30 | Cell, Tissue, and Organ Culture Techniques for Genetic Improvement of Cucurbits

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ABSTRACT. Cucurbit tissue, cell, and organ culture is an area of research that is growing rapidly. Several important advances have been made within the past several years. Embryo culture has been used to rescue the progeny of interspecific crosses of species of *Cucumis*. Axillary buds have been induced to produce multiple shoots in cucumbers (*Cucumis sativus*) and watermelons (*Citrullus lanatus*), and both organogenesis and embryogenesis have been accomplished from several tissue sources of cucurbits. Cucumber and muskmelon (*Cucumis melo*) protoplasts have been isolated from leaf mesophyll cells and grown into callus. Protoplast-derived callus has been induced to form embryos in cucumbers and muskmelons. No successful protoplast fusions have yet been reported. In anther culture experiments with squash (*Cucurbita pepo*), only roots have been induced to form. However, cucumber anthers formed callus that later produced mature embryos. These breakthroughs may soon make genetic manipulations in culture a useful tool in cucurbit breeding programs.

Eight decades after the first report of an attempt to grow plant tissue in culture (27), successful techniques have been developed for many crop species, especially solanaceous species like tobacco, *Nicotiana tabacum* L. Progress with other crop species, however, including the cucurbits, has been much slower. Several reviewers have covered the application of in vitro techniques to crop plant improvement (25, 34, 44, 45, 54, 55). For that reason, we will review the status of tissue culture systems only as they relate to cucurbits.

Research activity has increased recently in the area of cell, tissue, and organ culture of cucurbits. Much of the basic work has been done in other crop species, so that often it is necessary only to adapt the available techniques to the cucurbit species of interest. We know of no reports in which in

vitro culture of cucurbits has been used for selection. However, progress has been made in the areas of embryo culture, propagation, embryogenesis, organogenesis, protoplast fusion, and anther and pollen culture (Table 1).

Propagation

In vitro propagation via shoot tip culture has been used successfully in several cucurbit species. The advantage of in vitro methods over conventional vegetative propagation is that selected clones can be multiplied in a short period of time. Shoot tip culture has an advantage over regeneration from callus since it usually induces less genetic variability in the resulting clones. Thus, plants that are regenerated from callus tend to be less uniform than plants produced from axillary or apical buds.

Handley and Chambliss (28) cultured the axillary buds of cucumber, *Cucumis sativus* L., on a Murashige-Skoog (MS) medium (44) with 0.1 mg/l each of naphthalene acetic acid (NAA) and 6-furylamino purine (kinetin). They were able to produce both shoots and roots with little callus formation, and the plants were successfully transferred to soil. A fivefold increase in the number of plants obtained was estimated for bud culture over the traditional method of rooted cuttings.

Fortunato and Mancini (21) were able to increase that efficiency ten times by using enhanced axillary branching. With that technique many shoots can be produced from a single bud. Shoot proliferation in cucumber occurred on a medium containing 20 to 30 ppm of 2-dimethylallyl-amino-purine (2-iP), while elongation and rooting were achieved by transferring the shoots to a medium with 1.0 ppm indole-3-acetic acid (IAA), 1.0 ppm 2-iP, and 0.025 ppm gibberellic acid-3 (GA_3). More recently, Aziz and McCown (2) obtained single shoots from axillary buds of cucumber by culturing them on a medium containing benzyladenine (BAP).

Similarly, Barnes (4) stimulated axillary bud development in watermelon, *Citrullus lanatus* (Thunb.) Matsum. & Nakai, shoot tips with a high kinetin and low IAA medium. Those shoots likewise were induced to elongate and root on a medium containing IAA. Alternatively, axillary shoots from the first medium could be transferred back to the same medium, i.e., high kinetin and low IAA, where an average of 10.3 axillary shoots were obtained per shoot per cycle. A similar protocol has also been used (37) in the propagation of buffalo gourd, *Cucurbita foetidissima* HBK. The use of a high cytokinin/low auxin medium is fairly routine for shoot tip culture in most plant species.

Other components of the culture medium may also influence the multiplication of buds in culture. In particular, ammonium and nitrate sources of nitrogen in the commonly used MS medium may not be optimum for cucumber shoot tip propagation (38).

Table 1. Summary of research in cell and tissue culture of cucurbits

Species	Research findings	Reference
Propagation		
Cucumis sativus	Asparagine and glutamine stimulated shoot tip growth in culture better than the Murashige-Skoog nitrogen sources (ammonium + nitrate)	38
C. sativus	Axillary buds cultured to produce one shoot/bud	2, 28
C. sativus	Enhanced axillary branching used to produce a mean of 10 shoots/bud	21
Citrullus lanatus	Axillary buds from shoot tips developed into shoots in culture (mean of 4.5/shoot tip) that could then be subcultured into plantlets and grown into whole plants	4, 5
Cucurbita foetidissima	Apical shoot tips and axillary buds produced 4–9 multiple shoots in 4 weeks	37
Embryo and ovule culture		
Cucumis metuliferus × *C. africanus*	Embryos from interspecific cross cultured into plantlets	13, 16
C. metuliferus × *C. melo*	Embryos from interspecific cross cultured but failed to form growing points	32
C. metuliferus × *C. melo*	Embryos from interspecific cross cultured into plantlets and grown into whole plants	46
C. metuliferus × *C. melo*	Culture of immature seeds used to obtain an extra generation of plants per year	24
Cucurbita pepo × *C. moschata*	Embryos from flat seeds cultured into mature plants using sterile media and soil; obtained 45 plants from 65 embryos	58
C. pepo	Unfertilized ovules cultured; some haploid plants obtained	9
Floral bud culture		
Cucumis sativus and *C. melo*	Floral buds induced to develop on a modified White's medium. Hormonal control of sex expression was studied	22, 49
Organogenesis		
Cucumis sativus var. *hardwickii*	Cotyledon explants made from cotyledons 2.5–4 mm long formed buds in culture	14
C. sativus	Callus and roots (but not shoots) were obtained by culturing stem, root, tendril, petiole, leaf, flower, and seedling hypocotyl and cotyledon tissue	1
C. sativus	Callus, but not shoots, obtained from field-grown leaf tissue	60
C. sativus	Apical stem explants formed shoots in culture	39
C. sativus	Hypocotyl and cotyledon explants formed callus in culture; subcultured callus formed shoots	53
C. sativus	Hypocotyl and cotyledon explants formed buds in culture	15

(*continued*)

Table 1. (Continued)

Species	Research findings	Reference
C. sativus	Best growth of hypocotyl explants in culture occurred on medium containing glucose or sucrose plus galactose; subcultures grew fastest on medium containing stachyose or raffinose	26
C. sativus	Cotyledon but not hypocotyl explants from 28 lines formed shoots in culture	8, 59
C. sativus	Cotyledons formed best callus with NAA + BAP; root and shoot formation occurred at low NAA + BAP concentration	47
C. sativus	Callus initiated from cucumber fruit tissue	3
C. melo	Seedling root callus developed chorophyll best on a modifed White's medium with 4% sucrose added, but grew best if only 2% sucrose was added	7
C. melo	Cotyledon callus induced to form well-developed shoots at good level of efficiency	42
C. anguria var. *longipes*	Calli from stem segments produced roots at higher frequencies than leaf-derived calli; leaf-derived calli produced more shoots than stem-derived calli	23
Cucurbita pepo and *C. maxima*	Mature cotyledons produced callus and shoots while immature embryo cotyledons produced only callus	35
Embryogenesis		
Cucumis sativus	Embryoids produced from anthers developed into whole plants	36
C. sativus	Embryos developed from leaf callus grown in dark for 6 weeks	40
C. sativus	Embryos obtained from hypocotyl callus	51
C. sativus	Embryos obtained from protoplast-derived callus	31, 48
C. melo	Somatic embryos obtained from callus on hypocotyl explants	6, 7, 42
C. melo	Embryos obtained from protoplast-derived callus	43
Cucurbita pepo	Fruit tissue explants formed callus in culture; subcultured callus formed embryonic outgrowths that formed plantlets when subcultured	52
C. pepo	Occasional hypocotyl and cotyledon explants formed callus only after 3 months in culture; callus formed embryos that were grown into whole plants	29, 30
Protoplast culture		
Cucumis sativus	Leaf mesophyll protoplasts isolated and cultured into callus, but not into plantlets	11, 12

Table 1. (Continued)

Species	Research findings	Reference
C. sativus	Glycine found to have stabilizing effect on leaf protoplasts; embryos developed form protoplast derived calli on solid medium	48
C. sativus	Protoplasts isolated from cotyledons; embryos formed on a low auxin/high cytokinin solid medium	31, 56
C. melo	Protoplasts isolated from leaf mesophyll and grown into callus	41
C. melo	Protoplast derived calli form embryos	43
Anther and pollen culture		
Cucumis sativus	Anthers formed callus in culture; subcultured callus formed embryoids, then plantlets; the origin of embryoids (from either sporophytic or gametophytic tissues) was not checked	36
Cucurbita pepo	Stamens cultured at the uninucleate stage formed roots but not shoots	10

Embryo and Ovule Culture

Techniques for in vitro culture of embryos are potentially useful in two important areas of cucurbit breeding. Culture of immature embryos could be used to reduce the generation time of crops to permit more cycles of selection of generation advances. For example, an extra generation of cucumbers could be grown each year in the greenhouse using the technique (24). In addition, embryo culture might be used to rescue the normally inviable F_1 hybrids of interspecific crosses before the embryos abort. Wall (58) obtained 45 mature plants by culturing 65 embryos from flat seeds of the cross *Cucurbita pepo* L. × *C. moschata* (Duch. ex Lam.) Duch. ex Poir. He used a solid medium to grow the embryos for 7 to 12 days before transferring them to soil. Embryo culture permitted more plants of the interspecific hybrid to be obtained than if the usual process of using the rare, plump seeds were used (of 7000 seeds from interspecific crosses, only 4 were plump).

Viable embryos from the cross of *Cucumis metuliferus* Naud. × *C. africanus* L. f. were obtained by removing those longer than 3.0 mm from the fruits approximately 30 days after pollination and culturing them into plantlets (16). The reciprocal cross did not produce embryos larger than 3.0 mm, and those embryos could not be regenerated into plantlets. Additional research on embryo culture using the same cross identified the early heart stage as the optimum time for removing the embryos from ovules that might abort in advanced stages. Early heart stage embryos were 0.1 to 0.8 mm

long and were excised 17 to 22 days after pollination. The embryos were cultured into plantlets in 32% of the cases, whereas only 15% of the embryos at the late globular stage produced plantlets (13).

Embryo culture has also been used in an effort to rescue embryos from the cross of *Cucumis metuliferus* with the muskmelon, *C. melo* L. Embryos in culture developed cotyledons but not growing points (32). In another case, success has been reported in making the above cross using embryo culture (46), but the plants were not checked to verify that they were interspecific hybrids.

Fassuliotis (20) attempted to transfer nematode resistance from *C. metuliferus* into *C. melo* using the above technique, but only one of the many embryos cultured was raised into a fruit-bearing plant. It was, however, considered to be apomictic, not an interspecific hybrid.

Most recently, unfertilized ovules of *Cucurbita pepo* have been cultured in vitro to obtain haploid plants (9). Ovules excised one to two days before flowering were best for regenerating plants. Under optimum conditions, four to seven plantlets were obtained per 100 cultured ovules. The ovules then grew into plants after transfer to a hormone-free medium. Although most of the resulting plants were diploid, a few were haploid-diploid chimeras, or polyploids. Eventually, it may be possible to adapt these techniques for rescue of interspecific hybrids before the embryos abort, but that has not yet been attempted.

Organogenesis

A basic requirement for nearly all uses of cell and tissue culture techniques in crop improvement is the regeneration of whole plants from cultured protoplasts, cells, or tissues. Regeneration from callus is accomplished in one of two ways: embryogenesis or organogenesis. Organogenesis involves the adventitious regeneration of any plant organ, including leaves, shoots, flowers, and roots.

Most of the work on cucumber organogenesis has been difficult to repeat in other laboratories, and the results have often been unpredictable. There is no clear-cut protocol available yet, although work is proceeding in several locations that may solve that problem (Zamir Punja, pers. comm., 1986). Maciejewska-Potapczykowa et al. (39) were the first to report organogenesis from callus produced by cucumber stem pieces in culture, but they did not describe the methods for obtaining shoots, since that was not the objective of their experiments.

Many studies have been carried out on cucumber callus. Alsop et al. (1) obtained callus only on several organ explants with various concentrations of NAA and BAP. Some budlike knobs were observed in callus grown at 0.1

mg/l of NAA and 0.1 mg/l of BAP. Aziz and McCown (2) also described budlike nodules on callus from cucumber internode pieces but could only induce roots to form. Callus growth of muskmelon seedling explants was best on modified White's medium with 0.1 to 1.0 mg/l of NAA and 2% sucrose, although chlorophyll development was best if 40% sucrose was added (17). In another study, callus subcultures grew faster when stachyose or raffinose was used as a carbon source instead of sucrose, galactose, or glucose (21). These results can be understood in light of the fact that the cucumber is one of the few species that translocates carbohydrates primarily as stachyose (50).

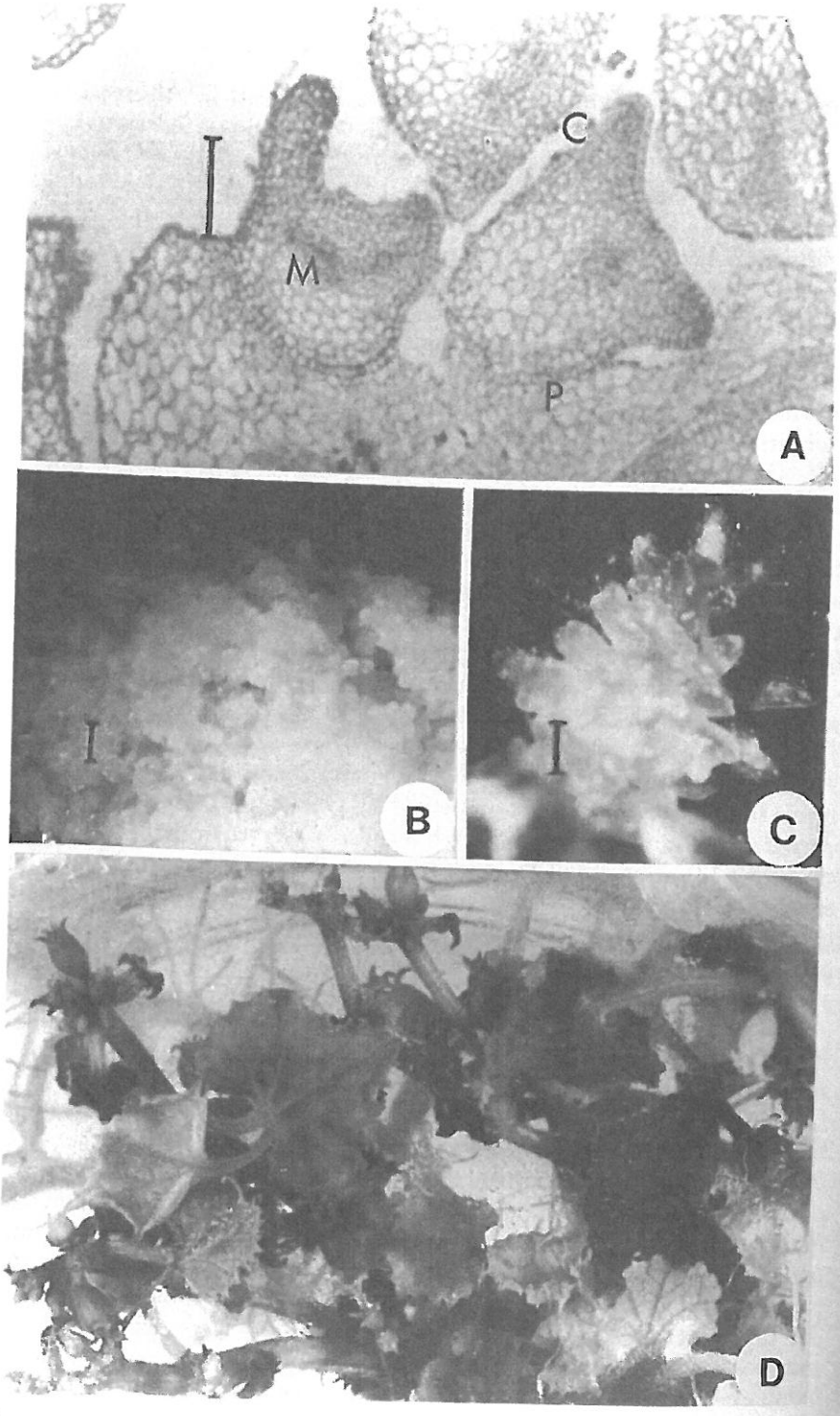
Others working with cucumbers have been able to produce buds, shoots, or both from callus formed on either hypocotyls or cotyledons (15, 47, 53, 59). Cotyledons presently appear to be the better explant for use in organogenesis experiments (8, 41, 59). Cotyledon callus, however, characteristically proliferates fibrous roots, whereas hypocotyl callus does not (47, 53). The proliferation may be caused by higher endogenous levels of auxin in the cotyledon tissue. Besides stems, cotyledons, and hypocotyls, other organs, including leaves (23, 60), fruit tissues (2), and embryos (14), have been used as explants for organogenesis in Cucurbitaceae.

Other factors are involved in organogenesis. Wehner and Locy (59) found a great deal of variation in the percentage of shoots produced among different cucumber genotypes. Some accessions from the United States Department of Agriculture germplasm collection had as many as 53% of cotyledon pieces forming shoots; while commercial cultivars in the United States never exceeded 13%. Other researchers have also observed differences among genotypes (8). The age of the fruit from which seed is obtained may also affect shoot production. Mature cotyledons that were explanted displayed a greater ability to form callus and differentiate into shoots and roots than cotyledons in formative stages (35).

Embryogenesis

Some of the earliest work in cucurbit tissue culture dealt with the formation of somatic embryos in culture (see Figure 1). Schroeder (52) was the first to recognize this phenomenon in cucurbits. Zucchini (*Cucurbita pepo*) pericarp tissue was grown on a Nitsch and Nitsch medium for 18 months. After that, spherical cell masses of friable callus were discovered that eventually produced torpedo-shaped embryoids. Finally, root hairs and leaf primordia developed on some of the embryoids, but none of the embryos developed further.

In 1972 Jelaska (29) observed somatic embryos in callus cultures from pumpkin (*Cucurbita pepo*) hypocotyls and cotyledons. No correlation was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found between the hormones used and the number of embryos produced. This is not surprising, since the media used were undefined, i.e., nutrient concentrations were not known. She attributed the ability of cotyledons to form embryos to physiological factors within the plant. Later Jelaska (30) reported organogenesis from the same cultures.

In the 1980's additional research on embryogenesis was reported for cucurbits. Blackmon et al. (7) and Blackmon and Reynolds (6) obtained somatic embryos from callus hypocotyl explants of muskmelon after two to three months on a modified Nitsch and Nitsch medium. No additional development of the embryos into plants was reported.

Lazarte and Sasser (36) were able to induce embryoid formation from anthers of cucumber on a modified Nitsch and Nitsch medium having raffinose as the carbon source. Callus was initiated with 0.1 mg/l BAP and 0.1 mg/l GA. Pieces of callus were then placed on a Nitsch and Nitsch medium with or without kinetin. Dark green areas of the callus differentiated into embryoids, which developed into embryos when all growth regulators were removed.

Malepszy et al. (40) were able to induce embryogenesis from leaf explants of cucumber with high frequency. They used a primary medium high in auxin and cytokinin followed by a secondary medium with the same level of cytokinin and no auxin. The highest number of embryos (220 from one explant) was obtained on a primary medium containing 1.2 mg/l 2,4,5-trichlorophenoxy acetic acid and 0.8 mg/l BAP. The paper did not mention whether the embryos were grown into mature plants.

Moreno et al. (42) could obtain only embryos from muskmelon hypocotyls, while only cotyledons underwent organogenesis. The embryos did not undergo further development. Rajasekaran et al. (51) were also able to obtain a few embryos from hypocotyl explants by using a medium high in 2,4-D medium. Both Orczyk and Malepszy (48) and Moreno et al. (41, 42) were able to adapt their embryogenesis techniques to later work on protoplast cultures, which will be discussed in the next section. Jia et al. (31) also obtained embryos from protoplast-derived cucumber callus. Cotyledon tissue, however, was used as the explant source instead of leaf tissue. A low auxin level and a high cytokinin level were used instead of the high auxin/low cytokinin ratio that is generally used to obtain somatic embryos. Embryos developed fully when placed on a half-strength MS medium de-

Figure 1. A. Heart-stage cucumber embryos developing on edges of callus from cotyledon explants. C, cotyledon, P, protoderm, and M, meristem tissue. Bar is 0.1 mm long. B. Embryogenic cucumber callus on Murashige-Skoog medium containing 2 mg/1 2, 4-D and 0.5 mg/l kinetin. Bar is 1 mm long. C. Cluster of late-stage cucumber embryos. Bar is 1 mm long. D. Cucumber plantlets rooting on Murashige-Skoog medium with no growth regulators.

void of exogenous hormones. Similar work has been done at the ARCO Plant Cell Research Institute by Trulson and Shahin (56). Embryos were initiated from cotyledon and root explants on MS medium containing 1 mg/l 2,4-D, 1 mg/l NM, and 0.5 mg/l BAP. After three weeks on the above medium, the callus was transferred to the same medium but without 2,4-D for two weeks of maturation. Plants were regenerated with high frequency when the callus was transferred to the same medium with no growth regulators.

Selection in Culture

Several potential uses exist for selecting cucurbits in in vitro culture, especially if single-cell cultures could be used. Plants could be selected for resistance to herbicides (e.g., atrazine and trifluralin), to high or low temperatures, to high salt (e.g., NaCl) concentrations, or resistance to diseases, if the techniques were available. Additional research is needed, however, before selection of single cells in culture will be a useful technique for cucurbit breeding. Until those advances have been made, shoot tip cultures could be used. Twenty or more shoot tips could be grown and tested in a single Petri plate, and the procedure for growing resistant shoot tips into whole plants has been demonstrated for both cucumbers (28) and watermelons (5).

Protoplast Culture

Recently, there has been increased interest in the use of plant protoplasts for genetic manipulation in culture. Protoplast fusion, i. e., the fusion of two cells to form a hybrid daughter cell, is one attractive use for the system. Crosses that are difficult or impossible to make by sexual methods because of incompatibility barriers may be possible through protoplast fusion if the techniques are available for the crop of interest.

It has been possible to isolate and grow cucumber protoplasts from leaf mesophyll cells (11) and to grow cucumber mesophyll cells into callus (12). These techniques have also been adapted for muskmelon (43), but until recently, the only morphogenic response has been root production in cucumber (12).

Embryos recently were obtained from callus grown from leaf protoplasts of cucumbers and muskmelons (31, 41, 48). Orczyk and Malepszy (48) found that glycine had a stabilizing effect on leaf protoplasts. They were able to transfer mini-calli to solid medium containing 0.8 mg/l 2,4-D and 2-iP, and later obtain embryos. The embryos grew into plants when trans-

ferred to a hormone-free medium. The efficiency of embryo and plantlet production was not given.

Moreno et al. (42) achieved both organogenesis and embryogenesis from the protoplast-derived calli of muskmelon. They found that an agitated liquid medium was better than a solid medium for obtaining embryos, and, under the best conditions, 1308.3 ± 322.7 embryos per replicate were obtained. Few of the embryos developed further into plantlets. Jia et. al. (31) and Trulson and Shahin (56) used a different approach, as mentioned previously, in that cotyledons were used as a protoplast source instead of leaf mesophyll. Both papers reported successful regeneration of embryos, several of which developed into mature plants. The use of cotyledons instead of leaf mesophyll was probably the key to their success, since cotyledon tissue is younger and more active metabolically.

There are no reports of the use of protoplast fusion for the interspecific hybridization of cucurbits. However, interspecific hybridization of cucumber with other *Cucumis* species would be useful to plant breeders, and attempts have been made using more conventional methods (32, 33). Possible applications include incorporation of root-knot nematode resistance from other *Cucumis* species into cucumbers and muskmelons. *Cucumis ficifolius* A. Rich. and *C. metuliferus* have the highest levels of resistance of 14 *Cucumis* species tested by Fassuliotis (18, 19). Thus far, however, plants have not been recovered from the interspecific crosses that have been attempted.

Anther and Pollen Culture

Successful culture of anthers or pollen grains and subsequent regeneration into whole plants would provide a method for production of haploid cells for selection, dihaploids (i.e., chromosome-doubled haploids to be used as inbred lines) for breeding programs, and as haploid plants for genetic studies (57). Little work has been done on anther culture in cucurbits. However, stamen cultures of squash (*Cucurbita pepo*) formed roots but not shoots if cultured when the microsporocytes were at the uninucleate stages (10). Also, a recent report has demonstrated successful embryogenesis from cultured anthers of cucumber (36). Anthers from plants of 'SMR 58' were cultured on several different media to produce callus, then embryoids, and finally plantlets. It was not determined whether the embryoids originated from sporophytic or gametophytic tissue.

In studies of hormonal control of sex expression in cucumbers (22) and muskmelons (49), floral buds have been induced to develop on a modified White's medium. That procedure has been used to provide a source of "clean" pollen for use in tissue culture studies.

Conclusions

There is much room for improvement in the area of in vitro culture of cucurbits. Embryo culture and anther and pollen culture have not been developed as fully as needed. However, where research has been done, success has been fairly rapid. Embryogenesis and regeneration of whole plants from protoplasts are now becoming routine. With those tools available, selection in culture and protoplast fusion may not be far off.

Literature Cited

1. Alsop, W. R., W. W. Cure, G. F. Evans, and R. L. Mott. 1978. Preliminary report on in vitro propagation of cucumber. Cucurbit Genet. Coop. Rep. 1: 1–2.
2. Aziz, H. A., and B. H. McCown. 1985. Hormonal response curves of shoot and callus cultures of cucumber (*Cucumis sativus* L.). HortScience 20: 540 (Abstract).
3. Aziz, H. A., B. H. McCown, and R. L. Lower. 1986. Callus initiation from cucumber (*Cucumis sativus* L.) fruits. Cucurbit Genet. Coop. Rep. 9: 3.
4. Barnes, L. R. 1979. In vitro propagation of watermelon. Scientia Hort. 11: 223–227.
5. Barnes, L. R., F. D. Cochran, R. L. Mott, and W. R. Henderson. 1978. Potential uses of micropropagation for cucurbits. Cucurbit Genet. Coop. Rep. 1: 21–22.
6. Blackmon, W. J., and B. D. Reynolds. 1982. In vitro shoot regeneration of *Hibiscus acetosella*, muskmelon, watermelon, and winged bean. HortScience 17: 588–589.
7. Blackmon, W. J., B. D. Reynolds, and C. E. Postek. 1981. Production of somatic embryos from callused cantaloupe hypocotyl explants. HortScience 16: 451 (Abstract).
8. Bouabdallah, L., and M. Branchard. 1986. Regeneration of plants from callus cultures of *Cucumis melo* L. Z. Pflanzenzucht. 96: 82–85.
9. Chambonnet, D., and R. Dumas de Vaulx. 1985. Obtention of embryos and plants from in vitro culture of underfertilized ovules of *Cucurbita pepo*. Cucurbit Genet. Coop. Rep. 8: 66.
10. Chang, Y.-F., and J. E. Lazarte. 1981. Anther culture of squash (*Cucurbita pepo* L.). HortScience 16: 451 (Abstract).
11. Coutts, R. H. A., and K. R. Wood. 1975. The isolation and culture of cucumber mesophyll protoplasts. Pl. Sci. Lett. 4: 189–193.
12. Coutts, R. H. A., and K. R. Wood. 1977. Improved isolation and culture methods for cucumber mesophyll protoplasts. Pl. Sci. Lett. 9: 45–51.
13. Custers, J. B. M. 1981. Heart shape stage embryos of *Cucumis* species more successful in embryo culture than advanced stage embryos. Cucurbit Genet. Coop. Rep. 4: 48–49.
14. Custers, J. B. M., and J. H. W. Bergovoet. 1980. In vitro adventitious bud formation on seedling and embryo explants of *Cucumis sativus* L. Cucurbit Genet. Coop. Rep. 3: 2–4.
15. Custers, J. B. M., and L. C. Buijs. 1979. The effects of illumination, explant

position, and explant polarity on adventitious bud formation in vitro of seedling explants of *Cucumis sativus* L. cv. 'Hokus'. Cucurbit Genet. Coop. Rep. 2: 2–4.
16. Custers, J. B. M., and G. van Ee. 1980. Reciprocal crosses between *Cucumis africanus* L. and *C. metuliferus* Naud. II. Embryo development in vivo and in vitro. Cucurbit Genet. Coop. Rep. 3: 50–51.
17. Fadia, V. P., and A. R. Mehta. 1976. Tissue culture studies on cucurbits: chlorophyll development in *Cucumis* callus cultures. Phytomorphology 26: 170–175.
18. Fassuliotis, G. 1967. Species of *Cucumis* resistant to the root-knot nematode, *Meloidogyne incognita acrita*. Pl. Dis. Reporter 51: 720–723.
19. Fassuliotis, G. 1970. Resistance of *Cucumis* spp. to the root-knot nematode, *Meloidogyne incognita acrita*. J. Nematol. 2: 174–178.
20. Fassuliotis, G. 1977. Embryo culture of *Cucumis metuliferus* and the interspecific hybrid with *C. melo*. 4th Ann. Colloquium Plant Cell Tissue Culture. Ohio State Univ., Columbus.
21. Fortunato, I. M., and L. Mancini. 1985. Tecnica sulla propagazione vegetativa in vitro di centriolo (*Cucumis sativus* L.). Annali della Facotadi Agraria, Universita de Bari 32: 651–659.
22. Galun, E., Y. Jung, and A. Lang. 1963. Morphogenesis of floral buds of cucumber cultured in vitro. Dev. Biol. 6: 70–387.
23. Garcia-Sogo, M., I. Granelli, B. Garcia-Sogo, L. A. Roig, and V. Moreno. 1986. Plant regeneration from explant-derived calli of *Cucumis anguria* L. var. *longipes*. Cucurbit Genet. Coop. Rep. 9: 108–110.
24. George, B. F., and L. V. Crowder. 1970. The use of a modified embryo culture technique in cucumber breeding. HortScience 5: 329 (Abstract).
25. Green, C. E. 1977. Prospects for crop improvement in the field of cell culture. HortScience 12: 131–134.
26. Gross, K. C., D. M. Pharr, and R. D. Locy. 1981. Growth of callus initiated from cucumber hypocotyls on galactose and galactose-containing oligosaccharides. Pl. Sci. Lett. 20: 333–341.
27. Haberlandt, G. 1902. Culturversuche mit isolierten Pflanzenzellen. Sitz-Ber. Mat.-Nat. Kl. Kais. Akad. Wiss. Wien 111: 69–92.
28. Handley, L. W., and O. L. Chambliss. 1979. In vitro propagation of *Cucumis sativus* L. HortScience 14: 22–23.
29. Jelaska, S. 1972. Embryoid formation by fragments of cotyledons and hypocotyls in *Cucurbita pepo*. Planta 103: 278–280.
30. Jelaska, S. 1974. Embryogenesis and organogenesis in pumpkin explants. Physiol. Pl. 31: 257–261.
31. Jia, S., Y. Fu, and Y. Lin. 1986. Embryogenesis and plant regeneration from cotyledon protoplast culture of cucumber (*Cucumis sativus* L.). J. Pl. Physiol. 124: 393–398.
32. Kho, Y. O., J. Franken, and A. P. M. den Nijs. 1981. Species crosses under controlled temperature conditions. Cucurbit Genet. Coop. Rep. 4: 56.
33. Kho, Y. O., A. P. M. den Nijs, and J. Franken. 1980. Interspecific hybridization in *Cucumis* L. II. The crossability of species, and investigation of in vivo pollen tube growth and seed set. Euphytica 29: 661–672.
34. Kleinhofs, A., and R. Behki. 1977. Prospects for plant genome modification by nonconventional methods. Ann. Rev. Genet. 11: 79–101.

35. Lange, N. E., and J. A. Juvik. 1986. Age dependence for organogenesis of seed explants from four *Cucurbita* accessions. Cucurbit Genet. Coop. Rep. 9: 93.
36. Lazarte, J. E., and C. C. Sasser. 1982. Asexual embryogenesis and plantlet development in anther culture of *Cucumis sativus* L. HortScience 17: 88.
37. Lee, C. W., and J. C. Thomas. 1985. Tissue culture propagation of buffalo gourd. HortScience 20: 218–219.
38. Locy, R. D., and T. C. Wehner. 1982. Cucumber shoot tip growth on 9 nitrogen sources in in vitro culture. Cucurbit Genet. Coop. Rep. 4: 20–22.
39. Maciejewska-Potapczykowa, W., A. Rennert, and E. Milewska. 1972. Callus induction and growth of tissue cultures derived from cucumber plant organs of four different sex types. Acta Soc. Bot. Pol. 41: 329–339.
40. Malepszy, S., and A. Nadolsky-Orczyk. 1983. In vitro culture of *Cucumis sativus*. I. Regeneration of plantlets from callus formed by leaf explants. Z. Pflanzenphysiol. 111: 273–276.
41. Moreno, V., M. Garcia-Sogo, I. Granelli, B. Garcia-Sogo, and L. A. Roig. 1985. Plant regeneration from calli of melon (*Cucumis melo* L. cv. 'Amarillo Oro'). Pl. Cell Tiss. Organ Cult. 5: 139–146.
42. Moreno, V., L. Zubeldia, B. Garcia-Sogo, F. Nuez, and L. A. Roig. 1986. Somatic embryogenesis in protoplast-derived cells of *Cucumis melo* L. *In* W. Horn, C. J. Jensen, W. Odenbach, and O. J. Schieder, eds., Genetic Manipulation in Plant Breeding. Proc. Int. Symp., Berlin, 1985. W. de Gruyter, Berlin.
43. Moreno, V., L. Zubeldia, and L. A. Roig. 1984. A method for obtaining callus cultures from mesophyll protoplasts of melon (*Cucumis melo* L.). Pl. Sci. Lett. 34: 195–201.
44. Murashige, T. 1974. Plant propagation through tissue cultures. Ann. Rev. Pl. Physiol. 25: 136–166.
45. Narayanaswamy, S. 1977. Regeneration of plants from tissue cultures. *In* J. Reinert and Y. P. S. Bajaj, eds., Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture. Springer-Verlag, New York.
46. Norton, J. D. 1980. Embryo culture of *Cucumis* species. Cucurbit Genet. Coop. Rep. 3: 34.
47. Novak, F. J., and M. Dolezelova. 1982. Hormone control of growth and differentiation in the in vitro cultured tissue of cucumber (*Cucumis sativus* L.). Biologia 37: 283–290.
48. Orczyk, W., and S. Malepszy. 1985. In vitro culture of *Cucumis sativus* L. V. Stabilizing effect of glycine on leaf protoplasts. Pl. Cell Rep. 4: 269–273.
49. Porath, D., and E. Galun. 1967. In vitro culture of hermaphrodite floral buds of *Cucumis melo* L.: microsporogenesis and ovary formation. Ann. Bot. 31: 283–290.
50. Pristupa, N. A. 1957. The transport form of carbohydrates in pumpkin plants. Fizcol. Rast. 6: 26–32.
51. Rajasekaran, K., M. G. Mullins, and Y. Nair. 1983. Flower formation in vitro by hypocotyl explants of cucumber (*Cucumis sativus* L.). Ann. Bot. 52: 417–420.
52. Schroeder, C. A. 1968. Adventive embryogenesis in fruit pericarp tissue in vitro. Bot. Gaz. 129: 374–376.
53. Sekioka, T. T., and J. S. Tanaka. 1981. Differentiation in callus cultures of cucumber (*Cucumis sativus* L.). HortScience 16: 451 (Abstract).

54. Skirvin, R. M. 1978. Natural and induced variation in tissue culture. Euphytica 27: 241–266.
55. Thomas, E., P. J. King, and I. Potrykus. 1979. Improvement of crop plants via single cells in vitro—an assessment. Z. Pflanzenzucht. 82: 1–30.
56. Trulson, A. J. and E. A. Shahin. 1986. In vitro plant regeneration in the genus *Cucumis*. Pl. Sci. 47: 35–43.
57. Vasil, I. K., and C. Nitsch. 1975. Experimental production of pollen haploids and their uses. Z. Pflanzenphysiol. 76: 191–212.
58. Wall, J. R. 1954. Interspecific hybrids of *Cucurbita* obtained by embryo culture. Proc. Amer. Soc. Hort. Sci. 63: 427–430.
59. Wehner, T. C., and R. D. Locy. 1981. In vitro adventitious shoot and root formation of cultivars and lines of *Cucumis sativus* L. HortScience 16: 759–760.
60. Wehner, T. C., and R. D. Locy. 1981. Tissue culture propagation of field-grown cucumber selections. Cucurbit Genet. Coop. Rep. 4: 20–22.