TISSUE CULTURE OF GLOXINIA

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Gloxinia, *Sinningia speciosa,* does not come true when propagated by seed and new plants of a given cultivar are primarily obtained by leaf cuttings and occasionally by stem cuttings. This research was undertaken to see if gloxinia could be propagated by tissue culture and if so, would it be possible to rapidly multiply it under controlled conditions. During the last few years a number of herbaceous plants have been propagated by tissue culture, such as asparagus (13), carnation (1, 4), cattleya (11), chrysanthemum (5, 6), cymbidium (7, 12), dahlia (8), iris (2), potato (9), rhubarb (14), and strawberry (3).

This study was done in cooperation with Dr. Toshio Murashige who graciously invited me to work in his well-equipped, tissue-culture laboratories at the University of California, Riverside.

In the preparation of the plant the shoots were cut back to the tuber when it was found that all of these shoots were reproductive. The ideal stage for taking vegetative shoots was when the new shoots were from 4 to 5 cm long. The shoots were rinsed with tap water to remove any

Table 1. Murashige and Skoog's High Salt Medium.

Chemical	Conc. mg / l
NH_4NO_3	— 1650
KNO_3	— 1900
$CaCl_2 \cdot 2H_2O$	— 440
$MgSO_4 \cdot 7H_2O$	— 370
KH_2PO_4	— 170
Na_2-EDTA	— 37.3
$FeSO_4 \cdot 7H_2O$	— 27.8
H_3BO_3	— 6.2
$MnSO_4\ 4H_2O$	— 22.2
$ZnSO_4\ 4H_2O$	— 8.6
KI	— 0.83
$NA_2MoO_4 \cdot 2H_2O$	— 0.25
$CuSO_4 \cdot 5H_2O$	— 0.025
$CoCl_2\ 6H_2O$	— 0.025

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continued

Other constituents included

i-inositol	—	100	adenine sulfate	—	0-80
kinetin	—	0-30	$NaH_2PO_4 \cdot H_2O$	—	0-170
IAA	—	0-10	thiamine.HCl	—	0.4
IBA	—	0-3	sucrose	—	30,000
NAA	—	0-10	Bactoagar	—	6,000-10,000

debris and the lateral leaves were cut off. The 10 to 15 mm long trimmed shoots were placed in an antioxidant solution until they were ready to be disinfected in a 5% liquid chlorine bleach solution for 5 minutes. The shoots were then rinsed several times with a sterile antioxidant solution. In the transfer chamber a 1 to 2 mm apical section of the shoot tip was excised and placed in the culture tube. Axillary buds from large shoots were tried but it was found that they tended to oxidize too rapidly.

The medium used was the Murashige and Skoog (10) high salt medium adjusted to pH 5.7.

The cultures were placed under standard conditions of 25° C with 16 hr of 100 ft-c of Gro-lux illumination per day.

A number of apical shoot tip experiments were conducted, among them a variation in concentration of different auxins, kinetin concentration, photoperiod, and light intensity. Other experiments were conducted on rooting shoots from these cultures *in vitro* and then transplanting them into pots.

Effect of NAA Concentration. Experiments were conducted on the effect of varying NAA concentration on the growth of excised gloxinia shoot tips. Tables 2 and 3 show the results of these experiments, 2 and 6 months, respectively, after the cultures were started.

Table 2. Effect of NAA Concentration on the Growth of Excised Gloxinia Shoot Tips (Kinetin, 10 mg/1).

NAA (mg/l)	$\bar{X}$ No. of Shoots	$\bar{X}$ No. of Tubers	$\bar{X}$ No. of Roots
0.0	15.0	2.0	0
0.1	9 1	2.3	0.6
0.3	2.3	0	0
1.0	2.2	0	0.8
3.0	1.3	0	9 8
10.0	0.3	0	2.8

The number of usable shoots for rooting was highest where there was an absence of NAA and decreased as NAA concentration increased. The cultures tended to be all callus as the NAA concentration increased, although callus was present in all cultures.

Table 3. Effect of NAA Concentration on the Growth of Excised Gloxinia Tips (Kinetin, 2 mg/1).

NAA (mg / l)	$\overline{X}$ No. of Shoots	$\overline{X}$ No. of Tubers	$\overline{X}$ No. of Roots
0.0	101.3	2.9	320.9
0.1	34.4	3.4	219.3
0.3	81.6	2.0	309.7
1.0	22.5	1.8	187.5
3.0	15.6	0.4	143.0

After 6 months the cultures also tended to have the most usable shoots where there was an absence of NAA and least where it was the highest.

Effect of IAA Concentration.

Table 4. Effect of IAA Concentration on the Growth of Excised Gloxinia Shoot Tips.

IAA mg / l	$\overline{X}$ No. of Shoots	$\overline{X}$ No. of Tubers	$\overline{X}$ No. of Roots
0	11.6	2.0	43.0
0.1	108.4	4.2	322.4
0.3	57.6	1.7	182.7
1.0	34.3	1.0	113.3
3.0	86.3	6.7	260.2
10.0	18.5	2.0	53.3

The IAA concentration was varied from 0 to 10 mg / l and after 5 months it was noted that the cultures with the largest number of usable shoots had 0.1 mg / l of IAA, (Table 4).

Effect of Kinetin Concentration.

Table 5. Effect of Kinetin Concentration on the Growth of Excised Gloxinia Shoot Tips (IAA, 0.1 mg/1).

Kinetin (mg / l)	$\bar{X}$ No. of Shoots	$\bar{X}$ No. of Tubers	$\bar{X}$ No. of Roots
0.0	1.0	0.3	5.7
0.3	1.2	0.7	5.2
1.0	3.3	1.2	5.5
3.0	5.0	1.2	8.4
10.0	68.8	6.6	168.0
30.0	229.3	17.3	225.8

The number of usable shoots, tubers and roots per culture increased as the concentration of kinetin in the medium increased. The cultures without kinetin had little or no growth and were approximately 5-10 mm wide. The original explant was readily visible in these cultures and the foliage was reddish green. As the concentration of kinetin increased the plant size also increased. The cultures with 30 mg / l of kinetin were approximately 25 x 65 mm and had green foliage.

Effect of Photoperiod. The excised shoot tips were grown under treatments from continuous darkness to that of continuous light with increments of 4 hr of light between each treatment. The cultures under total darkness were etiolated. The shoots were white and elongated with small yellow leaves; the callus was large and white. The cultures with 8 hr or less of light still showed some signs of etiolation such as their elongated stems and small leaf blades. As the length of the photoperiod increased the leaves tended to be thicker, stouter, larger and darker.

Effect of Light Intensity.

Table 6. Effect of Light Intensity on the Growth of Excised Gloxinia Shoot Tips.

Light Intensity (ft-c)	$\bar{X}$ No. of Shoots	$\bar{X}$ No. of Tubers	$\bar{X}$ No. of Roots	Callus Present
0	9.8	1.0	0	10 / 10
30	52.1	2.4	51.8	10 / 10
100	49.1	3.6	91.8	6 / 8
300	17.0	1.4	100.1	0 / 9
1000	1.2	0.3	0	0 / 10

The cultures growing without light were etiolated. The leaf blades were small (1 x 1.5 mm) and yellowish-green and there was a larger amount of tuber-like callus which was a translucent, whitish-yellow.

The plant and leaf size increased up to 300 ft-c of light and leaf color darkened with an increase in light intensity. Some of the newer leaves were quite large (20 x 25 mm) but the average leaf blade was 12 x 12 mm. The cultures under 1000 ft-c of light had small (1.5 x 2 mm), yellow or purple-brown leaves and had very little growth. Callus was present in all of the cultures growing without light and at 30 ft-c, but was absent in all of the cultures growing under 300 and 1000 ft-c of light.

Rooting of Cultured Shoots. Shoots 5-10 mm in length were transferred to media with varying concentrations of IAA (Table 7).

Table 7. Effects of IAA on the Rooting of Cultured Gloxinia Shoots.

IAA (mg / l)	$\bar{X}$ No. of Roots
0.0	6.6
0.1	7.8
0.3	11.9
1.0	14.9
3.0	11.0

The cultured shoots growing in the medium with 1 mg / l of IAA had the most rooting after 3 weeks.

Effect of Plant Part on Rooting. It was found that a single node section of a cultured shoot rooted and grew as well as an entire cultured shoot (Table 8) when transferred to a rooting medium.

Table 8. Effect of Plant Part as a Propagule.

Plant Part	$\bar{X}$ No. of Shoots	$\bar{X}$ No. of Tubers	$\bar{X}$ No. of Roots
Shoot	2.3	0.4	12.7
Single Node	2.1	0.6	22.9

After 4 weeks the cultures from the single node sections had approximately the same number of shoots and tubers but had a decidedly larger number of roots.

Effect of Transplanting Treatments. Rooted cultured shoots were removed from the culture tubes after 4 weeks. The shoots were

well rooted and were approximately 25 x 30 mm in size. The agar which was attached to the roots was carefully removed. The rooted shoots were transplanted in either a 1:1 peat and perlite soil mix or a 1:2 peat and vermiculite soil mix in peat pots. The transplants were placed in either an 80% shade saran tent, 4 mil polyethylene sheet tent, or under intermittent mist. All of these treatments were in shaded greenhouses. The plants were examined 2 weeks after transplanting (Table 9).

Table 9. Effect of Humidity Control and Soil Mix on the Growth of Transplanted Cultured Gloxinia.

Treatment	No. of Plts. Trans-planted	No. of Plts. Dead	Plant Injury *	Plt. Width (mm)
Saran shade				
— peat and perlite	10	0	0	40.0
— peat and vermiculite	10	0	0.4	42.0
Polyethylene tent				
— peat and perlite	10	1	0.5	51.1
— peat and vermiculite	10	0	0	50.0
Intermittent Mist				
— peat and perlite	10	0	0.8	26.5
— peat and vermiculite	10	0	1.0	33.5

* 0—no injury, 5—dead.

The plants under the saran shade in both soil mixes had good green color, were low and flush to the soil and were well rooted. The plants under the polyethylene tent all looked good, but were taller and spindlier than those under the saran shade or intermittent mist. The leaf color was a slightly lighter green. The plants were well rooted. The plants under the intermittent mist had fair to good growth but the leaves were dotted with small black necrotic spots. The plants were also well rooted. The soil mix used didn't appear to have any affect on the growth of the gloxinias.

Selected and desirable cultivars of gloxinias can be rapidly multiplied by the use of tissue culture. With the use of Murashige and Skoog's high salt medium plus other constituents it is possible to culture the shoot tip apex to produce large numbers of shoots. These cultured shoots and nodes will root readily. They are easily transplanted to artificial soil mixes under conditions of low light intensity and high humidity. It is possible to obtain 500 or more plants from a single shoot tip culture by using both shoots and single node sections.

Cultured plant parts can also be repeatedly recultured in a high shoot producing medium, thus increasing many fold a highly desirable cultivar of gloxinia in a short length of time.

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MODERATOR PINNEY: Unfortunately, we are out of time and so we will have no questions directed to Chiko. I do want to thank all of the speakers; they had interesting papers, they were well presented, and we kept right to the schedule. At this time I will turn the program back to you, Bill.

MODERATOR FLEMER: Once again, thanks to the participants of the Speaker-Exhibitor Symposium and to you, President Pinney for serving as moderator.

We will move on now to a subject to which I feel many of us in this Society pay too little attention, and that is the methods of keeping cost records in propagation. We do have an expert to talk to us on that subject; Ralph Shugert of Spring Hill Nurseries.

RALPH SHUGERT: Before I present my paper, I wish to take the prerogative as President of the International Society to extend to our members an invitation, and I have asked Editor Stoltz that this invitation be placed in the Proceedings of this meeting.

Williamsburg, Virginia's Colonial Capital, is only about 40 miles away. Many of you have visited Williamsburg. Others who have not are, no doubt, familiar with the restoration of Williamsburg to its 18th century appearance by Mr. John D. Rockefeller, Jr. The fame of Williamsburg, its historical heritage, buildings, gardens, craft program, collections of antiques, and research program are widespread.

Though perhaps not often thought of as such Williamsburg, including the campus of the College of William and Mary, is a vast arboretum containing one of the most outstanding collections of plant material in this country. This expanding collection utilizes, in the historic areas, only plants native to the region or known to have been introduced before 1800. The collection of native southeastern woody plants will soon include virtually all of the trees, shrubs, and woody vines native north of peninsular Florida. Outside the historic areas a wide variety of modern plant material is cultivated and much of it is labeled. Many plants used in Williamsburg are at their northern limits and are quite unfamiliar to most northern visitors. Many unusual plants are growing on the William and Mary campus including large specimens of *Sequoia sempervirens* and, perhaps, the largest Metasequoias in the U.S. The collection of boxwood is outstanding. Dr. J. T. Baldwin, Professor of Botany at William and Mary College is one of the world's leading authorities on *Buxus*.

All in all, the results of horticultural experimentation and introduction, whose roots go back over 300 years awaits you in Williamsburg. Bob McCartney, on behalf of the Colonial Williamsburg Foundation and its Department of Landscape Construction and Maintenance, extends to each of you an invitation to visit Williamsburg before you leave for home.