop improvement by tissue culture methods must be tempered by the realization that many essential in vitro techniques remain to be extended to crop species. Considering the advances achieved in the recent past, it is reasonable to assume that future research in this field will make considerable progress. As tissue culture capabilities increase, so will the prospects and opportunities to modify crops genetically.

Literature Cited

1. Bingham, E. T., L. V. Hurley, D. M. Kaatz, and J. W. Saunders. 1975. Breeding alfalfa which regenerates from callus tissue in culture. *Crop Sci.* 18:719-721.
2. Bottino, P. J. 1975. The potential of genetic manipulation in plant cell cultures for plant breeding. *Radiation Botany* 15:1-16.
3. Braun, A. C. 1955. A study on the mode of action of the wildfire toxin. *Phytopathology* 45:659-664.
4. Button, J. and C. E. J. Botha. 1975. Enzymatic maceration of *Citrus* callus and the regeneration of plants from single cells. *J. Expt. Bot.* 26:723-729.
5. Carlson, P. S. 1973. Methionine-sulfoximine-resistant mutants of tobacco. *Science* 180:1366-1368.
6. _____ and J. C. Polacco. 1975. Plant cell cultures: Genetic aspects of crop improvement. *Science* 188:622-625.
7. Chaleff, R. S. and P. S. Carlson. 1974. Somatic cell genetics of higher plants. *Annu. Rev. Genet.* 8:267-278.
8. _____ and _____. 1975. *In vitro* selection for mutants of higher plants. p. 351-363. *In* L. Ledoux (ed.) Genetic manipulations with plant material, Plenum Press, N.Y.
9. Coleman, R. E. 1970. New plants produced from callus tissue culture. Sugarcane Research 1970 Rpt., ARS, USDA, p. 38.
10. Cummings, D. P., C. E. Green, and D. D. Stuthman. 1976. Callus induction and plant regeneration in oats. *Crop Sci.* 16:465-470.
11. Gengenbach, B. G.. R. J. Miller, D. E. Koeppe, and C. J. Arntzen. 1973. The effect of toxin from *Helminthosporium maydis* (race T) on isolated corn mitochondria; swelling. *Can. J. Bot.* 51: 2119-2125.
12. _____ and C. E. Green. 1975. Selection of T-cytoplasm maize callus cultures resistant to *Helminthosporium maydis* race T pathotoxin. *Crop Sci.* 15:645-649.
13. Graebe. J. E. and G. D. Novelli. 1966. A practical method for large scale plant tissue culture. *Expt. Cell Res.* 41:509-520.
14. Green, C. E. Cell and tissue cultures of maize. *In* D. B. Walden, (ed.) Genetics and breeding of maize, John Wiley and Sons, N.Y. (In press)
15. _____ and R. L. Phillips. 1974. Potential selection system for mutants with increased lysine, threonine. and methionine in cereal crops. *Crop Sci.* 14:827-830.
16. _____ and _____. 1975. Plant regeneration from tissue cultures of maize. *Crop Sci.* 15:417-421.
17. Greyson, R. I. and D. B. Walden. 1972. The abphyl syndrome in *Zea mays.* I. Arrangement, number, and size of leaves. *Amer. J. Bot.* 59:466-472.
18. Heinz, D. J. 1972. Sugarcane improvement through induced mutations using vegetative propagules and cell tissue techniques. *In*: Mutation breeding of vegetatively propagated and perennial crops. GAO/IAEA, Vienna p. 53-59.
19. _____ and G. W. P. Mee. 1970. Colchicine-induced polyploids from cell suspension cultures of sugarcane. *Crop Sci.* 10:696-699.
20. Henke, R. R.. K. G. Wilson, and J. W. McClure. 1974. Lysine-methionine- threonine-isoleucine interactions in growth and development of *Mimulus cardinalis* seedlings. *Planta* 116:333-345.
21. Kao, K. N.. O. L. Gamborg, W. A. Keller, and R. A. Miller. 1971. Cell division in cells regenerated from protoplasts of soybean and *Happlopappus gracilis. Nature* 232:124.
22. Lim, S. M. and A. L. Hooker. 1972. Disease determinant of *Helminthosporium maydis* race T. *Phytopathology* 62:968-971.
23. Melchers, G. 1972. Haploid higher plants for plant breeding. *Z. Pflanzenzucht.* 67:19-32.
24. Nickell, L. G. and D. J. Heinz. 1973. Potential of cell and tissue culture techniques as aids in economic plant improvement. *In* A. M. Srb (ed.) Genes, enzymes, and populations. Plenum Press, N.Y. p. 109-128.
25. Saunders. J. W. and E. T. Bingham. 1972. Production of alfalfa plants from callus tissue. *Crop Sci.* 12:804-808.
26. Smith, H. H. 1974. Model systems for somatic cell plant genetics. *Bio Science* 24:269-276.
27. Vasil. I. K. and C. Nitsch. 1975. Experimental production of pollen haploids and their uses. *Z. Pflanzenphysiol.* 76:191-212.
28. Widholm, J. M. 1972a. Cultured *Nicotiana tabacum* cells with an altered anthranilate synthetase which is less sensitive to feedback inhibition. *Biochim. Biophys. Acta* 261:52-58.
29. _____ . 1972b. Anthranilate synthetase from 5-methyltryptophan-susceptible and -resistant cultured *Daucus carota* cells. *Biochem. Biophys. Acta* 279:48-57.
30. _____ . 1976. Selection and characterization of cultured carrot and tobacco cells resistant to lysine, methionine, and proline analogs. *Can. J. Bot.* 54:1523-1529.