OTIMIZING GRAFT UNION FORMATION BETWEEN SWEET POTATO (IPOMOEA BATATAS) AND BUSH MORNING GLORY (IPOMOEA CARNEA SSP. FISTULOSA) AS A PRELUDE TO CHIMERA DEVELOPMENT

A Senior Thesis

By

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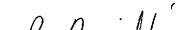

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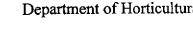

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ABSTRACT

Optimizing Graft Union Formation between Sweet Potato (*Ipomoea batatas*) and Bush Morning Glory (*Ipomoea carnea* ssp. *fistulosa*) As a Prelude to Chimera Development

Douglas W. Maxwell

Bush morning glory (*Ipomoea carnea* ssp. *fistulosa*) and ornamental sweet potato (*Ipomoea batatas* 'Blackie') were grafted using various procedures. Lanolin pastes containing 3% BA, 3% NAA, and a mixture of 3% BA / 3% NAA were tested as a means of promoting adventitious shoot development at the graft union. Excellent "take" was obtained with all graft types tested. The growth regulator treatments resulted in marked differences in callus formation at the wounded graft union. Lateral shoot development below the graft union also was affected by the growth regulator treatments. The highest quantity of callus was formed using the mixed paste while BA enhanced lateral shoot formation below the union. No adventitious shoot development occurred in or near the graft union. Results indicate that in vivo grafting of these two species may not be the best procedure for obtaining interspecific chimeras.

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CHAPTER I - INTRODUCTION

The myth of the Chimera dates back to the 9th century BC as Homer described the beast "in the fore part a lion, in the hinder a serpent, and in the middle a goat" (Bardi, 1997). In horticulture the chimera is no beast at all but a source of great variability in plants. The term chimera was coined in 1907 by H. Winkler as a plant composed of two genetically distinct tissue layers (Dermen, 1960).

The ways in which a chimera manifests itself in a plant are many. Variegation in foliage is the most common and most widely recognized form such as in varieties of *Dieffenbachia*, *Sansevieria*, Poinsettia, *Hosta*, *Saintpaulia*, and many more. This condition usually results from a spontaneous mutation in chlorophyll synthesis or plastid formation (Marcotrigiano, 1997). Other examples include thornlessness such as in blackberries (McPheeters & Skirvin, 1982), alterations in flower petal color as in African Violet (Lineberger & Druckenbrod, 1985), and others. To comprehend the nature of a chimera, one must first visualize cell tissue arrangement in plants.

In dicots, cells of a meristem are arranged in three layers (histogens) -- LI, LII, and LIII, outer to inner (Figure 1) (Tilney-Basset, 1986). Two and three layer meristems are typically found in

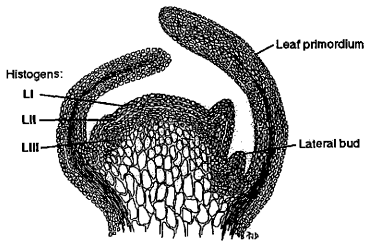

Figure 1 Meristem cross-section showing histogens in a dicot

monocots. These histogens are present in apical regions from which all organs are formed. Histogen expression in leaves and other plant organs varies among species, many expressing all three layers in the lamina from margin (LI) to midrib (LIII). When the genotype, and subsequently phenotype, of a layer is different from the others, this variability may be expressed throughout the plant.

In *Chlorophytum comosum*, for example, foliar variegation develops as alternating stripes lacking chlorophyll production. The result is green and white foliage. This condition is represented by the G-W-G or W-G-W notation. For example, a plant with a W-G-G histogen arrangement, as in the cultivar 'Vittatum,' would have a white LI, green LII, and green LIII. The leaf of this plant has white margins with a green midrib region. While this example is basic, the principle applies to a myriad of other chimeras.

Genotypic variation arising by mutation is the origin of a plant chimera. As a mutated cell divides in the apical dome, it becomes possible for an entire tissue layer to change in genotype. The likelihood that a chimera will be recognized once formed relies heavily on the phenotypic change. Variegation is easily noticeable while more discrete chemical changes often go unnoticed (Lineberger, 1997).

The degree to which a group of mutated cells overtakes a layer determines the phenotype of the plant as well as the overall stability of the condition. There are three types of chimeras which delineate these characteristics (Figure 2). Periclinal chimeras are the most stable and the most important horticulturally. This condition occurs when a cell mutates very near the crest of the apical dome so that the resulting layer is comprised completely of the new

genotype. This type of chimera can also be propagated vegetatively and still maintain the varied histogens.

Mericlinal chimeras also involve mutating cells in a single layer, but unlike periclinal chimeras, these do not produce entire layers of the new genotype. This condition forms as a cell mutates further from the apex so that cells of both genotypes are present in one layer. Mericlinal chimeras are often very unstable, reverting back to the original genotype.

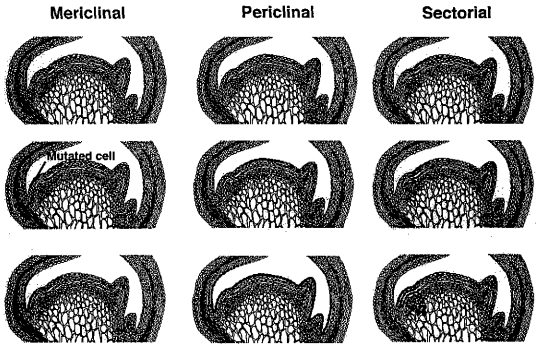

Figure 2 Development of chimeras

The third category is sectorial chimeras. Where the first two types are characterized by mutations in distinct layers, sectorial chimeras are comprised of genetically varied regions that extend through all three layers but do not cover the entire circumference. While this condition is technically a chimera, the shoots that arise from these regions are often of a single

genotype, depending on their origin. For example, a shoot arising from the portion of the circumference composed of mutated cells would consist only of those cells. While different from the original genotype, this shoot is not chimeral. Sectorial chimeras are also very unstable and are not important horticulturally other than for the genetic variation that results from mutant shoots.

Apart from naturally occurring chimeras, considerable effort has been directed toward experimental synthesis of custom chimeras between different genotypes, species, and even genera. These plants, primitively named graft-hybrid chimeras (Tilney-Basset, 1963), offer a wide range of phenotypic variation in theory to the control of horticulturists. The concept is basic. Determine a means of uniting tissues of two different genotypes in such a way that shoot meristems arise from a heterogeneous cell mixture. The resulting shoot then contains the tissues of the two distinct genotypes.

Unlike most chimeras, graft chimeras are rare (Marcotrigiano, 1986). These arise not from a single mutant cell but are produced as adventitious shoots proposed to be of multicellular origin. Neilson-Jones (1969) showed that shoot apical meristems arising from callus regions formed at the graft of two species can possess cells from both species.

Most reported cases of interspecific chimeras are members of the Solanaceae, primarily among the genus *Solanum*. However, genera in other families have been successfully grafted to form interspecific chimeras (Marcotrigiano and Goiun, 1984). Intergeneric graft chimeras are even more scarce. Examples include *Crataego-mespilus*, intergeneric graft chimera of *Mespilus germanica* over *Crataegus monogyna*, and *Laburnum adami*, graft chimera of *Cytisus purpureus* over *Laburnum anagyroides* (Dermen, 1960).

Other horticultural chimeras include examples such as the blackberry 'Thornless Evergreen' (McPheeters and Skirvin, 1982), the grape 'Meunier,' a number of poinsettia cultivars (Tilney-Bassett, 1986), *Ajuga* 'Burgundy Glow,' and pinwheel flowering african violets (Lineberger and Druckenbrod, 1985). Chimeric plants expressing variegation are the most commonly recognized; however, any number of mutations can stabilize themselves as plant chimeras.

Reaching back to ancient Greek mythology, the chimera is a unique condition in plants which yields unending phenotypic variation from chance and often unstable mutations. To harvest and eventually control this genetic variation offers a new realm of plant diversity. While today's botanists travel to the far corners of the world in search of new and unusual specimens for the garden, tomorrow's plant scientists may simply look within the plant. This mix of science, creativity, and chance is the true magnificence of the chimera.

Research goals

The goal of this study is to introduce novel forms of bush morning glory and sweet potato that are significant to the horticultural industry. To accomplish this, a procedure for creating interspecific chimeras *in vivo* between sweet potato and bush morning glory will be attempted. Finally, any chimeric shoots that develop will be identified and classified to give insight into the origin of plant chimeras in species of *Ipomoea* as well as members of the Convolvulaceae.

CHAPTER II - LITERATURE REVIEW

The experimental synthesis of plant chimeras is well documented in literature. Variables that have been investigated include synthesis procedures, plant growth regulator applications, as well as observations of success within certain plant families.

In vitro *synthesis*

Marcotrigiano and Gouin (1984) theorized two means of chimera formation in vitro. While neither procedure was successful in the synthesis of interspecific chimeras of *Nicotiana*, a review of the two is beneficial in understanding the goals of in vitro synthesis.

The "passive capture" technique occurs as developing cell meristems incorporate neighboring callus of a different genotype into its organization thus developing into a chimeric shoot. Alternatively, "active participation" synthesis utilizes a media containing a hormonal regime favoring organogenesis of both genotypes so that meristem formation occurs simultaneously creating, in some instances, chimeric shoots. These systems were tested by Marcotrigiano and Gouin using mixed callus on a solid medium as well as in suspension culture.

A novel application of grafting in a plant tissue culture systems was first developed by Murashige et al. as a means of virus cleansing in *Citrus* in the early 1970's (Murashige, 1972). Noguchi, Hirata, and Yagishita (1992) utilized a similar system of *in vitro* grafting to successfully synthesize interspecific chimeras of *Brassica*.

Seedlings of *Brassica oleracea* and *B. campestris* were approach grafted at the meristem and bound with surgical fiber under aespetic conditions and subsequently grown *in vitro* on half strength MS medium containing 0.2 mg/l IBA and 2mg/l BA until the graft union was secure. Cross sections of the grafted region were then cultured on full strength MS medium containing the same growth regulators to promote shoot formation. This means was capable of producing chimeras at a rated of 53%.

Micrografting has also been applied, although unsuccessfully, to chimera fomation in *Rubus* cultivars (Chen, et al., 1996) as a theoretical means of conferring pest resistance in the LI layer. Different from the previous study, researchers utilized an in vitro wedge graft bound by sterilized silicon rubber tubing to unite the two genotypes. While no chimeras were produced by these means, the study shows the possibilities of micrografting in graft union.

In vivo *synthesis*

Reciprocal slice grafts between species of *Nicotiana* proved to be a successful means of uniting two genotypes and obtaining chimeric shoots from the union (Marcotrigiano and Gouin, 1984). Mature plants are reciprocally grafted and bound with budding rubbers. The scion is cut to only a single node and lateral shoots are continously removed throughout the process. The grafted specimens are placed under intermittant mist until the graft is secure. Kaddoura and Mantell (1991), using a similar procedure in *Nicotiana-Solanum* grafts sited 15 days as the period necessary for the graft to secure itself.

Following grafting, the scion is decapitated leaving only a thin layer attached to the stock. A percentage of adventitious shoots arising the from the union of the two genotypes

have been observed as chimeric in both studies. Subsequently, growth regulator treatments were tested to determine effects on chimera generation.

The success of this system of *in vivo* grafting has secured its use in a number of research studies. However, it is limited to plants which are similar in stem width and morphology. The lack of variation and novel approaches in grafting techniques exhibited in published research has prompted a test of graft types in this study.

Plant Growth Regulator Applications

The endogenous hormone concentrations within plants vary among genotypes. The applications of certain plant growth regulators can enhance organogenesis and therefore give researchers advantages in plant customization. Two of the most important growth regulators are cytokinins and auxins favoring the formation of shoots and roots respectively. The application of these growth regulators to the decapitated grafts in chimera synthesis studies has produced conflicting results. For this reason, studies of these compounds has been included in this research.

Goffreda, et al. (1990) successfully produced interspecific chimeras of *Lycopersicon* simply using reciprocal splice grafts decapitated through the graft region without the application of any plant growth regulator. Endogenous levels of auxin and cytokinin were apparently adequate to favor the formation of callus and adventitious shoots at the site of decapitation. However, this is not always the case.

In *Solanum*, another Solanaceous plant, the auxin CPA increased the production of adventitious shoots at the graft union while BAP, a cytokinin, appeared to inhibit the

formation of adventitious shoots (Kaddoura and Mantell, 1991). While this seems to go contrary to the basic understanding of auxin/cytokinin interations, this study agrees with a similar test with different species of Solanum conducted by Clayberg (1975).

Marcotrigiano and Gouin (1984) found the same CPA (auxin) concentration to inhibit adventitious shoot production in their study of *Nicotiana* interspecific grafts, even though it did increase callus formation at the graft union. In fact, greatest chimeric shoot production was obtained without the formation of any callus and without the application of any growth regulator. Obviously, the effect of plant growth regulator treatments varies between plant genotypes.

Malinich and Lineberger (1987) found cytokinin (BA) applications to increase adventitious shoot production in African Violet inflorescences. Shoot proliferation increased with concentrations up to a 1% BA paste, the most concentrated application tested. It is based on this research that the cytokinin BA was first utilized in this study as a means of promoting adventitious shoot proliferation.

Successes

Most of the success stories in experimental synthesis of plant chimeras have been limited to herbaceous plants and to the families Solanaceae and Brassicaceae, genera including: *Solanum*, *Lycopersicon*, *Nicotiana*, and *Brassica*. A large number of horticulturally important crops are plant chimeras; however, their origin is usually spontaneous and not the result of experimental synthesis.

While there is considerable documentation on synthesis of chimeras, limitation to certain plant families has yielded only science with little success in applied uses. This research attempts to apply the principles discussed to the creation of a custom interspecific chimera between species in the Convolvulaceae.

CHAPTER III - MATERIALS AND METHODS

The starting point for the research was to identify a means for producing healthy grafts of sweet potato and bush morning glory. Secondly, tests were designed to determine optimal procedures for adventitious shoot formation near the graft union. Longer term steps include the establishment and identification of any chimeric shoots.

Plant material

Bush morning glory (*Ipomoea carnea* ssp. *fistulosa*) is an erect herbaceous tropical shrub reaching heights in excess of 6 feet. As with other members of the Convolvulaceae, clusters of funnelform lavender flowers adorn bush morning glory. This impressive floral show makes the plant a welcomed addition to any garden either as an annual in northern climes or a perennial in the south. However, its tall habit and rather lanky growth is a considerable drawback for inclusion in the home garden.

Since this plant is relatively new to the ornamental industry in the U.S., variation would definitely be beneficial. A dwarfed form or foliage interest would aid the success of this plant in the market. One means of accomplishing this is to create a chimera with a low growing related species with foliage interest. The species chosen is the ornamental sweet potato 'Blackie' (*Ipomoea batatas* 'Blackie').

'Blackie' is a low growing trailing sweet potato with maroon foliage and dissected margins. The plant requires similar cultural requirements as bush morning glory. Interspecific

chimeras between these two genotypes are proposed to offer plants which would be rapidly accepted in the home garden.

The cultivar 'Blackie' was chosen for a number of reasons. The variation in leaf color and morphology are characteristics of interest in the custom chimera desired. Also, the anthocyanin pigments which are found in the epidermal layer of this plant would provide a means of identification in the even chimeric shoots develop. This type of marker is advantageous in the designation and classification of plant chimeras (Marcotrigiano & Gouin, 1984).

Grafting

Three grafting techniques were performed to determine which methods resulted in the most favorable graft union. For each type, the sweet potato served as the scion and the bush morning glory as the stock. This arrangement was chosen for mechanical reasons since the trailing habit of sweet potato would not effectively support a bush morning glory scion. The graft types tested are (Figure 3):

- Wedge Graft (A)
- Side Veneer Graft (B)
- Topworking (C)

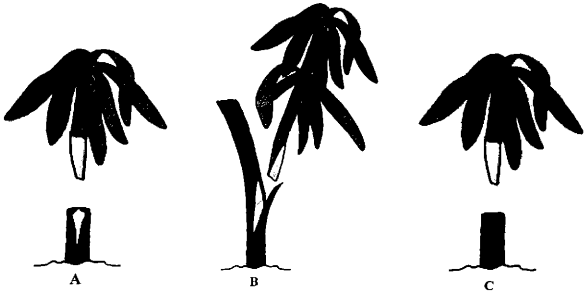

Figure 3 Graft types tested A: Wedge graft, B: Side veneer graft, C: Topworking

In the wedge graft, the scion was cut into a wedge and inserted into a V-shaped, complementary notch in the stock apex (A). This created a contact of cambium at one point. With the side veneer grafts, the stock is notched on the side to accept the scion again cut into a wedge shape (B). Cambium contact at two points was created by this method. The final graft type utilizes the hollow stem characteristic of bush morning glory. Topworking was accomplished by first removing the epidermis of the lower portion of the scion and inserting it into the stem of bush morning glory (C). Care was taken to match the stem diameters of both plants to create intimate contact around the entire circumference.

After each graft, the union was wrapped in parafilm M and placed under intermittent mist for two weeks. The plants were then moved and grown under reduced light for an additional week before moving to full sun. This allows the graft to secure itself and harden-off before subjected to traditional greenhouse growing conditions.

Shoot induction

Seven weeks following the initial grafting, the plant was decapitated by cutting through the graft union at approximately 45° away from the scion (Figure 4). This allows any latex produced by either species to drain away from the union so there is no interference with adventitious shoot formation. Only a small portion (≈5mm) of the scion was left attached to the stock, and a relatively large surface area of united stock and scion is exposed.

Figure 4 Decapitation of the scion

The wound region was then treated with anhydrous lanolin pasted and capped with aluminum foil. Variable growth regulator treatments including no treatment, 3% BA, 3% NAA, and 3%BA / 3%NAA were tested in a number of tests to determine the effects on callus formation and shoot development. Also, lateral shoots from the stock were removed to focus plant resources toward the graft union.

Statistical Analysis

Significant difference between means was determined using t-tests and nonparametric mean separation (Kolmogorov-Smirnov) in the case of callus growth. No difference in the mean separation at α=0.05 was seen between these two tests. The analysis package used was SPSS - Windows, Release 7.5 (SPSS, 1997).

Experiments

Test A (n=30)

Table 1

	Variables
Graft Types	Topworking
Growth Regulators	Lanolin paste only (control), 3% BA paste

Test B (n=18)

Table 2

	Variables
Graft Types	Wedge Graft, Side Veneer Graft, Topworking
Growth Regulators	Lanolin paste only (control), 3% BA paste
Notes	Replicates plant growth regulator test of Test A

Test C (n=52)

Table 3

	Variables
Graft Types	Wedge Graft, Side Veneer Graft, Topworking
Growth Regulators	Lanolin paste only (control), 3% BA paste, 3% NAA Paste, Mixed 3% BA / 3% NAA paste
Notes	Replicates graft test of Test B

Test D (n=33)

Table 4

	Variables
Graft Types	Wedge Graft, Side Veneer Graft, Topworking
Growth Regulators	Lanolin paste only (control), 3% BA paste, 3% NAA Paste, Mixed 3% BA / 3% NAA paste
Notes	Replicates growth regulator test of Test C

Shoot Induction

After decapitation, callus proliferation was observed at the graft union of several plants within days of plant growth regulator treatments. This observation was qualified using a subjective scale. Each plant received a numeric score ranging from 0 to 4 as outlined below:

Score	Observation
0	Necrotic graft union or plant death
1	No callus formation, healthy graft union
2	Slight callus formation, 1 - 2 small pockets
3	Moderate callus formation, 3+ pockets
4	Vigorous callus formation, union mostly to entirely covered by callus

Table 5 Subjective scale of callus observation

In Tests A and B, the 3% BA paste treatment produced significantly higher levels of callus proliferation at the graft union than did the control treatment of lanolin paste only (Figures 7 & 8, data presented at 4 weeks). Means separated by different letters are significantly

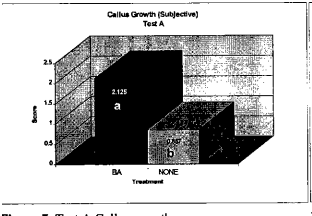

Figure 7 Test A Callus growth

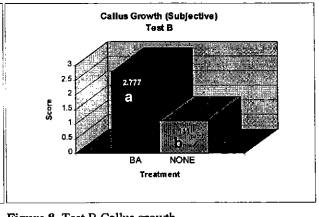

Figure 8 Test B Callus growth

CHAPTER IV - RESULTS AND DISCUSSION

Grafting

The results of the graft tests support earlier findings by Dukes, Jones and Schalk (1990) that sweet potato and bush morning glory are graft compatible. All three graft types tested proved to be viable means of uniting the two species. No significant difference in percent "take" was determined; however, greatest success was observed with the wedge and side grafts, often exhibiting greater than 90% success (Figures 5 & 6). Also, no advantage toward callus/shoot formation was determined by the use of any particular graft type tested.

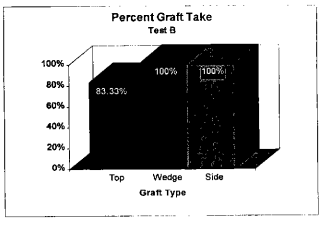

Figure 5 Test B Percent graft take

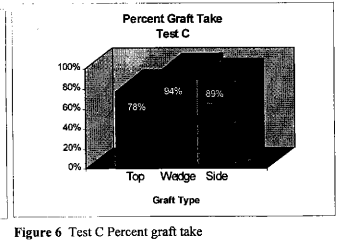

Figure 6 Test C Percent graft take

The high success rate of these grafts also might prompt their use in a curriculum designed toward teaching grafting methods. Both species are herbaceous which reduces the skill required to perform the grafts. Plus, the mix of plant habits and leaf color/morphology make the grafted specimens an interesting teaching aid.

different at α=0.05. Even higher degrees of callusing were observed in Tests C and D with the mixed treatment of 3% BA / 3% NAA paste (Figures 9 & 10). This treatment produced almost entirely vigorous callus formation and was easily statistically distinguishable from the control treatment. The individual 3% BA and 3% NAA treatments in these tests were insignificant.

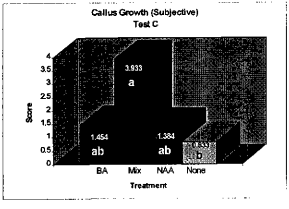

Figure 9 Test C Callus growth

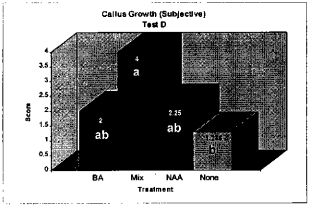

Figure 10 Test D Callus growth

While impressive callus proliferation was achieved through the growth regulator treatments, no adventitious shoots developed. Instead, the callus remained primarily unorganized. Lateral shoots developing from preexisting meristematic regions were counted and analyzed to determine the effect of the growth regulators. Data are presented in two time frames: from decapitation to four weeks and from four to eight weeks. After eight weeks, a majority of the plants had begun to decline rapidly.

No statistical difference between the treatments could be shown in any of the tests; however, some general observations may be made. First, the BA treatment seemed to

enhance shoot production in each of the tests (Figures 11 & 12). This follows the basic principle of cytokinin as a plant growth regulator. Secondly, auxin seemed to inhibit shoot formation. This was observed in the independent NAA treatment as well as the mixed paste (Figure 12).

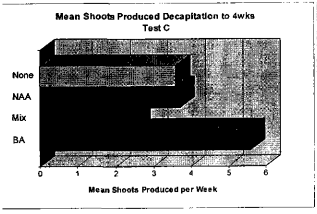

Figure 11 Mean shoots produced from decapitation to 4 weeks

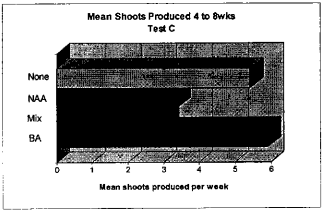

Figure 12 Mean shoots produced 4 to 8 weeks

Conclusions

It is clear that the experimental synthesis of custom chimeras is a very fickle science. A great deal of variation exists between methods of synthesis as well as plant families and even genotypes utilized. This study attempted to apply methods of synthesis via *in vivo* grafting to species of *Ipomoea.* One generalization that can be made fairly safely is that members of this genus, and possibly the Convolvulaceae as a whole, are less receptive to synthesis of chimeras than previously researched families such as Solanaceae.

The graft trials showed that the two species tested are highly graft compatible using a number of novel means. These grafting procedures might have promise in demonstrative applications.

The test of plant growth regulators clarified the basic nature of auxins and cytokinins in plant growth. A mix of the two (BA and NAA) encouraged callus proliferation by the highest degree of the treatments utilized. However, this treatment seemed to produce the fewest lateral shoots. Cytokinin, BA, encouraged the greatest lateral shoot development of the treatments tested. These results agree with those published by Marcotrigiano and Gouin (1984) studying *Nicotiana.*

Certainly more investigation is required toward the development of an interspecific chimera in the genus *Ipomoea.* Since no adventitous shoots were produced with any of the treatments, one must consider alternative synthesis means such as different growth regulator treatments, callus culture or micrografting.

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