7. Murashige, T. and F. Skoog. 1974. Plant propagation through tissue culture. Annu. Rev. Plant Physiol. 25:135–166.
8. Norstog, K. 1973. New synthetic medium for the culture of premature barley embryos. In Vitro 8:307–308.
9. Roberts, P. R. and H. J. Oosting. 1958. Responses of Venus Fly Trap *Dionaea muscipula* to factors involved in its endemism. Ecol. Monographs 28:193–218.
10. Rowland, J. T. 1975. Carnivorous seed plants: sources and references. HortScience 10:112–114.
11. Schwartz, R. 1974. Carnivorous plants. Praeger, New York.
12. Slack, A. 1980. Carnivorous plants. The MIT Press, Cambridge, Mass. p. 222–223.
13. Staba, E. J. 1969. Plant tissue culture as a technique for the phytochemist. Recent Adv. Phytochemistry 2:77–105.
14. Zimmerman, R. H. and O. C. Broome. 1980. Blueberry micropropagation. p. 44–47. In: Proc. Conf. Nursery Culture — Applications and Feasibility, April 21–22, 1980, Beltsville, Md., USDA, SEA, AR, NE-11.

J. Amer. Soc. Hort. Sci. 107(2):310–316. 1982.

Adventitious Bud Differentiation and Development in Leaf Cuttings of *Dionaea muscipula* Ellis Ex. L. (Venus Fly-trap) Cultured *in Vitro*[1]

Bruce J. Parliman,[2] Phillip T. Evans,[3] and Anthony R. Mazur[3]
Department of Horticulture, Clemson University, Clemson, SC 29631

Additional index words. tissue culture, Venus fly-trap, asexual propagation, 6-benzylamino purine, naphthaleneacetic acid, 6-(-γ,γ-dimethylallylamino)-purine

Abstract. **Excised whole leaves of *in vitro* cultured Venus fly-trap plants produced up to 15 adventitious bud- (AB) and lateral bud-derived (LB) plantlets in 60 to 90 days, when cultured on a modified Murashige and Skoog salt medium supplemented with naphthaleneacetic acid (NAA) at 1.9 mg/liter and 6(-γ,γ-dimethylallylamino)-purine (2iP) at 0.2 mg/liter and grown under a 16 hr photoperiod at 22°C. Leaves dipped for 10 seconds to 24 hr in various concentrations of 6-benzylamino purine (BA), 2iP, NAA and indolibutyric acid (IBA) improved the production of plantlets over undipped leaves. A 24 hr 2iP dip at 2.1 mg/liter produced the largest average number of adventitious and lateral bud-dervied plantlets. Lateral bud-derived plantlets are proposed to have arisen from nodes within the rhizomes of previously derived plantlets. Morphologically, the resulting chain-like growth habit of lateral bud-derived plantlets is very unusual and provides an increased number of plantlets for subcultures.**

Many herbaceous species (7, 9) are propagated from leaf cuttings (lamina or lamina and petiole) through adventitious bud formation. The Butterwort is commonly propagated by leaf cuttings and improved *in vitro* procedures have been reported (1). Explants of *Dionaea* have been propagated *in vitro* (4, 5, 19). Procedures for propagating *Dionaea* by leaf cuttings stuck in sphagnum moss have been described (25), but initial attempts to stimulate the development of adventitious buds on excised leaves grown *in vitro* were not successful (19). Cytokinins (7, 10, 16, 24), auxins (2, 27) abscissic acid (11), and temperature (10) have been found to stimulate adventitious bud production. Economou and Read (8) dipped leaves or leaf segments of several *Petunia hybrida* cultivars in BA solutions up to 800 mg/liter prior to culturing in a cytokinin-free medium and found significant increases in shoot numbers and ratings over nondipped leaves. To promote bud and shoot development on excised leaves of several Rieger begonia cultivars, Davies and Moser (7) used 12-hr petiole soaks and foliar sprays of 6-furfurylamino purine (kinetin), BA or 6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine (PBA) at 1.0 to 1000 μm/liter. In *Begonia* (21), *Lunaria annua* (20), and *Streptocarpus* (12), cytokinins alone had little effect on promoting adventitious buds and auxins either alone or in combination with cytokinins were needed. For *in vitro* cultures of *Hyacinthus orientalis* L. cv Pink Pearl (20) and several *Lilium* species (18, 27, 28), certain auxins have stimulated adventitious bulblet production on scales but cytokinins have had little stimulatory effect and only enhanced auxin activity when used at very low concentrations.

High concentration, auxin quick-dips have been used to promote the rooting of cuttings. As a growth regulator assay, Hitchcock and Zimmerman (14) quick-dipped leaf petioles in 1 to 8000 mg/liter of auxin. Hartman and Kester (9) described the use of a 5–sec stem dip of 500 to 10,000 mg/liter of auxin in a 50% alcohol solution. Ten second foliar sprays of 10,000 mg/liter of 3-indolebutyric acid (IBA) in a 50% alcohol solution (15) and 1000 to 3000 mg/liter of IBA (6) as foliar sprays have been used to promote rooting of woody cuttings and leaf cuttings of *Ficus pumila* L., respectively.

This investigation was conducted to identify optimum

[1]Received for publication July 2, 1981. Technical Contribution No. 1921, Agr. Expt. Sta., Clemson, SC 29631.

The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[2]Current Address: Research Horticulturist, USDA, SEA, AR, Plant Introduction Station, Glenn Dale, MD 20769.

[3]Graduate Research Assistant, Department of Agronomy and Soils, and Associate Professor of Horticulture, respectively.

[4]Trade names and company names are included for the benefit of the reader and do not infer any endorsement of preferential treatment of the product listed by the U.S. Department of Agriculture.

medium components, growth regulators, leaf dip periods and growth temperatures for stimulating rapid adventitious bud differentiation and shoot development in excised leaves of *Dionaea* cultured *in vitro*. The procedures are intended to provide an alternative source of large quantities of explants for *in vitro* culture, to develop a potential procedure for revitalizing continously-grown *in vtiro* cultures, and to provide an alternative system (19) for introducing *Dionaea* tissue into aseptic culture.

Materials and Methods

The basal culture medium consisted of half strength Murashige and Skoog (17) (MS) inorganic salts supplemented with Staba vitamins (26), 100 mg/liter myo-inositol, 100 mg/liter casein hydrolysate, 30 g/liter sucrose, 6.7 g/liter Difco Bacto Agar[4] and was adjusted to pH 4.9. All media were heated until agar dissolved and 20 to 30 ml were poured into 20 × 150 mm culture tubes. Cultures were covered with Bellco Kap-uts[4] and autoclaved for 16 minutes at 121°C at 1.3 kg/cm^2 (18 psi). Whole leaves were cut from rhizomes of parent stock plants grown *in vitro* at 23° to 26° for 60 to 90 days on basal medium supplemented with NAA at 1.9 mg/liter and 2iP at 0.2 mg/liter (19). Excised leaves were held in sterile, distilled, deionized water until placed in culture with the abaxial surfaces in contact with the medium. Cultures were grown under Cool White fluorescent bulbs (10 klx) on a 16-hr photoperiod at 8°, 15° or 20°.

In experiment 1 the basal medium was supplemented with a factorial combination of NAA at 0.02, 0.2, 1.9, 5.6, 12.1, or 18.6 mg/liter and 2iP at 0.06, 0.2, 0.6, 2.1 or 6.1 mg/liter. Only 7 ml of media were poured into 15 × 50 mm cultured tubes and capped with 20 × 20 mm glass tubes. Each treatment was repeated with six tubes each containing a single leaf. In experiments 2 through 5 the basal medium was supplemented with NAA at 1.9 mg and 2iP at 0.6 mg/liter. Half of the tubes in experiment 2 were also supplemented with 80 mg/liter adenine sulfate. Treatments in experiments 2, 3 and 4 were replicated with 24 leaves cultured in 12 tubes and experiment 5 was replicated with 40 leaves in 20 tubes.

In experiment 2 excised leaves were "quick-dipped" for 10 sec in concentrated solutions of BA or 2iP at 68, 270, 405, 607, 810, 1012 or 1400 mg/100 ml of 50% ethanol (9), a 2% dimethyl sulphoxide (DMSO) water solution or a water solution (3, 14). In experiment 3 excised leaves were dipped for 24 hr in BA at 0, 0.2, 0.7, 2.3, 6.8, 22.5, or 67.6 mg/liter of water or in 2iP at 0.2, 0.6, 2.0, 6.1, 20.3 or 61.0 mg/liter of water. Treatments were applied simultaneously and only one control was used.

Leaves from stock cultures were randomly placed across treatments and tubes were grown in a completely randomized block. Qualitative evaluations were made at 60 and 90 days for callus, root, rosette, leaf and plantlet production (volume and size) and for overall culture growth. Since *in vitro* rhizomes generally had two leaves, the number of plantlets per parent leaf was estimated by counting lamina. Characteristics were evaluated on a 0=dead, 1=poor to 9=excellent scale. Square root transformations were used on counted data and transformed means were converted for reporting by squaring.

Results

Whether from single or multiple cell origin, plantlets in this work were expected to be derived from leaves as independent adventitious buds (AB) (Fig. 1A and 1B). However, in mature cultures most plantlets were observed to arise as lateral bud-derived (LB) plantlets, which grew from initial AB plantlets (Fig. 1C and 1D) in long chain-like growth patterns. It is proposed that these LB plantlets develop from meristematic tissue at nodes within derived AB rhizomes rather than from adventitious buds. Regardless of origin, AB and LB plantlets consist of a single root and two leaves growing from a single rhizome. Anatomical and morphological studies are being conducted to determine the exact nature and origin of both the AB and LB plantlets but current data do not make a distinction (Fig. 1E and 1F).

The mean number of AB and LB plantlets and the overall mean rating for AB and LB production reflect treatment effects and only these data are presented. Evaluations conducted at 60 days reflected AB production while evaluations at 90 days reflected a large LB increase relative to all AB production. In experiments 2 through 5 leaves grown at 8° and 15°C often remained green throughout the duration of the tests but failed to produce significant numbers of plantlets. Only data from 60 day evaluations of cultures grown at 20° are presented beyond experiment 1.

Expt. 1. Media growth regulator supplements. After 60 days, excised leaves grown on a medium supplemented with NAA at 1.9 mg/liter produced the highest mean number of plantlets (Fig. 2A). Leaves on media supplemented with NAA at 0.2 or 5.6 mg/liter produced lower but similar mean values and leaves on media with other NAA rates produced considerably fewer plantlets. The highest overall ratings (Fig. 2B) were also found on a medium supplemented with NAA at 1.9 mg/liter. Leaves cultured in media supplemented with 2iP alone or in combination with NAA did not develop significantly increased plantlet production and the data are not presented.

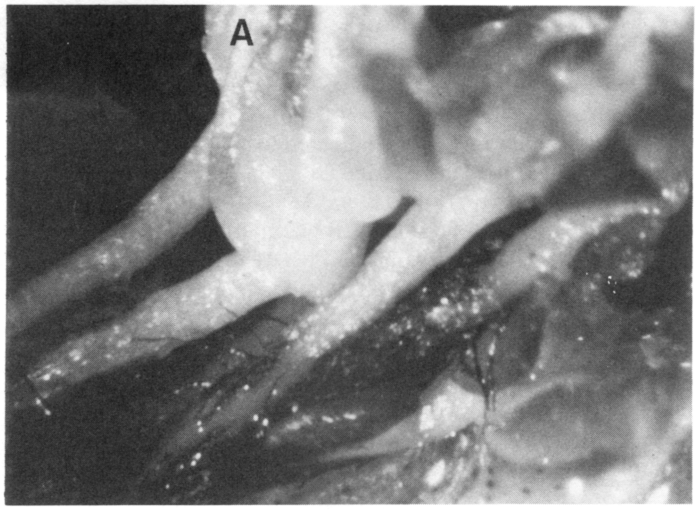
After 90 days plantlet production increased 5 to 10 fold over the 60 day values. When not used for subculture experiments (19), a few of the smallest cultures were allowed to grow for 120 days before being dissected. As many as 80 plantlets per excised leaf were found, which constitutes nearly a 20-fold increase over the 60 day values. At 60 days many AB plantlets were observed, but the rapid increases in the numbers of plantlets in a culture from 60 to 90 and 120 days reflect the initiation and growth of LB-derived rather than AB-derived plantlets.

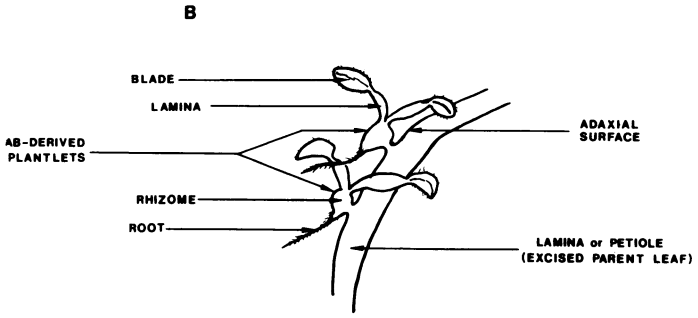
Expt. 2. Cytokinin quick-dips. No differences were found between 5 and 10 second dips. Leaves treated with high concentration, 10 sec 2iP dips resulted in higher overall AB- and LB-derived plantlet production ratings than with BA dips, but all quick-dips were generally ineffective in promoting large increases in AB- or LB-derived buds and no data are presented.

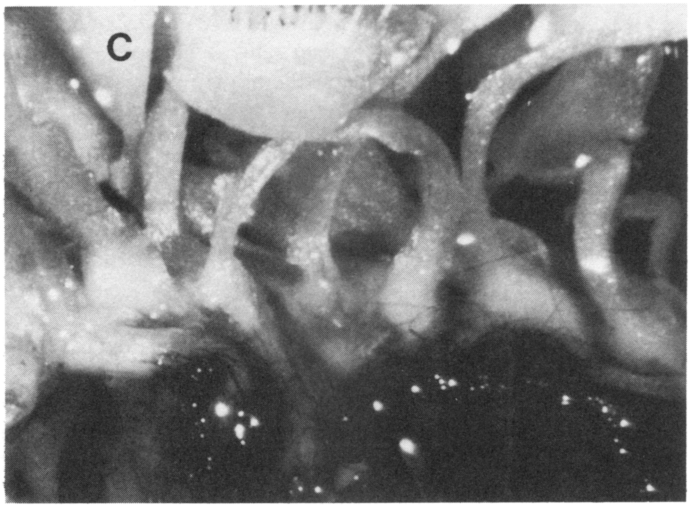
Expt. 3. 1 to 125 minute BA leaf dips. Leaves dipped for 5 and 25 minutes failed to produce significant numbers of plantlets. For 1 and 125 minute dips the means for BA at 0.0 and 6.8 mg/liter (Fig. 3A) and BA at 2.3 and 22.5 mg/liter (Fig. 3B) represented highest overall ratings, respectively. With the exception of the 1 min BA dip at 22.5 mg/liter, none of the experimental means were comparable to the means of either the media supplement treatments of Experiment 1 (Fig. 2B) or the 24 hr BA and 2iP dip treatments of Experiment 4 (Fig. 5).

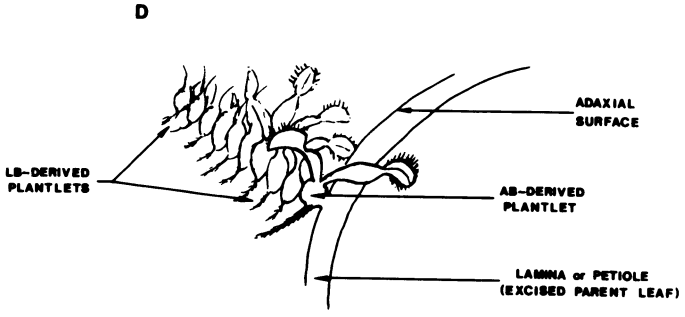
In the Fig. 4, 60 day means of the numbers of adventitious buds and plantlets are presented for leaves dipped in BA for 1 min. Means for 0.0 and 6.8 mg/liter BA dips were not different from each other, but the 6.8 mg/liter treatment was measurably larger than the other means. The BA at 6.8 mg/liter produced an average of 9.1 plantlets per leaf, which was about twice the optimum yield in Experiment 1 (Fig. 2A).

Expt. 4. 24 hour BA and 2iP leaf dips. Generally, BA treated leaves did not produce as many adventitious buds as did 2iP treated leaves (Fig. 5). The 2iP dips at 2.0 and 6.1 mg/liter were not different from each other but the 2.0 mg/liter mean was larger than all other ratings. Leaves treated with BA at 0.7 and 6.8 mg/









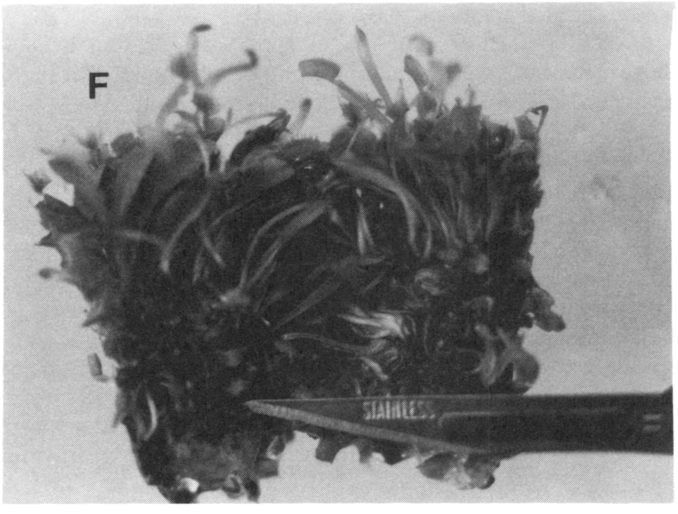


Fig. 1. Adventitious and lateral bud-derived plantlets arising from the adaxial surfaces of *in vitro* cultured leaves (Lamina or petiole) of *Dionaea*. (A) Photograph and (B) Schematic diagram of adventitious bud-derived plantlets arising directly from the adaxial surface of a leaf. (C) Photograph and (D) Schematic diagram of a long chain of lateral bud-derived plantlets. Individual plantlets have a single root and rhizome with one to two fully defined leaves. Depending on the age and number per culture, individual rhizomes ranged from approximately 1 to 3 mm in diameter. (E) One excised leaf at 60 days, surrounded by AB- and LB-derived plantlets. The parent leaf petiole width at its broadest point is approximately 7 to 8 mm. (F) Two excised leaves at 90 days surrounded by large numbers of AB and LB plantlets. Parental leaves are hidden or have degenerated. Roots appear as dark areas in the lower centers of each plantlet mass. The scalpel blade is 5 mm wide at its center.

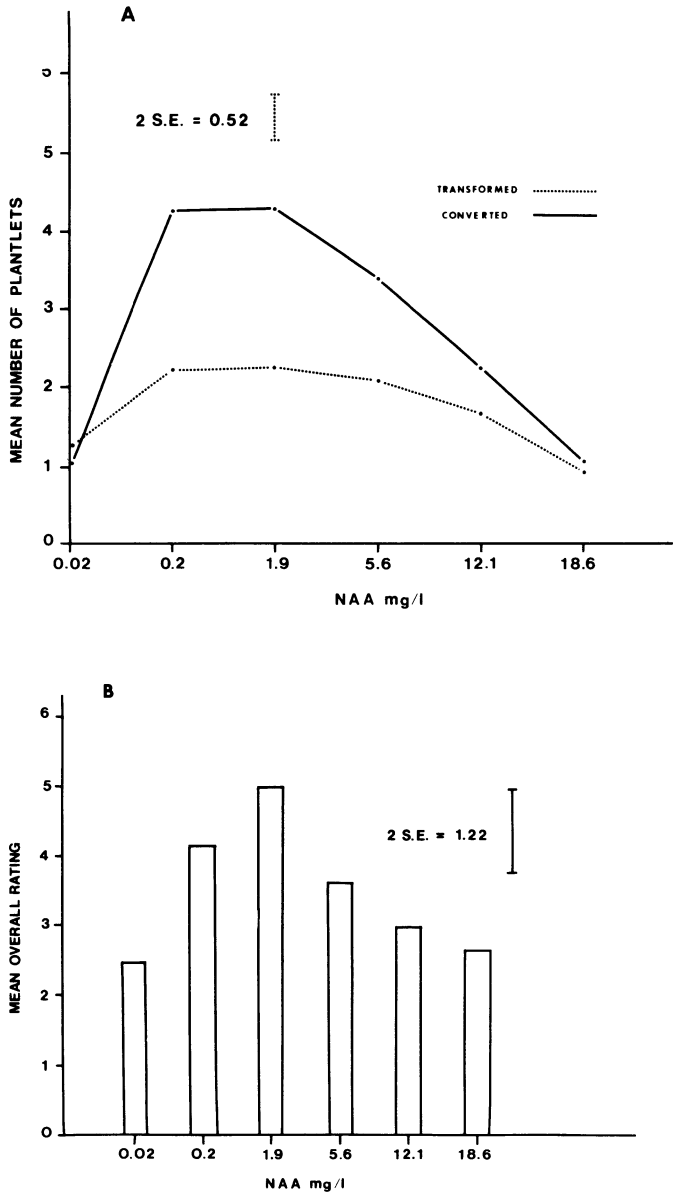


Fig. 2. Effects of supplemental media on excised leaves of *Dionaea muscipula* grown *in vitro* for 60 days at 20°C on modified 0.5 Murashige and Skoog medium and supplemented with six NAA concentrations. Means are calculated from 12 leaves cultured in 6 tubes.(A) The means of estimated numbers of single rhizome plantlets derived from adventitious and lateral bud origins. The standard error is calculated for transformed means and for reporting of means the transformed means are converted by squaring. (B) The mean overall ratings for adventitious and lateral bud-derived plantlet production and quality.

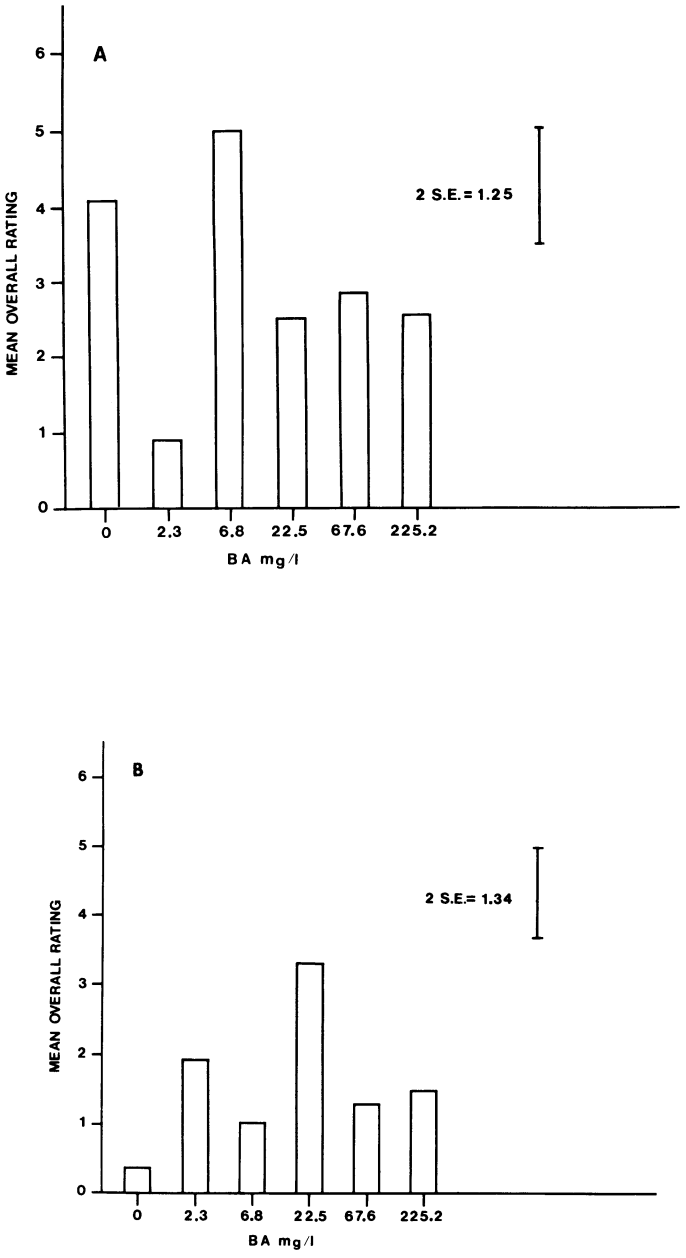


Fig. 3. Mean overall ratings of adventitious and lateral bud-derived plantlet production and quality for excised leaves of *Dionaea muscipula* dipped in varying concentrations of BA for (A) 1 minute, and (B) 125 minutes and grown *in vitro* for 60 days at 20°C on modified 0.5 Murashige and Skoog medium supplemented with NAA at 1.9 mg/liter and 2iP at 0.2 mg/liter. Means are calculated from 24 leaves cultured in 12 tubes.

liter and 2iP and 0.6 and 6.1 mg/liter produced similar but smaller overall ratings. With the exception of the BA at 0.2 mg/liter and the 2iP at 20.3 mg/liter, all other treatments produced significantly lower mean ratings.

In Fig. 6A and B the mean numbers of AB- and LB-derived plantlets are presented for leaves dipped in BA and 2iP for 24 hr. The means for leaves dipped in BA at 0.7 and 6.8 mg/liter (Fig. 6A) were not different from each other but the 0.7 mg/liter treatment produced more plantlets than other concentrations. The BA at 0.7 and 6.8 mg/liter treatments produced an average of 5.6 and 4.6 plantlets per leaf, respectively. These values are higher than the optimum means in Experiment 1 (Fig. 2A). The mean value of AB- and LB-derived plantlet production for 2iP at 2.1 mg/liter (Fig. 6B) was 15.1, which was larger than all other experimental mean values in this research. An average of 8.1 and 7.3 AB- and LB- plantlets were produced from leaves treated with 2iP at 0.6 and 6.1 mg/liter, respectively. These means were similar to each other but larger than any of the remaining treatments. Optimum production rates for 24 hr dip treatments were 2–3 times as large as those of Experiment 1 (Fig. 2A).

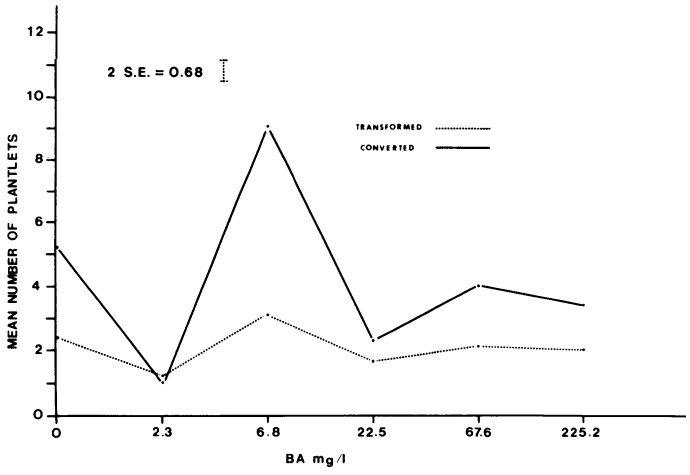


Fig. 4. The mean estimated numbers of single rhizome plantlets derived from adventitious and lateral bud origins. Excised leaves of *Dionaea muscipula* were dipped in varying concentrations of BA for one minute and grown *in vitro* for 60 days at 20°C on modified 0.5 Murashige and Skoog medium supplemented with NAA at 1.9 mg/liter and 2iP at 0.2 mg/liter. Means are calculated from 24 leaves cultured in 12 tubes. The standard error is calculated for transformed means and for reporting of means the transformed means are converted by squaring.

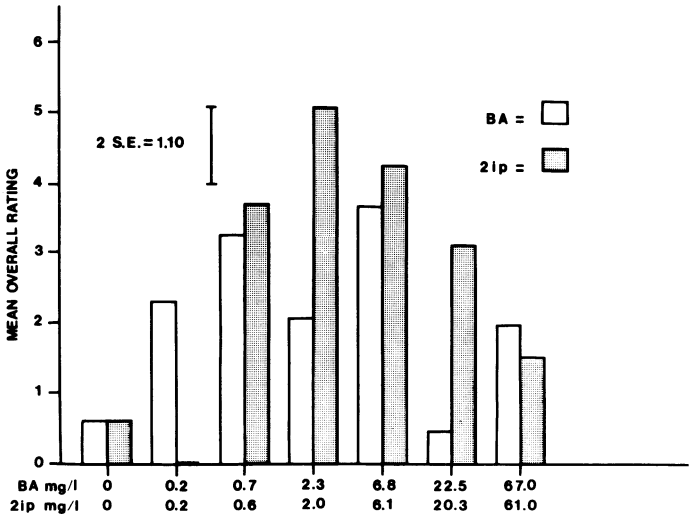


Fig. 5. The mean overall rating of adventitious and lateral bud-derived plantlet production and quality for excised leaves of *Dionaea muscipula* dipped in varying concentrations of BA and 2iP for 24 hours and grown *in vitro* for 60 days at 20°C on modified 0.5 Murashige and Skoog medium supplemented with NAA at 1.9 mg/liter and 2iP at 0.2 mg/liter. Means are calculated from 24 leaves cultured in 12 tubes.

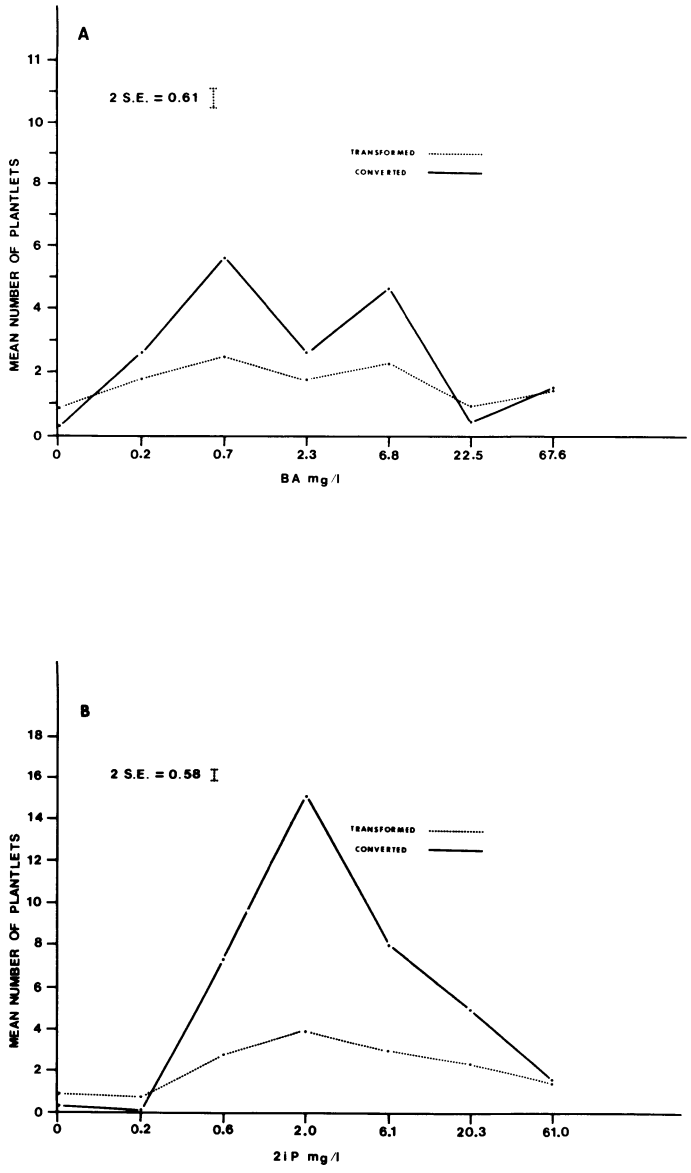


Fig. 6. The mean estimated number of single rhizome plantlets derived from adventitious and lateral bud origins. Excised leaves of *Dionaea muscipula* were dipped in varying concentrations of (A) BA and (B) 2iP for 24 hours and grown *in vitro* for 60 days at 20°C on modified 0.5 Murashige and Skoog medium supplemented with NAA at 1.9 mg/liter and 2iP at 0.2 mg/liter. Means are calculated from 24 leaves cultured in 12 tubes. The standard errors are calculated for transformed means and for reporting of means the transformed means are converted by squaring.

Discussion

When transferred to the plantlet growth medium of modified half-strength MS salts medium supplemented with NAA at 1.9 mg/liter and BA at 0.2 mg/liter (19), single AB- and LB-derived explants produced large cultures within 60 to 80 days. As compared to explants subcultured from the multiplication and growth medium, AB- and LB-derived explants took approximately 10 to 20 days longer to produce full grown cultures.

Excised *Dionaea* leaves produced the largest number of high quality AB- and LB-derived plantlets, when grown on a modified half-strength MS salt medium supplemented with NAA at 1.9 mg/liter and though of limited demonstrable value, 2iP at 0.2 mg/liter has been included. Except for the replacement of BA with 2iP, this is the same medium previously described for optimum plantlet growth (19). The similarities between these media can be interpreted in several ways: 1) adventitious bud production on excised

leaves can be stimulated by media modification; 2) adventitious bud production occurs regardless of the constitution of the medium and different media simply promote or retard initiation of LB-plantlets and the growth of both AB- and LB-derived plantlets; or 3) on an optimum medium excised parent leaves survive, grow and age physiologically to a point where they are capable of producing AB- and LB-derived plantlets. Theses possibilities could not be distinguished herein.

With the exception of the 1 min dip of BA at 6.8 mg/liter, leaves dipped in BA or 2iP for times ranging from 10 sec to 125 min (Expts. 2 and 3) generally did not respond to cytokinins by producing AB- or LB-derived plantlets. Problems with leaf burn, deformed growth, application difficulties and solution instability were common. Lower concentrations of BA or 2iP used in 24 hr dips remained in solution, were easier to accurately apply, and stimulated higher AB- and LB-derived plantlet production. Solutions of 2iP were found to be more active than BA in stimulating plantlet yield. The differential effectiveness of cytokinins in promoting adventitious buds on excised leaf tissue has been reported for many plants (7, 8, 16, 27).

Cool growth temperatures have been reported (2, 10, 16) to be effective in stimulating adventitious bud production on excised leaves of *Begonia, Streptocarpus*, and other plants, but this did not occur with bud production in *Dionaea*. Heide (10) hypothesized that lower growth temperatures reduced the auxin to cytokinin ratio to a point where adventitious buds in excised leaves would be promoted. However, in *Dionaea* leaves it appears that reduced adventitious bud production is due to slower metabolic processes in the leaf; leaves in cool temperatures simply did not grow.

Position, age, physiological condition, and scale segment origin have been identified as critical factors in stimulating adventitious bud production on leaves and scales of many lily species (18, 22, 28). It is hypothesized that age of excised leaves and culture longevity may be critical factors in the ability of *Dionaea* leaves to produce adventitious buds. Throughout this work it was observed that older outer leaves of parent cultures tended to produce more adventitious buds while large, young and rapidly expanding leaves tended to brown and die when excised. Also, small (under 7 to 10 mm in length), injured, or etiolated leaves and leaves without laminae rarely survived to produce adventitious buds.

Applegren and Heide (2) and Heide (12) working with *Streptocarpus* leaf discs, and Pierik and Steegmans (20) working with *Hyacinth* bulb segments, have found that auxins, not cytokinins (23), promote adventitious buds. These authors suggest that endogenous auxins and cytokinins are required for adventitious bud development, tissues stimulated by exogenous applications of auxins have a high and unlimiting supply of cytokinins, and that tissues stimulated by cytokinins have a high and unlimiting supply of auxins. In a preliminary test excised *Dionaea* leaves were dipped for 5 min in a factorial combination of BA at 1, 2.3, and 6.8 mg/liter and either NAA at 0.6, 1.9 and 5.6 mg/liter or IBA at 0.6, 2.0 and 6.1 mg/liter and grown in the modified half-strength MS medium for 60 days at 20°C. Very few AB- and LB-plantlets developed and parental leaves appeared to be burned by the auxin treatments. Those plantlets that did form were extremely small, being only 1 to 2 mm in height. The AB-derived plantlet production appeared to be stimulated while LB-plantlet production and growth was retarded. A limited number of AB-derived plantlets were formed along the basal cut surface of dipped leaves which is a phenomenon not seen in non-auxin treatments. A refinement of auxin application methods, types and concentrations may increase AB-and LB-derived plantlet production to the point where it may become a practical procedure.

With the exception of the 1 min BA control dip (Fig. 3A), none of the other control means in experiments 2 through 4 (Figs. 3B and 5) approached the values found in Experiment 1 (Fig. 2B). Experiment 1 was established using less media in smaller tubes than was used in Experiments 2 through 5. Over the 60 to 90 day growth periods, culture media in the smaller tubes were depleted while media volume remained nearly constant in larger tubes. These differences in media quantity and tube size may be the distinguishing factors between the means of Experiment 1 and those of the controls for Experiments 2 through 5 and may indicate new approaches to increasing adventitious bud production.

The 60 to 120 day propagation cycle required for these *in vitro* propagation procedures, coupled with the erratic behavior of excised leaves, make *Dionaea* leaf cuttings impractical for general multiplication purposes. Leaf cuttings may play a critical role in introducing specific *Dionaea* genotypes into aseptic cultures and in rejuvenating long-term *in vitro* cultures, but previously described *in vitro* procedures (19) increase populations at a faster rate.

Leaves of *Dionaea* appear to be excellent material for the study of adventitious bud differentiation and growth. The leaves do not initiate adventitious buds easily but do respond to specific treatments, large numbers of leaves can be tested over a short period of time, callus does not appear to be the origin of buds, and LB-derived plantlets are a relatively unique phenomenon. Further studies will be directed toward: increasing the frequency and speed with which AB-derived plantlets are produced, defining the physiological conditions of a receptive leaf, and describing anatomical and morphological origins and structures involved in AB- and LB-derived plantlet production.

Literature Cited

1. Adams, II, R. M., S. S. Koenigsburg, and R. W. Langhans. 1979. *In vitro* propagation of the Butterwort *Pinguicula moranensis* H. B. K. HortScience 14:701–702.
2. Appelgren, M. and O. M. Heide. 1972. Regeneration in *Streptocarpus* leaf discs and its regulation by temperature and growth substance. Physiol. Plant. 27:417–423.
3. Beatty, K. D., E. A. Rupert, and N. Dehgan. 1976. Doubling chromosome numbers of wheat rye amphiploids with colchicine, DMSO, and cold treatments. Wheat Information Service, Kihara Institute for Biological Research, Misima, Japan 43:10–12.
4. Beebe, J. D. 1980. Morphogenetic responses of seedlings and adventitious buds of the carnivorous plant *Dionaea muscipula* in aseptic culture. Bot. Gaz. 141(4):396–400.
5. Carroll, B. 1979. Propagating *Pinguicula lutea*. Carnivorous Plants Newsl. 8:58.
6. Davies, Jr., F. T. and J. N. Joiner. 1980. Growth regulator effects on adventitious root formation in leaf bud cuttings of juvenile and mature *Ficus pumila*. J. Amer. Soc. Hort. Sci. 105(1):91–95.
7. Davies, Jr., F. T. and B. C. Moser. 1980. Stimulation of bud and shoot development of Rieger *Begonia* leaf cuttings with cytokinins. J. Amer. Soc. Hort. Sci. 105:27-30.
8. Economou, A. S. and P. E. Read. 1980. Effect of benzyladenine pretreatments on shoot proliferation from petunia leaf segments cultured *in vitro*. Proc. Plant Growth Regulator Working Group. 7:96–103.
9. Hartmann, H. T. and D. E. Kester. 1975. Plant propagation, principles and practices, 3rd ed. Prentice Hall, Englewood Cliffs, N.J. p. 293–295.
10. Heide, O. M. 1965. Interaction of temperature, auxins, and kinins in the regeneration ability of *Begonia* leaf cuttings. Physiol. Plant. 18:891–919.
11. Heide, O. M. 1968. Stimulation of adventitious bud formation in

Begonia leaves by abscisic acid. Nature 219:960–961.
12. Heide, O. M. 1972. Regeneration of *Streptocarpus* leaf discs and its regulation by temperature and growth substances. Physiol. Plant. 27:417–423.
13. Hervás, J. P. and G. Giminéz-Martín. 1973. Dimethyl sulphoxide effect on division cells. Experientia 29(12):1540–1542.
14. Hitchcock, A. E. and P. W. Zimmerman. 1938. The use of green tissue test objects for determining the physiological activity of growth substances. Contrib. Boyce Thomp. Inst. 9:463–518.
15. McGuire, J. J., L. S. Albert, and V. G. Shutak. 1968. Effect of foliar applications of 3-indolebutyric acid on rooting of cuttings of ornamental plants. Proc. Amer. Soc. Hort. Sci. 93:699–704.
16. Murashige, T. 1974. Plant propagation through tissue cultures. Annu. Rev. Plant Physiol. 25:135–166.
17. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473–497.
18. Niimi, Y. and T. Onozawa. 1979. *In vitro* bulblet formation from leaf segments of lilies, especially *Lilium rubellum* Baker. Sci. Hort. 11:379–389.
19. Parliman, B. J., P. T. Evans, and E. A. Rupert. 1981. Tissue culture of single rhizome, adventitious bud- and lateral bud-derived explants of *Dionaea muscipula* Ellis ex. L., the Venus fly-trap, for rapid asexual propagation. J. Amer. Soc. Hort. Sci. 107:305–310.
20. Pierik, R. L. M. and H. H. M. Steegmans. 1975. Effects of auxins, cytokinins, gibberellins, abscisic acid and ethephon on regeneration and growth of bulblets on excised bulb scale segments of *Hyacinth*. Physiol. Plant. 34:14–17.
21. Ringe, F. and J. P. Nitsch. 1968. Conditions leading to flower formation on excised *Begonia* fragments cultured *in vitro*. Plant Cell Physiol. 9:639–652.
22. Robb, S. M. 1957. The culture of excised tissue from bulb scales of *Lilium speciosum* Thunb. J. Expt. Bot. 26:348–352.
23. Skoog, R. and C. O. Miller. 1957. Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. Symp. Soc. Expt. Biol. 11:118–131.
24. Skoog, R. and C. Tsui. 1948. Chemical control of growth and bud formation in tobacco stem segments and callus cultured *in vitro*. Amer. J. Bot. 35:782–787.
25. Slack, A. 1980. Carnivorous Plants. p. 222–223. The MIT Press, Cambridge, Mass.
26. Staba, E. J. 1969. Plant tissue culture as a technique for the phytochemist. *Recent Advances* in Phytochemistry 2:77–105.
27. Stimart, D. P. and P. D. Ascher. 1978. Tissue culture of bulb scale sections for asexual propagation of *Lilium longiflorum* Thunb. J. Amer. Soc. Hort. Sci. 103(2):182–184.
28. Takayama, S. and M. Misawa. 1980. Differentiation in *Lilium* bulbscales grown *in vitro*. Effects of activated charcoal, physiological age of bulbs and sucrose concentration on differentiation and scale leaf formation *in vitro*. Physiol. Plant. 28:121–125.

J. Amer. Soc. Hort. Sci. 107(2):316–319. 1982.

Seasonal Patterns in Chemical Composition of the Fruit of *Actinidia chinensis*[1]

Michael S. Reid,[2] David A. Heatherbell,[3] and Harlan K. Pratt[4, 5]

Plant Diseases Division, Department of Scientific and Industrial Research, Mt. Albert Research Centre, Auckland, New Zealand

Additional index words. Chinese gooseberry, kiwifruit, sugars, starch, organic acids, ascorbic acid, soluble solids, growth

Abstract. **Chinese gooseberry fruits or "kiwifruit" (*Actinidia chinensis* Planchon, cv. Bruno) were harvested for analysis at intervals throughout the season. On a fresh weight basis, immature fruit contained high concentrations of starch which was hydrolyzed after the fruit reached full size. Concurrently, there was a rapid increase in the concentrations of sucrose, glucose, and fructose, which were the major sugars present. This increase was reflected in a linear rise in soluble solids content of the fruit. Malic and quinic acid concentrations decreased during the early part of fruit growth, rose to a maximum after the fruit reached full size, and then declined slightly. The concentration of citric acid rose linearly during fruit growth, then fell gradually after the fruit reached full size. Ascorbic acid and amino nitrogen concentrations fell during the early part of fruit development, then remained relatively constant. The relationships between patterns of chemical change and the triple sigmoid growth curve of this fruit are discussed.**

The Chinese gooseberry or kiwifruit has become one of New Zealand's most important horticultural crops, second only to apples as an export earner. Schroeder and Fletcher (13) reviewed the history and horticulture of kiwifruit, and there have been a number of studies of anatomical and physiological changes during fruit development (6, 9, 11, 15) and of the chemical composition of the mature fruit (3, 5, 6, 8). Wright and Heatherbell (15) studied changes in some of the physio-chemical properties of the fruit during the later stages of development, and Heatherbell (8) has provided detailed information on the types and concentrations of sugars and non-volatile organic acids present in the mature fruit. Okuse and Ryugo (10) reported on compositional changes in the developing 'Hayward' cultivar from 3 weeks after full bloom until commercial harvest (27 weeks). Because kiwifruit may not attain eating ripeness until long after commercial harvest (11), we felt that studies of chemical changes in the fruit should be carried out over as long a period as possible. In this paper we re-

[1]Received for publication Jan. 22, 1981.

The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[2]Present address: Department of Environmental Horticulture, University of California, Davis, CA 95616.

[3]Present address: Department of Food Science, Oregon State University, Corvallis, OR 97331.

[4]On leave from: Department of Vegetable Crops, University of California, Davis, CA 95616.

[5]We thank Linda M. Withy and Carol A. Galvin for assistance with analytical procedures.