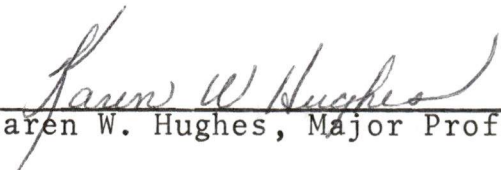
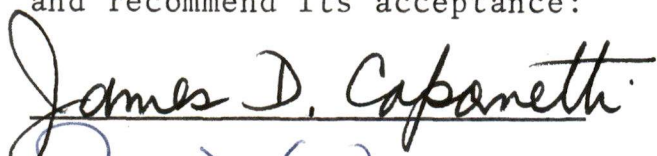
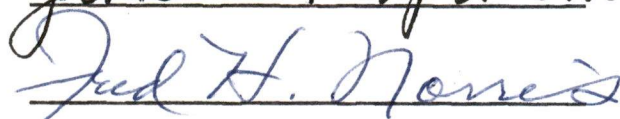


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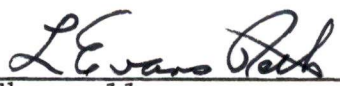
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Karen W. Hughes, Major Professor

We have read this thesis
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EFFECTS OF AUXIN AND CYTOKININS ON MORPHOGENESIS
OF THREE VARIETIES OF EPISCIA

A Thesis
Presented for the
Master of Arts in College Teaching
Degree
The University of Tennessee, Knoxville

Alta Elaine Pearson

December 1979

1405013

ACKNOWLEDGMENTS

The author would like to thank Dr. Karen W. Hughes of the Department of Botany, The University of Tennessee, Knoxville, for suggesting the topic of this study, her guidance during the research, and constructive criticism of this manuscript. Appreciation is also extended to Dr. Fred H. Norris and Dr. James D. Caponetti for advice and use of laboratory facilities when needed. Gratitude is extended to Janet Loy who, during her undergraduate research program in Dr. Hughes' laboratory, did a portion of one of the experiments.

ABSTRACT

The objective of this study was to determine the optimal auxin to cytokinin ratios for plant initiation from leaf blade and/or stolon explants of Episcia cupreata var. Chocolate Soldier, E. cupreata var. Silver Sheen, and E. dianthiflora. Knowledge of hormonal requirements necessary for morphogenesis would enable workers with these plants to propagate them more rapidly than is possible by conventional propagation techniques.

Blade and stem explants were cultured on modified Linsmaier and Skoog media with various combinations of auxin (IAA) and cytokinin (kinetin or 2iP). Plant-growth regulators were added to the medium at 0.0, 1.0, 2.5, 5.0, 10.0, or 15.0 mg/l. Shoots initiated from the explants were transferred to modified Knop's medium with sucrose where plantlets increased in size and produced roots. When plantlets were 2 to 3 cm tall, they were transplanted to soil.

Only low percentages of plant initiation were observed for E. dianthiflora on medium with IAA 5.0 mg/l and kinetin 2.5 mg/l and for E. cupreata var. Chocolate Soldier on media with IAA 1.0 mg/l or with IAA 5.0 mg/l and kinetin 1.0 mg/l. A high percentage of blade explants of E. cupreata var. Silver Sheen initiated shoots by 16 weeks on medium with IAA and 2iP at 1.0 mg/l. The different Episcia taxa have somewhat different media requirements for shoot initiation, and shoot

initiation was usually preceded by intensive callus formation. However, not all callus produced shoots. Very few variant cultured plants were observed for any of the Episcia taxa.

The results of this study indicate that Episcia can be propagated successfully in tissue culture; however, the requirements for shoot morphogenesis from explants varies with the taxon and the plant organ used for the explant.

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CHAPTER I

INTRODUCTION

Plant Tissue Culture

Plant tissue culture had its beginnings at the turn of the twentieth century with G. Haberlandt's speculations regarding cell totipotency. Haberlandt suggested that techniques for isolating and culturing plant tissues should be developed and postulated that if the environment and nutrition of cultured plant cells were manipulated, those cells could recapitulate the developmental sequences of normal plant growth (Haberlandt, 1902). Since then plant tissue culture techniques have become an important new series of tools for theoretical research of physiological processes in plant development. Murashige (1974) defined four major areas in which plant tissue culture techniques may be applied: (1) production of secondary plant products such as those used in the pharmaceutical industry, (2) genetic improvement of plants via somatic cell hybridization, (3) recovery of disease-free plants from stem tip culture, and finally (4) rapid clonal multiplication of selected varieties of plants. The fourth application is the one of significance to this study.

In vitro propagation techniques have been applied to numerous plant species (Murashige, 1974; Narayanaswamy,

1977). Major advantages to the horticultural nurseries include production of superior plants in relatively short periods of time as compared to normal propagation methods and the reduced space needed for maintaining stock plants (Oglesby, 1978). Also, cultured plants may tend to be healthier because of removal of surface contamination during the tissue culture process.

Plant-Growth Regulators

The study of plant hormones and their mechanisms of action has been greatly influenced by animal hormone research. Animal hormones have been defined as organic compounds synthesized in cells in one part of an organism and transferred to the site of action in another part of the organism where the substances are effective at low concentrations. Certain plant substances including the auxins and cytokinins also meet this definition (Noggle and Fritz, 1976; Goodwin and Mercer, 1972). These compounds are now referred to as plant-growth regulators rather than as plant hormones because the primary response is growth related. In addition, plant-growth regulators include synthetic compounds that elicit the same growth responses as endogenous regulators (Noggle and Fritz, 1976). The plant-growth regulators differ from the animal hormones in that animal hormones act on target organs generating specific responses. Plant-growth regulators may stimulate

many different plant tissues with a wide range of physiological responses. There is an overlap of function among the plant-growth regulators and interactions among the compounds produces typical plant growth (Leopold and Kreidmann, 1975; Lesham, 1973).

Several factors are important in the initiation of shoots and roots from excised plant tissues (Murashige, 1978). Among these, the interaction of growth regulators is considered especially significant. Skoog and Miller (1957) proposed that the ratio of the plant-growth regulators auxins and cytokinins determines the type of organ formation.

In this study the relative amounts of auxins and cytokinins in the plant initiation medium were selected as the factors to be manipulated in triggering organogenesis. Further information regarding these two particular groups of growth regulators follows.

Auxins. Experiments dealing with phototropic responses in plants (Went, 1927) culminated in the discovery and isolation of indoleacetic acid (IAA) from corn kernels (Haagen-Smit, et al., 1946). As discussed by Hess (1975), IAA was the first of the plant-growth regulators to be chemically defined and thus stimulated further hormone research. As a result, some physiological effects of auxins have been determined. Auxins elicit such responses as cell enlargement, inhibition of lateral bud

development, control of leaf abscission, promotion of cell division in cambial regions, and inhibition of growth in roots (Galston and Davies, 1970).

Auxins are a group of chemical compounds resembling IAA in either structure or function. Leopold and Kriedemann (1975) discussed early hypotheses that linked auxin activity to a chemical structure with an indole ring and carboxy-terminal side chain. Analyses of various auxins' chemical structures reveal that there are two charges on the molecule, a negative charge as found on the carboxyl group and a partial positive charge on another position in the molecule. A distance of 5.5 Å between the charges apparently is necessary for the molecule to have auxin activity (Porter and Thimann, 1965).

The mechanism of action of auxins at the cellular level is unresolved. There is a possibility that more than one mode of action is involved in eliciting the varied physiological responses. Auxins in stimulating prolonged growth appear to be involved in ribonucleic acid (Guilfoyle, et al., 1975) and/or protein synthesis (Ray, 1977).

Cytokinins. As early as 1892 a chemical regulator of cell division was predicted by Wiesner (Leopold and Kriedemann, 1975). Yet it was not until 1963 that zeatin, the first plant-derived cytokinin, was chemically characterized from corn endosperm (Letham, 1963). The group of cytokinins

embraces those chemical substances that promote cell division in plant tissues under specific bioassay conditions and generally affects growth and morphogenesis in a manner similar to 6-furfurylaminopurine (kinetin) (Burrows, 1975). Kinetin was the first cytokinin to be chemically defined (Miller, et al., 1955b) and was the growth promoting factor originally separated from breakdown products of herring sperm deoxyribonucleic acid (Miller, et al., 1955a). Kinetin has not been isolated from higher plant tissues but evidence suggests its presence at very low concentrations. The compound 6-(3-methyl-2-butenylamino) purine (2iP) is the most widespread endogenous cytokinin in plants (Noggle and Fritz, 1976).

Cytokinins are divided into two groups based on chemical structure (Leonard, 1974). One set of cytokinins with high activity is usually found to have an adenine molecule with an intact purine ring and an N⁶ substituent of moderate size (Skoog and Armstrong, 1970). The greatest activity is usually associated with molecules having a side chain length of 5 carbons and loss of activity occurs with a side chain length greater than 10 carbons (Skoog and Schmitz, 1972). Diphenyl urea and its derivatives constitute the other set of chemical structures exhibiting cytokinin activity (Leonard, 1974). The urea-type cytokinins' activity is generally less than the adenine category of cytokinins (Kende, 1971). Whether the purine

and urea cytokinins act upon the same active site in cells to promote the growth response is not known (Takahashi, et al., 1978).

The mechanism of action of the cytokinins at the cellular level is unknown. Certain cytokinins have been found to be part of the structure of specific transfer ribonucleic acids (Burrows, et al., 1971; Walker and Leonard, 1974; Vreman and Corse, 1978). Cytokinins incorporated in transfer ribonucleic acids are not restricted to the plant kingdom. Reports of exogenously applied cytokinin incorporation into transfer ribonucleic acid vary from as low as 1 cytokinin per 16,000 transfer ribonucleic acids (Armstrong, et al., 1976) to as high as 1 per 3600 transfer ribonucleic acids (Murai, et al., 1977). Most authorities agree that these low levels do not warrant the physiological effects generated. Cytokinin binding receptor sites have been identified on the ribosomes indicating the possibility of translational control by cytokinins (Berridge and Ralph, 1970; Fox and Erion, 1975; Klambt, 1976). Intact cytokinins are also incorporated into ribosomal ribonucleic acid by a 15 fold preferential uptake over transfer ribonucleic acid. It is not known whether this incorporation is a result of specific events involving the mode of cytokinin action (Murai, et al., 1978).

Gesneriaceae

Description. The family Gesneriaceae comprises mostly herbaceous plants and rarely shrubs or trees. The family is primarily tropical and subtropical in its distribution; no Gesneriads are native to the United States. Important ornamentals in this group include Sinningia (Gloxinia), Kohleria, Saintpaulia (African violet), Streptocarpus (Cape primrose), and Episcia (Flame violet).

The plants are characterized by pubescent leaves which are usually opposite. The bisexual flowers are showy and borne in a leaf axil or in a cymose inflorescence (Bailey, 1949; Lawrence, 1951).

Tissue culture applications. The primary goal in tissue culturing of the Gesneriaceae has been to provide a faster method of propagation than is usually obtained through typical propagation techniques. The genera that have been cultured in vitro most frequently are Sinningia, Streptocarpus, and Saintpaulia. (See Appendix A for a review of the work done with these genera.) More recently, work has been done with Episcia.

Johnson (1978a) used 1 cm² leaf explants of Episcia cupreata (Hook.) Hanst. var. Silver Queen. To initiate shoots the explants were placed on Murashige and Skoog (1962) medium (MS) with kinetin plus IAA or Napthalene

acetic acid (NAA). The growth regulators were added at 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 mg/l. With the basic medium plus NAA and kinetin, little callus was generated and some of the explants formed roots. On basic medium with IAA and kinetin, callus production was profuse and numerous shoots were produced in 6 to 8 weeks. The largest number of shoots (20-30 per explant) was produced on medium with 0.2 mg/l kinetin and IAA. When callus was subcultured in larger containers, several hundred shoots were produced per explant. Shoots 0.5 cm long were transferred to basic media without kinetin but including IAA or NAA at the same levels as for shoot initiation media. Roots did not form with IAA but formed on all media with NAA within 1 to 2 weeks. Clusters of 5 to 10 plantlets were transferred together to soil.

Bilkey and McCown (1979) reported 1-2 mm long stem tips were best for production of shoots when compared to 5 x 10 mm leaf sections. The following cultivars were selected for culturing: E. x wilsonii (Ember Lace), E. cupreata var. Silver Sheen, E. cupreata var. Ruby Red Dress, E. cupreata var. Cleopatra, and E. cupreata var. Tropical Topaz. After 4 weeks in culture, leaf sections produced pin-head sized growth of callus along cut surfaces near veins. When the callus was removed from the leaf and subcultured, growth increased. Stem tips of all cultivars except Tropical Topaz produced the greatest

initial amount of callus on MS medium with 0.1 mg/l NAA and 0.5 mg/l benzyladenine. Tropical Topaz performed best on a lower level of benzyladenine (BA), 0.01 mg/l. Callus was subcultured on fresh medium containing 0 - 5 mg/l of both NAA and BA. Subcultured callus growth was generally not affected by either NAA or BA, but growth was rapid, doubling in size as often as every 4 days. Neither NAA nor BA was essential for differentiation but a reduction of the cytokinin level stimulated plantlet development. The differentiated plantlets were observed after the third week of subculture and by the eighth week the container was reported full of plants. Rooting was induced by placing the plantlets on medium with 0.1 mg/l NAA and 0.01 mg/l BA or by placing 6 week old subcultures under lower light intensity.

Purpose

The purpose of this study was to identify optimum auxin and cytokinin levels for plant initiation from leaf blade and stolon explants of Episcia dianthiflora H. E. Moore and R. G. Wils. and two varieties of E. cupreata Hanst. (Chocolate Soldier and Silver Sheen). Data were sought as to the effectiveness of different cytokinins when used in combination with IAA upon differing plant tissues. The development of rapid propagation techniques for Episcia has commercial application due to the plant's popularity as a houseplant.

CHAPTER II

MATERIALS AND METHODS

Plant Material

Stolon and blade sections of Episcia dianthiflora H. E. Moore and R. G. Wils. and two varieties of Episcia cupreata Hanst. (Chocolate Soldier and Silver Sheen) were selected for culturing. These species were chosen because of their availability, and earlier experiments indicated E. cupreata had regenerative potential (Hughes, oral communication). Stock plants were maintained in growth rooms under a light intensity of $6530 \text{ ergs cm}^{-2}\text{sec}^{-1}$ with 14 hours of light and 10 hours of darkness at 25 degrees Centigrade.

Surface Sterilization

Surface sterilization of the plant tissue was accomplished by the following procedure: (1) The plant material was placed under running tap water for 1-1/2 hours. (2) The plant material was transferred to a 1/6 volume-by-volume chlorox solution for 20 minutes. (3) The plant material was rinsed several times in sterile double-distilled water. (4) The plant material was briefly immersed in 70 percent ethanol before sectioning and placing the tissue on plant initiation medium. In subsequent experiments in which leaf tissue alone was cultured

at optimum auxin to cytokinin ratios, plant tissues were placed in the chlorox solution for a 10-minute interval and the immersion in 70 percent ethanol was omitted.

Media

The medium used for callus production and plant initiation was a modified Linsmaier and Skoog medium (1965). (See Appendix B for formulation.) Auxin and cytokinin levels in the medium were varied to determine the optimum balance for plant initiation of each type of Episcia. The auxin used was 3-indoleacetic acid (IAA) and was obtained from Eastman Kodak Company, Rochester, New York, Lot #A3-D. IAA was added to the medium at 0.0, 1.0, 2.5, 5.0, 10.0, and 15.0 mg/l. The cytokinins used were 6-(3-methyl-2-butenylamino) purine (2iP) and 6-furfurylaminopurine (kinetin); they were obtained from Sigma Chemical Company, St. Louis, Missouri, Lot Numbers 66C-5020 and 96C-0115, respectively. The cytokinins were also used at 0.0, 1.0, 2.5, 5.0, 10.0, and 15.0 mg/l. Ten replicates were made of all possible combinations of IAA/2iP, IAA/kinetin, and IAA/2iP with kinetin at 1.0 mg/l. Twenty-five milliliters of medium were dispensed into 25 x 150 mm test tubes, plugged with cotton, capped with Bellco KAPUTS, and autoclaved at 121 degrees Centigrade and 15 lbs. in⁻² for 20 minutes. Media were allowed to cool and harden before use.

Cultured plantlets initiated roots on a modified Knop's medium (Nakosteen, 1978). The basic medium consisted of a mineral salt solution (Nitsch, 1951), ferric citrate, and a formulation of Nitsch's trace elements modified from Steeves (1955). Sucrose was added for some experiments. (See Appendix C for formulation.) Fifty milliliters of medium were dispensed into 125 or 250 ml Erlenmeyer flasks, plugged with cotton, and capped with aluminum foil. The medium was autoclaved for 20 minutes at 121 degrees Centigrade and 15 lbs. in⁻².

Experimental Procedure

The leaf blade and stem material were sectioned and placed on Linsmaier and Skoog media. The transfer procedures were carried out under sterile conditions in a biological safety hood. Blades were sectioned into 1 cm² pieces; a vein segment was included in each section. (See Figure 1.) Stolons were cut to yield cylinders from 0.5 to 1.0 cm in length. The blade sections were placed abaxial side downward on the modified Linsmaier and Skoog medium (LS). Stolon explants were placed so that either of the cut ends of the cylinder was in contact with the medium. Polarity of the stolon explant was randomized and thus, not a factor considered in this study. The tubes were re-plugged with cotton, capped with the Bellco KAPUTS and placed in a growth chamber. The growth chamber conditions included a light intensity of 5800 ergs cm⁻²sec⁻¹ with 18

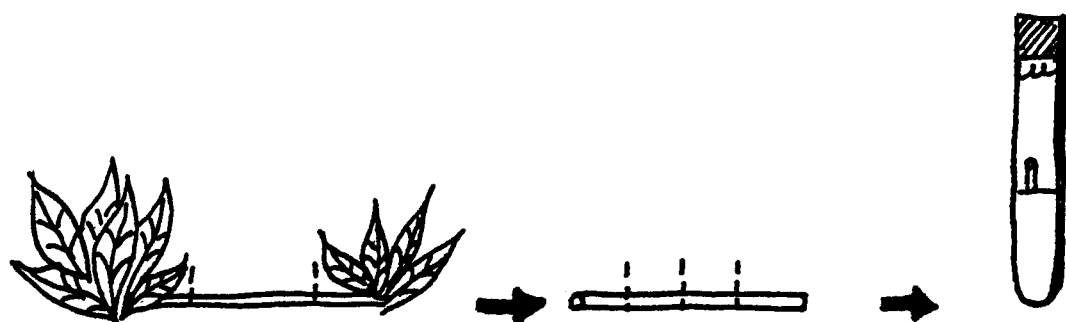
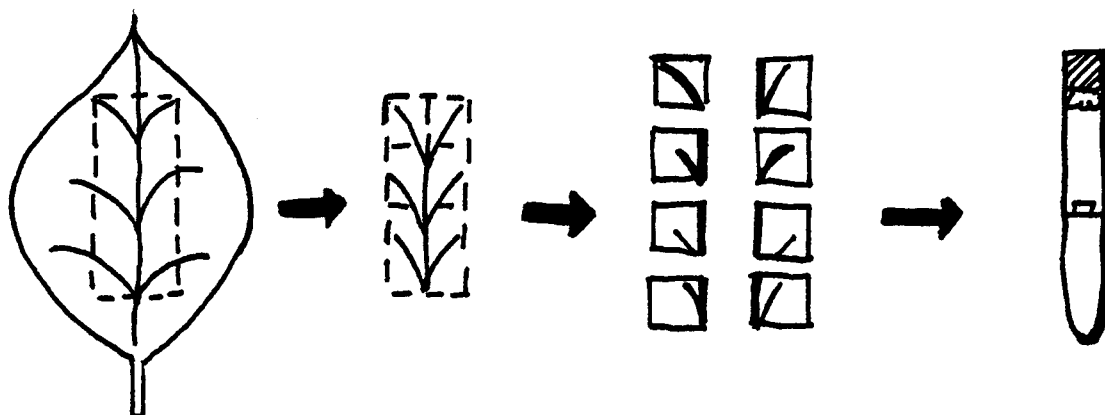


Figure 1. The placement of explants on media.

A. The inner portion of the leaf blade was cut into squares approximately 1 cm^2 and placed on media in test tubes.

B. The stolon explants were cut into cylinders approximately 1 cm in length and placed on media in test tubes.

hours of light and 6 hours of darkness at 25 degrees Centigrade. Observations were made of the tubes at 4-week intervals; most plant tissues were sacrificed at 16 weeks. Procedures were repeated at those levels demonstrating a high percentage of plantlet initiation and callus production. Repeated experiments were checked at 2-week intervals.

Cultures of blade and stem tissues for E. dianthiflora, E. cupreata var. Silver Sheen, and E. cupreata var. Chocolate Soldier were examined and data recorded on the percentage of tubes showing callus production, the average level of callus per tube, and the percentage of tubes showing shoot and/or root initiation. Level of callus growth per tube was evaluated qualitatively as 1.0, 2.0, or 3.0. A score of 1 indicated that callus had been initiated in the plant tissue. A score of 2 indicated callus production occurred over a large proportion of the plant tissue and portions of the blade or stem explant were still visible. A score of 3 was given when callus completely obscured the original explant.

Some plantlets initiated on the various LS media were transferred to flasks containing 50 ml of Knop's medium with sucrose and others to Knop's medium without sucrose. Plantlets were transferred from the Knop's medium to soil after roots had developed. The soil was kept moist, and the plantlets were maintained under glass

beakers for 1 to 2 weeks in the stock plant growth rooms; then beakers were removed.

Limited numbers of tubes with cultured plantlets of each Episcia taxa were available for subculture procedures. Callus which had already demonstrated shoot initiation, blades from initiated plantlets, or initiated shoots were selected for subculture. Transfers were made to basic LS medium without growth regulators, to medium with the same growth regulator levels as had initiated the shoots, or to medium with growth regulator levels that were optimum for Chocolate Soldier and Silver Sheen. These subculture procedures were done primarily to enhance shoot differentiation. Some tubes were maintained for several months without subculture. In the repeated experimental procedures at optimum growth regulator levels, some callus, prior to any shoot initiation, was subcultured on LS medium with the same auxin to cytokinin levels to observe if subculturing would hasten plantlet initiation.

A supplementary experiment was done to determine if contamination levels varied with the cultivar, different time exposures to the chlorox solutions, and the concentration of the chlorox solution. Using only blade tissue from the three taxa of Episcia, the leaves were washed with soap and placed under running water for 1-1/2 hours. Leaves of each type were placed in 0, 1/12, 1/6, and 1/3

volume-by-volume chlorox solutions for time intervals of 5, 10, and 20 minutes. The leaves were rinsed several times in sterile double-distilled water, sectioned, and cultured on Linsmaier and Skoog medium without growth regulators. Tubes were checked for contamination at 2, 4, and 6 weeks after culturing.

CHAPTER III

RESULTS

See Appendix D for summary Tables 1-10 of explant response at 16 weeks given for each growth regulator series and for each selected *Episcia* taxa. Tables 1-9 give the percentage of tubes callusing, the average amount of callus for all tubes at a particular growth regulator combination, and the percentage of tubes showing root and/or shoot initiation at 16 weeks. As mentioned previously in the chapter on Methods and Materials, some cultures were maintained past 16 weeks. Selection of which cultures were maintained was based on whether the explant had initiated shoots. In a few cases, cultures with only intensive callus formation were maintained. Transfers to basic medium without growth regulators, to medium at the same growth regulator levels, or to media with optimum growth regulator levels for Chocolate Soldier or Silver Sheen were carried out in an attempt to enhance shoot initiation. A few cultures were maintained on the original medium without subculture for comparative observations. Callus, blades of initiated plantlets, or shoots from the maintained cultures were subcultured. In some cases shoots and attached callus or callus and roots could not be separated and were subcultured together. Appendix D, Table 10, summarizes the results at 16 weeks

for the repeated experiments of blade explants on LS medium with optimum growth regulator levels of each of the selected Episcia taxa.

E. dianthiflora IAA/K Series (See Appendix D, Table 1)

Blade. Callus production from E. dianthiflora blade tissue occurred in media containing IAA from 2.5 to 10.0 mg/l and kinetin from 0.0 to 10.0 mg/l. One hundred percent of the tubes showed callus formation at the IAA to kinetin combinations 5.0/5.0 and 10.0/2.5 mg/l. An average callus level of 3.0 per tube occurred at the following IAA to kinetin combinations: 2.5/2.5, 10.0/0.0, and 10.0/5.0. Twenty to 25 percent of the tubes demonstrated shoot initiation in media containing IAA from 5.0 to 10.0 mg/l and kinetin from 1.0 to 10.0 mg/l. No tubes showed root initiation at 16 weeks.

Stem. Callus production from E. dianthiflora stem tissue occurred in media containing IAA from 0.0 to 15.0 mg/l and kinetin from 0.0 to 10.0 mg/l. One hundred percent of the tubes showed callus formation at the IAA to kinetin combinations 5.0/1.0 and 10.0/5.0 mg/l. An average callus level of 3.0 per tube occurred at the following IAA to kinetin combinations: 1.0/0.0, 1.0/2.5, 2.5/1.0, 2.5/2.5, 10.0/0.0. Twenty to 50 percent of the tubes demonstrated shoot initiation in media containing IAA from 1.0 to 10.0 mg/l and kinetin from 2.5 to 5.0 mg/l.

Roots were initiated at IAA to kinetin combinations 0.0/0.0, 5.0/1.0, and 15.0/0.0 mg/l; no callus production occurred at the 15.0/0.0 combination.

E. dianthiflora IAA/2iP Series (See Appendix D, Table 2)

Blade. Callus production from E. dianthiflora blade tissue occurred in media containing IAA/2iP combinations of 2.5/2.5 and 10.0/1.0 mg/l. Only 40 percent of the tubes showed minimal callus production on medium with IAA 2.5 mg/l and 2iP 2.5 mg/l. There was no shoot or root initiation by 16 weeks.

Stem. Callus production from E. dianthiflora stem tissue occurred in media containing IAA from 1.0 to 15.0 mg/l and 2iP from 0.0 to 15.0 mg/l. One hundred percent of the tubes showed callus formation at the IAA to 2iP combination 2.5/2.5 mg/l. An average callus level of 3.0 per tube was observed at IAA 15.0 mg/l and 2iP 5.0 mg/l. None of the tubes showed shoot and/or root initiation at 16 weeks.

E. dianthiflora IAA/2iP/Kinetin 1.0 mg/l Series (See Appendix D, Table 3)

Blade. Callus production from E. dianthiflora blade tissue occurred on LS media containing IAA from 2.5 to 5.0 mg/l, 2iP from 0.0 to 5.0 mg/l, and kinetin 1.0 mg/l. Eighty percent of the tubes showed callus production at the

IAA/2iP/kinetin combination of 5.0/0.0/1.0 mg/l. Average callus levels per tube were minimal. No shoots or roots were initiated by 16 weeks.

Stem. Callus production from E. dianthiflora stem tissue occurred in media containing IAA from 1.0 to 15.0 mg/l, 2iP from 0.0 to 10.0 mg/l, and kinetin 1.0 mg/l. One hundred percent of the tubes showed callus production at the IAA/2iP/kinetin combination of 5.0/0.0/1.0 mg/l. An average callus level of 2.0 per tube was obtained on medium containing IAA 15.0 mg/l, 2iP 10.0 mg/l, and kinetin 1.0 mg/l. No shoots and/or roots were initiated by 16 weeks.

E. dianthiflora Cultures Maintained Past 16 Weeks

Some cultures yielding shoots were maintained past 16 weeks for the E. dianthiflora IAA/kinetin series.

From stem tissue that initiated shoots on a medium containing IAA 1.0 mg/l and kinetin 2.5 mg/l, callus and shoots were transferred to basic medium lacking growth regulators where a few roots were produced. The culture remained in this state until 13 months after the explant had originally been placed in culture. The shoots were then transferred to medium containing IAA and 2iP 1.0 mg/l where they increased in size and produced new roots. The callus and roots were also transferred to medium with IAA and 2iP 1.0 mg/l where the roots were noted to callus.

From stem tissue that initiated plantlets on IAA and kinetin 2.5 mg/l, callus and shoots were subcultured on basic medium lacking growth regulators. After 3 months, some callus plus a few roots were transferred to a medium containing only IAA 1.0 mg/l; no change was noted after 2 months. Some callus and roots were also transferred from the medium lacking growth regulators to medium containing IAA 5.0 mg/l and kinetin 1.0 mg/l where the roots began to callus after 2 months. A blade from one of the initiated shoots was subcultured on medium containing IAA 5.0 mg/l and kinetin 1.0 mg/l and was observed to callus and to initiate a shoot from the blade edge. One of the shoots with associated roots and callus was transferred to medium with IAA 5.0 mg/l and kinetin 1.0 mg/l. After 2 months the original shoot had increased in size, and new shoots and roots had been produced. Another cluster of small initiated plantlets plus roots and callus when transferred to medium with IAA and 2iP 1.0 mg/l yielded multiple new shoots and roots; the transferred cluster of plants increased in size.

A blade from an initiated plantlet generated from stem tissue on medium with IAA 10.0 mg/l and kinetin 5.0 mg/l was placed on basic medium lacking growth regulators. The blade stayed green but did not callus; the blade was then transferred to medium containing IAA 5.0 mg/l and kinetin 1.0 mg/l. After 2 months, the leaf had turned

brown and callus had been produced.

Callus from blade explants that had exhibited shoots by 16 weeks were maintained in original tubes after transferring the initiated shoots to Knop's medium. Callus did not produce new shoots.

E. dianthiflora Optimum Level Experiment

E. dianthiflora blade tissue that was cultured at IAA 5.0 mg/l and kinetin 2.5 mg/l for 6 weeks showed no response, and the tubes were discarded.

E. cupreata var. Chocolate Soldier IAA/K Series (See Appendix D, Table 4)

Blade. Callus production from Chocolate Soldier blade tissue occurred in media containing IAA from 1.0 to 15.0 mg/l and kinetin from 0.0 to 2.5 mg/l. One hundred percent of the tubes showed callus formation at the IAA to kinetin combinations 10.0/0.0 and 15.0/0.0. An average callus level of 3.0 per tube occurred at the following IAA to kinetin combinations: 10.0/0.0 and 15.0/0.0. Twenty percent of the tubes demonstrated shoot and root initiation in medium containing IAA 1.0 mg/l as the only growth regulator. Twenty percent of the tubes showed shoot initiation alone in medium with IAA 5.0 mg/l and kinetin 1.0 mg/l. Root initiation occurred in media containing IAA from 1.0 to 15.0 mg/l and kinetin at 0.0 or 1.0 mg/l.

One hundred percent of the tubes demonstrated root initiation at IAA 10.0 or 15.0 mg/l and no kinetin.

Stem. Callus production from Chocolate Soldier stem tissue occurred in media containing IAA from 1.0 to 10.0 mg/l and kinetin 0.0 or 1.0 mg/l. One hundred percent of the tubes showed callus formation at the IAA to kinetin combinations 1.0/0.0 and 5.0/0.0. An average callus level of 2.8 per tube occurred in medium with IAA 5.0 mg/l and no kinetin. No tubes demonstrated shoot initiation by 16 weeks. From 20 to 60 percent of the tubes showed root initiation at the same growth regulator combinations that demonstrated callusing.

E. cupreata var. Chocolate Soldier IAA/2iP Series (See Appendix D, Table 5)

Blade. Callus production from Chocolate Soldier blade tissue occurred in media containing IAA from 0.0 to 15.0 mg/l and 2iP 0.0 to 15.0 mg/l. One hundred percent of the tubes showed callus formation at the IAA to 2iP combinations 1.0/0.0, 1.0/10.0, 2.5/0.0, 5.0/0.0, 5.0/1.0, 5.0/2.5, 5.0/5.0, 5.0/10.0, 5.0/15.0, 10.0/0.0, 10.0/1.0, 10.0/2.5, 15.0/0.0, 15.0/1.0, 15.0/2.5, 15.0/5.0, and 15.0/15.0. An average callus level of 3.0 per tube occurred at the following IAA to 2iP levels: 5.0/1.0, 5.0/2.5, 5.0/5.0, 10.0/1.0, 10.0/5.0, 15.0/0.0, 15.0/1.0, 15.0/2.5, and 15.0/5.0. There was no shoot initiation by 16 weeks. Twenty

to 100 percent of the tubes showed root initiation in media containing IAA from 1.0 to 15.0 mg/l and 2iP from 0.0 to 2.5 mg/l.

Stem. Callus production from Chocolate Soldier stem tissue occurred in media containing IAA from 1.0 to 15.0 mg/l and 2iP 0.0 to 15.0 mg/l. One hundred percent of the tubes demonstrated callus formation at the IAA to 2iP combinations 1.0/0.0, 1.0/1.0, 5.0/0.0, 5.0/1.0, 5.0/2.5, 10.0/0.0, 10.0/1.0, 15.0/0.0, and 15.0/1.0. An average callus level of 3.0 per tube occurred at the IAA to 2iP combinations 1.0/0.0 and 5.0/0.0. There was no shoot initiation by 16 weeks. Twenty to 100 percent of the tubes showed root initiation in media containing IAA from 1.0 to 15.0 mg/l and 2iP 0.0 or 1.0 mg/l.

E. cupreata var. Chocolate Soldier IAA/2iP/Kinetin 1.0 mg/l Series (See Appendix D, Table 6)

Blade. Callus production from Chocolate Soldier blade tissue occurred in media containing IAA from 1.0 to 15.0 mg/l, 2iP from 0.0 to 15.0 mg/l, and kinetin 1.0 mg/l. One hundred percent of the tubes showed callus production at IAA 2.5 mg/l, 2iP 0.0 mg/l, and kinetin 1.0 mg/l. An average callus level of 3.0 per tube occurred at the IAA/2iP/kinetin combinations 10.0/0.0/1.0 and 15.0/2.5/1.0. Only 10 percent of the tubes showed shoot initiation at

the IAA/2iP/kinetin combinations 1.0/0.0/1.0, 1.0/1.0/1.0, and 2.5/0.0/1.0. Twenty percent of the tubes showed root initiation at IAA 2.5 mg/1, 2iP 0.0 mg/1, and kinetin 1.0 mg/1.

Stem. Callus production from Chocolate Soldier stem tissue occurred on media containing IAA at 10.0 or 15.0 mg/1, 2iP from 0.0 to 5.0 mg/1, and kinetin 1.0 mg/1. Sixty percent of the tubes yielded callus at the IAA/2iP/kinetin combinations 10.0/5.0/1.0 and 15.0/2.5/1.0. An average callus level of 3.0 per tube occurred at IAA 10.0 mg/1, 2iP 1.0 or 2.5 mg/1, and kinetin 1.0 mg/1. There were no tubes with shoot and/or root initiation by 16 weeks.

E. cupreata var. Chocolate Soldier Cultures Maintained Past 16 Weeks

IAA/kinetin series. Shoots were produced exclusively from blade tissue by the end of 16 weeks of culture on medium with either IAA 1.0 mg/1 or on medium with IAA 5.0 mg/1 and kinetin 1.0 mg/1. Development of shoots from blade tissue after 16 weeks occurred on media with the IAA to kinetin combinations 2.5/0.0, 2.5/1.0, 10.0/0.0, and 15.0/1.0. After 16 weeks, shoots were also produced from stem explants on media with the IAA to kinetin combinations 1.0/1.0, 2.5/0.0, and 5.0/0.0.

The calluses produced from the original blade explants

were subcultured on fresh medium at the same growth regulator levels approximately 30 weeks after the experiment had started for the two IAA/2iP combinations 2.5/0.0 and 2.5/1.0. Both of these subcultures produced multiple shoots and roots. The shoot and associated callus produced from the blade explant on medium with IAA 10.0 mg/l and no kinetin was transferred to basic medium without growth regulators; the plantlet increased in size and produced roots. Callus from the culture on medium with IAA 10.0 mg/l and no kinetin was separated and subcultured on two different media. Callus placed on fresh medium with only IAA 10.0 mg/l produced a few short roots. Callus subcultured on basic medium without growth regulators increased in size, produced roots and a plantlet. The shoot produced from the blade explant on medium with IAA 15.0 mg/l and kinetin 1.0 mg/l was transferred to Knop's medium; the callus was subcultured to fresh medium with the same growth regulator levels. Short roots and a tiny plantlet were produced from the callus. Cultures of blade explants that had produced shoots by 16 weeks (1.0/0.0 and 5.0/1.0) were maintained. Shoots were transferred to Knop's medium. Callus from the culture on medium with IAA 1.0 mg/l and no kinetin when separated and subcultured on medium with the same IAA level and on medium with no growth regulators produced multiple shoots and roots. A shoot initiated from the original explant culture when subcultured on medium with

IAA 1.0 mg/l and no kinetin also produced multiple shoots and roots. Subculture of callus from the medium with IAA 5.0 mg/l and kinetin 1.0 mg/l to fresh medium with the same growth regulator levels produced a few small shoots and short roots.

A stem explant on medium with IAA and kinetin 1.0 mg/l yielded callus and one tiny shoot. The shoot with some callus was subcultured on fresh medium; the original shoot increased in size and new shoots and roots were produced. Callus from the stem explant culture was transferred to basic medium lacking growth regulators where new shoots developed. The shoot initiated on medium with IAA 2.5 mg/l and no kinetin from stem tissue was transferred to Knop's medium. The callus when subcultured on fresh medium with IAA 2.5 mg/l and no kinetin produced multiple shoots and roots. The shoot produced from stem tissue on medium with IAA 5.0 mg/l and no kinetin was transferred to Knop's medium. A blade from the initiated plantlet was subcultured on fresh medium and produced callus and a few short roots.

IAA/2iP series. Certain tubes with high levels of callus were not sacrificed after the experiment ended. In this series no shoot initiation was observed at the end of 16 weeks of culture, but shoots were produced later on media with the IAA to 2iP combinations 1.0/1.0, 5.0/0.0,

and 5.0/1.0. Both blade and stem explants produced shoots on medium with IAA 5.0 mg/l and no 2iP. A stem explant on medium with IAA 5.0 mg/l and 2iP 1.0 mg/l, even after subculture on fresh medium, exhibited no further growth; a blade explant on the same medium did develop a shoot. No comparison between blade and stem explants could be made for response past 16 weeks in culture on medium with IAA and 2iP 1.0 mg/l for only a blade explant culture was maintained. The initiated shoot was transferred to Knop's medium, and the callus was subcultured on fresh medium but failed to generate more shoots. A blade from the initiated plantlet from stem tissue on medium with IAA 5.0 mg/l and no 2iP did produce callus, roots, and a tiny shoot when subcultured on fresh medium. Subcultures of blades from other initiated plantlets only produced callus.

IAA/2iP kinetin 1.0 mg/l series. Multiple shoots were produced from blade tissue on medium with IAA 2.5 mg/l, 2iP 1.0 mg/l, and kinetin 1.0 mg/l after 16 weeks. Shoots were also produced after 16 weeks from blade tissue on medium with IAA 15.0 mg/l, 2iP 2.5 mg/l, and kinetin 1.0 mg/l. The shoots were transferred to Knop's medium. Callus from the medium with IAA 2.5 mg/l, 2iP 1.0 mg/l, and kinetin 1.0 mg/l was subcultured on fresh medium where multiple shoots and roots were produced.

Cultures that had produced shoots by 16 weeks were

maintained. The shoots were transferred to Knop's medium then to soil. Subculture of callus produced from blade tissue on medium with IAA 1.0 mg/l, no 2iP, and 1.0 mg/l kinetin yielded multiple shoots and roots on fresh medium with the same growth regulator levels and on medium lacking growth regulators. Subculture of a blade from an initiated plantlet produced some callus on fresh medium. Callus from blade tissue on medium with IAA 2.5 mg/l, no 2iP, and kinetin 1.0 mg/l when subcultured on fresh medium produced multiple shoots and roots. Blades from initiated plants when subcultured on fresh medium yielded some callus and a few roots. Blades subcultured on basic medium lacking growth regulators did not respond at all.

E. *cupreata* var. Chocolate Soldier Optimum Level Experiments
(See Appendix D, Table 10)

IAA 1.0 mg/l and kinetin 0.0 mg/l. Forty replicates of leaf blade explants were cultured on shoot initiation medium with IAA 1.0 mg/l. Subcultures of callus produced from some of the blade explants were made after 7 weeks to fresh medium with the same IAA level to observe if subculture would hasten shoot initiation. Other cultures were maintained on the original medium. Response after a total of 16 weeks in culture was recorded.

Approximately 98 percent of the tubes produced callus. The average amount of callus per tube for both subcultures

and maintained cultures was 2.8. Approximately 96 percent of the subcultures produced roots as compared to 76 percent of the maintained cultures. Four percent of the subcultures yielded shoots; no shoots were produced from the maintained cultures.

IAA 5.0 mg/l and kinetin 1.0 mg/l. Forty replicates of leaf blade explants were cultured on shoot initiation medium with IAA 5.0 mg/l and kinetin 1.0 mg/l. Subcultures of callus produced from the explants were made after 7 weeks to medium with the same growth regulator levels. Other cultures were maintained on the original medium.

Sixty percent of the tubes produced callus. The average callus per tube for subcultures was 2.4 and for maintained cultures 2.3. Approximately 7 percent of subcultures produced roots as compared to 20 percent of the maintained cultures. No shoots were produced from subcultures or maintained cultures by 16 weeks.

E. cupreata var. Silver Sheen IAA/K Series (See Appendix D, Table 7)

Blade. Callus production from Silver Sheen blade tissue occurred in media containing IAA 0.0 to 15.0 mg/l and kinetin 0.0 or 1.0 mg/l. One hundred percent of the tubes demonstrated callus formation at the IAA to kinetin combinations 5.0/0.0 and 5.0/1.0. An average callus level

of 3.0 per tube occurred at the IAA to kinetin combinations 2.5/0.0 and 10.0/0.0. On medium containing no growth regulators, 20 percent of the tubes showed shoot and root initiation and 60 percent of the tubes demonstrated only root initiation. Root initiation also occurred on media with IAA 5.0 or 10.0 mg/l and no kinetin.

Stem. Callus production from Silver Sheen stem tissue occurred in media containing IAA from 0.0 to 15.0 mg/l and kinetin 0.0 to 2.5 mg/l. One hundred percent of the tubes demonstrated callus formation at the IAA to kinetin combinations 2.5/0.0, 5.0/0.0, and 5.0/1.0. An average callus level of 3.0 per tube occurred at the IAA to kinetin combinations 0.0/0.0, 5.0/0.0, 15.0/0.0, and 15.0/1.0. On medium containing no growth regulators, 25 percent of the tubes showed shoot and root initiation and 25 percent of the tubes displayed only root initiation. Shoot and root initiation also occurred on medium with IAA 2.5 mg/l and no kinetin. Root initiation only was noted on media with IAA 2.5, 5.0, or 15.0 mg/l.

E. cupreata var. Silver Sheen IAA/2iP Series (See Appendix D, Table 8)

Blade. Callus production from Silver Sheen blade tissue occurred on media with all possible combinations of IAA and 2iP levels except 0.0/0.0. One hundred percent

of the tubes demonstrated callus production at the IAA to 2iP combinations 0.0/1.0, 0.0/2.5, 1.0/0.0, 1.0/1.0, 2.5/0.0, 2.5/1.0, 2.5/2.5, 2.5/5.0, 2.5/10.0, 5.0/2.5, 5.0/10.0, 5.0/15.0, 10.0/0.0, 10.0/2.5, 10.0/5.0, 10.0/15.0, 15.0/1.0, 15.0/5.0, 15.0/10.0, and 15.0/15.0. The following IAA to 2iP levels yielded an average callus level of 3.0 per tube: 1.0/0.0, 1.0/1.0, 1.0/2.5, 1.0/5.0, 1.0/10.0, 2.5/0.0, 2.5/1.0, 5.0/1.0, 5.0/15.0, 10.0/0.0, 10.0/1.0, 10.0/2.5, 10.0/5.0, 10.0/10.0, 15.0/2.5, 15.0/10.0, and 15.0/15.0. Twenty to 80 percent of the tubes showed shoot initiation at the IAA to 2iP combinations 0.0/2.5, 1.0/0.0, 1.0/1.0, 1.0/2.5, 1.0/5.0, 2.5/15.0, and 5.0/1.0. Twenty to 33 percent of the tubes showed only root initiation at the IAA to 2iP combinations 5.0/0.0, 15.0/0.0, and 15.0/2.5.

Stem. Callus production from Silver Sheen stem tissue occurred on media containing IAA from 0.0 to 15.0 mg/l and 2iP from 0.0 to 15.0 mg/l. One hundred percent to the tubes yielded callus at the IAA to 2iP combinations 1.0/2.5, 1.0/15.0, and 2.5/2.5. An average callus level of 3.0 per tube occurred at the IAA to 2iP combinations 1.0/0.0, 1.0/1.0, 1.0/2.5, 1.0/10.0, 1.0/15.0, 2.5/0.0, 2.5/2.5, 5.0/1.0, 5.0/2.5, 5.0/5.0, 5.0/10.0, 5.0/15.0, 10.0/0.0, 10.0/1.0, 10.0/2.5, 10.0/5.0, 10.0/10.0, 15.0/1.0, 15.0/5.0, 15.0/10.0, and 15.0/15.0. Only 20 percent

of the tubes showed shoot initiation on medium with IAA and 2iP 1.0 mg/l. Twenty percent of the tubes demonstrated root initiation on media with IAA 1.0 or 15.0 mg/l and no 2iP.

E. cupreata var. Silver Sheen IAA/2iP/Kinetin 1.0 mg/l Series (See Appendix D, Table 9)

Blade. Callus production from Silver Sheen blade tissue occurred on media with IAA from 0.0 to 15.0 mg/l, 2iP 0.0 to 15.0 mg/l and kinetin 1.0 mg/l. One hundred percent of the tubes showed callus production at the IAA/2iP/kinetin combinations 1.0/0.0/1.0, 1.0/2.5/1.0, 1.0/5.0/1.0, 1.0/15.0/1.0, 2.5/2.5/1.0, 2.5/5.0/1.0, 2.5/10.0/1.0, 2.5/15.0/1.0, 5.0/0.0/1.0, 5.0/1.0/1.0, 5.0/5.0/1.0, 10.0/0.0/1.0, 10.0/1.0/1.0, 10.0/2.5/1.0, 10.0/15.0/1.0, 15.0/0.0/1.0, and 15.0/15.0/1.0. An average callus level of 3.0 per tube occurred on media with the following IAA/2iP/kinetin combinations: 1.0/1.0/1.0, 1.0/2.5/1.0, 1.0/10.0/1.0, 2.5/0.0/1.0, 2.5/1.0/1.0, 2.5/2.5/1.0, 5.0/1.0/1.0, 5.0/5.0/1.0, 10.0/1.0/1.0, 10.0/2.5/1.0, 10.0/5.0/1.0, 10.0/10.0/1.0, 15.0/2.5/1.0, 15.0/5.0/1.0, and 15.0/10.0/1.0. Twenty to 25 percent of the tubes showed shoot initiation only at the IAA/2iP/kinetin combinations 1.0/0.0/1.0, 1.0/1.0/1.0, and 2.5/0.0/1.0. Twenty to 40 percent of the tubes showed shoot and root initiation on media with the IAA/2iP/kinetin combinations 1.0/1.0/1.0, 2.5/1.0/1.0,

and 2.5/2.5/1.0. Twenty to 100 percent of the tubes showed root initiation only on media with the IAA/2iP/kinetin combinations 1.0/1.0/1.0, 1.0/2.5/1.0, 1.0/5.0/1.0, 1.0/15.0/1.0, 2.5/0.0/1.0, 2.5/1.0/1.0, 2.5/2.5/1.0, and 10.0/2.5/1.0.

Stem. Callus production from Silver Sheen stem tissue occurred on media with IAA from 1.0 to 15.0 mg/l, 2iP 0.0 to 15.0 mg/l, and kinetin 1.0 mg/l. One hundred percent of the tubes showed callus production at the IAA/2iP/kinetin combinations 5.0/0.0/1.0, 5.0/1.0/1.0, 5.0/2.5/1.0, 5.0/5.0/1.0, 5.0/10.0/1.0, 5.0/15.0/1.0, 10.0/0.0/1.0, 10.0/1.0/1.0, 10.0/2.5/1.0, 10.0/5.0/1.0, and 15.0/2.5/1.0. An average callus level of 3.0 per tube occurred at the IAA/2iP/kinetin levels 1.0/0.0/1.0, 1.0/2.5/1.0, 1.0/10.0/1.0, 2.5/0.0/1.0, 2.5/1.0/1.0, 2.5/5.0/1.0, 2.5/10.0/1.0, 2.5/15.0/1.0, 5.0/2.5/1.0, 5.0/5.0/1.0, 5.0/10.0/1.0, 5.0/15.0/1.0, 10.0/10.0/1.0, 10.0/15.0/1.0, 15.0/0.0/1.0, 15.0/1.0/1.0, 15.0/2.5/1.0, 15.0/5.0/1.0, 15.0/10.0/1.0 and 15.0/15.0/1.0. Twenty percent of the tubes produced shoots on medium with IAA 2.5 mg/l, 2iP 5.0 mg/l, and kinetin 1.0 mg/l. From 20 to 33 percent of the tubes produced roots on media with the IAA/2iP/kinetin combinations 2.5/1.0/1.0, 2.5/2.5/1.0, 5.0/5.0/1.0, 15.0/2.5/1.0, and 15.0/5.0/1.0.

E. cupreata var. Silver Sheen Cultures Maintained Past
16 Weeks

IAA/kinetin series. No tubes were maintained past the 16 week experimental period.

IAA/2iP series. Tubes yielding shoots by the end of 16 weeks of culture were maintained. No further growth response was noted from the callus and only one new shoot was observed on medium with no IAA and 2iP 2.5 mg/l after approximately 7 months of culture. From the blade explant on medium with IAA 1.0 mg/l and no 2iP, a small plantlet cluster was subcultured on fresh medium. Multiple shoots, roots, and callus were produced and the original cluster increased in size. The original tube which was also maintained produced multiple shoots, roots, and callus. The culture of blade tissue on medium with IAA and 2iP 1.0 mg/l when kept in original tube for 7 months produced only two small shoots. Subculture of callus produced from blade tissue on medium with IAA 1.0 mg/l and 2iP 2.5 mg/l to medium without growth regulators showed no growth response and was further subcultured on fresh medium with IAA and 2iP 1.0 mg/l. This culture developed contamination and was discarded. Plantlets produced from a blade explant on medium with IAA 1.0 mg/l and 2iP 2.5 mg/l were subcultured on medium with IAA 1.0 mg/l and on medium with IAA and 2iP 1.0 mg/l. Plantlets subcultured on the medium with IAA only

increased in size and produced roots and callus. The plantlet subcultured on medium with both IAA and 2iP produced new shoots and callus. Blades from initiated plants on the medium IAA 1.0 mg/l and 2iP 2.5 mg/l were subcultured on medium with IAA 1.0 mg/l and on medium with both IAA and 2iP 1.0 mg/l. Callus and roots were produced from the blade on the medium with IAA alone; callus only was produced from the blade on medium with both IAA and 2iP. Subculture of callus tissue produced from blade explant on medium with IAA 1.0 mg/l and 2iP 2.5 mg/l to media with IAA 1.0 mg/l or IAA and 2iP 1.0 mg/l produced roots and some new callus tissue. The blade explant culture on medium with IAA 1.0 mg/l and 2iP 5.0 mg/l was maintained without subculturing for approximately one year. Small shoot clusters plus callus were then subcultured on medium with IAA 1.0 mg/l or medium with IAA and 2iP 1.0 mg/l. The shoots increased in size on the medium with IAA alone; new shoots and roots were produced from the shoot cluster with attached callus placed on medium with both IAA and 2iP. Blades from plantlets initiated on medium with IAA 1.0 mg/l and 2iP 5.0 mg/l when subcultured on media with either IAA 1.0 mg/l or IAA and 2iP 1.0 mg/l produced callus and on medium without 2iP roots were also produced. Shoots initiated from blade tissue on media with the IAA to 2iP combinations 2.5/15.0, 5.0/1.0, and 10.0/0.0 were transferred to Knop's medium.

Callus produced from the blade explants on medium with IAA 2.5 mg/l and 2iP 15.0 mg/l and on medium with IAA 5.0 mg/l and 2iP 1.0 mg/l did not produce further shoots or roots when subcultured on medium lacking growth regulators, or medium with IAA 1.0 mg/l, or on medium with IAA and 2iP 1.0 mg/l. The blade explant culture on medium with IAA 5.0 mg/l and no 2iP produced a few shoots after 16 weeks of culture.

Stem explants produced shoots only on medium with IAA and 2iP 1.0 mg/l by 16 weeks. This culture was maintained for 7 months in the original tube. The shoots turned brown and the tube was discarded. Stem explants on media with the IAA to 2iP combinations 1.0/0.0 and 2.5/2.5 did develop shoots after 16 weeks. Subculture of initiated shoots plus callus from medium with IAA 1.0 mg/l to fresh medium yielded new shoots and roots; the older shoots increased in size.

IAA/2iP kinetin 1.0 mg/l series. Tubes showing shoot initiation from blade tissue by 16 weeks were maintained. The shoot initiated on medium with IAA and kinetin 1.0 mg/l and no 2iP was transferred to Knop's medium. Further subculture of callus was not possible due to contamination. Cultures at the IAA/2iP/kinetin combinations 1.0/1.0/1.0 and 2.5/0.0/1.0 were maintained without subculturing for 6 months; no further production of shoots

occurred and the tubes were discarded. Plantlets initiated on medium with IAA 2.5 mg/l and 2iP and kinetin 1.0 mg/l were transferred to Knop's medium. Subculture of callus on media without growth regulators, or with IAA 5.0 mg/l and kinetin 1.0 mg/l, or IAA and 2iP 1.0 mg/l, or with IAA 1.0 mg/l did not produce further shoots. Small shoots, callus, and roots produced on medium with IAA and 2iP 2.5 mg/l, kinetin 1.0 mg/l were maintained for 6 months before discarding.

Stem tissue on medium with IAA 2.5 mg/l, 2iP 5.0 mg/l, and kinetin 1.0 mg/l produced shoots by 16 weeks. The shoots were transferred to Knop's medium. Subculture of callus on media lacking growth regulators, or containing IAA 1.0 mg/l or with IAA and 2iP 1.0 mg/l did not yield further shoots. Stem explants on media with the IAA/2iP/kinetin combinations 2.5/2.5/1.0, 5.0/2.5/1.0, and 5.0/5.0/1.0 were observed to produce small shoots by 6 months; the cultures were then discarded.

E. cupreata var. Silver Sheen Optimum Level Experiments
(See Appendix D, Table 10)

IAA 1.0 mg/l and 2iP 0.0 mg/l. Forty replicates of leaf blade explants were cultured on shoot initiation medium with IAA 1.0 mg/l. Subcultures of callus produced from the blade explants were made after 7 weeks to medium with the same IAA level. Other cultures were maintained on the original medium.

Approximately, 73 percent of the tubes produced callus. The average callus per tube for subcultures was 2.6 and for maintained cultures 1.7. One hundred percent of the subcultures produced roots as compared to 80 percent of the maintained tubes. Approximately 14 percent of the subcultured tubes and 7 percent of the maintained tubes produced shoots by 16 weeks.

IAA 1.0 mg/l and 2iP 1.0 mg/l. Thirty-eight replicates of leaf blade explants were cultured on shoot initiation medium with IAA and 2iP 1.0 mg/l. Subcultures of callus produced from the blade explants were made after 7 weeks to medium with the same growth regulator levels. Other cultures were maintained on the original medium.

One hundred percent of the tubes produced callus. The average callus per tube for both subcultures and maintained cultures was 3.0. Root tips or a few thin roots were noted by 16 weeks. Fifty-eight percent of the subcultures produced root buds as compared to 41 percent of the maintained cultures. Eight percent of the subcultures produced shoots by 16 weeks as compared to 50 percent of the maintained cultures.

Comparison of Contamination Levels

Contamination levels varied little for the three cultivars chosen for this study during the series of

experiments to determine optimum levels of growth regulators for shoot initiation. Overall loss of cultures to contamination was 8 percent. Episcia cupreata var. Silver Sheen for the three growth regulator series did show a 15 percent contamination level for stem cultures as compared to 6 and 5 percent levels for E. cupreata var. Chocolate Soldier and E. dianthiflora, respectively. Contamination levels for blade cultures were 6.5 percent E. cupreata var. Silver Sheen, 7 percent E. cupreata var. Chocolate Soldier, and 6 percent E. dianthiflora.

In the supplementary experiment carried out varying chlorox levels and time exposure of leaf tissue, E. dianthiflora blade tissue displayed the highest total amount of contamination (38 percent) with contamination occurring for the treatments 5 minutes at 1/6 and 1/12 volume-by-volume chlorox, 10 minutes at 1/6 volume-by-volume chlorox, and 20 minutes at 1/3 volume-by-volume chlorox. Blade tissue that had not been exposed to chlorox at all did not demonstrate contamination. E. cupreata var. Chocolate Soldier demonstrated 15 percent total contamination for all treatments. Contamination occurred for the treatments 5 minutes of exposure to 1/12 and 1/3 volume-by-volume chlorox. E. cupreata var. Silver Sheen demonstrated the lowest total contamination level (7 percent) for all treatments. Contamination occurred for the treatment with no chlorox exposure.

In the experimental procedures when blade tissue was cultured at the optimum auxin to cytokinin combinations, no contamination occurred for the first 7 weeks of culture for all three cultivars. Only after subcultures had been made did a few cultures demonstrate contamination.

Comparison of Rooting Media and Transfer to Soil

A few of the first shoots to be initiated were transferred to either Knop's medium plus 20 g/l sucrose or to Knop's medium without sucrose. Root growth occurred on both media. Roots were thin but numerous on medium lacking sucrose. Root growth appeared more vigorous on Knop's medium plus sucrose with the roots being thicker and branching extensively. Therefore, further initiated shoots were transferred to Knop's medium with sucrose exclusively.

After plantlets had generated roots on Knop's medium and were at least 3 to 5 cm tall, they were removed from the flasks. Agar medium was rinsed as much as possible from the roots with a gentle stream of tap water. Plantlets were potted on general greenhouse potting mixture of 2 parts soil to 2 parts perlite to 1 part sand and 1 part peat moss. The potting mixture also included 1/4 teaspoon of fertilizer with 6 percent nitrogen, 12 percent phosphorus, and 12 percent potassium per 6 inch pot. The plantlets were kept covered with beakers to maintain high humidity for 1 to 2

weeks. Only a few plants did not survive the transition to soil, and these were generally very small.

CHAPTER IV

DISCUSSION AND CONCLUSIONS

Comparison of Episcia Cultivars

E. cupreata var. Silver Sheen was the most responsive of the selected taxa to tissue culture techniques. Chocolate Soldier was similar in response but did not exhibit shoot production to the same extent as Silver Sheen. Of the three, E. dianthiflora was the least responsive with little callus production and low percentages of cultures differentiating shoots. In addition, E. dianthiflora stock plants were noted as much slower in propagating by runners than either Chocolate Soldier or Silver Sheen. The response of E. dianthiflora as compared to E. cupreata in these experiments confirm the observations of Murashige (1974) and Narayanaswamy (1977) that plant species which readily regenerate new plants in vivo are often easier to propagate in vitro.

Though many growth regulator combinations were permissive for callus production, shoot and root initiation from explants were observed less often. These results are in agreement with results obtained from other plant species (Street, 1979). Generally, rhizogenesis from primary blade and stem explants occurred more often than caulogenesis for the E. cupreata varieties. This has been found to be true

of most plant species (Street, 1979). Though shoot initiation occurred more often, E. dianthiflora stem explants did infrequently produce roots. Root initiation for all three cultivars was primarily on media with higher levels of IAA as compared to cytokinin concentrations. High auxin levels have been reported to favor root initiation (Murashige, 1974; Narayanaswamy, 1977; Kohlenbach, 1977).

Johnson (1978a) varied the auxin (IAA or NAA) levels in media to induce rooting from cultured E. cupreata var. Silver Queen shoots. She did not obtain root initiation with IAA but did with NAA. In this study, root initiation was observed from blade and stem explants and in subcultures of initiated shoots on media with IAA. The discrepancy of response to IAA is possibly due to the concentration used by Johnson (0.1-1.0 mg/l) as compared to the levels included in these experiments (0.0-15.0 mg/l). Butenko (1968) noted that NAA has a greater effect than IAA on development of roots.

As roots were obtained occasionally on LS media with IAA, shoots were transferred to Knop's medium where induction of roots was reliable for all Episcia taxa. Inclusion of sucrose in Knop's medium enhanced root development; however, shoots did not die on medium lacking sucrose as was observed by Start and Cummings (1976) with Saintpaulia shoots. Cultured Episcia plantlets apparently by this stage of development are capable of synthesizing carbohydrates necessary for growth.

With the exception of E. dianthiflora, higher percentages of blade explants responded with shoot initiation to a greater number of growth regulator combinations than did stolon explants. The variation of response of explants on the same medium may be due to differences in endogenous concentrations of auxins and cytokinins (Hall, 1976; Narayanaswamy, 1977; Street, 1979). Bilkey and McCown (1979) found that stem tip explants of Episcia yielded callus more readily than blade explants; subsequent subculture of callus led to shoot initiation. In this case, variation of response may also be due to the physiological age and extent of differentiation among the constituent cells of the primary explant (Murashige, 1974).

Blade explants of all three Episcia taxa demonstrated less shoot initiation when cultured a second time on the same media that had previously given the best results. In fact, E. dianthiflora showed no growth at all in the second experiment. Failure of the blade explants to show the same response could be a result of a change in the state of the stock plants (Murashige, 1974). Although leaves were selected from stock plants that appeared vigorous, several stock plants were dying for unknown reasons. An alternative explanation of the variable results of shoot production from the blade explants could be due to use of sterile technique! Ethylene produced in flaming flask openings has been reported to cause excessive callus formation and

decrease the number of cotton ovules forming fibers in response to IAA (Beasley and Eaks, 1979). There are a number of plant species where the action of ethylene has been noted as antagonistic to cell differentiation (Street, 1979). Care was taken in the repeated experiments to flame instruments and tube openings often as no alcohol was used in sterilizing explants. Possibly this procedure inhibited shoot initiation from the blade explants.

Individual blades from cultured Episcia plantlets did not prove satisfactory for subculture; some callus was produced but only occasional shoots were initiated. For E. dianthiflora and Silver Sheen, subculture of whole shoots proved to be the best source for yielding multiple new shoots. Subculture of Chocolate Soldier callus which had already produced shoots also demonstrated numerous new shoots; E. dianthiflora and Silver Sheen callus did not respond similarly. In this study subculture of Episcia callus prior to shoot initiation did not enhance shoot differentiation as was observed by Bilkey and McCown (1979).

No obvious changes of vegetative characteristics of cultured Silver Sheen or E. dianthiflora plantlets were observed. Less than 1 percent of cultured Chocolate Soldier plantlets showed a large variation from vegetative characteristics of stock plants. The variant shoots had leaves that were primarily pink and did not grow well or produce a vigorous root system when placed on Knop's medium.

Pink leaves suggest little or no chlorophyll present thus affecting photosynthesis. Bilkey and McCown (1979) and Johnson (1978a) also noted few variant plantlets produced from culture of Episcia. Variant plants produced in tissue culture are more likely to occur when cultures include high levels of auxin (Holdgate, 1977). Though high IAA concentrations did not produce plantlets that were variant in this study, the number of shoots produced from an explant at high IAA levels was much lower.

Contamination Problems

No general conclusions can be drawn from the results obtained from the contamination experiment tried in this study. Murashige (oral communication) has suggested that ethanol inhibits differentiation. The very low level of contamination observed in the repeated experiments of blade explants on media with optimum growth regulator levels indicates immersion of explants in 70 percent ethanol is not necessary. Contamination levels will vary due to the state of the stock plants, as well as to the sterile techniques of individuals carrying out the culture procedures. Ethylene production by flaming flask openings and possible subsequent inhibition of shoot initiation should be considered and be kept to a minimum. Omission of immersion of explants in 70 percent ethanol is recommended.

Conclusions

Only low percentages of blade or stem explants producing shoots were observed for E. dianthiflora and E. cupreata var. Chocolate Soldier by 16 weeks. E. cupreata var. Silver Sheen demonstrated a high percentage of blade explants producing shoots on media with IAA 1.0 mg/l or IAA and 2iP 1.0 mg/l at 16 weeks. Repetition of experiments at optimum growth regulator levels demonstrated a variation in shoot initiation response to results originally obtained. Once plantlets were obtained, subculture of shoots for Silver Sheen and E. dianthiflora produced new shoots. Chocolate Soldier callus, after demonstrating shoot initiation, also responded well to subculture procedures by producing multiple new shoots. Shoot initiation was usually preceded by intensive callus formation but not all callus produced plants. Subculture of callus prior to any shoot initiation was not found to enhance later shoot initiation. The different taxa demonstrated somewhat different media requirements for shoot initiation. Once shoots were initiated, they were relatively easy to transfer to Knop's medium. Addition of sucrose to the Knop's medium enhanced root development. Plantlets transferred easily to soil. Very little variation of vegetative characteristics of plants was observed for cultured plantlets.

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APPENDIXES

APPENDIX A

REVIEW OF TISSUE CULTURE APPLICATIONS OF MAJOR GENERA OF THE GESNERIACEAE

Sinningia

Sinningia does not always breed true when propagated by seed; new plants of a given cultivar are generally obtained by leaf and/or stem cuttings. Therefore, an added benefit to rapid propagation by in vitro techniques is one of getting the desired plant characteristics reliably.

Haramaki (1971) used shoot tip segments from 1-2 mm in length of Sinningia speciosa as an explant source. His best results for shoot initiation were obtained using Murashige and Skoog (1962) (MS) medium with inositol 100 mg/l, thiamine·HCl 0.4 mg/l, sucrose 30 g/l, IAA 0.1 mg/l, kinetin 30 mg/l, and agar 6-10 g/l. After approximately 5 months, shoots were transferred to a rooting medium containing IAA at 1 mg/l and lacking kinetin. Plantlets were transferred to pots with peat plus vermiculite or perlite after 1 month on rooting medium. From the results a single shoot tip had the potential for producing 500 or more plants.

Bigot (1974, 1975) tried stems, petioles, leaves, floral peduncles, receptacles, and floral pieces of Gloxinia hybrida as explant sources. Best shoot initiation was

obtained from floral peduncles and, to a lesser extent, from leaves. The shoot initiation medium contained MS medium with inositol 100 mg/l, various vitamins, sucrose 30 g/l, 6-benzylaminopurine (BAP) 1-2 mg/l, naphthalene-acetic acid (NAA) 0.2-1 mg/l and agar 8 g/l. Rooting medium included IAA 0.5 mg/l and BAP 50 µg/l. Plantlets were transferred first to vermiculite in flasks then to soil. Approximately 6 months were required for a mature plant in soil to be produced from the explant.

Grunewaldt (1977), using very similar growth regulator levels of 0.2-1 mg/l of IAA and BAP with a basal shoot initiation medium of Linsmaier and Skoog (1965) medium plus inositol 100 mg/l, thiamine 1 mg/l, 30 g/l sucrose, and agar 8 g/l, achieved plant initiation for several of the Gesneriads (Gloxinia hybrida, Saintpaulia ionantha, and Streptocarpus LINDL. x hybridus) from explants of blade and petiole sections. Grunewaldt observed only Gloxinia hybrida had the potential for plant initiation from peduncles.

Raman (1977) used pedicel segments of Sinningia speciosa that were 10-15 mm in length. On basal MS medium with vitamins and glycine, inositol 100 mg/l, and 3 percent glucose, NAA and BAP 1 mg/l or adenine 40 mg/l or adenine sulfate 50 mg/l and kinetin 1 mg/l were added. Equivalent levels of shoot production occurred on all three media. On the medium including NAA, roots were also produced; therefore,

plants could be transferred directly to soil. From the results obtained, a single pedicel had the potential for producing 300 plants in 5 to 6 months.

Johnson (1978b) used leaf blade sections (1 cm^2) of Sinningia speciosa as her explant source. Shoots were readily obtained at the cut ends of veins or over the central vein region when placed on MS medium with kinetin 0.3-0.7 mg/l and IAA 1.6-1.8 mg/l. The rooting medium contained IAA and kinetin at 0.2 mg/l. One to 10 plants were obtained per explant and a single leaf provided approximately 100 explants.

Streptocarpus

As with gloxinia, Streptocarpus seed stocks are usually mixtures of different colored lines. Vegetative multiplication is generally slow. For these reasons, Grunewaldt (1977) and Raman (1977) were interested in developing tissue culture methods for faster propagation of Streptocarpus. Grunewaldt also noted that the regeneration rate is of interest for in vitro selection of mutants. Grunewaldt's results indicated plant initiation from lamina and petiole sections on the same medium as used for gloxinia; no plantlets were obtained from peduncle explants. Raman (1977), on the other hand, used pedicels for his explant on the same basal medium as he had used for gloxinia with adenine 40 mg/l, or adenine sulfate 10 mg/l and kinetin 10 mg/l, or BAP 1 mg/l, or NAA and BAP 1 mg/l.

Shoots were obtained on all of the above media, but enhanced shoot and root production occurred on the medium including NAA. Initially, buds arose from both cut ends and later emerged from all surfaces of the pedicel. The maximum number of buds per segment of pedicel increased with the age of the pedicel prior to anthesis.

Appelgren and Heide (1972) observed responses to various plant growth regulators by Streptocarpus x hybridus "Constant Nymph" leaf discs. After 5 weeks of culture on filter paper in petri dishes wetted with Schott and Scraudolf (1967) minerals plus the particular hormone treatment, leaf discs were observed for the number of visible roots and buds. Results indicated auxins (IAA, indole-3-butyric acid, NAA, and 2,4-dichlorophenoxyacetic acid) generally promoted bud and root formation with optimum root induction 1 to 2 orders of magnitude higher than for bud formation. Cytokinins (adenine, BAP, kinetin, zeatin, and zeatin riboside) had only a small stimulatory effect at low concentrations and an inhibitory effect on bud formation at high concentrations. Gibberellic acid inhibited both bud and root formation. Abscissic acid plus optimum auxin levels enhanced bud formation.

Handro (1977) used tissue culture techniques as a tool for inducing floral buds from Streptocarpus nobilis C. B. Clarke leaf discs. One centimeter diameter discs were placed on medium containing Knop's macroelements

(Gautheret, 1959), microelements and vitamins (Nitsch and Nitsch, 1965), benzyladenine 350 $\mu\text{g/l}$, IAA 100 $\mu\text{g/l}$, adenine 1.5 g/l, sucrose 20 g/l, and agar 8 g/l. Cultures were kept under a short day (10 hr light) or a long day (16 hr light) cycle. The discs were harvested at certain intervals, fixed, dehydrated, stained, and sectioned for microscopic investigation. Results showed that buds formed on the proximal end of the severed midrib, cut edges of the disc, and on the epidermal surface. Floral buds developed on the short day discs and vegetative buds on the long day discs.

Saintpaulia

Mass propagation of Saintpaulia by tissue culture has definite appeal to the commercial horticulturists. Reports of the production of 20,000 plantlets from a single African violet leaf in 3 months (Bilkey and Hildebrandt, 1977) certainly indicate the valuable potential of this plant. Better control of overall quality has been predicted by using tissue culture methods as compared to leaf cuttings (McCown and Bilkey, 1977).

Rao and Morel (1973) used 0.5-1.0 cm cross sections of petiole tissue from Saintpaulia ionantha on MS medium supplemented with 1.0 mg/l Heller's micronutrient and iron solutions (Butenko, 1968), sucrose 3 percent and agar 6 percent. Agrobacterium tumefaciens T37 and B6 were used

to induce callus formation on some of the petiole tissues. Petiole cross sections were removed weekly and fixed for microscopic study. Results demonstrated that the epidermis of Saintpaulia petiole segments can give rise to buds when both the petiole and blade were excised together. Untreated petioles yielded no buds but petioles treated with Agrobacterium developed callus and buds. There was no root initiation.

Hughes, Bell, and Caponetti (1975) were the first to report production of haploid African violets. Intact buds were surface sterilized and anthers removed and placed on Blaydes' medium (Blaydes, 1966) supplemented with 0.8 percent agar. After 6 to 8 weeks plants were transferred to Skoog's modified medium (Skoog and Armstrong, 1974) supplemented with 2 mg/l IAA, 1 mg/l kinetin, and 0.8 percent agar. Plantlets increased in size and were again transferred to induce rooting. Optimum rooting medium included Skoog's minerals with 0.4 mg/l thiamine, 100 mg/l inositol, 30 g/l sucrose, 2 mg/l indolebutyric acid (IBA) and 0.8 percent agar. The rooted plants were transferred to wet sand and covered with beakers. Finally, plants were transferred to soil. Hughes (1977) reported the production of haploid and diploid plants on a modified Skoog's medium supplemented with IAA 5 mg/l. The explant sources for the haploid plants were anthers and for the diploid plants were petals and intact flower buds.

Protoplasts from these cultured plantlets were obtained using an enzyme mixture of 2 percent cellulysin, 0.25 percent macerozyme, hemicellulase, and pectinase and the sugars 0.27 M sorbitol and mannitol. Protoplasts could not be obtained from stock plants.

Grunewaldt (1976) initiated plants from Saintpaulia petiole cross sections and from leaf blades (1977). Up to 100 percent of the petioles yielded callus with 1/2 of the callusing segments producing plantlets without roots on a Linsmaier and Skoog medium (LS) supplemented with inositol, thiamine, coconut milk, 0.2 mg/l IAA, 0.1 mg/l 2,4-D, and 0.4 mg/l BAP, and agar. Roots were induced on the same basic medium with IAA as the only growth regulator. Explants from blades generated plants on LS medium supplemented with 0.2-1.0 mg/l IAA and BAP, 1 mg/l thiamine, 100 mg/l inositol, 8 g/l agar, and 30 g/l sucrose.

Start and Cummings (1976) used blades with attached petioles for explants to obtain shoot induction from Saintpaulia ionantha Wendl. The explants were placed upright with 1/4 the length embedded in the culture medium. The optimum medium for initiating shoots was a modified MS medium supplemented with 0.1 mg/l NAA, 5 mg/l BAP, and 125 mg/l adenine sulfate. Shoots formed first at the base of veins touching the medium and within 3 weeks shoots formed on the entire adaxial surface of the leaf. Few shoots formed on the abaxial leaf surface but those that did were

located near main veins. Plantlets were rooted on Morel and Muller (1964) medium with 16 g/l sucrose. Various concentrations of sucrose were tried in the rooting medium. Shoots died at sucrose levels 0-5 g/l and root growth was inhibited at 20-30 g/l. Optimum root growth occurred when sucrose levels were 12-20 g/l. Plants were observed to grow faster in larger culture vessels both on the shooting and rooting media.

Cooke (1977) found that 10 mm² leaf explants produced plantlets when placed on medium with MS salts, NaH₂PO₄·H₂O 170 mg/l, sucrose 30 g/l, inositol 100 mg/l, thiamine.HCl 0.4 mg/l, niacine 0.4 mg/l, pyridoxine.HCl 0.4 mg/l, IAA 2 mg/l, BAP 0.08 mg/l, and agar 9 g/l. Rooting medium was the same except growth regulators were omitted. Shoots formed after 30 days of culture with multiple shoots formed by 60 days. The average number of shoots per explant was 30. No root inhibition occurred as was noted earlier by Start and Cummings (1976) with the high level of sucrose. Plants flowered after transfer to soil in 3 to 6 months.

Bilkey, McCown, and Hildebrandt (1978) used 2 mm cross sections of petioles on MS medium with NAA 0.1 mg/l, benzyladenine 0.01 mg/l, sucrose 2.5 percent, and agar 0.8 percent to initiate shoots from Saintpaulia ionantha. Swelling of the petiole cross sections around the circumference was observed after 1 week of culture. Sparse callus

growth was noted on the surface of the explant with shoots being initiated within 6 weeks. Rooting medium was not necessary as cultures produced roots when placed under a lower light intensity for a few weeks before transplanting to soil. Bilkey and McCown (1978) also observed that container size is a variable to be considered in tissue culture. Twenty-five to 30 plantlets were generated from petioles on MS medium with 0.05 mg/l benzyladenine and 0.1 mg/l NAA. The plantlets were sectioned into four groups and placed on MS medium with 0.01 mg/l benzyladenine and 0.1 mg/l NAA in either 30 ml jars or 125 ml flasks. There was an 89 percent increase in the number of plants subcultured in flasks as compared to a 46 percent increase in the number of plants subcultured in jars; however, plantlets in jars demonstrated more advanced plantlet development and two times greater fresh weight.

APPENDIX B

LINSMAIER AND SKOOG MEDIUM

<u>For 1 Liter of Medium</u>	<u>mg/l</u>	<u>Company</u>	<u>Lot #</u>
Mineral Salts			
NH_4NO_3	1650.000	Fisher-Scientific	762744
KNO_3	1900.000	J. T. Baker	33642
H_3BO_3	6.200	Fisher-Scientific	736047
KH_2PO_4	170.000	J. T. Baker or Fisher-Scientific	28148 763449
KI	0.830	Fisher-Scientific	730784
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.250	Fisher-Scientific	737178
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.250	Fisher-Scientific	741161
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	440.000	Fisher-Scientific	761927
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	370.000	Fisher-Scientific	734823
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	16.900	Fisher-Scientific	735007
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	8.600	Fisher-Scientific	733217
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.025	Fisher-Scientific	780036
Na_2EDTA	37.300	Sigma Chemical	76C-0143
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	27.800	Fisher-Scientific	734553
Thiamine·HCl	0.400	Sigma Chemical	22C-3210
Myo-inositol	100.000	Calbiochem	601219
Auxin (IAA)	0.0-15.0	Eastman Kodak	A3-D
Cytokinin (2iP or kinetin)	0.0-15.0	Sigma Chemical	66C-5020 96C-0115

<u>For 1 Liter of Medium</u>	<u>mg/l</u>	<u>Company</u>	<u>Lot #</u>
Sucrose	20 g/l	Fisher-Scientific	782016
Agar	8 g/l	GIBCO or Difco	
pH	5.6-5.8		

Linsmaier and Skoog, 1965, *Physiol. Plant.* 18, 100-127.

APPENDIX C

MODIFIED KNOP'S MEDIUM

<u>For 1 Liter of Medium</u>	<u>mg/l</u>	<u>Company</u>	<u>Lot #</u>
Knop's Stock Solution	500.0 ml		
Double-distilled H ₂ O	500.0 ml		
Nitsch's Minor Elements	0.5 ml		
Ferric Citrate Solution	0.4 ml		
(Sucrose)	20.0 g		
Agar	8.0 g		
pH	5.5-5.8		

Knop's Stock Solution (Nitsch, 1951, AJB 38, 569)

Ca(NO ₃) ₂ ·4H ₂ O	0.500 g	Baker and Adamson	
KNO ₃	0.125 g	J. T. Baker	33642
MgSO ₄ ·7H ₂ O	0.125 g	Fisher-Scientific	734823
KH ₂ PO ₄	0.125 g	J. T. Baker or Fisher-Scientific	28148 763449
double-distilled H ₂ O	1000 ml		

Nitsch's Trace Elements (modified from Steeves, et al., 1955, AJB 42, 232-235)

H ₂ SO ₄	0.5 ml	Fisher-Scientific	776414
MnCl ₂ ·4H ₂ O	2.5 g	J. T. Baker	32739
H ₃ BO ₃	2.0 g	Fisher-Scientific	736047
ZnSO ₄ ·7H ₂ O	0.05 g	Fisher-Scientific	733217
CoCl ₂ ·6H ₂ O	0.03 g	Fisher-Scientific	741161

<u>For 1 Liter of Medium</u>	<u>mg/l</u>	<u>Company</u>	<u>Lot #</u>
CuCl ₂ ·2H ₂ O	0.015 g	J. T. Baker	22648
Na ₂ MoO ₄ ·2H ₂ O	0.025 g	Fisher-Scientific	737178
double-distilled H ₂ O	1000 ml		
Ferric Citrate Solution			
FeC ₆ H ₅ O ₇ ·5H ₂ O	25 g	Fisher-Scientific	743496
double-distilled H ₂ O	1000 ml		

APPENDIX D

SUMMARY TABLES OF RESULTS

Table 1. Episcia dianthiflora IAA/Kinetin Series.

IAA/K	Blade				Lateral Stem			
	% Tubes Callusing	Average Callus	% Tubes Roots	% Tubes Shoots	% Tubes Callusing	Average Callus	% Tubes Roots	% Tubes Shoots
0/0					40	1.0	20	
0/1								
0/2.5								
0/5								
0/10								
0/15								
1/0					20	3.0		
1/1					20	1.0		
1/2.5					20	3.0		20
1/5								
1/10								
1/15								
2.5/0					20	2.0		
2.5/1					20	3.0		
2.5/2.5	20	3.0			20	3.0		20
2.5/5								
2.5/10								
2.5/15								
5/0	50	1.0			40	1.0		
5/1	80	1.5			100	2.6	20	
5/2.5	75	2.0		25	50	1.0		
5/5	100	1.75		25	50	1.0		
5/10					20	1.0		
5/15								
10/0	50	3.0			60	3.0		
10/1	75	1.67		25	25	2.0		
10/2.5	100	2.6			60	2.0		
10/5	66	3.0			100	2.0		50
10/10	60	2.67		20				
10/15								
15/0							50	
15/1					60	1.0		
15/2.5					25	1.0		
15/5								
15/10								
15/15								

*Callus levels were evaluated qualitatively as 1, 2, or 3. A score of 1 indicated callus had been initiated; a score of 2 indicated callus occurred over a large proportion of the explant but portions of the explant were still visible; a score of 3 indicated callus completely obscured the explant.

Table 2. Episcia dianthiflora IAA/2iP Series.

IAA/K	Blade				Lateral Stem			
	% Tubes Callusing	Average Callus	% Tubes Roots	% Tubes Shoots	% Tubes Callusing	Average Callus	% Tubes Roots	% Tubes Shoots
0/0								
0/1								
0/2.5								
0/5								
0/10								
0/15								
1/0					20	1.0		
1/1					40	1.0		
1/2.5					50	1.0		
1/5					60	1.0		
1/10					20	1.0		
1/15								
2.5/0					50	1.0		
2.5/1					50	1.5		
2.5/2.5	40	1.0			100	1.0		
2.5/5								
2.5/10								
2.5/15								
5/0								
5/1								
5/2.5					50	1.0		
5/5								
5/10								
5/15								
10/0					80	1.0		
10/1	20	1.0			50	1.0		
10/2.5					40	1.5		
10/5					40	1.5		
10/10					20	2.0		
10/15					20	1.0		
15/0					40	1.0		
15/1					80	1.0		
15/2.5					20	1.0		
15/5					20	3.0		
15/10								
15/15					20	1.0		

Table 3. Episcia dianthiflora IAA/2iP/Kinetin 1.0 mg/l Series.

IAA/K	Blade				Lateral Stem			
	% Tubes Callusing	Average Callus	% Tubes Roots	% Tubes Shoots	% Tubes Callusing	Average Callus	% Tubes Roots	% Tubes Shoots
0/0								
0/1								
0/2.5								
0/5								
0/10								
0/15								
1/0								
1/1					25	1.0		
1/2.5								
1/5								
1/10								
1/15								
2.5/0	40	1.0			40	1.0		
2.5/1	40	1.0			20	1.0		
2.5/2.5					20	1.0		
2.5/5	20	1.0						
2.5/10								
2.5/15								
5/0	80	1.0			100	1.0		
5/1	40	1.0						
5/2.5								
5/5								
5/10					20	1.0		
5/15								
10/0					20	1.0		
10/1					40	1.0		
10/2.5					40	1.0		
10/5					60	1.0		
10/10								
10/15								
15/0					60	1.3		
15/1					60	1.0		
15/2.5					80	1.0		
15/5					80	1.5		
15/10					20	2.0		
15/15								

Table 4. *Episcia cupreata* var. Chocolate Soldier IAA/
Kinetin Series.

IAA/K	Blade				Lateral Stem			
	% Tubes Callusing	Average Callus	% Tubes Roots	% Tubes Shoots	% Tubes Callusing	Average Callus	% Tubes Roots	% Tubes Shoots
0/0								
0/1								
0/2.5								
0/5								
0/10								
0/15								
1/0	40	2.5	20	20	100	2.6	20	
1/1	80	1.3			40	1.5	20	
1/2.5								
1/5								
1/10								
1/15								
2.5/0	75	2.7	25		60	2.7	60	
2.5/1	80	2.3						
2.5/2.5	20	1.0						
2.5/5								
2.5/10								
2.5/15								
5/0	66	2.0	66		100	2.8	40	
5/1	60	2.7		20				
5/2.5	75	2.2						
5/5								
5/10								
5/15								
10/0	100	3.0	100		50	1.5	50	
10/1	66	2.5	33					
10/2.5	33	1.0						
10/5								
10/10								
10/15								
15/0	100	3.0	100					
15/1	50	2.5	25					
15/2.5	60	2.0						
15/5								
15/10								
15/15								

Table 5. *Episcia cupreata* var. Chocolate Soldier IAA/
2iP Series.

IAA/K	Blade				Lateral Stem			
	% Tubes Callusing	Average Callus	% Tubes		% Tubes Callusing	Average Callus	% Tubes	
			Roots	Shoots			Roots	Shoots
0/0								
0/1	80	2.3						
0/2.5	80	2.0						
0/5	40	1.0						
0/10								
0/15								
1/0	100	2.8	20		100	3.0	20	
1/1	50	1.0			100	2.8	40	
1/2.5	75	1.7	25					
1/5	20	1.4						
1/10	100	1.0						
1/15	75	1.0						
2.5/0	100	1.2	20					
2.5/1								
2.5/2.5								
2.5/5	20	1.0						
2.5/10								
2.5/15								
5/0	100	2.8	100		100	3.0	100	
5/1	100	3.0	80		100	2.8	20	
5/2.5	100	3.0	40		100	2.6		
5/5	100	3.0			60	2.7		
5/10	100	2.6			75	2.7		
5/15	100	2.8			75	1.3		
10/0	100	2.5	50		100	2.4	40	
10/1	100	2.8						
10/2.5	80	3.0						
10/5	80	1.8						
10/10	80	1.8			40	1.5		
10/15	80	1.8			25	1.0		
15/0	100	3.0	20		100	2.0	20	
15/1	100	3.0	20		100	1.5		
15/2.5	100	3.0			25	1.0		
15/5	100	3.0			40	1.0		
15/10	80	2.3			60	2.0		
15/15	100	1.0						

Table 6. *Episcia cupreata* var. Chocolate Soldier IAA/2iP/
Kinetin 1.0 mg/l Series.

IAA/K	Blade				Lateral Stem			
	% Tubes Callusing	Average Callus	% Tubes Roots	% Tubes Shoots	% Tubes Callusing	Average Callus	% Tubes Roots	% Tubes Shoots
0/0								
0/1								
0/2.5								
0/5								
0/10								
0/15								
1/0	60	1.8		10				
1/1	70	2.3		10				
1/2.5	50	1.8						
1/5	60	1.8						
1/10	50	1.0						
1/15	14	1.0						
2.5/0	100	2.6	20	10				
2.5/1	89	2.5						
2.5/2.5	70	2.0						
2.5/5	70	2.1						
2.5/10	63	1.0						
2.5/15	33	1.0						
5/0								
5/1								
5/2.5	25	1.0						
5/5	40	1.0						
5/10								
5/15	25	1.0						
10/0	20	3.0			40	1.0		
10/1	40	1.5			20	3.0		
10/2.5	25	2.0			20	3.0		
10/5	40	1.0			60	1.7		
10/10								
10/15								
15/0								
15/1	20	1.0						
15/2.5	40	3.0			60	1.8		
15/5	60	1.0			25	1.0		
15/10	60	2.0						
15/15	40	1.0						

Table 7. *Episcia cupreata* var. Silver Sheen IAA/Kinetin Series.

IAA/K	Blade				Lateral Stem			
	% Tubes Callusing	Average Callus	% Tubes Roots	% Tubes Shoots	% Tubes Callusing	Average Callus	% Tubes Roots	% Tubes Shoots
0/0	80	3.0	80	20	75	3.0	50	25
0/1								
0/2.5								
0/5								
0/10								
0/15								
1/0	20	2.0						
1/1	20	1.0						
1/2.5								
1/5								
1/10								
1/15								
2.5/0	25	3.0			100	2.6	40	20
2.5/1	25	2.0			50	1.0		
2.5/2.5								
2.5/5								
2.5/10								
2.5/15								
5/0	100	2.8	25		100	3.0	100	
5/1	100	1.4			100	2.3		
5/2.5								
5/5								
5/10								
5/15								
10/0	40	3.0	40					
10/1								
10/2.5								
10/5								
10/10								
10/15								
15/0	25	1.0			40	3.0	40	
15/1					20	3.0		
15/2.5					40	2.0		
15/5								
15/10								
15/15								

Table 8. *Episcia cupreata* var. Silver Sheen IAA/2iP Series.

IAA/K	Blade				Lateral Stem			
	% Tubes Callusing	Average Callus	% Tubes Roots	% Tubes Shoots	% Tubes Callusing	Average Callus	% Tubes Roots	% Tubes Shoots
0/0								
0/1	100	1.0						
0/2.5	100	1.6		20	50	2.0		
0/5	40	1.0						
0/10	40	2.0						
0/15	20	1.0						
1/0	100	3.0		80	80	3.0	20	
1/1	100	3.0		80	80	3.0		20
1/2.5	80	3.0		60	100	3.0		
1/5	60	3.0		40				
1/10	20	3.0			66	3.0		
1/15	60	2.0			100	3.0		
2.5/0	100	3.0			66	3.0		
2.5/1	100	3.0						
2.5/2.5	100	2.6			100	3.0		
2.5/5	100	2.8						
2.5/10	100	2.5			60	2.7		
2.5/15	80	2.8		20				
5/0	60	2.0	20					
5/1	80	3.0		20	40	3.0		
5/2.5	100	2.8			40	3.0		
5/5	60	2.3			40	3.0		
5/10	100	2.7			25	3.0		
5/15	100	3.0			25	3.0		
10/0	100	3.0			50	3.0		
10/1	75	3.0			40	3.0		
10/2.5	100	3.0			75	3.0		
10/5	100	3.0			40	3.0		
10/10	60	3.0			50	3.0		
10/15	100	2.8						
15/0	33	2.0	33		40	2.0	20	
15/1	100	2.8			40	3.0		
15/2.5	40	3.0	20		40	1.0		
15/5	100	2.0			20	3.0		
15/10	100	3.0			25	3.0		
15/15	100	3.0			50	3.0		

Table 9. *Episcia cupreata* var. Silver Sheen IAA/2iP/
Kinetin 1.0 mg/l Series.

IAA/K	Blade				Lateral Stem			
	% Tubes Callusing	Average Callus	% Tubes		% Tubes Callusing	Average Callus	% Tubes	
			Roots	Shoots			Roots	Shoots
0/0	25	1.0						
0/1								
0/2.5								
0/5								
0/10								
0/15								
1/0	100	2.4		25	50	3.0		
1/1	80	3.0	40	40				
1/2.5	100	3.0	100		75	3.0		
1/5	100	2.6	60		40	1.8		
1/10	80	3.0			20	3.0		
1/15	100	2.6	20		60	1.2		
2.5/0	80	3.0	20	20	50	3.0		
2.5/1	80	3.0	40	20	66	3.0	33	
2.5/2.5	100	3.0	60	40	80	2.8	20	
2.5/5	100	2.2			80	3.0		20
2.5/10	100	2.4			80	3.0		
2.5/15	100	2.8			75	3.0		
5/0	100	2.5			100	2.6		
5/1	100	3.0			100	2.8		
5/2.5	80	2.5			100	3.0		
5/5	100	3.0			100	3.0	20	
5/10	50	2.8			100	3.0		
5/15	80	2.3			100	3.0		
10/0	100	2.5			100	2.4		
10/1	100	3.0			100	2.6		
10/2.5	100	3.0	50		100	2.5		
10/5	80	3.0			100	2.8		
10/10	80	3.0			40	3.0		
10/15	100	2.4			60	3.0		
15/0	100	1.9			60	3.0		
15/1	80	1.5			40	3.0		
15/2.5	75	3.0			100	3.0	20	
15/5	25	3.0			75	3.0	25	
15/10	40	3.0			80	3.0		
15/15	100	2.4			80	3.0		

Table 10. Optimum level experiments.

Characteristics	E. dianthiflora		Chocolate Soldier		Silver Sheen	
	IAA 5.0 mg/l K 2.5 mg/l	IAA 1.0 mg/l K 0.0 mg/l	IAA 5.0 mg/l K 1.0 mg/l	IAA 1.0 mg/l 2iP 0.0 mg/l	IAA 1.0 mg/l 2iP 1.0 mg/l	
% Tubes with callus	0	97.5	60.0	72.5	100.0	
Average callus level per tube	0	2.8	2.4	2.1	3.0	
Callus level for subcultures	0	2.9	2.4	2.6	3.0	
Callus level for maintained cultures	0	2.7	2.3	1.7	3.0	
% Tubes with shoots	0	2.5	0	10.3	23.0	
% Subcultures with shoots	0	4.3	0	14.3	7.7	
% Maintained cultures with shoots	0	0	0	6.7	50.0	
% Tubes with roots	0	85.0	12.0	89.7	51.2	
% Subcultures with roots	0	95.7	6.7	100.0	57.7	
% Maintained cultures with roots	0	76.5	20.0	80.0	41.2	

VITA

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