This is to certify that the

thesis entitled

Adventitious Shoot Induction Of Selected Explants Of Kalanchoe Blossfeldiana Poellniz, Cultured In Vitro

presented by

Robert Carl Karp

has been accepted towards fulfillment
of the requirements for

M.S. degree in Horticulture

R. C. Lacy
Major professor

Date Oct. 19, 1977

O-7639

ADVENTITIOUS SHOOT INDUCTION OF SELECTED EXPLANTS OF *KALANCHOE BLOSSFELDIANA* POELLNIZ. CULTURED *IN VITRO*

By

Robert Carl Karp

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Horticulture

1977

ABSTRACT

ADVENTITIOUS SHOOT INDUCTION OF SELECTED EXPLANTS OF *KALANCHOE BLOSSFELDIANA* POELLNIZ. CULTURED *IN VITRO*

By

Robert Carl Karp

The adventitious bud technique when applied for mutation breeding *in vitro* may be a more efficient method than *in vivo* ones; thus, this study was undertaken to evaluate the potential of such systems for *Kalanchoe blossfeldiana* Poellniz.

Six explants (leaf discs, stem segments, leaf petioles, flower buds, pedicels, and peduncle segments) from five cultivars of *Kalanchoe blossfeldiana* were tested on modified Linsmaier and Skoog media with the plant hormones 6-benzyl aminopurine (6-BAP) and naphthalene acetic acid (NAA) in all combinations at the following levels: 0.01, 0.1, 1.0, and 10.0 mg/l. After twelve weeks, each explant was evaluated for adventitious shoot production followed by rooting of the plantlets which were subsequently grown to flowering for further evaluation.

All explants from each cultivar produced adventitious shoots on at least one media combination of 6-BAP and NAA. There were extensive differences in cultivar response relative to adventitious shoot production while in general, vegetative explants readily produced more shoots than reproductive ones. No single explant-media combination was found to be optimum for all cultivars but leaf discs at 1.0 and 10.0 mg/l 6-BAP in

in combination with 0.1 and 1.0 mg/l NAA was overall the best for the cultivars studied. With 'Mace', leaf discs at 1.0 mg/l 6-BAP and 1.0 mg/l NAA was the best explant source while with 'Solferinopurpur', the least productive cultivar tested, leaf petioles at 10.0 mg/l 6-BAP and 1.0 mg/l NAA was the most efficient combination. Stem segments at 1.0 mg/l 6-BAP and 0.1 mg/l NAA was the explant-media combination chosen for 'Sirius'. With 'Korall', stem segments at 10.0 mg/l 6-BAP and 1.0 mg/l NAA was the superior choice for adventitious plantlet production. Leaf discs of 'Granat' produced large numbers of shoots on many media combinations, the optimum ones being 1.0 mg/l 6-BAP and 1.0 mg/l NAA, 10.0 mg/l 6-BAP and 0.1 mg/l NAA, and 10.0 mg/l 6-BAP and 10.0 mg/l NAA.

Theoretically, the adventitious bud technique assumes that the plants originate from a single epidermal cell which assures that the regenerated plantlets are non-chimeric. In this study, no histology studies were undertaken to determine the origin of adventitious shoots but by observation of morphological plant characters, no visible chimeras were observed in the adventitious buds grown to flowering in the greenhouse. Thin-layer chromatography of flower pigment extracts and pollen viability tests revealed no significant differences between the parental plants and the regenerated ones thus indicating genotypic similarity which further supports the supposition that the adventitious plantlets in this study most likely were derived from a single cell or a few cells.

An *in vitro* system for mutation breeding with *Kalanchoe blossfeldiana* would appear to be a more efficient technique than *in vivo* systems in the production of new cultivars by requiring less time for shoot production, requiring considerably less space, and producing higher numbers of shoots.

To Kathy

ACKNOWLEDGMENTS

I would like to thank the members of my committee Dr. Robert Herner and Dr. P. S. Carlson for their assistance. Special thanks goes to Dr. K. C. Sink, Jr., my major professor, for his guidance and friendship. Special thanks to Mr. Jim Mikkelsen for the plant materials used in this study. My parents and especially my wife.

TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Kalanchoe blossfeldiana Poellniz. is currently increasing as a commercial flower crop in the United States. It is asexually propagated by stem tip cuttings and new cultivars are produced by conventional hybridization or mutation breeding. For mutation breeding, leaf petiole cuttings appear to be the appropriate plant organs to utilize. This is due to the fact that shoots originating from the petiole base are believed to have their origin from a single epidermal cell which leads to non-chimeric mutants.

The concept of adventitious shoots arising from a single cell has been reported for over 350 species, including both monocots and dicots. And, when implemented as the basis for mutation breeding has successfully resulted in new cultivars of a wide range or ornamentals including Begoniaceae, Compositae, Crassulaceae, and Gesneriaceae. For example, Broertjes (1969) showed with Streptocarpus that the adventitious technique was possible with leaf cuttings. Mutated and non-mutated shoots arose from single, epidermal cells but it was difficult to determine by histology precisely how many cells were involved. Of the mutants produced, 94.8 percent were solid or non-chimeral. Broertjes interpreted the resulting high mutation frequency and wide spectrum obtained to indicate reduced diplontic selection; thus inferring that one or a few initial cells gave rise to the adventitious buds. The chimeras probably resulted from the instability of the genome induced by irradiation.

Recently various explants of plant species have been placed *in vitro* for mutation breeding to give more efficient results in terms of shoot yields. Broertjes et. al. (1976) compared *in vivo* and *in vitro* mutation breeding systems in *Chrysanthemum morifolium*. On detached leaves, shoots originated from callus at the petiole base or occasionally on callus formed on the upper part of the roots. Following irradiation with X-rays, the few shoots obtained from the main adventive shoots on laterals were chimeric indicating that they originated from more than one callus cell. Pedicels were chosen as the explant for *in vitro* irradiation after screening various explants since their adventitious shoots came directly from the epidermis and 100 percent of the explants irradiated or not, produced high numbers of shoots. Of all plants produced, only one was chimeric.

In vivo mutation breeding has been accomplished with *Kalanchoe blossfeldiana* utilizing the adventitious bud technique with leaf petiole cuttings (Broertjes and Leffring, 1972). Young leaves produced the highest number of adventitious shoots but shoot production was low and even lower (below 1) following irradiation. No chimeras were observed after considerable backpruning and histological observations providing further evidence that adventitious shoots were formed from single epidermal cells.

The present study was undertaken to determine for *in vitro* mutation breeding, which explant and *in vitro* media combination would promote optimum adventitious shoot production for *Kalanchoe blossfeldiana*. As well, to determine if desirable genetic variability possibly present in chimeric form, exists in intact plants that could be released through *in vitro* culture of various plant organs and their respective histogenic layers.

LITERATURE REVIEW

Kalanchoe blossfeldiana Poellniz. is a pot plant crop commercially propagated asexually by stem tip cuttings and leaf petiole cuttings for mutation breeding. The number of shoots from leaf petiole cuttings varies between cultivars, is generally low, and a slow propagation method but more importantly, for a mutation breeding project the adventitious plantlets appear to originate from a single, epidermal cell (Broertjes and Leffring, 1972).

There are over 350 species, both monocots and dicots, capable of forming adventitious buds from leaves with the list being incomplete since with many species the technique has not been applied (Broertjes et. al., 1968). There is great potential for mutation breeding systems and already within the following families of ornamentals the adventitious bud technique has been used for producing new cultivars: Begoniaceae, Compositae, Crassulaceae, and Gesneriaceae.

Broertjes (1969) with Streptocarpus and by using the adventitious bud technique with leaf cuttings was able to induce mutations and lend evidence of single cell and epidermal origin of the shoots. It was difficult to determine histologically the number of cells involved in the formation of adventitious buds but through mutation induction and a high number of solid mutants, 94.8 percent, it was felt that the shoots were single cell and epidermal in origin. Further proof of the solid mutants resulted when the mutants reproduced by leaf cuttings did so true to type. The high mutation frequency and wide mutation spectrum indicated reduced

diplontic selection which points to one or a few cells involved in adventitious bud formation. The instability of the genome was the explanation for the chimera formation.

Broertjes (1972a) working with Achimenes, a member of the Gesneriaceae, used the adventitious bud technique to produce mutants. With Achimenes, depending on the time of year and the dose of irradiation, detached leaves will form adventitious shoots, shoots and rhizomes, or rhizomes only and regardless of whether shoots or rhizomes were formed almost all were non-chimeric. Ninety-six percent of the mutants were solid adding support to the assumption that the plantlets were produced from single cells.

The in vivo adventitious bud technique has been utilized by Broertjes (1972b) with Saintpaulia leaves in an extensive mutation breeding project. Using two types of irradiation, few chimeras were produced and histological work suggested that the adventitious plantlets were single cell in origin. Broertjes felt that diplontic selection was restricted in the adventitious bud technique since even slow growing cells were able to express themselves as dwarf mutants which represented a large percentage of the mutated population.

Another mutation breeding project utilizing the adventitious bud technique with Saintpaulia was undertaken by Sparrow et. al. (1960). The results obtained were similar to Broertjes (1972b) in that after irradiating with X-rays, 154 mutants were obtained from leaf petiole cuttings and all but one being non-chimeric. The authors felt that from these results one cell forms a meristem which forms a whole plant. No histological study or propagation of mutants was undertaken to completely identify the nature of the mutants.

Warfield (1973), using EMS, a chemical mutagen, induced mutations using the adventitious bud technique and Saintpaulia leaves. His results agreed with Broertjes (1972b) and Sparrow et. al.'s)1960) work though no discussion of chimeras was included.

Begonia hiemalis Fotsch. is propagated mainly by leaf petiole cuttings utilizing the adventitious bud technique which Doorenbos and Karper (1975) have used in mutation breeding experiments by exposing intact leaf petiole cuttings to X-rays. Doorenbos and Karper (1975), at the levels used, found only 0.0—0.4 percent chimeras varying with cultivars which led them to assume that both mutated and non-mutated plants originated from single cells. Spontaneous mutation in a later stage of development was the explanation for chimera formation.

A comparison of the adventitious bud technique both in vivo and in vitro in a mutation breeding system of Chrysanthemum morifolium Ramat., 'Bravo' was done by Broertjes et. al. (1976) showing that with detached leaves (in vivo), the shoots originated from callus at the base of the petiole or occasionally on callus formed on the upper part of the roots. After irradiation with X-rays, it was found that the few shoots obtained, either the main adventive shoot or its laterals, were chimeric in nature which led the authors to believe that more than one callus cell was involved in the formation of a shoot. After preliminary screening, pedicels were chosen as the explant source in vitro because they formed adventitious shoots directly from the epidermis, 100 percent of the explants, irradiated or not, produced shoots, and they were produced in relatively high numbers. The control produced a high

average of adventitious shoots per explant. Only one plant was chimeric and along with mutants being propagated by shoot cuttings (in vivo) or by in vitro using pedicel explants resulting in true to type progeny which lends support to the single cell to whole plant theory.

MATERIALS AND METHODS

Five cultivars of Kalanchoe blossfeldiana were used in this study. 'Mace' which has very large obvate leaves with slight serration and small reddish-orange flowers; 'Korall' and 'Granat', hybrids that have serrated leaves with red flowers and less serrated leaves and red flowers, respectively. 'Sirius' is a hybrid that has leaves similar to 'Granat' but with orange flower color and 'Solferinopurpur' has small, roundish leaves, is self branching and has magenta colored flowers.

Vegetative and reproductive explants were used as the explants in this study. Vegetative explants were obtained from plants maintained under artificial long days by providing supplemental incadescent light 200 lux from 10 P.M. to 2 A.M. nightly from September 15 to April 15. The vegetative plant tissues were 12 mm leaf discs punched with a cork borer from the second to third leaf subtending the shoot apex; using two leaf discs from each leaf from five plants to give ten discs per test medium. Leaf petioles were obtained from one to four leaves subtending the shoot apex of the plant. Five petioles were tested per media combination; each originating from a different leaf and the slices were approximately 10 mm long and 5-10 mm in diameter. They were placed basal end down on the culture media. Stem segments, from 8-12 mm in diameter, were also used as an explant by placing nine internode segments on each test medium. Young, healthy green tissue was chosen and the position of stem segments on the intact plant was noted for experimental purposes.

The floral organs were initiated on greenhouse grown plants by providing a short day treatment with black cloth from 5 P.M. to 8 A.M. each day for a five week period. Kalanchoe blossfeldiana has a dichasial floral structure and the influorescence is supported by the peduncle. The peduncle explants, 5-10 mm thick, were placed basal end down, nine per test culture medium. Pedicel sections were used as nine explants per test medium each placed on its epidermis. Nine immature unopened flower buds were also cultured per test medium.

All plant tissues or organs were surface disinfected in 20% commercial sodium hypochlorite solution for fifteen minutes and rinsed twice in sterile distilled water. Prepared explants were placed on Linsmaier and Skoog (1965) medium with 2% sucrose, 0.8% agar, adjusted to pH 5.5—5.6 prior to autoclaving. The growth regulators tested included 6-benzyl aminopurine (6-BAP) and naphthalene acetic acid (NAA) at all combinations of 0.01, 0.1, 1.0, and 10.0 mg/l. Penicillin-G (5 mg/l), gentamicin sulfate (10.0 mg/l), and tetracycline (4 mg/l) were filtered, sterilized and added to the cooled medium (40°C) after autoclaving to control bacteria (Power et. al., 1976). The autoclaved medium was added, 25 mls, to sterile plastic petri dishes, 100 x 15 mm, and the explants were subcultured every four to five weeks.

The dishes were placed under 1000 lux continuous cool white fluorescent illumination at room temperature (26° C$\pm$2) for twelve weeks. The adventitious shoots were directly rooted in Jiffy Mix in covered glass containers in about three weeks under 10,000 lux of cool white fluorescent light, sixteen hours per day. Rooted plantlets were placed in cell packs filled with Jiffy Mix for two weeks under the same environmental conditions

used for rooting. The plantlets were subsequently placed on the mist bench for one week and to greenhouse conditions for further growth. To induce flowering of the plantlets, six weeks of nine-hour daily photoperiod was provided by blackcloth treatment.

Evaluation of regenerated plantlets was by visual inspection of morphological characters and thin-layer chromatography of flower pigment extracts. The pigment extract was made by grinding 0.5 g of freshly opened corolla tissue with a mortar and pestle in 5 mls of acidified methanol. The supernatant was collected and the remaining tissue was resuspended and pulverized in 5 mls of acidified methanol. The supernatant was again collected and both portions passed through a glass filter by vacuum. The extract was centrifuged 2000 x g, reduced in volume and stored at 10°C. Twenty µls of the extract were spotted, dryed, and run on cellulose thin-layer plates in butanol:formic acid:water (40:10:50) solution using the upper phase. Pollen viability was determined by placing dehisced anthers on a microscope slide and adding 1 percent aceto-carmine stain. Only round, dark stained grains were scored as viable.

RESULTS

Shoots were produced from all plant tissues and organs obtained from the five cultivars studied. With 'Mace', leaf discs produced the highest average number of shoots, 5.6, at 1.0 mg/l 6-BAP and 1.0 mg/l NAA followed by stem segments with an average of 3.5 on 0.01 mg/l 6-BAP and 0.1 mg/l NAA medium (Table 1, see Appendix Table 1). Leaf petioles produced the next highest number of shoots and averaged 2.7 on 0.1 mg/l 6-BAP and 0.01 mg/l NAA medium. 'Mace' peduncle sections averaged 1.5 plants per section on 1.0 mg/l 6-BAP and 1.0 mg/l NAA levels. Flower buds produced an average of 1.5 at 10.0 mg/l 6-BAP and 0.01 mg/l NAA with pedicel sections the highest average of 0.4 was produced on the medium 0.1 mg/l 6-BAP and 1.0 mg/l NAA.

Stem segments explants of 'Sirius' yielded shoots on seven different media combinations of 6-BAP and NAA; the highest average was 16.2 from 1.0 mg/l 6-BAP and 0.01 mg/l NAA (Table 1, see Appendix Table 2). Leaf discs produced shoots on six different media combinations with an average of 7.6 per leaf on 10.0 mg/l 6-BAP and 0.1 mg/l NAA. The other vegetative explant, leaf petiole discs, produced shoots on only one combination, 1.0 mg/l 6-BAP and 10.0 mg/l NAA with an average of 2.4. The floral explants also produced shoots with pedicels producing shoots on six different media combinations and the highest average 2.6, on 1.0 mg/l 6-BAP and 1.0 mg/l NAA. Peduncle tissue produced shoots at one

Table 1.--The effect of 6-BAP and NAA combinations on shoot formation from each explant and cultivar.

Cultivar	Plant Organ*	Average Number of Shoots Hormone Concentration (mg/l 6-BAP/NAA)															
		0.01/0.01	0.01/0.1	0.01/1.0	0.01/10.0	0.1/0.01	0.1/0.1	0.1/1.0	0.1/10.0	1.0/0.01	1.0/0.1	1.0/1.0	1.0/10.0	10.0/0.01	10.0/0.1	10.0/1.0	10.0/10.0
'Mace'	LD	**				0.3	1.2	1.7	0.1		4.4	5.6	2.6		0.9	5.1	2.9
	SS		3.5***							1.2	0.9	2.3		0.9		0.1	
	LP					2.7	0.3										2.5
	FB							0.1					0.1	1.5			
	PE							0.4				0.1				0.6	
	PU										1.1	1.5					
'Sirius'	LD										1.9	2.7		1.8	7.6	0.6	2.2
	SS	3.7				5.6	2.1			16.2	1.3					1.7	0.1
	LP												2.4				
	FB							0.1				0.9	1.4	1.3	1.3		
	PE				0.4						2.2	2.6	0.7			0.2	0.1
	PU	0.1	1.0							0.9	1.2	0.5	0.7				0.3
'Granat'	LD						1.4	4.7	3.8	7.2	8.3	19.3	8.0	9.7	19.4	0.1	15.0
	SS	1.7	0.1				0.3						2.8				
	LP					0.2	1.5	0.1	1.7		1.0	24.0	2.2		0.0****	6.7	2.0
	FB							0.3			2.1	0.9	1.7		0.5	12.2	
	PE						0.8	1.3	0.4		4.5	0.6	0.5			12.0	
	PU						0.5	0.2		0.3	5.5	6.2					
'Solferinopurpur'	LD								0.8	0.8			1.0		0.6	3.0	3.6
	SS	0.5	0.5					1.4		1.0	0.9	2.4		1.2	2.4		
	LP			2.0			0.0****				0.0****	1.2		2.3	0.2	10.2	
	FB			1.1			0.9	0.2	0.1				0.9				2.4
	PE			0.3	5.8		0.4	1.0		0.7	1.8	2.9	0.9			0.0****	
	PU									0.8				0.3			
'Korall'	LD							1.3	0.2		4.2	7.8				0.8	0.7
	SS	0.2	3.3				1.0	1.1	0.1	2.1	1.5	5.7	7.5			19.4	
	LP								1.4		5.2	8.0	17.0		0.7	1.6	1.2
	FB								0.7			3.9	1.0	1.9		5.8	2.8
	PE			1.3					0.9		0.5	3.0	0.9	10.0		5.1	6.0
	PU							0.1			1.0		0.7				

*LD = leaf discs
SS = stem segments
LP = leaf petioles
FB = flower buds
PE = pedicel sections
PU = peduncle sections

** No shoot formation

*** Average = total shoots ÷ total number of uncontaminated explants.

**** Shoots were formed but too immature to count.

more media combination than pedicel tissue 7, but the average was only 1.2 on 1.0 mg/l 6-BAP and 0.1 mg/l NAA. Flower buds averaged 1.4 at 1.0 mg/l 6-BAP and 10.0 mg/l NAA and shoots were produced on four other medias.

'Granat' was the most prolific shoot producer of all cultivars tested (Table 1, see Appendix Table 3). Leaf discs produced an average of 19.4 on the 10.0 mg/l 6-BAP and 0.1 mg/l NAA medium and on ten other combinations. Stem explants produced shoots on four different combinations with an average of 2.8 on 1.0 mg/l 6-BAP and 10.0 mg/l NAA. Petiole explants produced shoots on ten media combinations averaging 24.0 per segment on 1.0 mg/l 6-BAP and 1.0 mg/l NAA. Flower buds produced the highest average 12.2, on 10.0 mg/l 6-BAP and 1.0 mg/l NAA. Pedicels produced shoots on seven different combinations with 10.0 mg/l 6-BAP and 1.0 mg/l NAA having an average of 12.0 per section. Peduncle tissue averaged 6.2 shoots on 1.0 mg/l 6-BAP and 1.0 mg/l NAA and produced shoots on four other media combinations.

'Solferinopurpur' produced the least number of adventitious shoots *in vitro* among the cultivars studied (Table 1, see Appendix Table 4). Leaf discs averaged 3.6 on 10.0 mg/l 6-BAP and 10.0 mg/l NAA; stem sections 2.4, on 10.0 mg/l 6-BAP and 0.1 mg/l NAA while, overall, shoots were induced on eight medias. Petioles produced shoots on six medias; the highest for this cultivar was an average of 10.2 on 10.0 mg/l 6-BAP and 1.0 mg/l NAA levels. Pedicels produced the most shoots, an average of 5.8 at 0.01 mg/l 6-BAP and 10.0 mg/l NAA levels and also on eight other growth regulator combinations. Flower buds produced shoots on six different combinations, the best being 10.0 mg/l 6-BAP and 10.0 mg/l NAA which resulted in an average of 2.4 Peduncle tissue was the lowest,

giving shoots at only two levels with 1.0 mg/l 6-BAP and 0.01 mg/l NAA being optimum with an average of 0.8. Four of the six optimal concentrations of the 'Solferinopurpur' plant organs had 10.0 mg/l 6-BAP with various NAA levels; the other two levels were at 0.01 mg/l and 1.0 mg/l 6-BAP.

'Korall', similarly, produced shoots from both vegetative and floral explants (Table 1, see Appendix Table 5). Of the vegetative explants, the stems produced shoots on the most combinations 10, and was the most prolific at 10.0 mg/l 6-BAP and 1.0 mg/l NAA averaging 19.4. Leaf petioles averaged 17.0 shoots per explant at 1.0 mg/l 6-BAP and 10.0 mg/l NAA. Shoots were also produced on six other combinations with leaf petiole discs. The levels 1.0 mg/l 6-BAP and 1.0 mg/l NAA averaged 7.8 from leaf discs while shoots were formed on five other combinations. 'Korall' pedicels produced shoots on eight media combinations with 10.0 mg/l 6-BAP and 0.01 mg/l NAA averaging 10.0 per explant. Adventitious buds occurred on six different media combinations when flower buds were used; the combination 10.0 mg/l 6-BAP and 1.0 mg/l NAA producing an average of 5.8. Of all the explants, peduncle tissues produced shoots on the fewest combinations, three, and the lowest average, 1.0, on 1.0 mg/l 6-BAP and 0.1 mg/l NAA. In four of those six combinations the NAA level was either 1.0 or 10.0 mg/l.

Visual evaluation of the flowering plantlets revealed no visible modification in plant morphology when compared to the parents. Leaf shape, amount of leaf serration, plant habit, flower shape, and flower color were considered during evaluation and no differences were found.

By using a completely randomized design, choosing three adventitious plants per organ per three different hormone combinations, it was found with 'Granat' that there were no significant differences in pollen viability. Likewise with the thin-layer chromatography (TLC), all pigment extracts were freshly prepared for each replicate and three replicates were made of all the plant organs of 'Granat' and no qualitative differences in the resolved TLC pattern of the pigments was observed.

DISCUSSION

The primary objective of the study was to determine which explant of Kalanchoe was most suited for in vitro mutation breeding. There are several criteria which need to be considered as guidelines in establishing the appropriate explant. Firstly, to identify the explant that would yield the highest number of adventitious shoots on a given media combination. The average shoot production in this study was the total number of shoots arising divided by the number of uncontaminated explants that did or did not form shoots. Secondly, consistency of shoot production, as indicated by a low standard deviation which is calculated from the shoot forming explants, is as important as the first criterion. Preference is given to the plant tissue or organ that produces shoots on a high percentage of the explants and with even distribution. Thirdly, the growth stage of the shoots after a period of time, as reflected in the percentage rooted, is important since those plantlets which are relatively more developed will root readily thus ensuring a high survival rate. The five Kalanchoe cultivars studied herein varied considerably with respect to these criteria.

Using the above criteria, each cultivar warrants individual discussion since they did not have identical optimum explant-media combinations. As seen in Table 2, with all the cultivars the choice of the explant is evident, one being leaf discs of 'Mace' at 1.0 mg/l 6-BAP

Table 2.--Optimal *in vitro* plant organ-hormone combination of the cultivars tested of *Kalanchoe blossfeldiana* Polleniz.

Cultivar	Plant Part	Hormone Combination (mg/l) 6-BAP	NAA	Total Number of Shoots	Average*	Standard Deviation	Percent Explants With Shoots	Percent Rooted
Mace	Leaf Disc	1.0 and	1.0	45	5.6	± 3.2	100%	81%
Solferinopurpur	Leaf Petiole	10.0 and	1.0	51	10.2	± 6.4	40%	84%
Sirius	Stem Segment	1.0 and	0.01	81	16.2	±11.1	100%	78%
Korall	Stem Segment	10.0 and	1.0	136	19.4	±13.5	71%	53%
Granat	Leaf Disc	1.0 and	1.0	116	19.3	± 5.3	100%	65%
Granat	Leaf Disc	10.0 and	0.1	194	19.4	±15.8	70%	48%
Granat	Leaf Disc	10.0 and	10.0	150	15.0	± 5.4	100%	22%

* Average = total shoots ÷ total uncontaminated explants

and 1.0 mg/l NAA since the average was the highest for the explants tested with a low standard deviation 3.2. All the explants produced shoots and a substantial number of the adventitious plantlets rooted (81%). With 'Solferinopurpur' (Table 2), though shoot production was low, leaf petioles at 10.0 mg/l 6-BAP and 1.0 mg/l NAA was the best combination with an average of 10.2, standard deviation 6.4, but a low percentage of explants with shoots (40%) and the percent rooted was high, 84%. With 'Sirius' (Table 2), stem segments at 1.0 mg/l 6-BAP and 0.01 mg/l NAA was the choice since there was a high average of 16.2 but also a high standard deviation of 11.1. All the explants produced shoots and 78% of the plantlets rooted. 'Korall' presents a clear choice (Table 2) since no other plant organ approaches the high average, 19.4, though the standard deviation is high but a high percentage of the explants produced shoots and the rooting problem should be corrected by minor modifications in the rooting procedure. Leaf discs of 'Granat' are the explant choice and the media combination is not critical. The three most productive are represented showing that the averages are high (Table 2), the standard deviation fairly low with a high percent of explants with shoots.

By comparing the same explants of the different cultivars, trends in response to the media formulation is evident, the most obvious being that explant variation occurred. This was not unexpected since there were differences in shoot production and their response to the various media combinations. Leaf discs had averages from 3.6 to 19.4 (Table 3) and most of the shoot production occurred on the 1.0 and 10.0 mg/l 6-BAP combination with either 0.1 or 1.0 mg/l NAA. Stem segments,

Table 3.--The highest average and corresponding hormone combination of the cultivars

Plant Organ	Cultivar	Hormone Combination (mg/l) 6-BAP / NAA	Highest Average* of each cultivar
Leaf Discs	'Mace'	1.0/ 1.0	5.6
	'Sirius'	10.0/ 0.1	7.6
	'Granat'	10.0/ 0.1	19.4
	'Solferinopurpur'	10.0/10.0	3.6
	'Korall'	1.0/ 1.0	7.8
Stem Segments	'Mace'	0.01/ 0.1	3.5
	'Sirius'	1.0/ 0.01	16.2
	'Granat'	1.0/10.0	2.8
	'Solferinopurpur'	10.0/ 0.1	2.4
	'Korall'	10.0/ 1.0	19.4
Leaf Petioles	'Mace'	10.0/10.0	2.5
	'Sirius'	1.0/10.0	2.4
	'Granat'	1.0/ 1.0	24.0
	'Solferinopurpur'	10.0/ 1.0	10.2
	'Korall'	1.0/10.0	17.0
Flower Buds	'Mace'	10.0/ 0.01	1.5
	'Sirius'	1.0/10.0	1.4
	'Granat'	10.0/ 1.0	12.2
	'Solferinopurpur'	10.0/10.0	2.4
	'Korall'	10.0/ 1.0	5.8
Pedicels	'Mace'	10.0/ 1.0	0.6
	'Sirius'	1.0/ 1.0	2.6
	'Granat'	10.0/ 1.0	12.0
	'Solferinopurpur'	0.01/10.0	5.8
	'Korall'	10.0/ 0.01	10.0
Peduncle Segments	'Mace'	1.0/ 1.0	1.5
	'Sirius'	1.0/ 0.1	1.2
	'Granat'	1.0/ 1.0	6.2
	'Solferinopurpur'	1.0/ 0.01	0.8
	'Korall'	1.0/ 0.1	1.0

*Average = total shoots ÷ total number of uncontaminated explants

in general, were not as productive as leaf discs and there is no significant trend in media response where all appear to produce plantlets. Except for 'Granat', there was less production at 10.0 mg/l 6-BAP and all combinations of NAA. Petioles, the explant used by Broertjes and Leffring (1972) for Kalanchoe were the optimal explant to use in vivo but produced few shoots as was the case with the in vitro work where they were the least productive of the vegetative explants. In this work, low levels of BAP and low levels of NAA at the high 6-BAP levels inhibited shoot production. 6-BAP at 1.0 and 10.0 mg/l in combination with 0.1 and 1.0 mg/l NAA were the combinations which produced shoots. On all but one cultivar 'Granat', there was low shoot production with flower buds and the hormone levels which produced adventitious shoots was from the 1.0 mg/l 6-BAP and 0.1 mg/l NAA through the 10.0 mg/l 6-BAP and 10.0 mg/l NAA levels. The highest average of each cultivar utilizing pedicel explants ranged from 0.6 to 12.0 which occurred at the 1.0 and 10.0 mg/l 6-BAP levels in combination with 0.1 and 1.0 mg/l NAA. Peduncle explants generally produced few shoots with the high of each cultivar ranging from 0.7—5.5, mostly below 1.0 with the best response occurring at 1.0 mg/l 6-BAP in combination with 0.1 and 1.0 mg/l NAA. Of the floral organs, pedicels were the best explant source but when compared to the optimal vegetative plant organ, leaf discs, the vegetative one is the choice overall. For the cultivars tested, the highest average was with leaf discs at 1.0 and 10.0 mg/l 6-BAP in combination with 0.1 and 1.0 mg/l NAA.

Differences in shoot production between the cultivars was apparent when all the plant organs of the cultivars are compared (Appendix Table 6). With 'Mace', leaf discs were the only productive explant source,

the other plant parts being similar in average to one another. The highest average of 'Sirius' was 16.2 with stem segments while leaf discs produced an average of 7.6 with the four remaining plant organs averaging between 1.0 and 2.0. 'Granat' plant organs, except for stem segments, had relatively high averages, above 6.0. Leaf discs produced large numbers of adventitious shoots at many combinations but all the plant organs of the cultivar were productive. With 'Solferinopurpur', all plant organs were consistent regarding shoot production, between 2.0 and 3.0, except for peduncle tissue. As with 'Solferinopurpur', all plant organs of 'Korall', except peduncle tissue, adventitious shoot production was between 5.5 and 19.4 consistent within the cultivar. Adventitious shoot production between cultivars varied which was not unexpected. Within each cultivar there was consistency of shoot production even between vegetative and floral plant tissues except for outstanding examples.

With Kalanchoe, the response of a particular plant organ to a specific combination of growth regulators in the media was not always consistent. Parts of this study were repeated by utilizing explants taken from parent plants grown at different seasons in the greenhouse that plant tissues respond somewhat differently in vitro. The results indicated where temperature and light intensity affected the physiological condition of the parent plants that this could account for the differences in in vitro shoot production. This parallels Mikkelsen's work (1976), where differences in shoot production of Begonia hiemalis Fotsch. both 'Peach' and 'Rose' was apparent between the winter and summer months. Physiological age of the parent plants is also a prime concern since older

tissues tend to produce fewer adventitious shoots. As Kalanchoes age, the plants tend to become day neutral (Schwabe, 1969) causing flowering which could alter the endogenous hormone levels resulting in a varied response. There is a need to find the optimal environmental conditions and age of parent plants to secure a consistent media combination response.

New Kalanchoe cultivars are predominantly produced through hybridization followed by asexual propagation when a desirable seedling is found. This shows that most Kalanchoe cultivars are probably very heterozygous genetically which is advantageous for a mutation breeding program for new potential combinations of germplasm. In Broertjes and Leffring's in vivo work (1972) with leaf petioles of Kalanchoe no chimeras were found and in this in vitro work no variability was released regardless of explant origin (epidermis, vascular, or cortex). With the observation of morphological characters, thin-layer chromatography of flower pigment extracts, and pollen viability, all the plants appeared genetically similar to their respective parents. As reported by Skirvin and Janick (1976) with scented Pelargonium spp., an asexually propagated crop, variation can be released dependent on the explant being derived from histogenic layers. Thus, leading one to believe that after years of asexual propagation, complex chimeras have formed along with inherent cell variation. With Kalanchoe, though not the primary aim of this study, the explants utilized represented two histogenic layers in origin and they released no variability through in vitro culture. Although an asexually propagated crop, the original hybrid plants are of seedling origin from plant breeding programs. Thus,

because they are of recent origin, the histogenic layers are most likely identical and complex chimeras have not arisen either by point mutation or by ploidy changes.

The overall results indicate that an *in vitro* mutation breeding system may be feasible with *Kalanchoe*. Even cultivar differences in terms of shoot response and variation with respect to optimum media required, the number of shoots was generally greater than *in vivo* systems. Along with less space needed for propagation of adventitious shoots and less time to produce a plantlet to flower, preliminary screening of cultivars, in addition to those tested, indicated that an *in vitro* mutation breeding system for *Kalanchoe blossfeldiana* should be a practical system for producing new cultivars.

APPENDIX

Table A1.--In vitro reaction, total number of shoots, and average of the plant organs of 'Mace' at all combinations of 6-BAP and NAA tested.

Hormone Concentration (mg/l) 6-BAP	NAA	LEAF DISCS			STEM SEGMENTS			LEAF PETIOLES			FLOWER BUDS			PEDICELS			PEDUNCLE SEGMENTS		
		Reaction *	Total Shoots	Ave.**	Reaction	Total Shoots	Ave.	Reaction	Total Shoots	Ave.	Reaction	Total Shoots	Ave.	Reaction	Total Shoots	Ave.	Reaction	Total Shoots	Ave.
0.01 and	0.01	X	0	0	X	0	0	N	0	0	X	0	0	C	0	0	R,N	0	0
0.01 and	0.1	N	0	0	S,R,N	23	3.5	N	0	0	N	0	0	N	0	0	X	0	0
0.01 and	1.0	N	0	0	R,N	0	0	N	0	0	R,N	0	0	N	0	0	R,N	0	0
0.01 and	10.0	R,C,N	0	0	R,N	0	0	R,C,N	0	0	R,N	0	0	N	0	0	R,N	0	0
0.1 and	0.01	S,N	3	0.3	N	0	0	S,N	8	2.7	C	0	0	C	0	0	C	0	0
0.1 and	0.1	S,N	5	1.2	N	0	0	S,N	1	0.3	C	0	0	N	0	0	N	0	0
0.1 and	1.0	S,N	17	1.7	N	0	0	R,N	0	0	S,N	1	0.1	S,N	3	0.4	N	0	0
0.1 and	10.0	S,R,C,N	1	0.1	N	0	0	N	0	0	R,C,N	0	0	C	0	0	C	0	0
1.0 and	0.01	N	0	0	S,N	10	1.2	N	0	0	X	0	0	N	0	0	C	0	0
1.0 and	0.1	S,N	44	4.4	S,N	6	0.9	N	0	0	N	0	0	N	0	0	S,N	10	1.1
1.0 and	1.0	S,N	45	5.6	S,C,N	16	2.3	N	0	0	N	0	0	S,N	1	0.1	S,N	14	1.5
1.0 and	10.0	S,N	29	2.6	C	0	0	C,N	0	0	S,R,C,N	1	0.1	R,C,N	0	0	C,N	0	0
10.0 and	0.01	N	0	0	S,N	7	0.9	N	0	0	S,N	9	1.5	C	0	0	C	0	0
10.0 and	0.1	S,N	7	0.9	N	0	0	C,N	0	0	X	0	0	X	0	0	C	0	0
10.0 and	1.0	S,N	36	5.1	S,N	1	0.1	N	0	0	N	0	0	S,N	3	0.6	N	0	0
10.0 and	10.0	S,N	23	2.9	C,N	0	0	S,C,N	15	2.5	N	0	0	C,N	0	0	C,N	0	0

* S = shoots
R = roots
C = callus
N = pre-shoot and pre-root nodules
X = no reaction

** Average = total shoots ÷ number of uncontaminated explants.

Table A2.--In vitro reaction, total number of shoots, and average of the plant organs of 'Sirius' at all combinations of 6-BAP and NAA tested.

Hormone Concentration (mg/l) 6-BAP	NAA	LEAF DISCS			STEM SEGMENTS			LEAF PETIOLES			FLOWER BUDS			PEDICELS			PEDUNCLE SEGMENTS		
		Reaction*	Total Shoots	Ave.**	Reaction	Total Shoots	Ave.	Reaction	Total Shoots	Ave.	Reaction	Total Shoots	Ave.	Reaction	Total Shoots	Ave.	Reaction	Total Shoots	Ave.
0.01	0.01	N	0	0	S,N	33	3.7	N	0	0	X	0	0	X	0	0	S,N	1	0.1
0.01	0.1	N	0	0	R	0	0	N	0	0	X	0	0	X	0	0	S,N	7	1.0
0.01	1.0	R	0	0	R,N	0	0	R,N	0	0	R,N	0	0	R,N	0	0	R,N	0	0
0.01	10.0	R,N	0	0	R,N	0	0	R,N	0	0	R,N	0	0	S,R,N	2	0.4	C,N	0	0
0.1	0.01	X	0	0	S,N	28	5.6	X	0	0	X	0	0	X	0	0	N	0	0
0.1	0.1	C,N	0	0	S,N	15	2.1	N	0	0	N	0	0	N	0	0	N	0	0
0.1	1.0	R,N	0	0	R,N	0	0	N	0	0	S,R,N	1	0.1	R,N	0	0	R,N	0	0
0.1	10.0	R,C,N	0	0	R,N	0	0	C,N	0	0	R,N	0	0	R,N	0	0	R,N	0	0
1.0	0.1	N	0	0	S,N	84	16.2	N	0	0	X	0	0	X	0	0	S,N	7	0.9
1.0	0.1	S,N	15	1.9	S,N	12	1.3	N	0	0	N	0	0	S,N	18	2.2	S,N	10	1.2
1.0	1.0	S,C,N	11	2.7	C	0	0	N	0	0	S,N	8	0.9	S,N	21	2.6	S,N	4	0.5
1.0	10.0	N	0	0	R,N	0	0	S,N	12	2.4	S,R,N	13	1.4	S,C,N	6	0.7	S,N	5	0.7
10.0	0.01	S,N	11	1.8	N	0	0	N	0	0	S,N	12	1.3	X	0	0	X	0	0
10.0	0.1	S,N	53	7.6	X	0	0	N	0	0	S,N	9	1.3	N	0	0	N	0	0
10.0	1.0	S,N	4	0.6	S,N	17	1.7	N	0	0	C,N	0	0	S,N	2	0.2	N	0	0
10.0	10.0	S,C,N	18	2.2	S,N	1	0.1	C,N	0	0	C,N	0	0	S,C,N	1	0.1	S,C,N	2	0.3

* S = shoots
R = roots
C = callus
N = pre-shoot and pre-root nodules
X = no reaction

** Average = total shoots ÷ number of uncontaminated explants.

Table A3.--In vitro reaction, total number of shoots, and average of the plant organs of 'Granat' at all combinations of 6-BAP and NAA tested.

Hormone Concentration (mg/l) 6-BAP	NAA	LEAF DISCS			STEM SEGMENTS			LEAF PETIOLES			FLOWER BUDS			PEDICELS			PEDUNCLE SEGMENTS		
		Reaction*	Total Shoots	Ave.**	Reaction	Total Shoots	Ave.	Reaction	Total Shoots	Ave.	Reaction	Total Shoots	Ave.	Reaction	Total Shoots	Ave.	Reaction	Total Shoots	Ave.
0.01 and	0.01	X	0	0	S,N	12	1.7	N	0	0	X	0	0	X	0	0	X	0	0
0.01 and	0.1	N	0	0	S	1	0.1	N	0	0	X	0	0	X	0	0	N	0	0
0.01 and	1.0	R,N	0	0	R,N	0	0	N	0	0	R,N	0	0	R,N	0	0	R,C,N	0	0
0.01 and	10.0	R,N	0	0	N	0	0	R,N	0	0	R,N	0	0	R,C,N	0	0	C,N	0	0
0.1 and	0.01	C,N	0	0	X	0	0	S,N	1	0.2	N	0	0	X	0	0	X	0	0
0.1 and	0.1	S,N	11	1.4	S,N	2	0.3	S,N	4	1.5	N	0	0	S,N	7	0.8	S,N	2	0.5
0.1 and	1.0	S,R,C,N	42	4.7	R,N	0	0	S,N	1	0.1	S,N	3	0.3	S,R,N	12	1.3	S,R,C,N	2	0.2
0.1 and	10.0	S,N	38	3.8	C,N	0	0	S	7	1.7	R,C,N	0	0	S,C,	3	0.4	C,N	0	0
1.0 and	0.01	S,N	43	7.2	N	0	0	N	0	0	N	0	0	N	0	0	S	2	0.3
1.0 and	0.1	S,C,N	83	8.3	N	0	0	S,N	5	1.0	S,N	17	2.1	S,N	41	4.5	S,N	22	5.5
1.0 and	1.0	S,N	116	19.3	N	0	0	S,N	72	24.0	S,C,N	7	0.9	S,C,N	5	0.6	S,N	31	6.2
1.0 and	10.0	S,C,N	48	8.0	S,C,N	17	2.8	S,C,N	11	2.2	S,C,N	10	1.7	S,C,N	4	0.5	C	0	0
10.0 and	0.01	S,N	97	9.7	X	0	0	N	0	0	X	0	0	X	0	0	X	0	0
10.0 and	0.1	S,N	194	19.4	N	0	0	S,N	too immature		S,N	4	0.5	N	0	0	N	0	0
10.0 and	1.0	S,C,N	1	0.1	N	0	0	S,N	27	6.7	S,N	110	12.2	S,N	96	12.0	C,N	0	0
10.0 and	10.0	S,C,N	150	15.0	C	0	0	S,C	10	2.0	S,C,N	0	0	C	0	0	C,N	0	0

* S = shoots
R = roots
C = callus
N = pre-shoot and pre-root nodules
X = no reaction

** Average = total shoots ÷ the number of uncontaminated explants.

Table A4.--*In vitro* reaction, total number of shoots, and average of the plant organs of 'Solferinopurpur' at all combinations of 6-BAP and NAA tested.

Hormone Concentration (mg/l) 6-BAP	NAA	LEAF DISCS			STEM SEGMENTS			LEAF PETIOLES			FLOWER BUDS			PEDICELS			PEDUNCLE SEGMENTS		
		Reaction*	Total Shoots	Ave.**	Reaction	Total Shoots	Ave.	Reaction	Total Shoots	Ave.	Reaction	Total Shoots	Ave.	Reaction	Total Shoots	Ave.	Reaction	Total Shoots	Ave.
0.01	and 0.01	X	0	0	S,N	4	0.5	N	0	0	X	0	0	X	0	0	X	0	0
0.01	and 0.1	X	0	0	S,N	4	0.5	N	0	0	X	0	0	X	0	0	X	0	0
0.01	and 1.0	C,N	0	0	N	0	0	S,N	6	2.0	S,R,N	10	1.1	S,R,N	3	0.3	N	0	0
0.01	and 10.0	C,N	0	0	C,N	0	0	C,N	0	0	R,C,N	0	0	S,N	29	5.8	N	0	0
0.1	and 0.01	N	0	0	C	0	0	N	0	0	X	0	0	N	0	0	X	0	0
0.1	and 0.1	N	0	0	N	0	0	S,N	too immature		S,C	6	0.9	S,C	3	0.4	C	0	0
0.1	and 1.0	N	0	0	S,N	7	1.4	N	0	0	S,N	1	0.2	S,N	8	1.0	N	0	0
0.1	and 10.0	S,R,C,N	4	0.8	C	0	0	C	0	0	S,C,N	1	0.1	C,N	0	0	N	0	0
1.0	and 0.01	S,N	6	0.8	S,N	9	1.0	N	0	0	X	0	0	S,N	4	0.7	S,N	7	0.8
1.0	and 0.1	N	0	0	S,C,N	8	0.9	S,N	too immature		X	0	0	S,N	11	1.8	N	0	0
1.0	and 1.0	N	0	0	S,N	12	2.4	S,N	6	1.2	N	0	0	S,C,N	23	2.9	N	0	0
1.0	and 10.0	S,N	4	1.0	C	0	0	C,N	0	0	S,N	7	0.9	S,C,N	7	0.9	N	0	0
10.0	and 0.1	N	0	0	S	11	1.2	S	7	2.3	X	0	0	X	0	0	S,N	3	0.3
10.0	and 0.1	S,N	4	0.6	S,N	22	2.4	S,N	1	0.2	N	0	0	N	0	0	N	0	0
10.0	and 1.0	S,C,N	15	3.0	N	0	0	S,C,N	51	10.2	C,N	0	0	S,C,N	too immature		C,N	0	0
10.0	and 10.0	S,C,N	18	3.6	C,N	0	0	C,N	0	0	S,C,N	22	2.4	C,N	0	0	N	0	0

* S = shoots
R = roots
C = callus
N = pre-shoot and pre-root nodules
X = no reaction

** Average = total shoots ÷ number of uncontaminated explants.

Table A5.--In vitro reaction, total number of shoots, and average of the plant organs of 'Korall' at all combinations of 6-BAP and NAA tested.

Hormone Concentration (mg/l) 6-BAP	NAA	LEAF DISCS			STEM SEGMENTS			LEAF PETIOLES			FLOWER BUDS			PEDICELS			PEDUNCLE SEGMENTS		
		Reaction*	Total Shoots	Ave.**	Reaction	Total Shoots	Ave.	Reaction	Total Shoots	Ave.	Reaction	Total Shoots	Ave.	Reaction	Total Shoots	Ave.	Reaction	Total Shoots	Ave.
0.01 and	0.01	N	0	0	S	1	0.2	N	0	0	X	0	0	X	0	0	X	0	0
0.01 and	0.1	X	0	0	S,N	23	2.3	X	0	0	R,C	0	0	C	0	0	X	0	0
0.01 and	1.0	R,N	0	0	N	0	0	R,N	0	0	R,N	0	0	S,R	9	1.3	N	0	0
0.01 and	10.0	R,N	0	0	R,N	0	0	R,N	0	0	N	0	0	N	0	0	R,N	0	0
0.1 and	0.01	R,N	0	0	R,N	0	0	N	0	0	X	0	0	X	0	0	X	0	0
0.1 and	0.1	N	0	0	S,N	5	1.0	N	0	0	N	0	0	N	0	0	N	0	0
0.1 and	1.0	S,R,N	9	1.3	S,N	8	1.1	N	0	0	N	0	0	N	0	0	S,N	1	0.1
0.1 and	10.0	S,R,N	2	0.2	S,R,N	1	0.1	S,N	7	1.4	S,R,N	6	0.7	S,R,N	8	0.9	R,C,N	0	0
1.0 and	0.01	N	0	0	S,N	15	2.1	N	0	0	N	0	0	C	0	0	C,N	0	0
1.0 and	0.1	S,N	25	4.2	S,N	12	1.5	S,N	21	5.2	N	0	0	S,N	4	0.5	S,N	9	1.0
1.0 and	1.0	S,N	47	7.8	S,N	34	5.7	S,C,N	32	8.0	S,N	35	3.9	S,N	27	3.0	N	0	0
1.0 and	10.0	C,N	0	0	S,C,N	15	7.5	S,C,N	51	17.0	S,N	9	1.0	S,N	6	0.9	S,N	6	0.7
10.0 and	0.01	N	0	0	X	0	0	X	0	0	S,N	17	1.9	S,N	50	10.0	X	0	0
10.0 and	0.1	N	0	0	N	0	0	S,N	3	0.7	N	0	0	N	0	0	N	0	0
10.0 and	1.0	S,N	5	0.8	S,N	136	19.4	S,C,N	8	1.6	S,N	35	5.8	S,N	36	5.1	N	0	0
10.0 and	10.0	S,C,N	6	0.7	C	0	0	S,N	6	1.2	S,C,N	17	2.8	S,C,N	30	6.0	C,N	0	0

* S = shoots
R = roots
C = callus
N = pre-shoot and pre-root nodules
X = no reaction

** Average = total shoots ÷ number of uncontaminated explants.

Table A6.--Highest average of each plant part of each cultivar and the corresponding hormone combination.

Cultivar	Plant Organ*	Hormone Combination (mg/l) 6-BAP	NAA	Average**
'Mace'	LD	1.0	1.0	5.6
	SS	0.01	0.1	3.5
	LP	0.1	0.01	2.7
	FB	10.0	0.01	1.5
	PE	10.0	1.0	0.6
	PU	1.0	1.0	1.5
'Sirius'	LD	10.0	0.1	7.6
	SS	1.0	0.01	16.2
	LP	1.0	10.0	2.4
	FB	1.0	10.0	1.4
	PE	1.0	1.0	2.6
	PU	1.0	0.1	1.2
'Granat'	LD	10.0	0.1	19.4
	SS	1.0	10.0	2.8
	LP	1.0	1.0	24.0
	FB	10.0	1.0	12.2
	PE	10.0	1.0	12.0
	PU	1.0	1.0	6.2
'Solferinopurpur'	LD	10.0	10.0	3.6
	SS	10.0	0.1	2.4
	LP	10.0	1.0	10.2
	FB	10.0	10.0	2.4
	PE	0.01	10.0	5.8
	PU	1.0	0.01	0.8
'Korall'	LD	1.0	1.0	7.8
	SS	10.0	1.0	19.4
	LP	1.0	10.0	17.0
	FB	10.0	1.0	5.8
	PE	10.0	0.01	10.0
	PU	1.0	0.1	1.0

* LD = leaf disc
SS = stem segment
LP = leaf petiole
FB = flower bud
PE = pedicel
PU = peduncle segment

** Average = total shoots ÷ total number of uncontaminated explants

BIBLIOGRAPHY

BIBLIOGRAPHY

Broertjes, C. 1969. Mutation breeding of Streptocarpus. Euphytica 18:333-339.

Broertjes, C. 1972a. Mutation breeding of Achimenes. Euphytica 21:48-63.

Broertjes, C. 1972b. Use in plant breeding of acute, chronic, or fractionated doses of X-rays or fast neutrons as illustrated with leaves of Saintpaulia. Thesis Wagenigen: Agric. Res. Rep. 776, 74 pp.

Broertjes, C., B. Haccius, and S. Weidlich. 1968. Adventitious bud formation on isolated leaves and its significance for mutation breeding. Euphytica 17:321-344.

Broertjes, C. and L. Leffring. 1972. Mutation breeding of Kalanchoe. Euphytica 21:415-423.

Broertjes, C., S. Roest, and G. S. Bokelmann. 1976. Mutation breeding of Chrysanthemum morifolium Ram. using in vivo and in vitro adventitious bud techniques. Euphytica 25:11-19.

Doorenbos, J., and J. J. Karper. 1975. X-ray induced mutations in Begonia hiemalis. Euphytica 24:13-19.

Linsmaier, E. M., and F. Skoog. 1965. Organic growth factor requirements of tobacco tissue cultures. Physiologica Plantarum 18:100-127.

Mikkelsen, E. P. 1976. Histology of adventitious shoot and root formation on leaf-petiole cuttings of Begonia hiemalis Fotsch. cv. 'Aphrodite Peach'. M.S. thesis, Michigan State University.

Power, J. B., E. M. Frearson, D. Gearge, P. K. Evans, S. F. Berry, C. Hayward, and E. C. Cocking. The isolation, culture, and regeneration of protoplasts in the genus Petunia. Plant Science Letters 7:51-56.

Schwabe, W. W. 1969. Kalanchoe blossfeldiana Poellniz. In L. T. Evans (ed.). The induction of flowering. MacMillan. Australia. pp. 227-247.

Skirvin, R. M. and Jules Janick. 1976. Tissue culture-induced variation in scented Pelargonium spp. J. Amer. Soc. Hort. Science 101(3):281-290.

Sparrow, A. H., R. C. Sparrow, and L. A. Schairer. 1960. The use of X-rays to induce somatic mutations in Saintpaulia. African Violet Magazine 13:32-37.

Warfield, D. 1973. Induction of mutations in african violet (Saintpaulia ionantha Wendl) by ethyl methanesulfonate. Hort. Science 8(1):29.