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JOHN HART: How do we control genetic vulnerability of the plants we produce by tissue culture?

BRENT McGOWN: This technique can be misused like any other technique in propagation. It is a point of professionalism. We can use it just as readily for good benefit. We can produce multiple genotypes for forest planting in culture easier than by standard means. It is, therefore, possible to protect against the introduction of single genotypes that would be vulnerable to plant pest problems.

DISEASE-FREE PLANTS THROUGH MICROPROPAGATION[1]

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Asexual or vegetative propagation of plants is practiced when the qualities of elite clones are not maintained in seedling progenies. Asexual propagation also perpetuates any systemic infection caused by viruses or vascular wilt organisms. Micropropagation can eliminate such infections and, in some cases, is the only available method for obtaining healthy plants. Four methods of micropropagation currently are being practiced. Three of these have limited usefulness due to the possibility of propagating somatic mutations. In as much as current and future investigations could change this, they will be briefly discussed. The fourth, tip meristem culture, has been used for a quarter of a century to eliminate viruses from infected plants and is the basis for the commercial production of cultured car-

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nations, chrysanthemums and geraniums.

Callus tissue can be induced on many plant species, aseptically removed, and cultured under in vitro conditions. Culture of such tissue provides healthy plants but problems associated with ploidy and mutation restricts its usefulness to those crops where those abnormalities can be detected and rogued. It appears to be a promising method for propagating conifers (1,2,12) and poplars (14). In addition, it was physiological studies with callus culture which led to the development of defined media and identification of the proper environmental conditions essential for successful conditions. Since the individual cells of the culture are similar they have the potential to develop into a complete plant as was postulated by Haberlandt (4). This was accomplished some 50 years later by Muir *et al.* (8).

Anther and pollen culture have intrigued plant scientists since the late 1940's. If anthers or pollen of certain plant species are placed on media containing appropriate mineral and organic constituents then exposed to the correct environmental conditions, plantlets will develop. These plantlets are haploid but can be restored to the diploid condition with colchicine. Since few viruses pass through pollen most of these plants will be healthy but different from the parent. While this technique will not provide duplicates of the parent, it does have great potential for developing parental lines of hybrids for such crops as corn, *Zea mays* L. It also is being used for crop improvement since the Chinese have developed new cultures of rice, tobacco and wheat from pollen cultures (C. Nitsch, personal communication).

Regeneration of plants from protoplasts presently is one of the more exciting areas of research. While successful regeneration of plants has been limited to a few species the possibilities appear to be unlimited. Already scientists in Kansas have developed a new potato cultivar from the high quality 'Russet Burbank' with a yield potential twice that of the parent (A. Ayers, personal communication). Isolation of protoplasts involves the separation of leaf cells, generally mesophyll, and the enzymatic removal of the cell walls (13). Properly protected against plasmolysis, these naked protoplasts will develop into complete plants when exposed to the correct combinations of media constituents and environmental conditions. Since the regenerated plantlets may differ from the parent plant and viruses can be retained by the protoplast, this method presently is more useful for developing new cultivars than for obtaining healthy plants from diseased cultivars.

Tip meristem culture represents a micro method of propagation from shoot apices. In 1941, Dimock (3) obtained healthy plants by propagating the shoot apices from chrysanthemums infected with *Verticillium albo-atrum* Reinke and Berth. This study led Yoder Bros. Inc. to establish their disease-free chrysanthemum program. Subsequently, Limasset and Cornuet (6) found the concentrations of viruses were progressively lower in serial sections approaching the apical meristem. In about half of the cases no virus could be detected in the apical meristem itself. This prompted Morel and Martin (7) to postulate that virus-free plants identical to the "mother plant" might be obtained by meristem culture. This technique involved the excision of the apical dome (ca. 0.1 mm × 0.25 mm) which is composed of rapidly dividing cells and covered with bud scales. The dome, generally including 1 or 2 leaf primordia, is aseptically removed then placed on an appropriate medium and, if optimum conditions prevail, will develop into plantlets. Using the tip meristem technique, Morel and Martin obtained virus-free plantlets but were unable to induce rooting. These plantlets were then grafted to virus-free plantlets and developed into healthy plants of the mother plant genotype. Presently, tip meristem culture is the most useful method to produce genetically identical healthy plants from the diseased mother plant and is the only method by which healthy plants have been obtained from some infected cultivars of strawberries, potatoes and many floral crops. Some examples of economically important crops where healthy clones have been obtained from infected cultivars by tip meristem culture are listed in Table 1.

Using tip meristem culture, healthy plants have also been obtained from virus-infected cultivars of at least 15 different species of floral crops (11). The apparent greater success of tip meristem culture in floral crops may reflect emphasis, although most floral cultivars are relatively easy to propagate vegetatively. Species or cultivars which are easy to propagate by routine methods generally respond more favorably to tip meristem culture than species or cultivars which are difficult to propagate. Once we understand such physiological factors as dormancy and juvenility and define the correct cultural constituents and environmental conditions, it seems likely that tip meristem culture can be used to produce healthy plants from most species of higher plants.

TIP MERISTEMS WORK IN MISSOURI

We became interested in tip meristem work, primarily as a means of obtaining healthy clones from tree cultivars infected with viruses which could not be eliminated with heat therapy. We have had a virus certification program for nursery fruit trees

Table 1. Some horticultural and forest species in which complete, virus-free plants have been obtained by tip meristem culture from previously infected clones.

Plant	Disease	Reference
Vegetable		
Cauliflower	turnip mosaic	*J. Hort. Sci.* 49:273
Sweet potato	internal cork	
	rugose mosaic	*Japan Agric. Res.* Quart 6:1-7
	feathery mottle	
Potato	paracrinkle, PVX	*Ann. Appl. Biol.* 45:422
	PVX, PVS	*Advan. Hort. Sci.* Appl. 1:144
	PVX, PVY, PVS	*Proc. 10th Intern. Botan. Congr.*, *Edinburgh*, 485 pp.
	PVS, PVM, PVX PVS	*Phytopathology* 58:199
	Spindle tuber (viroid)	*Phytopathology* 60:1857
Fruit		
Strawberry	vein banding, crinkle yellows complex	*Plant Dis. Rptr.* 47:298
Banana	cucumber mosaic	*Phytopathology* 64:320
Gooseberry	vein banding	*J. Hort. Sci.* 43:289
Raspberry	mosaic	*Ann. Phytopath.* 3:493-501
Forest		
Cassava	unidentified	*In Vitro* 8:421
Poplar	unidentified	*In Vitro* 7:269

for a quarter of a century and, although healthy clones of most popular cultivars are available, occasionally all clones of a cultivar may be infected with one or more viruses. The intense interest in the early coloring, spur and compact types of fruit trees compound this condition since these types generally arise as mutations, natural or radiation-induced, on trees which may be infected. Heat treatment followed by propagating the shoot tips (50 to 70 mm) on healthy seedlings will result in healthy trees but certain viruses, such as the stem grooving or brown line (SGV), are not affected by that. In *Prunus* material successful shoot tip grafting is so difficult to achieve that this method is not very useful. Our work thus far has stressed apple although some preliminary studies on a *Prunus* clone infected with the green ring mottle virus appear promising.

Our work started in 1976 when we were unable to locate any trees of a certain high quality cultivar which were not infected with SGV. Within a month we developed a defined medium (Table 2) which supports the growth of meristems up to 5 cm in six weeks. All attempts to induce rooting failed so we investigated the possibility of grafting which has been used to obtain health citrus trees (9). In our studies it was shown (5) that the meristem can be grafted directly to the hypocotyl of a germinating seedling from which the cotyledons and epicotyl had been aseptically removed. Meristems from plantlets developed from meristems also can be used. These would permit additional therapy such as the use of malachite green which en-

abled Norris (10) to eliminate potato virus X from the infected cultivar, Green Mountain. Using meristem grafting we have obtained several plants from two apple cultivars which were infected with SGV. Preliminary tests indicate that these plants are free from SGV. If further tests corroborate these observations and the cultivar's characteristics are maintained, these plants will replace the infected ones and the cultivars will enter our certification program.

Table 2. Composition of nutrient solution for apple meristem culture.

MINERAL SALTS

Macroelement	mg/l	Micro elements	mg/l
NH_4NO_3	1650	H_3BO_3	6.2
KNO_3	1900	$MnSO_4 \cdot 4H_2O$	22.3
$CaCl_2 \cdot 2H_2O$	440	$ZnSO_4 \cdot 4H_2O$	8.6
$MgSO_4 \cdot 7H_2O$	370	KI	0.83
KH_2PO_4	170	$Na_2MoO_4 \cdot 2H_2O$	0.25
Na_2-EDTA	37.3	$CuSO_4 \cdot 5H_2O$	0.025
$FeSO_4 \cdot 7H_2O$	27.8	$CoCl_2 \cdot 6H_2O$	0.025

ORGANIC CONSTITUENTS

Vitamins	mg/l	Growth Regulators	mg/l	Carbon Source	mg/l
Nicotinic acid	1	Benzyladenine	0.1126	Sucrose	20
Thiamine HCl	10	Gibberellin A_3	0.03		
Pyrodoxine HCl	1				
i-inositol	100				

pH adjusted to 5.7 with NaOH or HCl.

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HOUCHANG KHATAMIAN: Strawberries are small plants and can be heat-treated in a growth chamber. Can we heat-treat the explants or do we have to grow small plants?

DAN MILIKAN: I think the thing to do is to heat-treat to remove the virus diseases. If you run into a virus, such as the stem grooving virus, which resists the heat treatment we have been successful in the grafting of the meristems on to a seedling.

RON GIROUARD: Are you limited to only one season for your buds?

DAN MILIKAN: We have taken apple buds year round. Terminal buds grow best. I feel that the bud scales are the source of the inhibiting factors, not the meristem.

THE LABORATORY OF MICROPROPAGATION AT CESENA, ITALY

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In 1976, a growers' cooperative (Centrale Ortofrutticola alla Produzione) in Cesena, Italy, began planning a tissue culture laboratory for large-scale production of several horticultural crops. Dr. Carmine Damiano, of the Istituto Sperimentale per la Frutticoltura in Rome, was instrumental in designing and organizing the laboratory and in consulting on problems once tissue culturing got under way. P. Boxus, of Gembloux, Belgium, also served as a consultant on the project.