THE EFFECT OF N^6 BENZYL-ADENINE AND INDOLE BUTYRIC ACID ON THE PROPAGATION OF *PEPEROMIA ARGYREIA* CV 'WATERMELON' AND *P. CAPERATA* CV 'EMERALD RIPPLE'

by

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THE EFFECT OF N^6 BENZYL-ADENINE AND INDOLE BUTYRIC ACID ON THE PROPAGATION OF *PEPEROMIA ARGYREIA* CV 'WATERMELON' AND *P. CAPERATA* CV 'EMERALD RIPPLE'.

INTRODUCTION

Plantlets do not easily regenerate from leaf cuttings of certain species and cultivars of Peperomia and other herbaceous plants. Such plants are therefore usually propagated by means of tip (stem) cuttings from which it is relatively easy to obtain both shoots and roots. Plantlets originating from tip cuttings however frequently do not develop into an appealing final product as compared to plants obtained from leaf cuttings.

It is often necessary to use growth regulators to stimulate both root and shoot development on leaf cuttings of cultivars that do not easily form these organs. Various results have been obtained from the use of growth regulators. Results obtained depend on many factors.

The objectives of this study were to characterize the effects of N^6 benzyl-adenine (BA) and indole butyric acid (IBA) on shoot and root development of leaf cuttings of Peperomia argyreia cv. 'Watermelon' and P. caperata cv. 'Emerald Ripple' and to enhance shoot development of the former.

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LITERATURE REVIEW

Uses and Appearance of *Peperomia*

Peperomias, members of *Piperaceae*, are highly decorative foliage plants native chiefly of Tropical America (42).

These plants vary considerably in appearance thus offering a great diversity of leaf size, shape, color and texture. They are used mostly as indoor foliage plants or as ground covers for shady frostless spots (20,42). The plants are mostly dwarf and compact and rarely exceed 30 cm in height e.g. *P. caperata*. However some species such as *P. rotundifolia pilosor* have thread-like trailing stems and are well suited for hanging baskets.

Most common peperomias have ovate leaves, but some have cordate, peltate or lanceolate leaves. The leaf edges are entire, leaf surfaces can be smooth or with trichomes. The length of the leaf varies from 2 to 13 cm. Leaf color may vary from green or striped, marbled or margined with pale green, yellow, creamy-white, red or gray, and the petioles of some kinds are red or pinkish (45).

The flower stalk inconspicuous in many forms appears as a rattail like spike. Most flowers are odorless; *P. resedeflora*, however is known for its fragrant white blossoms (45).

Propagation

Methods of Propagation

Various methods are utilized in the vegetative propagation of Peperomia. Among these are tissue culture, leaf cuttings, leaf discs and tip cuttings.

Some species easily regenerate plantlets in tissue culture while others do not. For instance P. scandens and P. longifolia are reported to be easily propagated by means of tissue culture (14,24), while P. eburnea is reported to have produced only callus and roots (24). Peperomia caperata is also reported to have regenerated plantlets in vitro (5). The medium utilized in most cases contains, among other substances, auxins and cytokinins. Ginseng powder was utilized to obtain consistent regeneration of plantlets of P. viridis (20). Leaf squares were cultured on a Murashige and Skoog's medium supplemented with 2 mg/ℓ kinetin and 1 mg/ℓ naphathaleneacetic acid. The addition of 100-500 mg/ℓ of raw ginseng powder increased shoots per explant and improved the quality of the regenerated plantlets.

The leaf cutting is probably the most common method of propagation among most members of this genus (8). This method is utilized for those species that do not have chimeras. In one experiment adventitious shoots were induced on leaves, leaf pieces and shoot axes of three cultivars each of two types of P. obtusifolia, P. glabella and P. scandens chimeras with green and white leaves (4). The white-green

variegations were not initiated by genes forming genetically uniform cell material but originated from genetic differences existing in the shoot apex in the apical layers. This indicates that to regenerate variegated types of Peperomia, the leaf cutting must possess both the genetic ability and inability to form chlorophyll. Other than tissue culture methods, propagation by leaf cutting supplies the most plants from the least propagating material (7).

Some studies have been done to compare the standard leaf-plus-petiole cuttings with leaf sections of P. hederafolia. The rates of root and shoot development and leaf production, 5 weeks after cutting insertion, differed little between the 4 leaf-plus-petiole types compared (in 1, 2, 3 or 4 cm of petiole was retained)(3). However, with the leaf sections, those in which almost all or half the area was retained (the petiole and leaf base being removed) produced considerably more shoots and longer roots than any of the leaf plus petiole cuttings. Leaf production after potting was markedly greater for leaf cuttings of the two largest sizes than for the leaf-plus-petiole cuttings.

Leaf discs can be utilized in the propagation of Peperomia. The greatest advantage of this method is the numerous plantlets that can be obtained from one leaf (18). Leaf disks can be cultured in petri dishes or similar covered containers and thus require no special propagating facilities. Numerous disks can be made to fit into a small area thus extensive

propagating space is not essential. Less frequent watering is required by using covered Petri dishes (25).

The regeneration potential of a leaf disc depends upon the length and diameter of the leaf vein (32). Maximum leaf surface should be left on cuttings for fastest and best rooting response since any reduction on leaf surface is detrimental to the rooting process. Leaves provide carbohydrates and hormones essential for root development (22).

Tip cuttings are a popular way of producing Dracaena, Aglaonema, Peperomia, Dieffenbachia and many others (7). They have been observed to produce desirable plants in a limited time period. The disadvantages of this method are the reduced number of cuttings that can be obtained from a stock plant and the final product is not as attractive as plants produced from other methods, such as leaf cuttings.

Factors Influencing Vegetative Propagation of Peperomia

The effects of selected growth regulators such as cytokinins and auxins on the propagation of Peperomia argyreia and P. caperata and possible mechanism of action of these growth regulators will be reviewed. Environmental factors will also be reviewed because these factors are known to influence the propagation of all plants in general and the effectiveness of growth regulators is dependent on them.

Growth Regulators

Growth regulators have, in most cases, a strong influence on propagation of Peperomia and other herbaceous plants. However, some cases have been reported where no benefit or only partial benefit was achieved from the use of GRs. The effect of GRs on plant propagation seems to be species dependent (9,17,24). The two most commonly used growth regulators in enhancing plant regeneration are the auxins and cytokinins. There is nearly always a relationship and interaction between auxin and cytokinins in propagation of plants. For clarity, their effects will be reviewed separately.

Auxins

'Auxins' are defined as "organic substances which at low concentrations (< 0.001 M) promote growth (cell enlargement) along the longitudinal axis, when applied to shoots of plants freed as far as practical from their own inherent growth promoting substance, and inhibit the elongation of roots" (40). It is used also as a generic term for compounds characterized by their capacity to induce elongation and other processes in a manner similar to indole-3-acetic acid (29).

Various results have been obtained from the application of synthetic auxins in propagation of Peperomia. Treatment of cuttings of P. hederafolia with 0.2% IBA increased root and shoot development (3). The influence of two commercial growth substances, namely Hormodin 2[a] and Patio[b], on the

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[a]Hormodin 2: 0.3% IBA

[b]Patio: 0.07% IBA, 0.05% NAACT (naphaleneacetimide), 0.05% NAA, 2.0% captan and 2.0% thiram

rooting of several foliage plants including Peperomia 'Marble' and P. 'Variegate' was investigated (1). The effect on rooting was scored as 1 for roots and 3 for heavy rooting. Peperomia 'Marble' had scores of 2.5 for both the control plants and ones treated with Hormodin 2 and 2.7 for Patio. Peperomia 'Variegate' had scores of 2.0 for the control and for both Hormodin 2 and Patio.

In another experiment, the interaction of 4 Chlrophenoxy-isobutyric Acid (PCIBA) and IBA on root and bud formation of Peperomia caperata leaf cuttings was evaluated (38). Both of these substances promoted root and shoot formation. However, PCPIBA was less effective than IBA at the same concentrations in producing roots and shoots. It was observed in this study that even though auxin is known to inhibit bud formation, low concentrations of IBA and PCPIBA both promoted bud formation. This increase in bud formation was thought to be an indirect rather than direct effect since all cuttings that had large numbers of buds also had large numbers of roots, and buds always appeared after roots were formed. Roots are said to be important sites of cytokinin synthesis (23); large numbers of roots might lead to higher cytokinin levels which in turn would promote bud formation.

Auxins are generally used to stimulate rooting during propagation. Reviews regarding the role of auxins in root development are available (36,40). It is now well accepted and has been confirmed many times that endogenous or exogenous auxin is essential for initiation of adventitious roots.

Basically two hypotheses have been proposed to explain the mechanism of action of auxins. In the first hypothesis auxins are said to affect gene activity and in the second membrane permeability (29).

Like in any other hormone, these mechanisms of action, are said to involve some form of amplification of the initial triggering response. This conclusion was made because hormones are active in extremely small concentrations.

Cytokinins

Cytokinins are naturally occurring or synthetic substances that induce cell division in certain excised plant tissues in the presence of auxin (40). Chemically, cytokinins are said to be N^6 substituted adenine types of compounds and close analogs (29).

Cytokinins are generally closely associated with bud initiation (9,31,36). Relatively high concentrations of cytokinins together with low concentrations of auxins are said to promote bud initiation whereas low concentrations of cytokinins and high concentrations of auxin favor root initiation (18). Various results have been obtained from the use of cytokinins on propagation of Peperomia and other herbaceous plants. Benzyl adenine is reported to have prevented rooting of leaf cutting of P. griseo-argenta Yuncker thus impairing the regenerative process (32). In another study five concentrations of N^6 benzyl-adenine (BA)(0, 25, 50, 100 and 200 ppm) were sprayed to leaf cuttings of P.

polybotrya after sufficient rooting had taken place. Plantlet production was found to be greatest at the 25 and 50 ppm concentrations of BA (8). It was concluded that low concentrations of BA could increase plantlet production. Kinetin and BA however inhibited initiation of roots and buds on leaf squares of P. sandersii (17). This was in contrast to the effect of these two compounds on leaf squares of Begonia rex where rooting was similarly inhibited but bud formation was markedly promoted.

There is not much literature available relating to the use of cytokinins in the propagation of Peperomia or specifically on the stimulation of bud and shoot development of this genus. However considerable amount of work has been done with other herbaceous species (9,33,44).

For instance a study was carried out (9) to characterize the effects of three types of cytokinins namely 6-furturylamino purine (kinetin), 6-benzylaminopurine (BA) and 6-(benzylamine)-9-(2-tetrahydropyranyl)-9H-purine (PBA) on leaf cuttings of Rieger elatoir begonias (Begonia bertini 'compacti' x B. socotrana cvs Aphrodite Cherry Red and Schwabenland Red). In 'Aphrodite Cherry Red' BA and PBA enhanced bud and shoot regeneration while kinetin showed no activity. All the cytokinins tested reduced shoot development in 'Schwabenland Red'. Once optimum shoot development was reached, further increased PBA reduced bud and shoot weight. PBA has been observed to be the most active cytokinin (19,35,44) on bud regeneration

of leaf cuttings. The structure of PBA is similar to that of BA except that the hydrogen on nitrogen 9 is replaced by a nonpolar ring structure of tetrahydropyran (43). The activity of cytokinins is said to vary with length, degree of unsaturation and substitutions on the side chain (29).

Cytokinins in general have many physiological effects on seed plants. Some of the striking effects as related to excised tissues is the induction of cell division, regulation of differentiation and delay of leaf senescence (29,43).

The role of cytokinins in differentiation and morphogenesis was demonstrated very well with tobacco callus tissue (37). Cytokinins are required for both initiation and continuation of cell division (43). The availability of cytokinins has made possible the culturing of many tissues. Some of these tissues grow actively in vitro with the addition of only a few organic compounds as well as cytokinin.

The other dramatic effect of cytokinin and one which may have direct consequence on propagation of leaf cuttings is delay of senescence in plant tissues. The most prominent changes that occur in the senescent leaf are declines in the proteins and nucleic acids and an irreversible yellowing due to loss of chlorophyll (29).

The effect of cytokinins in delaying senescence is a result of nutrient mobilization to areas treated with a cytokinin. This was demonstrated by (30) applying radioactive amino acids and nonradioactive kinetin to leaves. It was

found that radioactive amino acids migrated and accumulated in the kinetin treated parts of the leaves.

It is appropriate to mention at this point that applied cytokinins have been observed to be rather immobile.

The mechanism of action of cytokinins is not yet well known. Many tRNA molecules are however known to contain a cytokinin at their anticodon (15). These cytokinin moieties are therefore thought to have an important regulatory function for tRNA in the process of protein synthesis at the translational level. It is however not known how this regulatory function is achieved. It is thought it may be by preventing a wrong set of three nucleotides from being recognized by a codon.

In another experiment, the effect of BA on the fixation of carbon dioxide in barley leaves was studied (34). This work probably gives some possible biochemical explanation of BA induced "toxicity". Most of the information found in the literature about the mechanism of action of BA gives only biochemical explanations of how BA may enhance growth and not how it could inhibit it.

Environmental Factors

Light. The influence of the intensity, day length, and wavelength of light on plant propagation are well documented (10,18,22,32). Light is required for the propagation of leafy cuttings. It is essential for the production of carbohydrates and auxins (22). Growth of Peperomia is reported

to be optimum under production at in the range of 20-30 klux (6,22).

Studies on the regeneration of P. griseo-argenta (32) indicated that regeneration was superior under continuous illumination. Response under short periods of illumination or darkness was inferior. Regeneration in light of low intensity was found to be slight. In another experiment, uninterrupted illumination extended the life span of leaf cuttings of P. magnoliaefolia and that of seven other horticultural plants (46).

The effect of daylength on propagation seems to be dependent upon the species (18). Short or long days can either enhance or inhibit root and shoot initiation. Furthermore, photoperiod may have no effect on plant regeneration. This was actually found to be true for Peperomia griseo-argenta (32).

Red light is reported to have enhanced the regenerative response whereas infra-red light inhibited it. (32). The effects observed with red light suggest that the phytochrome system may be involved in the regeneration of Peperomia leaf cuttings.

Temperature. Day air temperatures of 25° to 35°C with night temperatures of 22°C are said to be in the best range for the propagation of most foliage plants (22). In fact, most of the papers reviewed reported temperatures within this range. For example, root grade[C] quality of P. obtusifolia was

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[C]Root grade: 1, no roots; 3, heavy rooting.

optimum (3.0) in media maintained at 25°C, 2.3 at temperatures of 15-25°C, and 2.1 at 30°C (2). However, the optimum temperature for rooting of leaf cuttings of P. griseo-argenta Y. was reported as being between 15° and 25°C (32).

An interaction of temperature and growth regulators was observed in Begonia leaf cuttings (19). High temperature by itself, suppressed bud formation, antagonized the promotive effect of cytokinins on this process and the inhibitory effect of the same growth regulator on root formation. The auxin effect, which was opposite to that of cytokins, was enhanced by high temperatures. However, it is reported elsewhere (18) that excessively high air temperatures tend to promote bud development in advance of root development.

Humidity. An intermittent mist water spray over the cuttings in the rooting bed is said to be very effective aid in rooting of leafy cuttings of many kinds of plants (18). Such sprays provide a film of water over the leaves and cuttings thus lowering their temperature and increasing the humidity around the leaves. This reduces both transpiration and respiration rate of the leaves.

The primary function of mist is to provide a thin, continuous film of water over the entire cutting surface (22). It is therefore recommended that the mist systems should operate the minimum time required to maintain this water film. Interval timers are usually used to provide minimum cycles of mist while the system is on to reduce water usage and

leaching of nutrients from foliage (22). It is further recommended that mist systems should be adjusted for air movement, temperature, humidity and light intensity to prevent desiccation and leaching of the foliage.

Adequate moisture in the media is essential for optimum root growth.

Propagating media. Several kinds of rooting media can be utilized in the propagation of Peperomia and other herbaceous plants. An ideal rooting medium is said to be one that provides sufficient porosity to allow good aeration and has a high water holding capacity and yet is well drained and free of harmful fungi and bacteria (18,22). It must be firm enough to support cuttings without deep sticking of cuttings and of a consistency to allow cuttings to be easily removed after development of root systems (22). This is of particular importance in research when data is taken on root development.

The rooting medium is said to affect the type of root system arising from cuttings (18). For instance, cuttings when rooted in sand produce long unbranched coarse and brittle roots and when rooted in a mixture such as sand and peat moss or perlite and peat moss develop roots that are well branched, slender and flexible, a type more suited for digging and repotting.

It is advisable that the media have a water holding capacity of 50 to 75 percent so that frequent irrigations will not be necessary when a mist system is not used. The water conductivity should at least be 10 cm/hr when cuttings are under

mist or watered heavily so it will not become saturated. Aeration is also important and it is recommended that the medium have 15 to 25 percent pore space to allow water flow and ensure gas exchange for an ample oxygen supply around root surfaces (22).

The pH level of the media should be between 5.5 and 6.5 for satisfactory rooting of most foliage plants (18).

Carbon Dioxide

Photosynthetic rates of cuttings, during the rooting period, are reported to be relatively low and current photosynthate supply may limit adventitious root formation on some cuttings (10,13). Under normal propagation CO_2 enrichment should increase photosynthetic rates and hence the supply of photosynthate in cuttings. There is evidence that the rooting percentage, number of roots per cutting or root weight per cutting of several species is promoted by CO_2 enrichment (10,16,27,28). However rooting of some species or varieties does not respond to high CO_2 (27).

In one study CO_2 enrichment (1200 ul CO_2/liter air) during rooting increased the number of roots per cutting from 7.4 to 12.0 in Peperomia glabella A. Dietr. 'Variegata'. CO_2 enrichment increased length and weight of root systems and fresh and dry weight of whole cuttings in P. glabella Fuchsia magellanica Lam., Peperomia nivalis Mig., Hemigraphis alternate T. Anderson, and Begonia x argenteo-guttata V. Lemoine but not in Osmnthus heterophyllus P.S. Green

'Rotundifolills', Ficus pumila L., and Pelargonium x hortorum L.H. Bailey 'Sprinter Scarlet'. After four weeks of growing at 330 ul Co_2/liter, only P. nivalis retained the size differential due to CO_2 enrichment (12).

Generally it is recommended that CO_2 concentrations of up to 1500 ppm will increase plant growth and decrease the time required to propagate foliage plants (22). Atmospheric content of CO_2 is normally 300 parts per million, but in closed greenhouses the level may drop to 100 ppm or less during daylight hours. CO_2 addition is therefore only helpful during daylight hours when vents are closed and temperatures are optimum (22).

Spacing

Spacing of cuttings varies with size and type of plant material and size of root ball to be produced. Single eye cuttings with leaves such as those taken for Philodendron should be spaced approximately 5 x 5 cm (7). Small tip cuttings should also be stuck at that spacing. Larger tip cutting, such as some Dieffenbachias should be placed about 8 x 8 cm. Sufficient space should be maintained between plants so that light will be available to leaves and to permit air movement between plants. Sufficient space should also be allowed so rooted cuttings can be removed without damaging roots. This is especially important for research purposes when data is to be collected on root development.

Other Factors

Other factors such as age of leaf, condition of stock plant, time of year cuttings are obtained have often been found to influence plant regeneration in most of the literature reviewed. These factors were considered when conducting the experiments in this project.

MATERIALS AND METHODS

Experiment #1

Growth Regulators

In experiment one, N^6 benzyl-adenine (BA) and indole butyric acid (IBA) were used in combination and singly to induce shoots and roots in Peperomia argyreia cv 'Watermelon' and P. caperata cv 'Emerald Ripple' leaf cuttings. Crystalline forms of BA and IBA were dissolved in 10 ml of 95% ethyl alcohol and the volume made up to 1 litre with distilled water. Five concentrations of each growth regulator including the control were prepared. These were 0, 25, 50, 100 and 200 ppm. Each concentration of BA was combined with each concentration of IBA resulting in twenty-five combinations. These twenty-five treatments of growth regulator were then applied to each cultivar.

The growth regulators were applied using a dilute soak method. Leaf cuttings were allowed to remain in solution for a period of 24 hours prior to planting. This was done in the greenhouse where conditions were optimum for a slow and steady uptake of growth regulator. The average room temperature was 20°C with an average relative humidity of 70%. The greenhouse was white washed to provide shady conditions.

Plant Materials

Two cultivars of Peperomia namely P. argyreia cv 'Watermelon' and P. caperata cv 'Emerald Ripple' were selected for the experiment. These cultivars were selected because they are frequently difficult to propagate. Four centimeter leaf and petiole cuttings were taken from the stock plants in the month of August.

Environmental Conditions

Light. A 75% shade black polyethelene material Lumite fabric was installed over the propagating bench to provide a light intensity of about 30 klux. Light intensity was monitored by use of a YSI Model 654 radiometer.

Temperature. Average night and day temperatures were in the range of 20° to 27°C throughout the experiment. The temperatures were recorded by use of a Cole Palmer hydrothermometer, model 836800. The temperature in the greenhouse was controlled by a Groton T.M. climate controller.

Humidity. A 5 second/6 minute cycle mist was applied to the leaf cuttings during daylight hours until sufficient rooting had taken place. The duration of the misting cycle was adjusted for air movement, temperature, humidity and light intensity. This was done to prevent unnecessary spoiling of leaves.

Propagating Media

A 1:1 peat and perlite mixture amended with 2.04 kg of dolomite per 0.056 cubic meters of media was used for rooting

the cuttings. The media was drenched with a fungicide (benlate) before inserting the cuttings. The cuttings were planted in flats containing the media at a spacing of 5 x 5 cm at a depth of 2 cm.

Experimental Design and Data Collected

A randomized complete block design was used for the experiment. This had 3 replications with 4 leaf cuttings per treatment. Each treatment was therefore a mean of 12 observations.

After twelve weeks data was collected on shoot and root development of the leaf cuttings. The number of shoots, buds and their fresh weight grams per cutting was used as a measure of shoot development. Root development was evaluated as total root length, root, root index and root dry weight in grams per cutting. The total root length was estimated using a modified line intersect method (39) where:

$$\text{Root Length (R)} = \frac{11}{14} \times \text{Number of intercepts (N)} \times \text{Grid Unit.}$$

Root index was a visual ranking, 0 to 5, based on degree of root development (26).

Experiment #2

In experiment two, some modifications were made in the procedure. These modifications were based on the results obtained in experiment one. The following modifications were made in procedure.

Growth Regulator

From experiment one we inferred that there was little if any benefit from using IBA to increase rooting in 'Watermelon' leaf cuttings. Therefore BA alone was used to enhance shoot development. The growth regulator was applied with a talc carrier. BA was measured, brought into solution with 95% ethyl alcohol and weighed talc added. The slurry was mixed until it became a dry powder (9). Six concentrations of BA including the control were used. These were 0%, 0.003%, 0.01%, 0.3%, 0.6% and 1.0% BA. The petioles were dipped into the BA talc mixture and planted. After ten weeks half of the experiment was sprayed, until run off, with a 100 ppm BA foliar spray. The spray was made by dissolving 100 mg of BA in 20% ethyl alcohol and 0.3 ml of 0.5% Tween 20 as surfactant.

Light

From experiment one, the mean light intensity during the course of the experiment was about 27 klux. This was lower than we had planned it to be i.e. 30 klux. We therefore substituted a 75% shade polyethelene material for a 50% shade.

Plant Materials

The leaf cuttings for the second experiment were taken in Spring (May 24th) from the same stock plants as in the first experiment.

Fresh media, mixed in the same proportions and amended with dolomite as in experiment one, was used. The rest of the experimental conditions remained similar to the previous experiment.

A completely randomized block was utilized with 3 replications of 6 cuttings each. The mean values per cutting therefore reflected an average of 18 cuttings.

The experiment was conducted for 14 weeks after which data were collected. Shoot development was evaluated as in experiment one.

RESULTS

It is well known that various cultivars and species of plants respond differently to specific growth regulators. It was interesting to note that with regard to shoot and root development the two cultivars were quite dissimilar.

Experiment #1

Shoot Development

The results of experiment one showed that there was a significant difference between the cultivars in the mean number of shoots and buds per cutting and fresh weight of buds per cutting (Table 1). P. caperata cv 'Emerald Ripple' had both a higher mean number of shoots per cutting and fresh weight of shoots per cutting than P. argyreia cv 'Watermelon' for most of the treatment combinations.

Table 1. Cultivar differences in shoot development.

Cultivar	Shoot Development	
	Mean number of shoots and buds/cutting	Mean freshweight/ cutting (g)
'Emerald Ripple'	2.59* a	0.12 a
'Watermelon'	0.97 b	0.03 b

*Mean separation by Duncan Multiple Range test, 5%.

The cultivar 'Emerald Ripple' initiated shoots much earlier than 'Watermelon'. The shoots in the former were visible in some of the treatments 6 weeks after planting. At 10 weeks 40% of all 'Emerald Ripple' treatments had visible shoots while only 6% of all 'Watermelon' treatments had shoots. 'Emerald' shoots were generally more developed in size than 'Watermelon' shoots.

The Effect of Growth Regulators on Shoot Development

The effect of growth regulators on shoot development was analyzed in such way as to enable us to characterize the effect of N^6 benzyladenine (BA) on shoot development at various levels of indole butyric acid (IBA) (Table 2 and Fig. 1).

The effect of BA on shoot development of the two cultivars at various IBA levels, was statistically significant only when evaluated as mean freshweight per cutting in grams. The two cultivars of Peperomia responded very differently to the growth regulators.

The cultivar 'Emerald Ripple' generally showed an increase in mean shoot freshweight per cutting with increasing concentrations of BA at all levels of IBA except at 25 ppm IBA and when no IBA was applied (Fig. 1). The optimum combined treatment which gave the best results for this cultivar was a concentration of 100 ppm BA and 100 pm IBA. In fact the best overall result seemed to have been at this same

Table 2. Effect of BA on shoot weight/cutting at various levels of IBA.

BA(ppm)	'Watermelon' shootweight/cutting(g)	'Emerald Ripple' shootweight/cutting(g)
	IBA 0 ppm	
0(control)	0.017 a*	0.195 b
25	0.082 ab	0.167 ab
50	0.007 a	0.156 ab
100	0.162 b	0.079 a
200	0.025 a	0.094 a
	IBA 25 ppm	
0	0.089 a	0.141 b
25	0.029 a	0.056 a
50	0.016 a	0.055 a
100	0.026 a	0.037 a
200	0.040 a	0.151 b
	IBA 50 ppm	
0	0.000 a	0.116 ab
25	0.002 a	0.052 a
50	0.023 a	0.158 b
100	0.0003 a	0.062 a
200	0.077 a	0.042 a
	IBA 100 ppm	
0	0.016 a	0.101 ab
25	0.005 a	0.025 a
50	0.010 a	0.134 b
100	0.074 a	0.183 b
200	0.005 a	0.133 b
	IBA 200 ppm	
0	0.005 a	0.246 c
25	0.010 a	0.05 a
50	0.035 a	0.144 ab
100	0.012 a	0.145 ab
200	0.030 a	0.177 bc

*Mean separation by Duncan Multiple Range test, 5%.

Fig. 1. Effect of BA on bud and shootweight/cutting at various levels of IBA.

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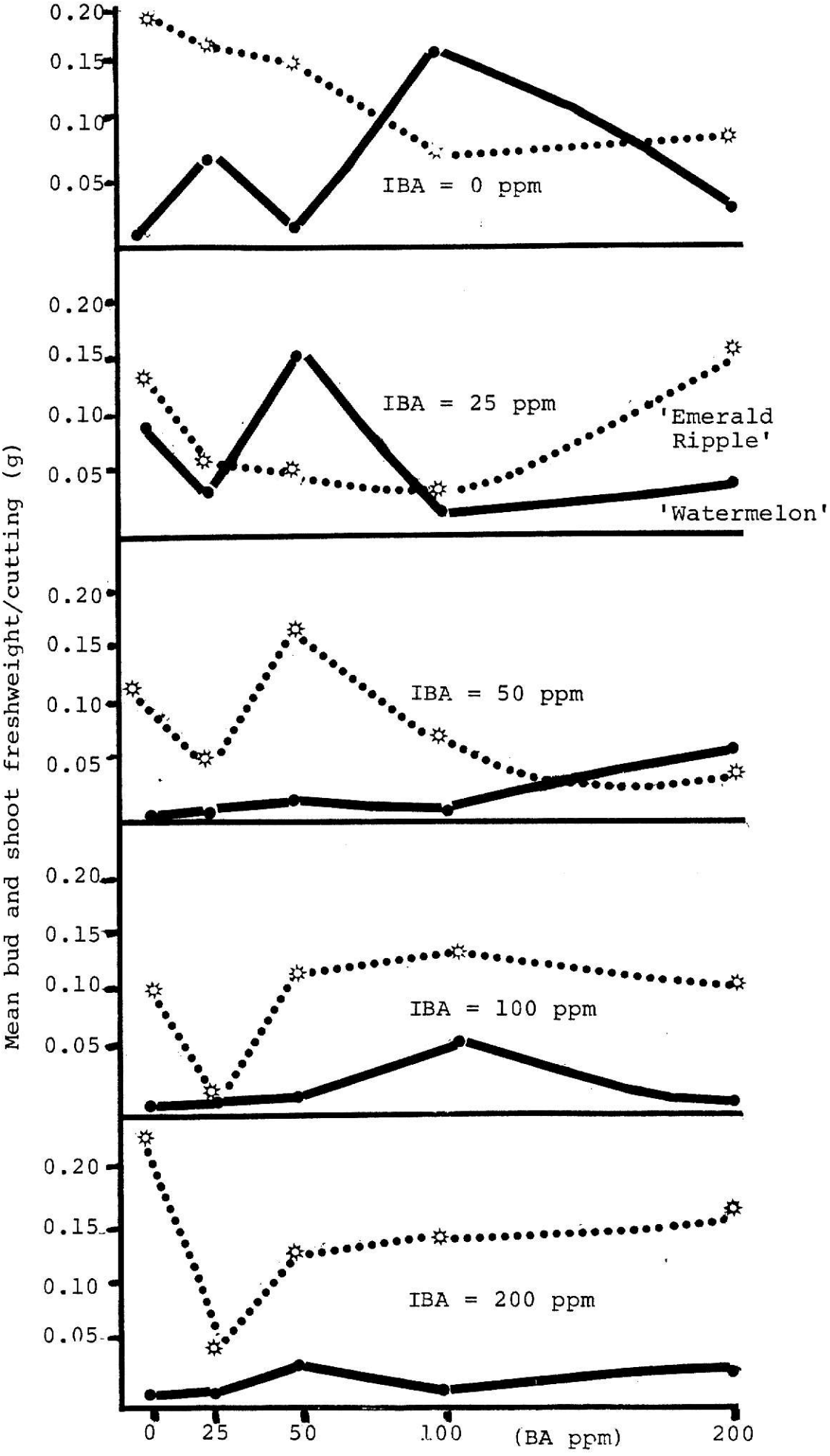

Mean bud and shoot freshweight/cutting (g)
0.20
0.15
0.10
0.05
IBA = 0 ppm
IBA = 25 ppm
'Emerald Ripple'
'Watermelon'
IBA = 50 ppm
IBA = 100 ppm
IBA = 200 ppm
0
25
50
100
200
(BA ppm)

concentration of IBA. Increasing concentration of BA above 100 ppm usually resulted in a decreased mean shoot freshweight per cutting.

In the cultivar 'Watermelon', in addition to having an overall lower mean shoot freshweight per cutting over all concentrations of BA and at various levels of IBA, the results of the effect of BA were significantly different only when IBA was not applied. In fact when no IBA was applied we seem to have obtained the best result in shoot development with increasing BA concentrations (Fig. 1).

In summary, at the end of Experiment 1 the 'Emerald Ripple' cuttings had well developed shoot systems satisfactory for repotting while the shoot system of 'Watermelon' was still very undeveloped.

Root development

The results of the experiment showed significant cultivar differences in root development, 'Watermelon' having a more developed root system than 'Emerald Ripple' (Table 3).

Table 3. Cultivar differences in root development.

Cultivar	Root Development		
	Mean Total Root Length per Cutting (cm)	Root Index	Mean Dry Root Weight per Cutting (g)
'Watermelon'	24.42 a*	3.63 a	0.028 a
'Emerald Ripple'	13.55 b	2.82 b	0.021 a

*Mean separation by Duncan Multiple Range test, 5%.

Roots were visible in the leaf cuttings of both cultivars 4 weeks after planting. The cultivar 'Emerald Ripple' had a more branched root system than 'Watermelon'. The latter had longer roots which were less branched. 'Watermelon' roots had a higher overall mean dry weight but the cultivar difference for this measurement was statistically insignificant.

The Effect of Growth Regulators on Root Development

The results of the effect of GRs on root development were analyzed in such a way as to characterize the effect of IBA on root development of the two cultivars at different levels of BA.

The effect of IBA on root development of the two cultivars at various levels of BA was found to be statistically significant only when the response was evaluated as mean total root length per cutting in grams.

Increasing IBA concentrations seem to have resulted in some slight increase in root development for both cultivars. However, the mean total root length of the non auxin treated cuttings were not significantly different from the treated cuttings in some instances (Fig. 2). Further increase in IBA concentration above 100 ppm usually resulted in a decline in the root development of the two cultivars except when the concentration of BA was 25 ppm. In this case further increases of IBA seem to have resulted in further increase in root development.

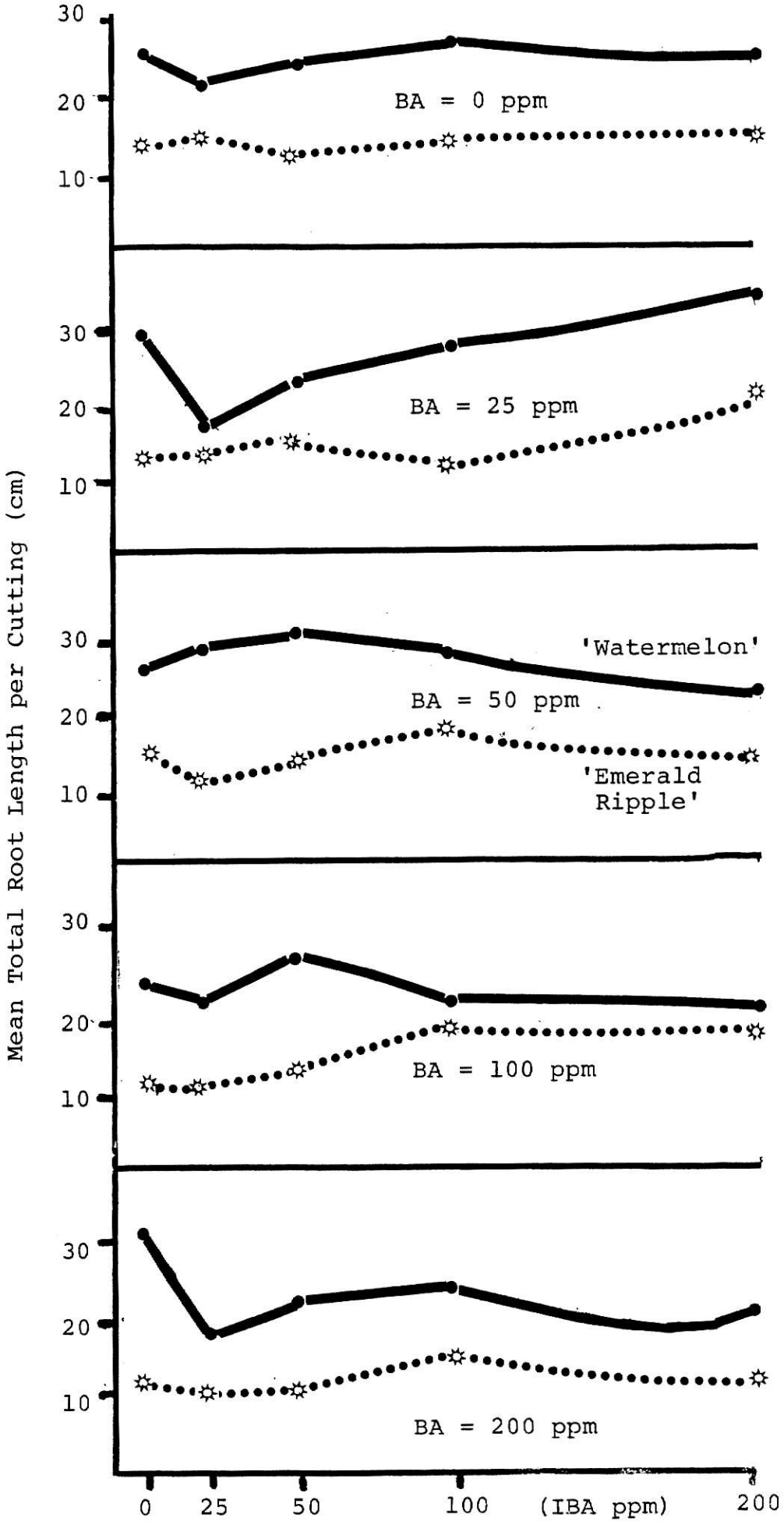
In summary, the root systems of the two cultivars were sufficiently developed for plant survival.

Table 4. Effect of IBA on Total Root Length at Various Levels of BA.

IBA	'Watermelon' Total Root Length (cm)	'Emerald Ripple' Total Root Length (cm)
	BA = 0 ppm	
0(control)	24.43 ab*	12.83 ab
25	21.16 a	13.96 ab
50	23.56 ab	11.83 a
100	26.86 b	13.16 ab
200	24.26 ab	14.76 b
	BA = 25 ppm	
0	30.40 bc	13.96 a
25	17.90 a	12.96 a
50	22.40 a	14.46 a
100	28.20 b	11.23 b
200	34.06 c	20.70 b
	BA = 50 ppm	
0	24.16 a	15.13 ab
25	26.90 ab	10.30 a
50	29.70 b	13.10 ab
100	26.70 ab	16.40 b
200	22.86 a	12.10 ab
	BA = 100 ppm	
0	23.40 a	11.63 a
25	20.90 a	10.15 a
50	24.76 a	12.20 a
100	20.50 a	17.90 b
200	19.40 a	17.36 b
	BA = 200 ppm	
0	32.70 d	11.8 a
25	16.80 a	11.16 a
50	23.70 bc	11.70 a
100	24.10 c	15.46 a
200	20.66 ab	12.76 a

*Mean separation by Duncan Multiple Range test, 5%.

Fig. 2. Effect of IBA on total root length at various levels of BA.


Mean Total Root Length per Cutting (cm)
30
20
10
BA = 0 ppm
BA = 25 ppm
'Watermelon'
BA = 50 ppm
'Emerald Ripple'
BA = 100 ppm
BA = 200 ppm
0
25
50
100
(IBA ppm)
200

Experiment #2

The results of the second experiment are shown in Figure 3. Application of increased BA concentrations appeared to reduce both number of shoots and buds and their freshweight per cutting. This trend was observed in treatments in which BA was applied in talc and those to which a 100 ppm BA foliar spray was later applied to half of treatments which had received BA in talc.

Table 5. Effect of BA applied in talc carrier on shoot development of 'Watermelon'

BA Treatment %	Number of buds and shoots/cutting	Freshweight of buds and shoots/cutting (g)
0 (control)	1.33 a*	0.415
0.003	1.27 a	0.296
0.01	1.44 a	0.218
0.03	0.50 b	0.14
0.6	0.38 b	0.14
1.0	0.33 b	0.06

*Mean separation by Duncan Multiple Range test, 5%.

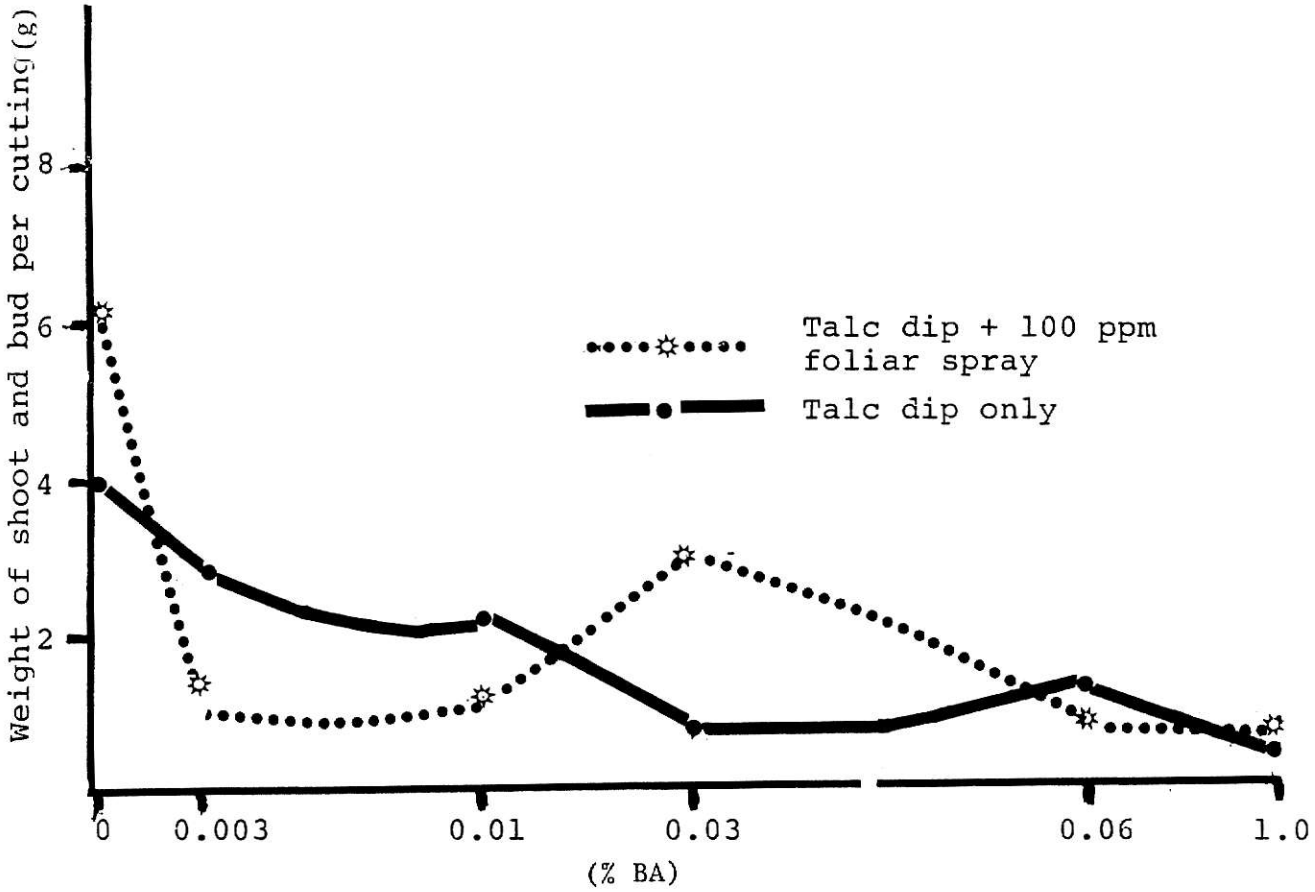
Table 6. Effect of BA applied in talc carrier and as 100 ppm foliar spray on shoot development of 'Watermelon'

BA Treatment %	Number of buds and shoots/cutting	Freshweight of buds shoots/cutting (g)
0 (control)	2.44	0.74 a*
0.003	2.38	0.14 b
0.01	1.22	0.13 b
0.03	0.83	0.29 b
0.60	0.77	0.09 b
1.00	0.22	0.09 b

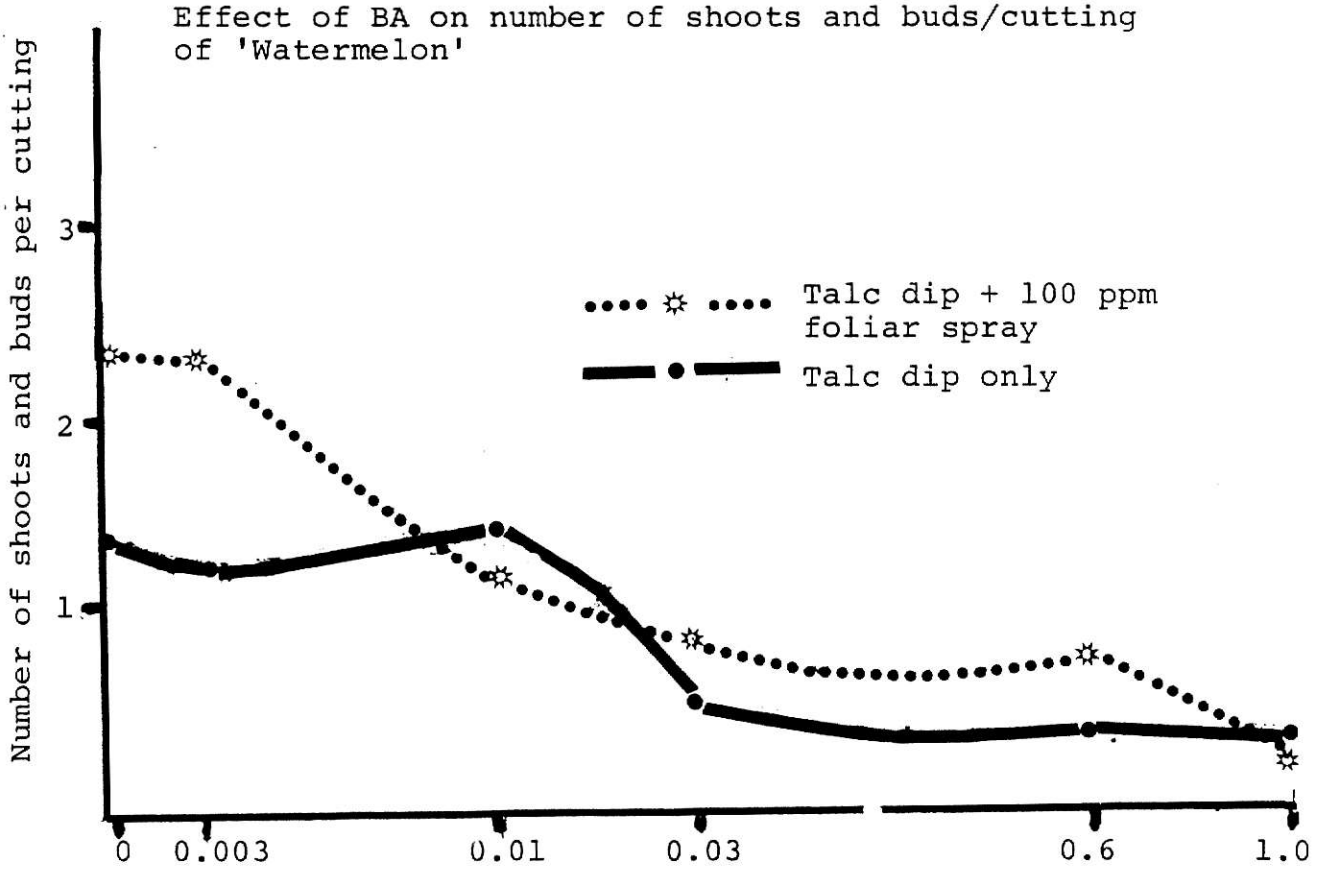
*Mean separation by Duncan Multiple Range Test, 5%.

Fig. 3. Effect of BA on shoot development of 'Watermelon'

Effect of BA on fresh shoot and bud weight/cutting of 'Watermelon'



Effect of BA on number of shoots and buds/cutting of 'Watermelon'



DISCUSSION

Our results regarding the effect of cytokinins on shoot development of P. argyreia seem to correlate with that of other workers (17) who found that increasing cytokinin concentration inhibited bud and shoot development of the cuttings. The results of the second part of the experiment confirmed what can be termed as the 'differential effect of cytokinins on shoot development'.

The effects of auxins and cytokinins on the propagation of Peperomia and other herbaceous plants seem to vary considerably among genuses, species of the same genus and cultivars of same species. This fact has been observed by many workers (9,17,24,32). However little attempt has been made to explain the reasons for this variation in response.

Endogenous hormones are thought to play an important role in determining the nature of response resulting from applied auxins and cytokinins (24). Theoretically one could say that no benefit can be obtained by applying growth regulators to a leaf cutting that has optimum inherent levels of the growth hormone. It is possible that such 'unjudicious' application of growth regulator can bring about inhibition of growth as toxic levels may be reached with very minute concentrations of the growth regulator.

If all workers performed such experiments under uniform environmental conditions and used similar methods, the basis of variation in response could possibly be attributed to genetic physiological and biochemical differences among the different plant categories.

Furthermore, if an attempt was made to categorize plants with reference to levels of endogenous hormones present and the amount needed to bring about a specific response, it would be possible to predict, with more confidence, the effect of growth regulators on propagation. Such a category would have practical implications as trial and error attempts to stimulate plant regeneration could be avoided. Plant materials such as leaf cuttings of P. argyreia are good investigational materials because they do not easily initiate shoots and need to initiate both roots and shoots for survival.

Generally a leafy plant cutting should initiate roots and then shoots when an optimum concentration of applied growth regulators and all endogenous hormones has been achieved. Applied growth regulator(s) should therefore have beneficial effect only when the level(s) of endogenous hormone(s) is below optimum.

From our results it may be said that P. caperata cv. 'Emerald Ripple' leaf cuttings may have adequate levels of endogenous auxins and cytokinins because they easily initiated both roots and shoots even when very little quantities of growth regulator were applied. Another possibility is

that this cultivar may have the capacity to conjugate excess applied growth regulator thus freeing itself of toxic or inhibitory effects which could result from excess growth regulator. On the other hand leaf cuttings of P. argyreia cv 'Watermelon' may have had high levels of endogenous auxins because they seemed to easily initiate more roots than shoots. Roots are thought to be sites of cytokinin synthesis (23). Applied cytokinins could result in super optimal levels of the hormone, thus inhibiting bud and shoot growth. Peperomia argyreia cv 'Watermelon' cuttings could further lack the ability to detoxify excess growth regulator. However further research is required before definite reasons for the 'differential effect of cytokinins on shoot development' may be put forward.

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THE EFFECT OF N^6 BENZYL-ADENINE AND INDOLE BUTYRIC ACID ON THE PROPAGATION OF *PEPEROMIA ARGYREIA* CV 'WATERMELON' AND *P. CAPERATA* CV 'EMERALD RIPPLE'

by

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AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT

The objective of this study was to characterize the effect of N^6benzyl-adenine (BA) and indole-butyric acid (IBA) on shoot and root development of leaf cuttings of Peperomia argyreia cv 'Watermelon' and P. caperata cv 'Emerald Ripple'.

In the first experiment five concentrations of each growth regulator were applied to both cultivars as a 24 hr petiole soak giving twenty five combined treatments. 'Emerald Ripple' easily iniated both roots and shoots in response to the combined treatments of cytokinin and auxin whereas 'Watermelon' developed more roots than shoots.

In the second experiment BA was applied in a talc carrier to petiole bases of leaf cuttings of 'Watermelon' in an attempt to stimulate shoot development. A further application of BA was applied as a 100 ppm foliar spray to half of the treatments after sufficient rooting had taken place. Increasing BA concentrations were found to be inhibitory to shoot development of 'Watermelon' peperomia.