PROBLEMS POSED BY MICROPLANT MORPHOLOGY

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Micropropagation by *in vitro* techniques has the potential of replacing conventional methods of propagation for many horticultural crops But several serious problems must be solved before micropropagation becomes profitable on a large commercial scale. One serious problem is the high rate of loss of cultured plants of many species when they are transferred to the greenhouse or to the field. These plants are characterized by rapid and severe desiccation unless they are nurtured in a protective environment for the first 1 to 2 weeks after they are removed from culture. Studies indicate that abnormalities in the morphology of cultured plants may cause this desiccation by blocking water flow through the plant Morphological abnormalities of the root-shoot junction, leaf anatomy, and the cuticular surface have special relevance to the problem of desiccation

The root-shoot junction has been shown to be morphologically abnormal in cultured plants in several species. In *Pelargonium* × *hortorum* vascular cylinders of multiple shoots converged inside a small knob of callus at the base of the shoots (8). The vasculature became interconnected, forming knots and blocked passageways. A complete disjunction of shoots and roots has been reported in primary explants of *Asparagus officinalis* (1) causing them to wilt severely upon transfer to the greenhouse. In cultured cauliflower plants vascular connections were narrow and ill-formed at the time the plants were transferred to the greenhouse (5) After three weeks in the greenhouse, the vascular connections were greater in number and size. Significantly greater water flow occurred from the roots to the shoots at this time compared to the water flow when the plants were first removed from culture. Thus, any hinderance to water flow between roots and shoots contributes to decreased survival rate since water stress in the leaves increases, and the plants wilt and often die.

The leaf anatomy of cultured plants also has been shown to be abnormal in several cases In *Asparagus officinalis* (6) leaves on cultured plants did not exhibit ferning characteristic of the species and survival of the transferred plants was very low Increasing the light intensity *in vitro* to 10,000 lux prior to transplanting resulted in normal leaf formation and an increase to 95-100 percent survival when plants were transferred to the greenhouse.

The leaves of cultured plants often resemble those grown in shade and those under low water stress. Brainerd *et al.* (3) measured smaller palisade cells, larger intercellular spaces and decreased stomatal frequency in cultured plum plants compared to their counterparts grown in the greenhouse and in the field. Further studies indicated that the leaves of some species were functionally, as well as structurally, anomalous. Leaves of cauliflower (12) and apple (2) had impaired stomatal functioning, with stomates remaining open under conditions of high water stress. They also had reduced photosynthetic capacity (5). These leaves would be more likely to be rapidly injured under the conditions of stress to which plants are exposed when transferred from culture to greenhouse.

Leaves of several species in culture also had altered surface wax characteristics compared to those of plants grown in the greenhouse or in a growth chamber. In cauliflower, cabbage, carnation and other normally glaucous plants, the leaves in culture were glossy rather than glaucous. Under the scan-

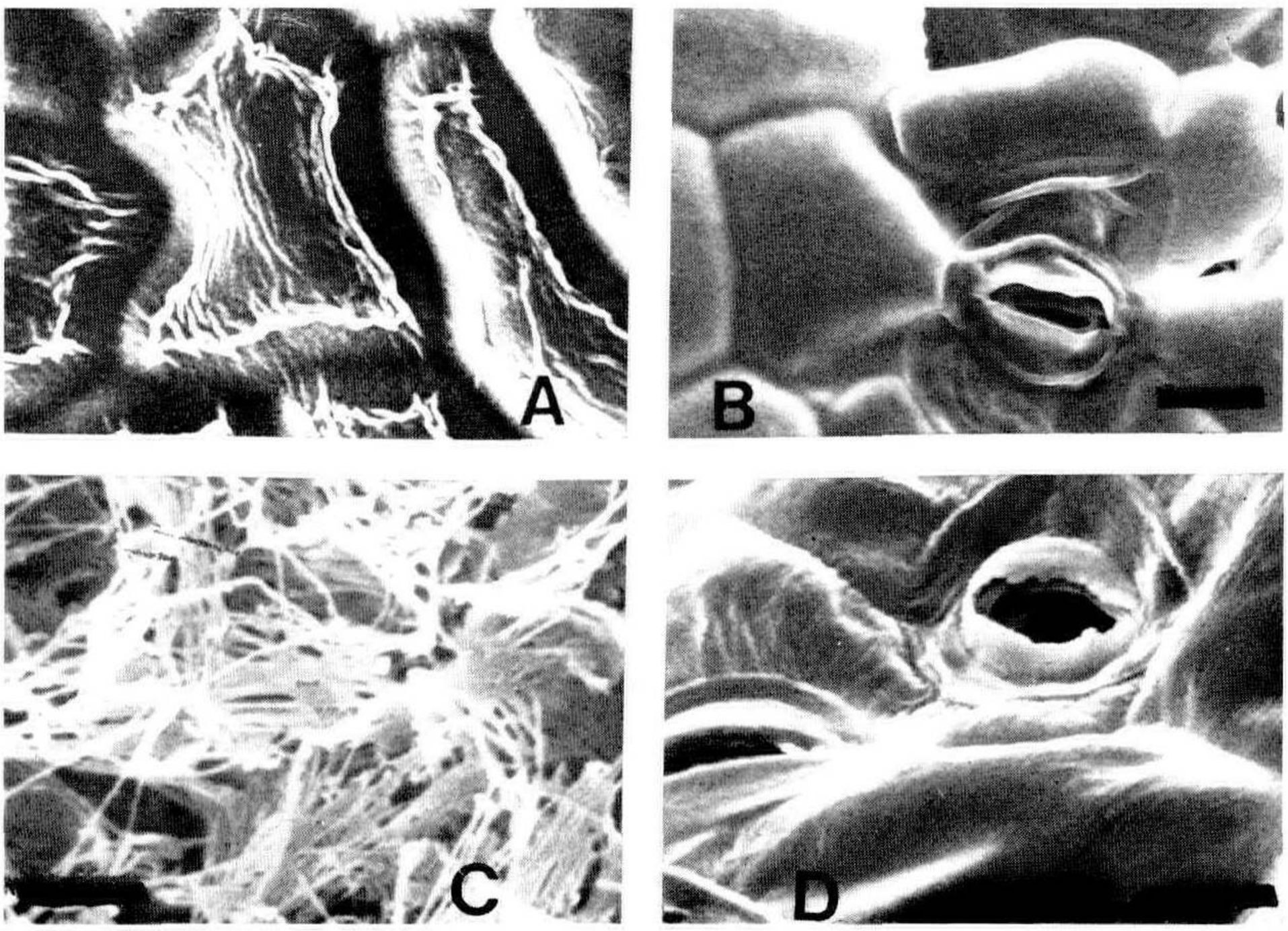

Figure 1. A. Abaxial leaf surface of *Dieffenbachia* 'Perfection Compacta' grown in the greenhouse. Ridges on surface are presumably wax. Bar = 20 μm.
B. Abaxial leaf surface of *Dieffenbachia* 'Perfection Compacta' grown in culture. Bar = 20 μm.
C. Adaxial surface of cabbage plant grown in the greenhouse. Wax covers the stomates. Bar = 5 μm.
D. Adaxial surface of cabbage plant grown in culture. Bar = 10 μm.

ning electron microscope (SEM) the cuticular surfaces were smooth, lacking the typical crystalline structure of plants grown in the greenhouse (Figure 1) (4,11,12). In a few cases wax has been observed on cultured plants of carnation (11) and cabbage (10) This wax had an abnormal crystalline structure which may indicate an alteration in chemical composition as well (7).

Measurements of the amounts of surface wax supported observations using SEM, with plants in culture having significantly less wax than those in the greenhouse (10). After transfer to the greenhouse new leaves formed normal amounts of wax with typical crystalline structure. The leaves formed in culture, however, showed reduced wax and altered crystalline structure as long as they remained on the plants.

Implications of reduced wax formation are severe Since it is the wax component that determines water flow through the cuticle (9) lack of wax would be expected to result in greater water loss. This was shown to be the case in cultured plants of cabbage (10). As the plants became hardened off, their rate of water loss decreased, approaching that of plants grown in a growth chamber

Thus, there are several morphological problems that demand attention because they adversely affect the survival of cultured plants when they are transferred to the greenhouse. Presently, these problems are being dealt with at the time the plants are transferred to the greenhouse. Cultural conditions are adapted to the morphology and physiology of the cultured plants in order to reduce stress resulting from lower relative humidity and increased light intensity Such measures as shade, mist benches and humidity tents greatly increase the survival rate of plants transferred to the greenhouse.

More effort is needed to determine the conditions that can be altered in the culture environment that will result in plants more adapted to growth in the greenhouse. The proper selection of nutrient components and growth regulators can often reduce or entirely eliminate the callus formed between roots and shoots. Direct vascular connections would allow unimpeded water flow to the leaves Altering environmental conditions in the culture room could induce the formation of leaves more suitable for growth in conditions of high light intensity and low relative humidity Presently some measures cannot be used. For example, increasing the light levels often causes damage to the plants while still in culture. Efforts should be directed to develop techniques to lower the relative humidity *in vitro* without increasing contamination since it was shown that lowering the relative humidity restores normal stomatal functioning (2) and induces the formation of wax (10). These

measures must be adopted to commercial production if the potential of mass propagation by *in vitro* techniques is to be realized

LITERATURE CITED

1 Aynsley, J and M Marston 1975 Aerial plantlet formation in *Asparagus officinalis* L *Sci Hort* 3 149-155

2 Brainerd, K and L Fuchigami 1981 Acclimatization of aseptically cultured apple plants to low relative humidity *J Amer Soc Hort Sci* 106 515-518

3 Brainerd, K , L Fuchigami, S Kwiatkowski, and C Clark 1981 Leaf anatomy and water stress of aseptically cultured 'Pixy' plum grown under different environments *HortScience* 16 173-175

4 Grout, B 1975 Wax development on leaf surfaces of *Brassica oleracea* var Currawong regenerated from meristem culture *Plant Sci Lett* 5 401-405

5 Grout, B and M Aston 1977 Transplanting of cauliflower plants regenerated from meristem culture I Water loss and water transfer related to changes in leaf wax and to xylem regeneration *Hort Res* 17 1-7

6 Hasegawa, P , T Murashige, and F Takatori 1973 Propagation of asparagus through shoot apex culture II Light and temperature requirements, transplantability of plants, and cyto-histological characteristics *J Amer Soc Hort Sci* 98 143-148

7 Jeffree, C , E Baker, and P Holloway 1975 Ultrastructure and recrystallization of plant epicuticular waxes *New Phytol* 75 539-549

8 Pillai, S and A Hildebrant 1969 Anatomical changes accompanying differentiation of geranium callus *in vitro Bull Torrey Bot Club* 96 96-100

9 Schonherr, J 1976 Water permeability of isolated cuticular membranes the effect of cuticular waxes on diffusion of water *Planta* (Berl) 131 159-164

10 Sutter, E 1981 Abnormalities in *Chrysanthemum morifolium* Ramat plants regenerated from long-term tissue culture and the formation of epicuticular wax in *Dianthus caryophyllus* L and *Brassica oleracea* var *capitata* L plants grown in tissue culture Ph D Thesis Cornell University, Ithaca, New York

11 Sutter, E and R W Langhans 1979 Epicuticular wax formation on carnation plantlets regenerated from shoot tip culture *J Amer Soc Hort Sci* 104 493-496

12 Wardle, K , A Quinlan, and I Simpkins 1979 Abscisic acid and the regulation of water loss in plantlets of *Brassica oleracea* L var *botrytis* regenerated through apical meristem culture *Ann Bot* 43 745-752

QUESTION AND ANSWER SESSION

MICHAEL DIRR: Has *Pyrus calleryana* been propagated by tissue culture?

DICK ZIMMERMAN: I believe that Microplant Nurseries in Oregon is doing it.

LEN STOLTZ: I have been working with *Pyrus calleryana* 'Aristocrat' and our multiplication rate has not been satisfactory. Rooting and greenhouse establishment are also problem areas

BRUCE BRIGGS· Question for Ellen Sutter. Have you had an occasion, as we do in the lab, to take the lids off the tubes and expose the plantlets to air for 4 to 5 days before planting? The plants harden off and do better. Is wax forming, or are the stomates working better?

ELLEN SUTTER. I have not done that; however, other research has shown that stomates function normally after 4 to 5 days. I am sure that wax starts to form very early

CHARLES HEUSER. Question for Ellen Sutter. Have you tried high agar during the rooting process and does it modify the cuticle?

ELLEN SUTTER· We tried that and the plants did not do too well. We are trying PEG as an osmoticum. We put mums in the greenhouse after being on PEG and they died faster than the controls.

PAUL READ: We have looked at the root morphology as the agar concentration is increased and have observed changes. This may account for the better success we and other people have had with direct sticking micropropagated plants.

VOICE: Is it feasible to do tissue culture on a small scale and if so what about the availability of supplies?

PAUL READ· It is certainly possible on a small scale Len Stoltz made a presentation in St. Louis a couple of years ago which suggests that it was possible to get started on a small scale with a minimal cost (Editor's Note. See *Proc. Inter. Plant Prop. Soc.* 29· 375-381, 1979). When starting, I recommend starting slow to learn the process You can buy prepackaged recipes from companies such as GIBCO and Flow Labs, to name two.

RANDALL STRODE We have gone through an evolution. Initially we started with prepackaged media. We now have better trained people and prepare our own media from scratch. There is nothing wrong with the prepackaged media. They are very good. I should point out that we initially began our operation in a pilot lab and then progressed to a 2000 sq ft operation

BEN DAVIS: Where does one obtain training in tissue culture?

PAUL READ· You need to learn it from a good person. That might be at your nearby university or the course at the Cell Science Center in Lake Placid.

PETER VERMEULEN: Is it possible to publish a digest of literature that has been done and is now ongoing? Also what is being done with coniferous evergreens?

PAUL READ: Tissue culture is a field that is developing so rapidly that I find it impossible to keep up with what is happening. This also raises the question of whether you, as a nurseryman, should have a tissue culture lab. Currently many nurserymen do not propagate all their plants. Maybe on a cost effective basis, tissue culture propagation should be left to selected producers.

DICK ZIMMERMAN: It is not enough to know the literature but also who is doing it on a commercial scale because their results are not in the scientific literature.

STEVE McCULLOCH: Referring to the question that Peter Vermeulen asked I would like to refer him to an abstract by Dr. McCown at the American Society for Horticultural Science Meeting at Atlanta last year. It was one of the first attempts to propagate a wide range of conifers. At present he has been successful with *Thuja*. We have also done *Thuja* and it comes quite easy.

PROGRESS IN BREEDING AROIDS

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We have been conducting breeding studies within the genera *Dieffenbachia* and *Aglaonema*, which are both members of the *Araceae* family. Two goals of this program are: A) development of new and better cultivars for commercial production in Florida and; B) to study the biology of their reproductive mechanisms and how it relates to other tropical plants. Our studies have included more than 50 distinct types of dieffenbachia and 15 different aglaonemas. The following discussion will center on the more important factors we have discovered which affect the breeding potential within these genera.

Stock plants are grown in greenhouses or shaded slat sheds with light intensities of 1500-2500 foot candles and a temperature regime of 65-95°F. Under these conditions, most dieffenbachia tend to produce a seasonal flush of blooms from April through June. Aglaonemas usually begin to flower in May and continue through June. However, some plants that we wanted to hybridize never seemed to bloom concurrently.