GENETIC, MOLECULAR AND BREEDING STUDY OF COLEUS (*Solenostemon scutellarioides* (L.) CODD) DURING GROWTH AND DEVELOPMENT

By

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To my family and friends for their love and support.

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Solenostemon scutellarioides (syn. *Coleus blumei* Benth.), commonly known as coleus, is a member of the Lamiaceae family and is highly prized for its vibrant and colorful foliage. In the last decade, coleus has gained popularity in the floriculture industry with the introduction of a large number of vegetatively propagated varieties with novel foliage colors. Besides novel foliage colors, opportunities exist to increase their commercial value by manipulating plant architecture. A breeding program was initiated at the University of Florida with focus on two main objectives: (1) the development of new trailing types with bright foliage colors, and (2) the development of new varieties with improved vigor, foliage color quality and delayed flowering induction for production companies and consumers. At the completion of the breeding program, in regards to new trailing types, several with bright orange colors were successfully produced and are currently being trialed in the greenhouse. Over a three year period, twenty-one new varieties were released from the breeding program with improved vigor, foliage color quality, and delayed flowering induction. During the development of new varieties with improved color quality, many varieties in the program were observed to become dark red when exposed to high light intensities. As a lack of information existed to explain color change in coleus, a focus on a third objective was pursued. A study was conducted on two related coleus varieties, 'Royal

Glissade' and 'UF06-1-6', to determine the physiological and molecular mechanisms involved in this color change phenomenon. Light intensity was found to affect coloration by inducing the accumulation of anthocyanin pigments in the leaves. Expression of the structural genes *PAL*, *CHS*, *F3H*, *DFR*, *ANS*, and *UFGT* and regulatory genes *AN1* and *AN2* were analyzed. *DFR* and *UFGT* were found to play an important role in foliage coloration in coleus.

CHAPTER 1
LITERATURE REVIEW

Introduction and Rationale

The genus *Solenostemon* is a member of the Lamiaceae family and contains more than 500 species (Lebowitz, 1985), one of which is coleus ((*Solenostemon scutellarioides* (L.) Codd)); (syns *Coleus blumei* Benth.; *Coleus scutellarioides* (L.) Benth.). Coleus is a versatile annual plant that has been common in the commercial bedding plant industry for decades and is highly prized for its vibrant and colorful foliage. Original species utilized for cultivation and hybridization of coleus in the early 1800s were relatively simple in regards to diversity of color patterns, leaf shape, and growth habit (Stout, 1916). The first ornamental coleus was introduced into Europe from Java by Dutch horticulturist M.J.A. Willink in 1851, and due to its rapid growth rate, easy propagation, and superior performance in the landscape, coleus quickly gained popularity as a bedding plant (Lebowitz, 1985).

In 1877, William Bull introduced 150 new varieties of coleus with colorful variegations and offered a wide selection of coleus seeds for sale at 0.43 (U.S) per seed (Pedley and Pedley, 1974). With high prices possible for a garden plant, the fever to breed new varieties of coleus ensued, with main developments centering on bright colors, unique leaf shapes and distinctive plant forms (Pedley and Pedley, 1974). However, at the turn of the century, the quality of coleus declined since coleus was being developed primarily as a seed crop and only those plants that produced large amounts of seeds were favorable. Thus, the types of coleus that soon became available had foliage leaves that were dull in color and growth habits that were stunted due to early flowering (Pedley and Pedley, 1974). In the early 1940s, coleus breeding was revived again due to a growing interest in developing coleus as a commercial bedding crop. By 1982, the Bedding Plants, Inc season sales summary listed coleus as the tenth most important bedding

plant grown in the United States (Lebowitz, 1985). In the last decade, coleus has gained further popularity in the floriculture industry with the introduction of a large number of vegetatively propagated varieties with novel foliage colors. Besides foliage color, opportunities exist to increase the commercial value of coleus with regard to two key areas: (1) the manipulation of plant architecture by developing new trailing varieties with bright foliage colors, and (2) the development of new varieties with improved vigor, color quality, and delayed flowering for production companies and consumers.

Trailing growth habit in coleus is desirable as it increases its utility for use in mixed containers, in hanging baskets, and as groundcovers in the landscape. Few trailing varieties exist and those available are generally dark green or red in foliage color. Little information is available regarding the genetics of trailing growth habit in coleus and the origins of current trailing types remain unclear. Furthermore, no known research to date has been published on the development of new trailing varieties with bright foliage colors. In order to develop new varieties with these desirable characteristics, an understanding of the inheritance and the genetics controlling trailing growth habit is required.

The success of ornamental plants for the commercial market depends on certain characteristics desired by both production companies and consumers. In past breeding efforts, coleus was primarily bred for novel foliage color with upright growth habits for use in the home garden. For coleus, desirable characteristics that are in current demand include improved vigor, color quality, and late flowering induction. Plants with good overall plant vigor have fast growth rates for rapid turnover and the ability to produce maximum cuttings for production (Wilkins, 1988). In addition to vigor, color quality is desirable in coleus because consumers purchase varieties based on aesthetic appeal (Catanzaro, 2005) and faded colors decrease its product value

(Oren-shamir, 2003). Coleus with late flower induction is desirable because varieties that flower early have greater landscape maintenance and a decrease in foliage colors quality during seed production. Considering these traits, a coleus breeding program was initiated in 2003 at the University of Florida to develop new cultivars through traditional breeding methods for improved vigor for commercial production, better foliage color quality and delayed flowering induction for consumers.

In the process of developing new coleus varieties with improved color quality for the landscape, many varieties were observed to become dark red when exposed to high light intensities. Two related varieties Royal Glissade (RG) and UF06-1-6 (UF) (a seedling produced by self-pollinating RG) showed this specific color change when examined under direct light and shade conditions. As a lack of information exists to explain the physiological and molecular mechanisms for color change in coleus foliage, a broad-based approach was pursued to provide a base level of information to explain the physiological and molecular mechanisms involved in color change.

Literature Review

Coleus Ploidy and Cytology

The genus *Solenostemon* (syn. *Coleus*) contains several well-known species that are grown as sources for medicinal compounds (*C. ambionicus* and *C. forskohlii*), as food (*C. tuberosus*), and as foliar ornamentals (*C. frederici* and *C. blumei*). The most popular species in this genus, *S. scutellarioides* (syn. *C. blumei* (Benth.), commonly known as coleus, has been in cultivation since the 1800s for its vibrant and colorful foliage. Although more than 500 varieties exist on the commercial market, little is known about its genetics and breeding (Lebowitz, 1985). Diploids, triploids, and tetraploids exist and chromosome counts for several *Solenostemon* species suggest basic chromosome numbers of x = 6, 7, and 8 (Darlington and Wylie, 1955; De wet, 1958). The

majority of cytological studies on *Solenostemon* report a 2n=48 chromosome number (Reddy, 1952). Hakeem and Rife (1966) found the appearance of multivalents during meiosis in certain Solenostemon cultivars with 2n=48 chromosomes and suggested polyploidy for this species. Based on their findings and the fact that genetic experiments produced simple diploid ratios, they further suggested that varieties belonging to *S. scutellarioides* were allotetraploids. The most current ploidy number for coleus, as proposed by Galbraith (1983), indicated that all cultivated hybrid varieties under *S. scutellarioides* are tetraploids, with n = 24 based on findings from rapid flow cytometry.

Coleus Genetics

Most of the literature on coleus genetics was generated in the early to mid-1900s, with D.C. Rife (Genetics Laboratory – Ohio State University) being the major contributor in providing the inheritance of several traits (Rife, 1939; Rife, 1940; Rife, 1944; Rife, 1945). Most of the traits studied in coleus are simply inherited and controlled by a single gene (Lebowitz, 1985). Since coleus is highly prized for its foliage, many of the genetic studies in the early 1900s predominantly focused on the genetics relating to leaf color and shape. In regards to foliage color, green coloration produced by chlorophyll pigments is dominant to dilute yellow-green (Boye and Rife, 1938) and the lack of chlorophyll (albinism) is controlled by recessive genes (Rife, 1948). Solid purple on the lower leaf epidermis is dominant to green (unpigmented) lower epidermis (Rife 1944), and red stems and veins are dominant to green ones (Rife, 1948). The bright red and purple coloration found in coleus is produced primarily by anthocyanins, with purple color conferring dominance (Rife, 1948). The amount and distribution of anthocyanins in the leaves (leaf color) is controlled by an incomplete dominant gene (*N*) (Rife, 1948). In addition, a dominant gene (*S*) is responsible for dark splotches in the upper surfaces whereas the recessive allele produces no splotches (Boye, 1948). In regards to leaf shape, traits that are

dominant include crinkled leaf surfaces (Rife, 1940) and narrowed leaves (Rife, 1972).

Trailing Growth Habit

Plant architecture is the three-dimensional organization of the plant body (Godin, 2000). For above ground parts of the plants, plant architecture includes the branching pattern, as well as size, shape, and position of the leaves and flower organs (Reinhardt and Kuhlemeier, 2002). The progressive development of additional shoots, which occurs by lateral branching, establishes a particular pattern of branched growth habit (Gifford and Foster, 1989). Efforts to manipulate plant architecture by increasing branching in plants have improved their value as commercial crops. For seed crops such as guar [*Cyamopsis tetragonoloba* (L.) Taub.], varieties with high amount of branching have the potential for high seed yield and are thus considered more desirable than erect or branching varieties (Liu, 2006). In red clover (*Trifolium pratense* L.), a lack of persistence has led to a high susceptibility to root rot and a short life span. Changing the architecture to improve the persistence of existing commercial varieties has increased their asset value to farmers (Mirzaie-Nodoushan, 1999). For coleus, the manipulation of plant architecture to develop new varieties with a more branched habit, i.e. trailing types, is desirable as it increases their utility for use as groundcovers, in hanging baskets, and in mixed containers.

The genetics controlling growth habit have been investigated in a limited number of plants. Branching types in guar is grouped into four basic classifications: erect (single primary axis), erect branching (one or more branches), basal branching (branches at base of primary axis) or branching (branching throughout the primary axis). Erect branching and branching is dominant to basal branching in guar and is controlled by a single gene (Liu, 2006). In the diploid red clover, prostrateness is partially dominant to erectness with strong evidence for a dominance x dominance epistasis controlling dominant to erectness and prostrateness controlled by a few genes (Mirzaie-Nodoushan, 1999). In tetraploid, cultivated peanut (*Arachis hypogaea* L.), two

types of growth habits, runner (trailing) and bunch (erect), exist with alleles Hb1 and Hb2 affecting the growth habit. In one variety of peanuts, at least three dominant alleles, in any combination [(Hb1, Hb1, Hb2, Hb2);(Hb1, Hb1, Hb2, hb2); and (Hb1, hb1, Hb2, Hb2)], are required to produce a runner. Two, one, or no dominant alleles are necessary to produce bunched (Ashri, 1968). In the narrow-leafed lupin (*Lupinus angustifolia* L.), three loci are involved in the determination of restricted branching (lack of branching) (Adhikari, 2001). Branching in petunia is controlled by the DECREASED APICAL DOMINANCE (DAD) genes and mutations of these genes result in plants with an increase in basal axillary branches and a decrease in height (Simons, 2007). Although hundreds of different types of coleus exist with an extensive variation in growth habit (Boye and Rife, 1938), information on the genetics for trailing growth habit is scarce.

Contrasting views have been proposed as to the origins of trailing types since most modern day coleus varieties were the result of interspecific hybridizations (Lebowitz, 1985). During the early 1920s, a different form of coleus was discovered and classified as *Coleus rehneltianus*. It was characterized as a ground cover with a low and scrambling habit, and rooted where the trailing stems touched the earth (Pedley, 1974). According to Bailey Hortorium (Lebowitz, 1985), current trailing varieties were developed from interspecific crosses between *C. scutellarioides* and *Coleus pumila* Blanco. *C. pumila* was believed to be identical to *C. rehneltianus* because its habit was trailing as compared to *C. scutellarioides* with an erect growth habit (Pedley, 1974).

The most well-known commercial trailing variety currently on the market is ‘Red Trailing Queen’ (RQ). RQ is a dark-purple leaved cultivar with lateral branches that extend more than three feet in length. Although desirable for its trailing habit, scarce information on its genetics is

available as only two short references have been found for this variety in works by Rife (1948) and Reddy (1952). Rife (1948) observed that plants of the 'Trailing Queen' variety were usually highly sterile, whether selfed or crossed, and progeny were only produced when 'Trailing Queen' was used as the maternal parent. Reddy (1952) studied four clones of "Trailing Queen' and suggested that "Trailing Queen" belonged to *C. rehneltianus* because it was highly branched and had flowers conspicuously larger than other varieties assigned to *C. blumei*.

Flowering

Flowering is not preferred in coleus because flowers decrease its foliage quality. The production of flowers often slows plant growth due to the remobilization of stored reserves from the leaves to the flowers and seeds, and increases landscape maintenance (Pedley and Pedley, 1974). The flowering response in plants is regulated through a complex interaction of several defined or overlapping genetic pathways. Two genetic pathways include photoperiod (Lin, 2000) and the circadian rhythm (Samach and Coupland, 2000; Thomas, 2006). Other factors affecting flowering responses in plants are light quality and quantity (Thomas, 2006).

Flowering responses in plants are often synchronized by night length and can be divided into five main groups (Thomas and Vince-Prue, 1997): short-day plants (SDP, flowers when night length exceeds a critical length); long-day plants (LDP, flowers when night length is shorter than the critical length); day-neutral plants (DNP, flowers irrespective of day/night length); intermediate-day plants (flower only when day length is neither too short or long); and ambiphotoperiodic-day plants (dual day length requiring plants, requires both SD and LDs in a sequence). Most crops that are grown for spring or summer production display a flowering response to day length and exhibit a photoperiodic response (Mattson and Erwin, 2005).

Variations in flowering times are apparent in many plant species. *Lathryus odoratus* has three flowering response classes: those that flower in the winter (DNP), in the spring (facultative

LDP) and in the summer (obligate LDP) (Ross and Murfet, 1985). Evaluations for variability in flowering traits for 66 accessions of rose clover (*Trifolium hirtum* All) showed a range of differences in time to reach maturity from 162 to 183 days, with three different maturing groups observed: early, midseason, and late (Nunes and Smith, 2003). Researchers have described long-day, short-day, and day-neutral photoperiodic responses in Coleus (Lebowitz, 1985). Coleus (*Coleus blumei* Benth.) is a day-neutral plant, while *Coleus frederici* and *C. blumei* x *C. frederici* (designated as *C. hybridus*) flower when the critical day length is above 12 hr and just below 13 hr (Halaban, 1968). For varieties of *C. blumei* developed in the breeding program at UF, early, mid, and late flowering varieties have been observed.

The high amount of variation in flowering responses is attributed to several interacting factors relating to environmental stimuli and the genetics within the plant. Photoperiod, i.e. the synchronization of flowering responses to day length, is a major factor in regulating flowering responses (Thomas, 2006). Photoperiodic changes are also closely linked to an internal oscillator within the plant known as the circadian clock (Halaban, 1968) which controls the daily rhythms in gene expression and behavior and act as a timer in photoperiodic response (Samach and Coupland, 2000). Other stimuli affecting the regulation of flowering responses in plants are light quality and quantity. Effects of light quality have been shown through studies of sun versus shade plants. Under a shade canopy, plants show a range of responses to the changes in red (R) to far-red (FR) ratio of ambient light (Thomas, 2006). Light under shaded conditions mimics short end-of-day FR treatments and phytochromes act as photoregulators (Thomas, 2006). Light quantity responses are linked to photosynthesis and the availability of assimilates and is particularly important during early development of plants (Thomas, 2006).

C. blumei are day-neutral plants and three main classes of flowering responses (early, mid, and late flowering) are found within the species (Lebowitz, 1985). In a breeding program which selects late-flowering varieties based on phenotypic characteristics, it is important to isolate those cultivars that are late-flowering and use these as breeding lines to generate new populations with late flowering responses.

Foliage Color

Anthocyanin synthesis and production. Color pigmentation in plants has been the subject of applied research for more than four centuries (Mol et al. 1998), and although the anthocyanin biosynthetic pathway has been extensively characterized in flowers (Grotewold, 2006) only a moderate amount of information is available for leaves. Plant pigmentation is due to the accumulation of flavonoids (including anthocyanins), carotenoids, and betalains (Mol et al. 1998). Flavonoids are considered to be the most important plant pigments as they provide most of the colors in the visible spectrum (Koes et al. 2005). The bright red and purple coloration found in coleus foliage is produced primarily by anthocyanin pigments, whereas green and yellow colorations are produced by chlorophyll and carotenoids, respectively (Lebowitz, 1985).

The flavonoid pathway is part of the larger phenylpropanoid pathway, which also produces a range of other secondary metabolites, such as lignins, stilbenes, flavonoids and hydroxycinnamic acids (Davies, 2004). The phenylpropanoid pathway starts from phenylalanine, itself derived from the shikimate/chorismate pathways (Davies, 2004). The first general enzyme of the flavonoid biosynthetic pathway is phenylalanine ammonia lyase (PAL), whereas the enzyme chalcone synthase (CHS) provides the first committed step towards the synthesis of anthocyanins (Grotewold, 2006) (Figure 1-1). CHS catalyzes the condensation of malonyl-CoA to yield naringenin chalcone (Schijlen, 2004) and chalcone provides the precursors for all classes of flavonoids, which includes the flavonols and anthocyanins (Grotewold, 2006). Naringenin

chalcone is isomerized by chalcone isomerase (CHI) to form flavanone naringenin and subsequent hydroxylation of flavanone to dihydroflavonols is carried out by the enzyme flavanone-3-hydroxylase (F3H) (Schijlen, 2004). The dihydroflavonols represent one branch point in the pathway where fine control of gene expression occurs, as they are the substrates for production of both the colored anthocyanins and the colorless flavonols (Davies, 2003). The next enzyme in the pathway, dihydroflavonol 4-reductase (DFR) provides one entry point to the biosynthesis of anthocyanins, and depending on the plant species, DFR can utilize as a substrate one or all three of the dihydroflavonols produced by F3H to form the corresponding leucoanthocyanidins (Grotewold, 2006). The conversion of leucoanthocyanidins to anthocyanidins is carried out by the next enzyme anthocyanidin synthase (ANS). The last enzyme in the anthocyanin biosynthetic pathway is UFGT. UFGT catalyzes the formation of anthocyanidin 3-glucoside from anthocyanidin under cytosolic neutral conditions (Saito, 2002). The pseudobase form of anthocyanidin 3-glucoside is then transported into the vacuoles for storage (Saito, 2002).

Two groups of genes involved in the regulation of anthocyanin biosynthesis in plants are the structural and regulatory genes. The structural genes encode the enzymes that directly participate in the biochemical reactions in the formation of anthocyanins and the regulatory genes encode the proteins that regulate expression of the structural genes (Gong, 1997). The regulatory genes also function in controlling the spatial and temporal accumulation of pigments (Gong, 1997). In maize, the R and C1 gene families encode transcription factors related to the MYC and MYB families that regulate the expression of the structural genes (Gong, 1997). In snapdragon, the pattern of red anthocyanin pigmentation is regulated by the product of the *delila* gene, which exhibits homology to the products of the R gene in maize (Gong, 1997). Six

anthocyanin genes are known to regulate the expression of some of the structural genes in *Petunia hybrida* (Quattrocchio et al., 1993). AN1 encodes a basic helix-loop-helix (bHLH) protein that activates transcription of DFR and its expression is regulated by AN2 and AN4 (Spelt et al., 2000). AN2 is expressed primarily in the petal limb and encodes a MYB domain transcription factor (Quattrocchio et al., 1999) that is functionally interchangeable with C1 from maize (Quattrocchio et al., 1998). AN4 regulates the expression of structural genes primarily in the anthers and it also encodes a MYB domain transcription factor (Spelt et al., 2000). The activity of AN1, AN2, or AN4 appears to be regulated post-transcriptionally by a cytosolic WD40 repeat protein encoded by AN11 in petunia (Spelt et al., 2002). In *Perilla frutescens*, a species closely related to coleus, three independent genes (A, B, and K) promote the formation of anthocyanin in different parts of the plant. Gene A controls anthocyanin production in leaves and stems, gene B promotes only in the stem, and gene K is essential for the expression of gene A in the epidermis of leaves (Gong, 1997). However, the molecular basis of genetic control by genes A, B, and K in anthocyanin production in Perilla remains unclear (Yamazaki et al., 2003).

Physiological factors affecting plant coloration: co-pigmentation and pH. Once anthocyanins are deposited in the vacuoles, several factors known to affect plant coloration include the physiological factors of co-pigmentation and vacuolar pH, and the environmental factor of light intensity. Co-pigmentation and vacuolar pH have been extensively investigated in flowers but little information exists for this phenomenon in leaves. Two types of co-pigmentation that exists include intermolecular and intramolecular co-pigmentation. Intermolecular co-pigmentation involves the interaction of anthocyanins with other non-colored flavonoids (flavonols, carotenoids, or metals) (Grotewold, 2006). Non-colored flavonoids provide ‘depth’ to many white or cream flowers (Grotewold, 2006). In intramolecular co-pigmentation,

anthocyanin chromatophores are covalently modified by organic acids, and together with the formation of stacked complexes, result in an increase in pigmentation and change in hue color (Grotewold, 2006). Here are a few special points to keep in mind when reviewing your thesis or dissertation:

Vacuolar pH provides the proper hue for anthocyanin coloration, and color expression of anthocyanins is highly influenced by pH (Torskangerpoll and Andersen, 2005; Grotewold, 2006). In general, the vacuolar lumen in every cell type is more acidic than the surrounding cytoplasm. Torskangerpoll and Andersen (2005) examined the color variation of three anthocyanins (cyanidin-based) at fourteen pH-values between 1.1 and 10.5 and found that hues changed with pH levels. The three pigments had reddish hues at the lowest pH values and changed toward more bluish tones with increasing pH levels around 7. In morning glory (*Ipomea tricolor*) petals, the vacuolar pH is relatively low when the flower buds open, resulting in a red color. Upon further maturation, the vacuolar pH increases and the petals acquire a strong blue color (Yoshida et al. 1995). In *P. hybrida*, wild-type flowers stay on the reddish side of the color spectrum due to an acidic vacuolar pH (Quattrocchio et al., 2006). Genetic screens of *P. hybrida* mutants have identified several genes (PH1 to PH7) involved in vacuolar acidification. PH1 to PH7 genes, when mutated, result in the 'blueing' of flowers (Mol et al. 1998; Grotewold, 2006; Quattrocchio et al., 2006) without loss or alteration of anthocyanin composition and content (Mol et al. 1998). Mutations in the genes ANTHOCYANIN 1 (AN1), AN2, and AN11 also cause an increase in pH of petal extracts as well as loss of anthocyanin pigments. AN1, encoding a basic helix-loop-helix protein (bHLH), was shown to activate anthocyanin biosynthesis and vacuolar acidification in petals through its interactions with two distinct MYB proteins encoded by AN2 and PH4 (Quattrocchio et al., 2006).

An environmental factor affecting plant coloration: light intensity. Light intensity is an environmental factor that is known to affect coloration in plants. For coleus varieties, foliage colors usually darken or fade under high light intensities depending on the variety. The physiological and molecular mechanisms for foliage color change have not been investigated in coleus, but have been for other plant species.

Light exposure has been shown to be a prerequisite for significant anthocyanin synthesis in vegetative tissues (Steyn, 2002). Generally, high light intensity is required to induce anthocyanin synthesis while anthocyanin levels in plants may vary with respect to light exposure levels (Steyn, 2002). In *Pennisetum setaceum* cvs 'Rubrum' and 'Red Riding Hood', pigmentation is highly dependent on light for induction, with colors of both cultivar ranging from pale green in low light to dark purple in high-light environments (Beckwith et al., 2004). Light-dependent anthocyanin production has also been observed in young *Brassica oleracea* L. seedlings (Rabino, 1986), in maize (Pietrini, 2002) and in apples (Merzlyak, 2000). In some cultivars of apples, sun-exposed sides have considerable amounts of accumulated anthocyanins, whereas shaded sides have an absence of pigments (Merzlyak, 2000). *Viola cornuta* L. cultivar 'Yesterday, Today and Tomorrow' (YTT) have flowers that undergo a dramatic anthocyanin-based ontogenetic color from white to lavender to deep purple over five to eight days (Farzad, 2002). Appearance of color in YTT is regulated by anthocyanin synthesis at the level of transcription. Light irradiation leads to the transcriptional activation of specific anthocyanin biosynthetic genes, resulting in increased amounts of malvidins (Farzad, 2002). Light is necessary for the appearance of deep purple color in YTT, as evidenced by a lack of color change in flowers maintained in the dark (Farzad, 2002).

The induction of anthocyanin synthesis in plants exposed to high light intensities may play a functional role in the protection of the photosynthetic apparatus against photoinhibition. The first step in photosynthesis is the capture of light photons by chlorophyll (Harpaz-Saad, 2007). When the absorbed light exceeds the capacity of the plant to use the trapped energy through photosynthesis, damage to the photosystems occurs (Huner, 1998). This damage, termed photoinhibition, can become irreversible and lead to significant decreases in plant productivity (Huner, 1998). Anthocyanins have been shown to absorb the excess light energy that would otherwise be intercepted by chlorophylls and protect leaves from photoinhibitory damage during periods of high light intensity (Neill, 2003). The photoprotective role of shielding excess light from chlorophyll by anthocyanins has been shown in *Begonia pavonina* (Gould et al., 1995), *Galax urceolata* (Hughes, 2005), and *Cornus stolonifera* (Field et al., 2001).

Summary

The economic success of many ornamental plants on the commercial market requires novel or improved characteristics that not only make them distinct from current varieties but also increase their product value. With regard to coleus, high genetic variability exists to allow for the manipulation of certain traits such as plant architecture (trailing growth habit, vigor, branching, and late flowering) and foliage color quality. For the success of the coleus breeding program, the development of new varieties with these ideal characteristics and the understanding of the genetics involved in the control of these traits are therefore essential.

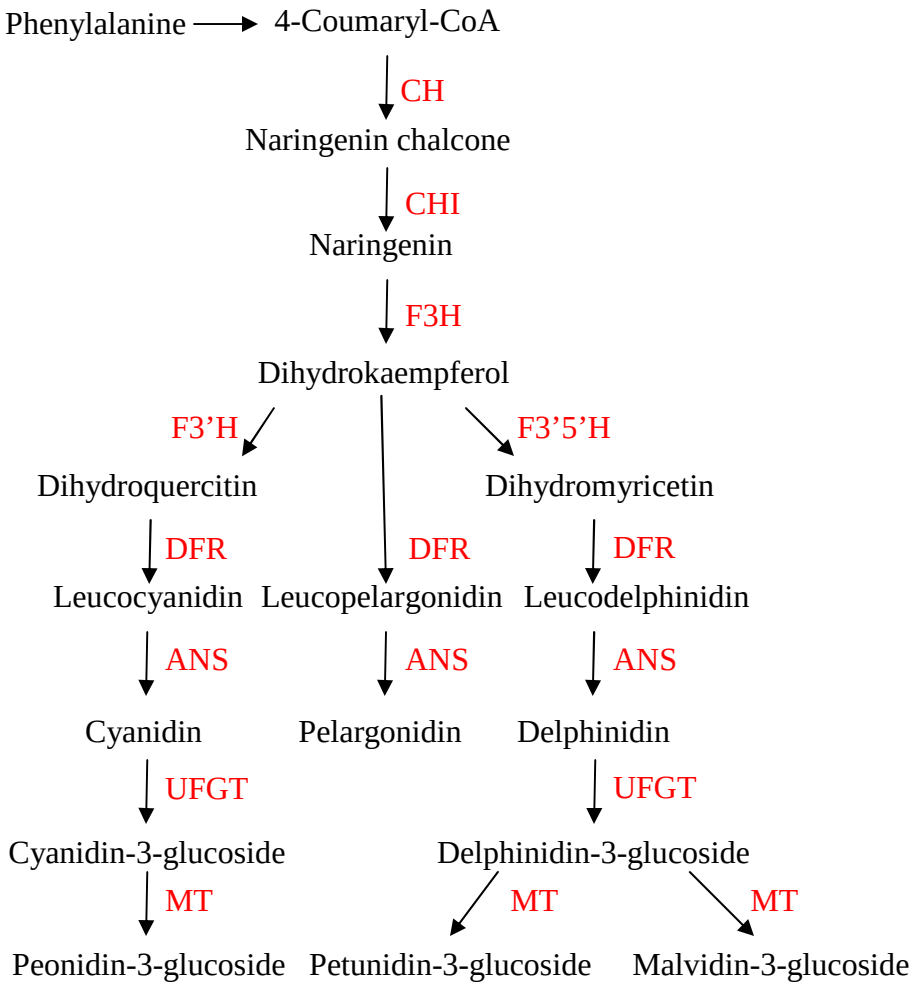

Figure 1-1. General anthocyanin biosynthetic pathway.

CHAPTER 2
THE GENETICS OF TRAILING HABIT AND THE DEVELOPMENT OF NEW COLEUS (*Solenostemon scutellarioides* (L.) CODD) VARIETIES WITH TRAILING HABIT AND BRIGHTER FOLIAGE COLOR

Introduction

Solenostemon scutellarioides (L.) Codd, commonly known as coleus, is a member of the Lamiaceae family and is valued for its vibrant and colorful foliage (Pedley and Pedley, 1974). Its status as a common household bedding plant dates back to its introduction in England from its native origin of Indonesia in the early 1800s (Stout, 1987). More than 300 species and several hundred varieties exist for the genus *Solenostemon* and commercial cultivars are characterized by their leaf colors, leaf shape, growth habit, and flowering characteristics (Lebowitz, 1985). Most brightly-colored coleus varieties commonly found on the market have vigorous growth habits and are mainly planted in the landscape as tall bedding plants. Few trailing habit varieties exist and those available have foliage that is generally dark green or red. Trailing growth habit in coleus is desirable as it increases its utility as an ornamental plant for use in mixed containers, hanging baskets, and as groundcover in the landscape. Varieties with trailing habits are characterized by having an extensive lateral branching pattern in which there is a reduction in apical dominance and a larger quantity of axillary meristems (Davies, 1995). The manipulation of plant architecture has been achieved during the improvement of many crop species. For example, in red clover, a lack of persistence, i.e. creeping thin stems and more adventitious roots, is a major limitation to its widespread acceptance as a forage crop (Smith and Bishop, 1993). Changing the architecture of existing commercial red clover cultivars and developing ones with an increase in persistence have increased their use by farmers (Mirzaie-Nodoushan, 1999).

The genes controlling growth habit vary for many plant species because variations in plant architecture reflect diverse growth and reproductive strategies (Goncharov, 1999). In narrow

leafed lupin, *Lupinus angustifolia* L., restricted branching (lack of branching) is a simply inherited recessive trait (Adhikari, 2001). In red clover, *Trifolium pratense* L., a dominance x dominance epistasis controls prostrateness, with prostrateness being partially dominant to erectness (Mirzaie-Nodoushan, 1999). Although hundreds of different types of coleus exist with an extensive variation in growth habit (Boye and Rife, 1938), information on the genetics for growth habit is limiting because most of the genetic studies in coleus have focused mainly on its leaf color (Boye and Rife, 1938; Rife, 1938; Lebowitz, 1985) and leaf shape (Rife, 1972; Rife and Hakeem, 1966). Types of growth habit observed in coleus are upright or erect habit and trailing habit, with varying levels for each habit type. Such variations in habit are the result of the high level of heterozygosity found in coleus.

Contrasting views have been proposed as to the origins of trailing types since most modern day varieties were the result of interspecific hybridizations (Lebowitz, 1985). During the early 1920s, a different form of coleus was discovered and classified as *Coleus rehneltianus*. It had a low, scrambling habit, seldom reached more than 25cm in height, and rooted where the trailing stems touched the earth (Pedley, 1974). According to Bailey Hortorium (1976), current trailing varieties were developed from interspecific crosses between *Coleus scutellarioides* and *Coleus pumila* Blanco. *C. pumila* had a trailing habit rather than erect like *C. scutellarioides* and was believed to be identical to *C. rehneltianus* (Pedley, 1974).

The majority of cytological studies on *C. scutellarioides* report a 2n=48 chromosome number (Reddy, 1952; Morton, 1962; Hakeem and Rife, 1966). Multivalents appear during meiosis in cultivars with 2n=48 chromosomes and indicate polyploidy. Since many genetic experiments produce simple diploid ratios (Rife, 1948 and 1952) the species is thought to be an allotetraploid (Lebowitz, 1987). Allotetraploids arise through the combination of chromosome

sets from two or more species (Poehlman and Sleper, 1995). Contrasting views on chromosome numbers have been reported for trailing coleus varieties. Scheel (1931) reported an n=24 for *C. rehneltianus* while Reddy (1947), from observations of three clones from a variety named 'Trailing Queen' (*Coleus blumei*), reported chromosomes numbers of n=24 +1 and n=36. Rife (1948) observed that plants of the 'Trailing Queen' variety were usually highly sterile, whether selfed or crossed and only progeny were produced when 'Trailing Queen' was used as the maternal parent. The cause for high sterility in 'Trailing Queen' was not investigated further by Rife or others.

The aim of this research was to provide further information on the genetics for trailing growth habit in coleus. Understanding the inheritance of trailing habit will provide useful information for the development of new coleus cultivars with desirable habit which can be combined with foliage color. Commercial cultivars 'Red Trailing Queen' (RQ) and 'Sedona' (S) were used as parental lines for the production of F1 hybrids in the breeding program (Figure 2-1). RQ is a dark purple-leafed cultivar and was chosen for use as the genetic source for trailing habit. RQ has a low growing habit and lateral branches that extend more than three feet in length, providing for good ground coverage. S is a standard upright cultivar and is one of the few commercially available plants that has bright orange foliage. Both cultivars flower profusely and flowering induction occurs year-round, facilitating hand-pollinations in the greenhouse. Seedlings resulting from RQ self-pollination were mostly all trailing but dark in foliage color while seedlings resulting from S self-pollination were mostly upright and had bright foliage color. Thus, both parental lines possessed high levels of homozygosity for use as breeding materials for the analysis of trailing habit (RQ) and for the introgression of orange color (S).

Little information is available regarding the genetics of trailing growth habit in coleus. Furthermore, no known study to date has been published on the development of new trailing varieties with bright foliage colors through the method of controlled hand-pollinations. In order to develop new varieties with these desirable characteristics, an understanding of the inheritance for trailing growth habit is required. Through this research, new relevant information on the known trailing variety (RQ) is provided. The main objectives of this study were to: (1) use RQ and S as breeding parents to produce hybrids, (2) determine the genetics for trailing habit and isolate hybrids with trailing habit, (3) introgress orange color into trailing habit types, and (4) develop new coleus varieties with trailing habit and bright foliage colors.

Materials and Methods

Plant Materials and Growth Conditions for Breeding

RQ, a commercial variety with desirable branching growth habit and S, a commercial variety with desirable orange color, were obtained from Proven Winners, Inc. Stock plants of RQ and S were grown in three-gallon pots filled with soilless medium (Fafard lightweight mix # 2; Apopka, Fl) in a greenhouse under standard conditions (21-25^{o}C). Cuttings were propagated from each variety and rooted in a mist house, with misting set for 20 seconds every 30 min. After rooting, 10 clonal cuttings from each parental line were transplanted to 15.2cm pots and irrigated as needed with liquid fertilizer with 150ppm nitrogen (15-5-15, N-P_2O_5-K_2O – Scotts, Apopka, Florida) in the greenhouse. 10 clonal plants were routinely grown from each parental line to ensure an adequate number for a continual supply of flowers for pollinations. The clonal plants were grown until flowering induction occurred at approximately six weeks after potting. After flowering, fertilization was stopped and the plants were irrigated with only water as needed. Not fertilizing was the most effective means to generate seeds because plants grown under lush conditions did not produce as many seeds as those grown under a somewhat stressed condition.

Analysis for Ploidy Level Differences among Parental Genotypes and Hybrids

Flow cytometry and comparative cell size were used to assess differences in ploidy levels for RQ, S, and F1 plants. For flow cytometry analysis, fresh apical meristematic tissues (~20 mg) were chopped and nuclei extracted in 1ml lysis buffer and DAPI (4',6-diamidino-2-phenylindole). DAPI is a DNA-specific stain which forms a fluorescent complex by binding to the minor grove of A-T rich sequences (Kapuscinski, 1995). Large tissue debris was removed by filtration and the extraction solution subsequently used for analysis, with a minimum of 2500 nuclei counted per extraction solution. Samples were analyzed with a PA1 Ploidy Analyzer (Partec, Münster, Germany) and DNA quantified using a DPAC software provided by the same company. A one-parameter histogram was obtained and ploidy level differences were analyzed by comparing the mean DNA content of each sample.

Comparative cell size analysis was performed by measuring the length and width of fresh pollen grains and stomates, and the length of glandular trichomes. It has been shown that ploidy level differences can be determined by measuring these parameters in which cell size often increases with increases in ploidy level (Ojiewo, 2006; Kelly, 2002). Guard cells and glandular trichomes were obtained from epidermal peels as previously described by Reddy (1952). Measurements (in microns) were taken at a magnification of 4X for glandular trichomes and at 40X for pollen grains and stomates using a compound light microscope (Nikon Labophot-2, Japan). Data shown are the means of 25 pollen grains and stomatal cells and twenty glandular trichomes for RQ, S, and five F1 plants. A t-test (Excel) was used to determine significance at the 0.1 level.

Pollen Viability and Microsporogenesis Analysis

Pollen viability estimates were calculated by counting the number of pollen grains that stained fully in aceto-carmine out of a total of 100. Pollen was collected from anthers of three

flowers from each variety and the mean value, generated as a percentage, was determined. Pollen estimates and photographs of pollen grains were taken at a magnification of 10X using a compound light microscope (Nikon Labophot-2). Aceto-orcein smears were used for the study of microsporogenesis in RQ to determine the cause for low pollen quality. Four anthers were smeared in aceto-orcein without prior fixation, covered immediately with a cover slip, and sealed with bees-wax. After sealing, the slide was gently pressed between two sheets of blotting paper and immediately viewed under a dissecting scope. Approximately 300 pollen mother cells from RQ were examined. Photographs of cells undergoing meiosis were taken at a magnification of 100X with a camera magnification of 4000X. Methods used for pollen viability estimates and microsporogenesis analysis were previously described by Reddy (1952).

Pollination Methodology

All pollinations, selfs and reciprocal crosses, were performed in the morning (8-11am) in a greenhouse using the following tools: round-tip paintbrush for pollen collection, tweezers for emasculation, permanent marker for marking pollinated flowers and 95% ethanol. Self-pollinations were made by emasculating flowers (pre-anthesis) in the morning and immediately pollinating the same flower with pollen collected from the same plant. Pollinated flowers were marked and seeds collected approximately three weeks later when the seeds and calyx (seed capsule) were dark brown in color. Cross-pollinations were performed by emasculating flowers (pre-anthesis) in the morning with tweezers. To prevent self pollen from contaminating the flowers, the tweezers were dipped in alcohol after all emasculations. Pollen from the second parental line was collected and immediately brushed on the stigma of the emasculated flowers from the first parental line. Corollas of pollinated flowers were marked with a fine-tipped marker and pollinations were made on a daily basis until 50-100 seeds were collected from each parental line. Seeds were collected from RQ selfs, S selfs, and the cross RQ (♀) x S (♂) while no seeds

were collected from the S (♀) x RQ (♂) cross. The F2 and F3 population were generated by selfing F1 and F2 plants, respectively.

Growth of Seedlings

Seeds collected from self and cross pollinations were sprinkled on the top surface of flats filled with soilless media (Fafard 2-mix, Apopka, Florida) and germinated in a mist house with mist cycles set at 20 seconds for every 30 minutes. Seedlings were removed from the mist house after 90% of seeds had germinated (10-14 days), moved to a polycarbonate shade house, and afterwards were transplanted one per cell into a 72 cell-pack tray. After three additional weeks, they were transplanted to 15.2cm pots and irrigated as needed with liquid fertilizer at150ppm nitrogen (15-5-15, N-P_2O_5-K_2O – Scotts, Apopka, Florida) at every other irrigation. The plants were grown for an additional six weeks and used for subsequent data collection.

Rating Scale for Growth Habit and Foliage Color Brightness

Growth habit was rated on a visual 1-5 rating scale, where 1 = upright, 2 = semi-upright, 3 = prostrate, 4 = semi-trailing, and 5 = trailing. The 1-5 rating scale was used because this scale has been used for other plants and all five growth habit types have been observed in coleus (Pedley, 1974). Growth habit was characterized as the height and branching pattern of the plant, in which a plant with upright growth habit was tall and had poor branching and a plant classified as trailing was short and had excellent branching. Foliage color was rated on a visual 1-5 rating scale, where 1 = very dark (color as dark RQ), 2 = dark (color slightly lighter than RQ), 3 = medium (color between RQ and S), 4 = bright (color slightly darker than S), and 5 = very bright (color as bright as S).

Rating Scale for Genotypic and Chi-square Analysis

The 1-5 scale previously used above to rate growth habits in the RQ self, S self and F1 populations where 1 = upright, 2 = semi-upright, 3 = prostrate, 4 = semi-trailing, and 5 = trailing

was modified to analyze genotypic ratios for the three populations. Using a tetraploid model, a plant observed as having an upright growth habit (rating of 1) had a designated quadraplex genotype of AAAA, assuming that upright growth habit was dominant to trailing. Therefore, five genotypic classes were observed. Based on the 1-5 scale, 1 = upright (AAAA), 2 = semi-upright (AAAa), 3 = prostrate (AAaa), 4 = semi-trailing (Aaaa), and 5 = trailing (aaaa). Chi-square (X^2) goodness-of-fit analysis based on a tetraploid model as illustrated for chromosomal segregation by Burnham (1962) was used to analyze the growth habit segregation for all populations and to determine the genotype of RQ and S.

Chi-square Test for Homogeneity

After the chi-square goodness-of-fit test was applied to the RQ selfed, S selfed, and F1 population (as described above), the genotypes of RQ and S were determined. To further support that the phenotypes matched the given genotypes, three plants from the F1 (or F2) population from each genotypic group (AAAA, AAAa, AAaa, Aaaa, and aaaa) were selfed. A chi-square test for homogeneity based on an r x c table, as previously described by Cochran (1954), was performed to obtain a better estimate of the population proportion and to determine whether frequency counts were distributed identically across the different populations. The value from the chi-square test for homogeneity was calculated according to Equation 2-1

$$X^2 = \sum \frac{(\text{observed} - \text{expected})^2}{\text{expected}}, \quad (r-1)\,(c-1)\ df \qquad (2\text{-}1)$$

where r = rows and c = columns. The X^2 values for each of the three plants in each genotypic group were summed to give an overall total and a P-value was obtained.

Introgression of Color Using the Selfing Methods

Thirty F1 plants characterized with semi-trailing habit were selected for use as lines to introgress bright color. A backcross method was initially chosen to introgress colors using S as

the backcross parent. Since progeny with bright colors were produced by just selfing several F1 plants, selfing was used as the preferred method. The F1 plants were selfed over a one month period and seeds collected to generate an F2 population. F2 plants exhibiting both trailing habit and bright foliage color were selected in the breeding program as potential new varieties.

Results

Determination of Ploidy Level Differences

Since RQ and S were used for the first time as breeding parents, flow cytometry and comparative cell size analysis was used to determine their cross-compatibility by observing if any ploidy level differences were present. Several F1 plants produced from crosses of RQ and S were also analyzed to determine if they had the same ploidy level as the parental lines. It is well known that as ploidy levels increase in a species, nuclear DNA content also increases (Sugiyama, 2003). A DNA content value of 112.4 and 110.4 were obtained for RQ and S, respectively. Ranges of DNA content values from 91.4 to 118.4 were observed for the F1 plants. Results from flow cytometric analysis indicated that no significant differences in ploidy levels were observed among RQ, S, and the F1 plants tested (Table 2-1).

The applicability of pollen, stomatal and glandular trichome length as indirect methods to further support the similar ploidy levels found for RG, S, and several F1 plants was investigated (Table 2-2). Comparative cell size analysis has been shown to differentiate ploidy levels between species, where increases in ploidy levels correlate with increases in cell size (Aryavand, 2003; Reddy, 1952). Pollen grain lengths of 37.6µM and 38.7µM were measured for RQ and S, respectively. Among five F1 plants tested, a range of 36.9µM to 40.8µM was measured. In regards to stomatal cells, a length of 29.2µM for RQ and 31.8µM for S was observed, with ranges of 30.4µM to 33.6µM measured for the F1 plants. No significant differences were found when glandular trichomes were measured for all of the varieties. RQ and S did not differ in the

size of their trichomes, with a length of 50.8µM and 49.7µM measured for RQ and S, respectively. Among the F1 plants, lengths of 48µM to 52.3µM for glandular trichomes were measured. No significant differences were also found when the widths of pollen grains and stomatal cells were investigated. Results from the t-test indicated no significant differences in ploidy levels based on comparative cell size analysis existed between RQ, S, and the F1 plants (Table 2-2), thus further supporting the results determined from flow cytometry analysis that RQ and S have the same ploidy. Further segregation analysis suggests that both are tetraploids (discussed later).

Pollen Viability and Microsporogenesis Analysis

RQ and S were shown to not differ in ploidy levels and thus were sexually compatible. However, differences in seed set and fertility were observed when self and reciprocal cross-pollinations were performed on RQ and S. From self-pollinations, low seed set was observed in RQ while higher seed set was observed in S. Of a maximum of four seeds that can be produced in a flower, only one seed was normally produced in RQ while three to four seeds were often produced from S (personal observation). Furthermore, self-pollinations were often unnecessary for S because seed set occurred spontaneously. Many species have low seed set, and pollen quality and/or pollen sterility due to non-functional pollen have been reported as factors affecting seed set for some plant species (Byers, 1995; McDade and Davidar, 1984). In order to determine if the low seed set observed in RQ was due to poor pollen quality, pollen viability estimates and microsporogenesis analysis were investigated (Table 2-3 and Figures 2-2 and 2-3).

A low pollen viability of 14% was observed for RQ while significantly higher pollen viability of 98% was observed for S (Table 2-3). When pollen viability of five F1 plants was compared, a wide range from a low pollen viability of 17% to a high of 87% was observed. When RQ pollen grains were viewed under a light microscope, many were not stainable (Figure

2-2a), whereas S pollen grains were fully stainable (Figure 2-2b). The shapes of pollen grains of F1 plants with low pollen viability were also consistent with those observed in RQ (data not shown). Microsporogenesis analysis showed the occurrence of micronuclei in RQ pollen mother cells due to the failure of unpaired chromosomes (enclosed in circle) to migrate to either pole (Figure 2-3a). Furthermore, the amount of irregular chromosomes segregating to either pole resulted in tetrads with microspores of different sizes (Figure 2-3b). Results from pollen viability estimates, pollen shape and microsporogenesis analysis suggest that the low seed set observed in RQ is likely due to poor pollen viability and quality.

Growth Habit Segregation

In order to determine how trailing habit was inherited in coleus, photographs showing the various growth habit forms observed when selfing RQ (Figure 2-4), S (Figure 2-5), and in the F1 population (Figure 2-6) were taken. Furthermore, the different growth habit types of all populations were rated on a visual 1-5 scale and their distribution analyzed (Figure 2-7). A distribution of five different growth habit types was observed in RQ selfed population (Figure 2-4). Of 96 selfs evaluated, 1 had an upright growth habit, 19 were semi-upright, 53 were prostrate, 21 were semi-trailing, and 2 were trailing. The two progeny that were characterized as having trailing growth habit had a habit even more trailing than RQ, suggesting that RQ was not completely homozygous for trailing habit. In the S selfed population three different growth habit types were observed (Figure 2-5). Of 100 progeny evaluated, 20 were upright, 57 were semi-upright, and 23 were prostrate. The 20 progeny with upright growth habits were taller and had poorer branching than S, suggesting that S was not an upright variety as previously suggested. Four growth habit types were observed in the F1 population (Figure 2-6). Of the 250 F1 plants evaluated, 22 were upright, 108 were semi-upright, 94 were prostrate, and 26 were semi-trailing. None of the F1 plants had trailing growth habits as observed in the RQ selfed population while

those that were upright were similar to the plants in the S selfed population characterized as having upright growth habits. The continuous distribution for growth habit types for RQ self, S self, and F1 population is shown in Figure 2-7.

Determination of Parental Genotypes

A chi-square test for goodness-of-fit (X^2) was used to test hypotheses of possible genotypes for RQ and S. For RQ, all types of growth habits were observed in the selfed population. Since both the quadraplex (AAAA) and nulliplex (aaaa) forms were present, the genotype proposed for RQ was the duplex form (AAaa). In regards to S, since only upright, semi-upright, and prostrate growth habits were observed in the selfed population, the triplex form (AAAa) was proposed. A chi-square value of 2.02 (P = 0.73) were calculated for RQ (Table 2-4) and a chi-square value of 2.14 and a P-value of 0.34 were calculated for S (Table 2-5). If RQ (AAaa) was crossed to S (AAAa), the F1 population should theoretically segregate in a 1:5:5:1 ratio. A chi-square value of 2.51 (P = 0.47) was calculated for the F1 population (Table 2-6). Results from the chi-square indicated a goodness-of-fit for the observed ratios in the RQ selfed, S selfed and F1 populations and thus supported the genotypes proposed for RQ (AAaa) and Sedona (AAAa).

To further support that the phenotypes matched their assigned genotypes (e.g. a plant characterized as having a semi-upright growth habit had the triplex (AAAa) genotype), plants from each of the five genotypic classes were selected. Three plants from each of the five genotypic classes were selfed and segregation for growth habit in the F2 population was tested against the chi-square test for goodness-of-fit. However, for the upright (AAAA) and trailing (aaaa) growth habit class, plants from the F2 generation were used instead, and the F3 population analyzed.

Chi-square data for the plants used to match phenotypes with their assigned genotypes are shown in Table 2-7. For the first genotypic class, if the plants with upright growth habits have a quadraplex genotype (AAAA), then all of the progeny selfed from the plants should all segregate for upright growth habits. The F3 populations produced from each of three F2 plants all had upright growth habits. For genotypic class 2, if the plants characterized as being semi-upright have a triplex genotype (AAAa), the progeny should be expected to segregate in a 1:2:1 ratio, with the upright, semi-upright, and prostrate growth habit observed. Based on the data from the chi-square test, the F2 population for each plant segregated according to the expected ratio. X^2 values of 1.84 (P = 0.34), 0.34 (P = 0.18), and 0.92 (P = 0.63) were observed. For genotypic class 3, progeny from plants with a duplex genotype (AAaa) should segregate in a 1:8:18:8:1 ratio with all growth habits observed. X^2 values of 4.04 (P = 0.40), 6.41 (P = 0.17), and 4.93 (P = 0.29) were obtained for plants in genotypic class 3. Plants tested in genotypic class 4 are semi-trailing with a simplex genotype (Aaaa). Progeny population resulting from selfing plants with a simplex genotype (Aaaa) should segregate in a 1:2:1 ratio for prostrate, semi-trailing, and trailing growth habits. When a chi-square test was tested for goodness-of-fit, X^2 values of 1.22 (P = 0.57), 0.52 (P = 0.77), and 1.69 (P = 0.43) were obtained. Lastly, for genotypic class 5, when plants with trailing growth habit (genotype aaaa) are selfed, the progeny population should be expected to segregate only for trailing growth habits. When the progeny population was evaluated, all plants had trailing growth habit. Data from the X^2 and P-values indicated that a goodness-of-fit was observed for all of the plants tested in each of the five genotypic classes. Data from the chi-square test for homogeneity, listed as the total X^2 and P-value in Table 2-7, also further supported the results from the Chi-square test.

Segregation for different growth habit types was observed in the selfed population of RG and S. Five different growth habit types were found in RQ progeny but only three growth habits were found in S. Since all five different growth habit types were observed in RQ selfed progeny, a duplex genotype (AAaa) was proposed. For S, since it segregated in a 1:2:1 ratio for upright, semi-upright, and prostrate growth types, a triplex genotype (AAAa) was proposed. For each of the self populations of RQ and S, the number of progeny characterized with each growth habit type constituted the 'observed' values, and these observed values were then compared to the 'expected' values. Expected values were the numbers of progeny expected to be seen if a duplex (AAaa) and a triplex (AAAa) were selfed. If the P-value obtained was less than 1, then the genotypes proposed for RQ and S were correct. The chi-square test was conducted for the F1 and F2 populations and because a goodness-of-fit was also observed, the data further supported the genotypes proposed for RQ and S. The data for growth habit had a goodness-of-fit to a tetraploid model, and genetics for growth habit in coleus appear to be controlled by a single tetrasomic locus gene with completely additive effects.

Foliage Color Brightness

Mean rating values were calculated for foliage color brightness in the RQ self, S self, F1 and the combined F2/F3 populations (Figure 2-8). A mean value of 4.1 was observed for the S self population while a mean value of 1 was observed in the RQ selfed population. Progeny produced from selfing RQ were all dark purple and green in color, with the two colors varying slightly in degree of intensity. For the S selfed population, brighter colors were observed, with colors ranging from yellow, orange, red, green, and mottled yellow/orange. In the F1 population, some combinations of foliage colors were observed which were not present in either the RQ or S selfed population. Foliage colors ranged from dark purple (similar to RQ), purple with large green margins, mottled light purple/green, mottled brown/green, and medium red (similar to

some progeny produced from selfing S) (Figure 2-9). The introgression of bright foliage color was observed in the F1 population because some individuals had bright green color in their foliage which was not observed in the RQ selfed population. Many F1 plants had foliage colors that were darker than S but still brighter than that of RQ. In addition, purple color from RQ appeared to be dominant and the orange color from S to be recessive because all of the F1 plants had some form of purple in their foliage. When the means for color brightness were compared, color brightness improved slightly in the F1 population. The mean rating value for color brightness of 1.3 was slightly higher than that of the RQ selfed population but was still lower than that of the S selfed population. In the combined F2/F3 population produced from selfing the thirty F1 and F2 plants for genotypic analysis, the mean rating value for color brightness was 2.65. Foliage color brightness was further improved in the next generation, however although the value of 2.65 was higher than in the RQ selfed and F1 population, it still remained lower than that of the S selfed population.

Introgression of Color

The backcross method was initially chosen to introgress color genes from S into F1 plants characterized with semi-trailing growth habits. However, when F1 plants were selfed for genotypic analysis, bright color as seen in S was observed in individuals in the F2 populations. Thus, the backcross method was not needed to introgress color into trailing types and the selfing method was used instead. Thirty F1 plants with semi-trailing growth habit and color brightness ratings of two or higher were self-pollinated to produce an F2 population. Foliage color observed in the F2 and F3 populations ranged from purple (as seen in RQ), green, mottled pink with green margins, mottled green/red, to orange color (as seen in S) (Figure 2-10). Furthermore, many F2 plants with bright foliage color also had trailing growth habits and one F2 seedling was produced

which had trailing habit equivalent to RQ and bright orange color similar to that of S (Figure 2-11).

Discussion

The use of flow cytometry and analysis of comparative cell size as methods to determine ploidy level differences have been investigated for many plant species (Dolezel, 2005). If S was a tetraploid and RQ either a diploid or triploid, then for a significant difference in ploidy level to occur, S would need to have at minimum doubled the chromosome count of RQ (Reddy, 1952). In a study conducted by Reddy (1952), size of pollen, stomatal cells, and glandular hairs were measured to differentiate diploids, triploids, and tetraploids in coleus. It was observed that diploid species had smaller pollen, guard cells and glandular hairs compared to triploids, which were slightly larger. For tetraploids, the sizes of the cells were measured to be twice the size of those measured in the diploids. Results from flow cytometry and comparative cell size analysis in this study suggest that RQ, Sedona, and F1 plants derived from crossing the two parental lines were of the same ploidy level.

A difference in ploidy level did not appear to be the factor contributing to the low seed set in RQ and the cross-incompatibility found in S(♀) x RQ(♂) but not in RQ(♀) x S(♂). Based on initial observations when self-pollinations were made in RQ and S, low seed set was found in RQ while higher seed set was found in S (personal observations). Similarly in a study conducted on *Eupatorium resinosum* species by Byers (1995), seed set (number of seeds and heads in each cross) was used as a measure of compatibility, with a cross considered compatible if there was at least one seed produced per head. Low seed set for this species was suggested to be due to poor pollen quality. Low pollen quality in terms of viability and micronuclei in pollen mother cells in RQ appear to have been factors contributing to its low seed set and unsuccessful seed set when RQ was used as the pollen donor in the cross S(♀) x RQ(♂). Poor pollen quality as a result of the

formation of micronuclei from irregular meiosis has been observed in other species such as *Paspalum maritimum* (Adamowski, 1999), *Clarkia exilis* (Vasek, 1961), and *Pfaffia tuberosa* (Taschetto, 2004). Cross-compatibility between RQ and S was examined, but the low seed set in RQ as a plausible result of self-incompatibility was not investigated in this study. Further examination of self-incompatibility due to incompatibility alleles or failure of proper pollen tube growth may offer a better understanding of the low seed set found in RQ.

The genetics of trailing habit in coleus appear to be controlled by a single gene expressed though additive gene action. If one assumes that upright growth habit is dominant to trailing with additive effects, the addition of one upright allele should enhance the expression for upright growth habit. Complete dominance is not suggested for the gene controlling trailing habit in coleus because F1 plants with genotypes AAAA, AAAa, AAaa, and Aaaa would all have upright growth habits, and they did not. Therefore, incomplete dominance is proposed with the effect of the allele A (for upright growth habit) being additive. Growth habits controlled by the interaction of several genes have been observed for other polyploids. In hexaploid wheat, one, two, or occasionally three dominant genes S controlled spring growth habit while recessive alleles at those loci controlled the winter growth habit (Akerman and MacKey, 1949). Pugsley (1971) later supported this claim and concluded that any of three dominant genes or their combinations controlled the spring growth habit. For coleus, trailing growth habit (aaaa) is qualitatively inherited and controlled by a single gene with additive effects and incomplete dominance.

The genetics for foliage color was not the main focus of this work, but significant observations were recorded. Rife (1948) stated that six multiple alleles, designated the purple series, affected the amount and distribution of anthocyanin. Their interactions resulted in nine phenotypic variations ranging from uneven purple, brown, and to green with purple color being

dominant (Rife, 1948). In support of his results, purple color from RQ was observed to be dominant to the orange color from S as all of the F1 plants predominantly had purple color in their foliage. Foliage color brightness was improved in the F1 population as the mean rating score for color was higher than the RQ self plants but lower than S self plants. Color brightness was further improved in the F2 population when colors similar to those seen in S were observed.

In the process of analyzing genetic segregation for trailing growth habit, separate selections were made for the development of varieties with both trailing habit and bright foliage color. The F1 population showed a slight introgression of brighter color due to the parental line S. However, all F1 plants were still relatively dark in color with remnants of purple foliage color and no plants had orange foliage color similar to S. In the F2 generation, plants with trailing habit and brighter foliage colors were identified (Figure 2-11). The goal of developing a plant with both trailing habit and bright orange foliage color was achieved, as one seedling in particular, ‘Copper Penny’, an F2 seedling selfed from the F1 plant H05-66, possessed these two desirable characteristics. When ‘Copper Penny’ was further selfed, F3 plants with all trailing and bright orange foliage colors were produced. Thus, in three generations, near homozygosity for trailing habit and orange foliage color was reached (Figure 2-11). These new varieties are currently being evaluated outdoors for garden and landscape performance for color stability and landscape use as groundcovers.

Figure 2-1. Parents used in breeding crosses to develop new trailing types with bright foliage color. A) Commercial variety ‘Red Queen’ with desirable trailing habit and dark purple foliage. B) A close-up of ‘Red Queen’ foliage. C) Commercial variety ‘Sedona’ with upright growth habit and desirable bright orange foliage. D) A close-up of ‘Sedona’ foliage.

Table 2-1. DNA counts for 'Red Queen' (RQ) and 'Sedona' (S) and 14 hybrids produced from the cross (RQ) x (S).

Cultivar Name	DNA Content[z]
RQ	112.39
S	110.39
H1	114.51
H2	105.38
H3	117.50
H4	114.03
H5	115.13
H6	102.05
H7	118.38
H8	97.32
H9	104.84
H10	115.14
H11	91.43
H12	101.63
H13	112.02
H14	116.06
Mean	109.26
Std Deviation	7.92

[z] DNA counts generated using a ploidy analyzer (Partec). No significant differences in ploidy levels were observed when all varieties were compared to each other.

Table 2-2. Comparative cell size analysis for 'Red Queen' (RQ), 'Sedona' (S), and five F_1 plants.

	Pollen size (uM)[xz]		Guard cell size (uM) [xz]		Glandular trichomes (uM) [yz]
Plant	length	width	length	width	length
RQ	37.6 ± 7	24.7 ± 4	29.2 ± 3	20.5 ± 2	50.8 ± 5
S	38.7 ± 6	25.3 ± 4	31.8 ± 2	22.8 ± 2	49.7 ± 4
H1	38.2 ± 6	25.4 ± 4	30.4 ± 3	21.6 ± 2	53.6 ± 5
H2	36.9 ± 5	25.5 ± 3	32.7 ± 3	21.4 ± 2	48.0 ± 4
H3	39.0 ± 6	25.8 ± 5	31.3 ± 3	20.3 ± 2	52.3 ± 5
H4	40.8 ± 7	26.2 ± 4	33.6 ± 4	22.5 ± 3	51.7 ± 6
H5	39.6 ± 4	25.2 ± 4	32.6 ± 4	21.5 ± 2	50.9 ± 4

[x] Measurements (u) were taken at a magnification of 40X using a compound light microscope (Nikon Labophot-2). Data are shown as the mean of 25 pollen grains and stomatal guard cells.
[y] Measurements (u) were taken at a magnification of 4X.Data are shown as the mean of 20 glandular trichomes.
[z] No significant differences were observed for the varieties and F_1s tested when the means and standard deviations were compared.

Table 2-3. Pollen viability estimates for ‘Red Queen’ (RQ), ‘Sedona’ (S), and five F_1 plants.

Genotype	Pollen viability % [y]
RQ	14%
S	98%
H1	87%
H2	49%
H3	20%
H4	33%
H5	17%

[y] Estimates were calculated by counting the number of pollen grains that stained fully in aceto-carmine out of 100. A wide range of pollen viability was observed for RQ (14%) and S (98%).

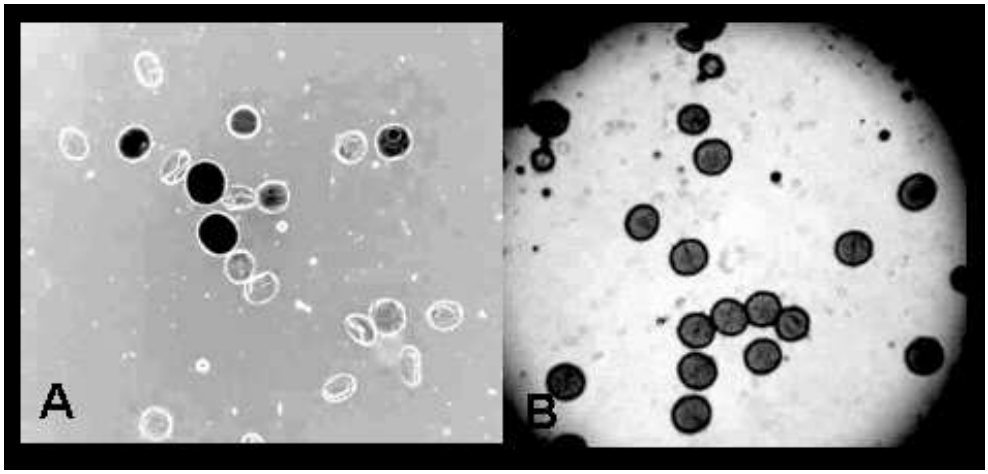

Figure 2-2. Pollen grains of ‘Red Queen’ (RQ) and ‘Sedona’ (S) stained with aceto-carmine. A) RQ pollen grains showing many not stainable. B) S pollen grains showing many fully stainable. Photographs were taken at a magnification of 10x using a compound light microscope (Nikon Labophot-2).

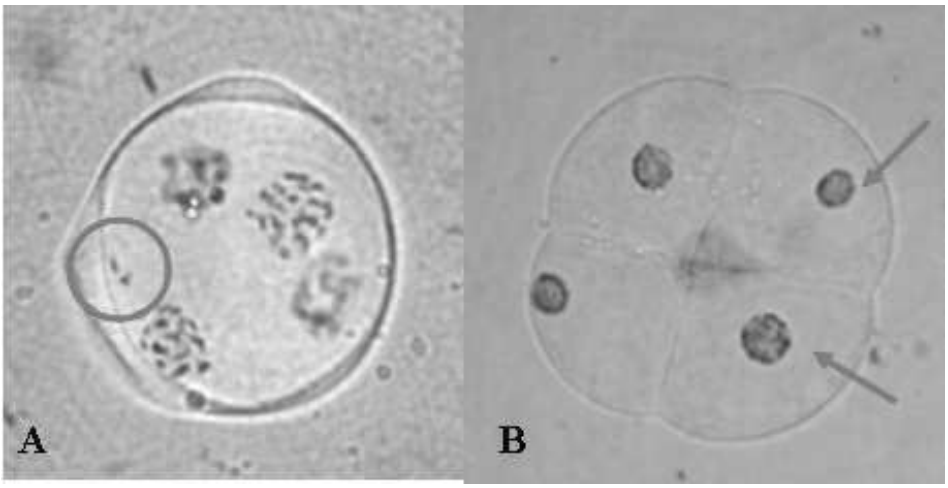

Figure 2-3. Microsporogenesis analysis of ‘Red Queen’ pollen mother cells. A) telophase II with some micronuclei. B) tetrad with microspores of different sizes. Photographs were taken at a magnification of 100X with a camera magnification of 4000X (Nikon Labophot-2).

Figure 2-4. ‘Red Queen’ selfed population. The plants in the selfed population were rated on a visual 1-5 scale, where 1 = upright and 5 = trailing. Five different growth habit types were observed. A) 1 = upright growth habit. B) 2 = semi-upright growth habit. C) 3 = prostrate growth habit. D) 4 = semi-trailing growth habit. E) 5 = trailing growth habit.

Figure 2-5. ‘Sedona’ selfed population. The plants in the selfed population were rated on a 1-5 scale, where 1 = upright and 5 = trailing. Three main growth habit types were observed. A) 1 = upright growth habit. B) 2 = semi-upright growth habit. C) 3 = prostrate growth habit.

Figure 2-6. F1 population produced from the cross ‘Red Queen’ x ‘Sedona’. The plants were rated on a 1-5 rating scale, where 1 = upright and 5 = trailing. Four main growth habit types were observed. A) 1 = upright growth habit. B) 2 = semi-upright growth habit. C) 3 = prostrate growth habit. D) 4 = semi-trailing growth habit.

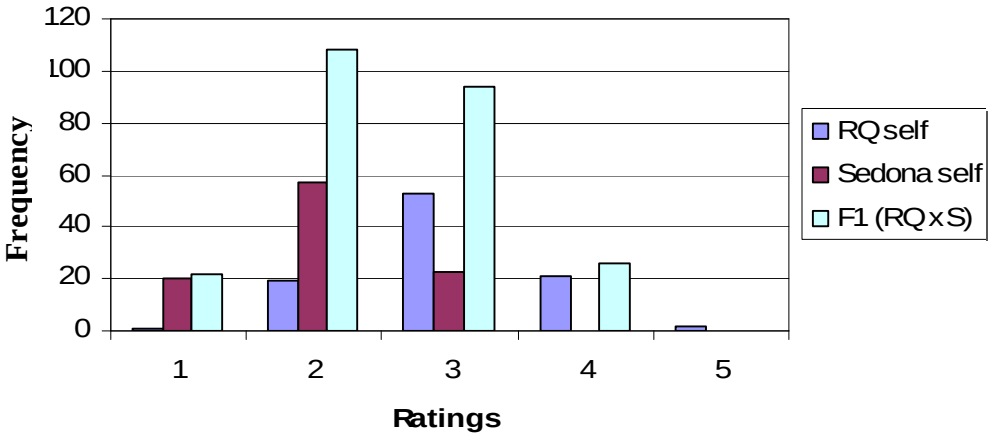

Figure 2-7. Distribution of the 'Red Queen' (RQ) selfed, 'Sedona' (S) selfed, and F1 population for growth habit. Growth habit was rated on a 1-5 scale, where 1 = upright, 2 = semi-upright, 3 = prostrate, 4 = semi-trailing, and 5 = trailing. The distribution of growth habit in the F1 population was between the distribution observed in the self populations of RQ and S.

Table 2-4. Chi-square test for 'Red Queen' (RQ) selfed population.

Genotype [x]	Segregation Ratio	Observed #	Expected #	X^2	P
1. AAAA	1	1	2.7	2.02	0.73 [y]
2. AAAa	8	19	21.3		
3. AAaa	18	53	48.0		
4. Aaaa	8	21	21.3		
5. aaaa	1	2	2.7		
Total	36	96	96.0		

[x] Five genotypic classes were observed for the five different growth habit types observed. Genotypic class 1 = upright (AAAA genotype), class 2 = semi-upright (AAAa genotype), class 3 = prostrate (AAaa genotype), class 4 = semi-trailing (Aaaa genotype) and 5 = trailing (aaaa genotype).

[y] The P-value of 0.73 supports the genotype (AAaa) proposed for RQ.

Table 2-5. Chi-square test for ‘Sedona’ (S) selfed population.

Genotype[x]	Segregation Ratio	Observed #	Expected #	X^2	P
1. AAAA	1	20	25	2.14	0.34 [y]
2. AAAa	2	57	50		
3. AAaa	1	23	25		
Total	4	100	100		

[x] Three genotypic classes were observed for the three different growth habit types observed. Genotypic class 1 = upright (AAAA genotype), class 2 = semi-upright (AAAa genotype), and class 3 = prostrate (AAaa genotype).
[y] A P-value of 0.34 supports the genotype (AAAa) proposed for S.

Table 2-6. Chi-square test for the F_1 population.

Genotype [x]	Segregation Ratio	Observed #	Expected #	X^2	P
1. AAAA	1	22	20.8	2.51	0.47 [y]
2. AAAa	5	108	104.2		
3. AAaa	5	94	104.2		
4. Aaaa	1	26	20.8		
Total	12	250	250.0		

[x] Four genotypic classes were observed for the four different growth habit types observed. Genotypic class 1 = upright (AAAA genotype), class 2 = semi-upright (AAAa genotype), class 3 = prostrate (AAaa genotype), and class 4 = semi-trailing (Aaaa genotype).
[y] A P-value of 0.47 supports the genotypes proposed for ‘Red Queen’ (AAaa) and ‘Sedona’ (AAAa).

Table 2-7. Chi-square test and test for homogeneity of the F_1 and F_2 plants.

Genotypic class	Pedigree	Upright	Semi-upright	Prostrate	Semi-trailing	Trailing	$X^{2\,y}$	P^{y}
1	H38-8	12	0	0	0	0	0.00	-
	H65-4	14	0	0	0	0	0.00	-
	H234-1	21	0	0	0	0	0.00	-
Total [z]							N/A	
2	H32	7	19	5	0	0	1.84	0.39
	H34	7	10	11	0	0	3.43	0.18
	H38	7	13	4	0	0	0.92	0.63
Total							5.19	0.20
3	H65	1	13	28	9	3	4.04	0.40
	H85	0	2	22	13	2	6.41	0.17
	H187	0	3	18	8	2	4.93	0.29
Total							15.38	0.08
4	H57	0	0	7	9	4	1.22	0.57
	H66	0	0	5	9	3	0.52	0.77
	H69	0	0	15	28	9	1.69	0.43
Total							3.43	0.50
5	H69-4	0	0	0	0	32	0.00	-
	H69-18	0	0	0	0	23	0.00	-
	H69-21	0	0	0	0	23	0.00	-
Total							N/A	

[x] Three plants from each of the five genotypic class were selfed in order to support the phenotypes assigned to each genotype.. The five genotypic classes were: 1 = upright (AAAA genotype), class 2 = semi-upright (AAAa genotype), class 3 = prostrate (AAaa genotype), class 4 = semi-trailing (Aaaa genotype), and class 5 = trailing (aaaa genotype).

[y] Values for the chi-square test are listed as X^2 with the P-values given for each plant within the genotypic class.

[z] Values for the chi-square test for homogeneity are listed as the total X^2 and the P-values for each genotypic class.

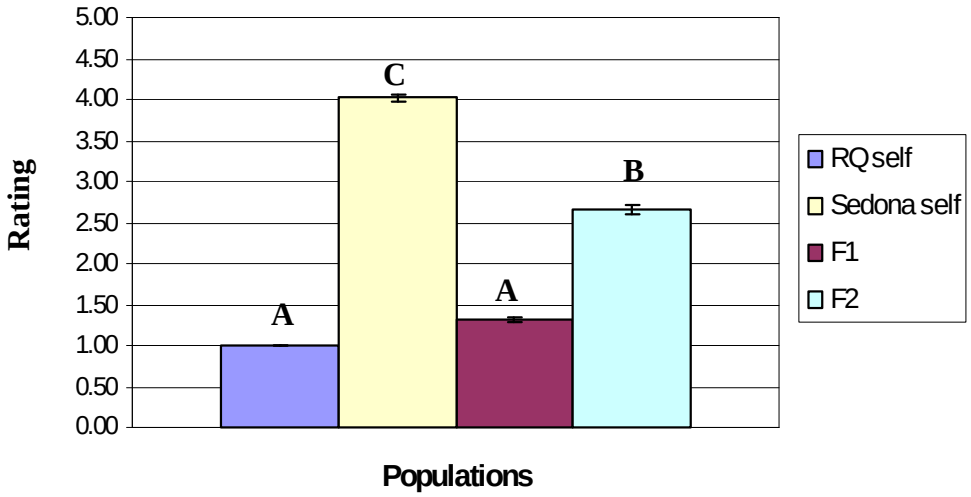

Figure 2-8. Mean rating values for color brightness for 'Red Queen' (RQ) selfed, 'Sedona' (S) selfed, F1, and the F2/F3 population. Color brightness was rated on a 1-5 scale where 1 = very dark (color as dark as or darker than RQ), 2 = dark (color slightly lighter than RQ), 3 = medium (color in between RQ and S, 4 = bright (color slightly darker than S), and 5 = very bright (color as bright as or brighter than S).

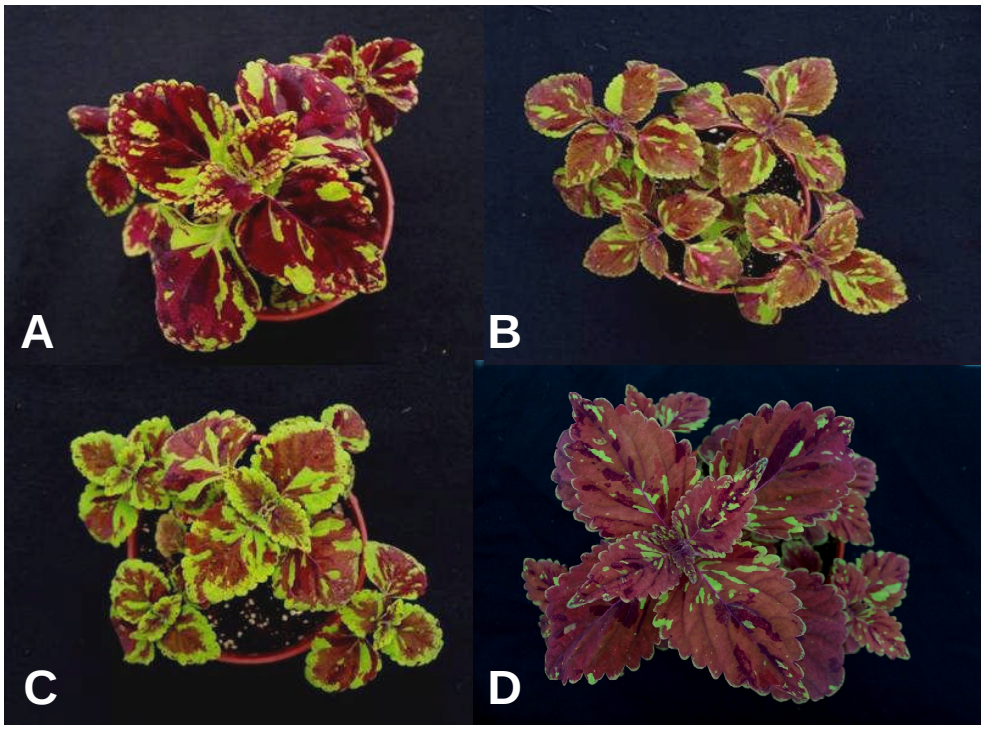

Figure 2-9. F1 plants produced from the cross ‘Red Queen’ (RQ) x ‘Sedona’ (S) with brighter foliage colors. (A-D) Foliage color was brighter than RQ but still darker than S and new color combinations were also observed which were not seen in the RQ and S self populations.

Figure 2-10. F2 and F3 plants (produced from selfing F1 and F2 plants, respectively) with brighter foliage color than RQ and as bright as S. A) plant in a hanging basket. B) variety with orange foliage color. C) close-up of plant with pink foliage color. D) close-up of plant with green foliage color.

Figure 2-11. Development of ‘Copper Penny’ with trailing habit and orange color. A) ‘Red Queen’. B) ‘Sedona’. C) F1 variety H66. D) F2 variety ‘Copper Penny’. E) F3 seedlings from selfing ‘Copper Penny’. F) Close-up picture of one seedling in F3 population.

CHAPTER 3
INFLUENCE OF LIGHT INTENSITY ON COLEUS FOLIAGE COLOR CHANGES

Introduction

Solenostemon scutellarioides (L.) Codd, commonly known as coleus, is a bedding plant valued primarily for its vibrant colorful foliage and not for its floral characteristics (Lebowitz, 1985). In plants, coloration of different organs such as flower, fruit and leaf is due to the accumulation of betalains, carotenoids or flavonoids (anthocyanins) (Mol, 1998). Anthocyanins are the major pigments that impart the wide range of red and purple colors observed in coleus leaves (Lebowitz, 1985). The synthesis of anthocyanins and color change in vegetative tissues due to light have been investigated at the physiological and molecular level for many plant species such as maize (Singh, 1999), *Perilla frutescens* (Gong, 1997), and in bilberry (Jaakola, 2004) but limited work has been done in coleus.

Anthocyanins are water-soluble pigments which are derived from a branch of the flavonoid synthesis pathway for which diagrammatic representations are available in numerous publications (Schijlen, 2004; Mol et al., 1998). The three predominant anthocyanin pigments found in plants are pelargonidins, cyanidins, and delphinidins (Holton and Cornish, 1995). The genes involved in the control of anthocyanin synthesis and hence coloration in plants (Quattrocchio et al., 1993) have been divided into two types: structural and regulatory genes. Most of the structural genes have been cloned from several model plants, including maize, *Antirrhinum*, *Petunia*, and *Arabidopsis* (Schijlen, 2004). These encode phenylalanine ammonia lyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI), dihydroflavonol reductase (DFR), flavanone-3-hydroxylase (F3H), flavonoid 3'-hydroxylase (F3'H), flavonoid 3',5'-hydroxylase (F3,5'H), anthocyanidin synthase (ANS), and UDP-glucose:flavonoid 3-O-glucosyltransferase (UFGT) (Holton and Cornish, 1995). Regulatory genes which control

expression of these structural genes include the R/B and C1/Purple (PI) families in maize (Dooner et al., 1991), DELILA and ELUTA genes in snapdragon (Goodrich et al., 1992), and the ANTHOCYANIN genes (AN1, AN2, AN6, AN10, and AN11) in petunia (Quattrocchio et al., 1993). Characterization of the AN1, AN2, and AN11 genes showed that they are transcription regulators (de Vetten et al., 1997). AN1 encodes the basic helix-loop-helix (bHLH) family of transcription factors, AN2 is a MYB transcription factor, and AN11 encodes a WD-40 repeat protein.

Foliage color may also be dependent on other factors such as co-pigmentation and vacuolar pH (Mol et al., 1998). Co-pigmentation involves the interaction of anthocyanins with other non-colored flavonols (e.g. flavonols), carotenoids, or metals (e.g. Mg^{2+}), and the modification of anthocyanins due to interactions with other molecules results in increased anthocyanin pigmentation and hue changes (Grotewold, 2006). Acidification of the vacuole results in red color in Petunia flowers; several mutants (ph1-7) with dark blue colors had high pH in the petal extracts without a change in anthocyanin composition and quantity (van Houwelingen et al., 1998).

The types of pigments involved in the coloration of coleus foliage have been investigated (Lebowitz, 1995) but little is known about their genetic control. The bright red and purple colors observed in the leaves are produced primarily by anthocyanin pigments, with most of the pigments composed of a complex of cyanidin and glucose components (Lebowitz, 1985). Green coloration is mainly due to chlorophyll pigments (Rife, 1948). As mottled coleus cultivars age, the content of carotene and xanthophyll in leaves increases (Lebowitz, 1985).

The regulation of anthocyanin synthesis in vegetative tissues by light has been well-documented in many plant species (Meng, 2004). A proposed function for anthocyanins is the

photoprotection of vegetative tissues that are predisposed to photoinhibition (Steyn, 2002), as they can significantly modify both the quantity and quality of light incident on chloroplasts (Krol et al., 1995). Under low light levels, foliage of *Pennisetum setaceum* cvs 'Rubrum' and 'Red Riding hood' is light-purple or green while under high irradiance leaves become dark purple (Beckwith, 2004). Mutants defective in the production of anthocyanins have been shown to be more sensitive to UV-B damage in which bleaching of the foliage is observed (Delpech, 2000).

In a majority of plants being developed in the coleus breeding program at UF, foliage color becomes dark red when plants are exposed to high light intensities. In this study, two related coleus varieties 'Royal Glissade' (RG) and 'UF06-1-6' (UF) (a seedling produced by self-pollinating RG) showing different color change in response to light intensity changes were examined under direct light and shade conditions in the greenhouse. As a lack of information exists to explain the physiological and molecular mechanisms for color change in coleus foliage, a broad-based approach was pursued in this study to provide a base level of information for this phenomenon. The main objectives of this study were to determine: (1) the effect of light intensity on foliage coloration, (2) the effect of anthocyanin and chlorophyll accumulation on foliage coloration, and (3) the regulation of genes involved in anthocyanin synthesis in coleus.

Materials and Methods

Plant Materials and Growth Conditions

Vegetative cuttings with two nodes were taken from stock plants of RG and UF (72 per variety) grown in a glass greenhouse under standard conditions at 23 + 2^{o}C. Cuttings were removed from the mist house after rooting (~ ten days), transplanted into 12.7cm azalea pots, and grown under 25% shade in the greenhouse for an additional three weeks. After three weeks when six leaf nodes were present on the plants, 64 plants that were uniform in color and size were

chosen from each variety and randomly assigned to two light treatment groups: direct light and shade in a glass greenhouse. A blackcloth was used to set the shade treatment.

Experimental Design

Light experiments for this study were conducted over a period of three weeks because RG and UF had a complete foliage color change from an initial green to red within this period in preliminary experiments. Two independent replicated light experiments were conducted at two different times. Experiment 1 (E1) was conducted from 8 March – 29 March, 2007 and Experiment 2 (E2) was conducted from 16 May – 6 June, 2007. Prior to the start of E1 and E2, plants of RG and UF were initially grown under 25% shade (moderate light) and moved to either 50% shade (low light) or direct light (high light). In E1, the moderate light intensity was 250$\mu mol^{-2}s^{-1}$, and plants were then transferred to a low light (150$\mu mol^{-2}s^{-1}$) or high light (350$\mu mol^{-2}s^{-1}$). In E2, the moderate light was 340$\mu mol^{-2}s^{-1}$, and plants were then transferred to the low light (225$\mu mol^{-2}s^{-1}$) or high light (450$\mu mol^{-2}s^{-1}$). Thus, in this study, RG and UF plants were exposed to two initial and four basic light intensity levels, from the lowest light intensity of 150$\mu mol^{-2}s^{-1}$in E1 to the highest at 450$\mu mol^{-2}s^{-1}$in E2.

The experimental design used for E1 and E2 was a split-split plot, with the two main treatments being direct sun and shade and the sub-treatments being time after the initiation of the experiment. Each main treatment was divided into four blocks, two plants per block, with a total of eight plants sampled for each variety per data collection time. The data collection time was divided into four weekly intervals, designated as weeks 0, 1, 2, and 3. Leaf tissue at the fourth node from the apex was collected in the morning (~ 10am) at each weekly interval for the extraction of RNA and other analyses (anthocyanin and chlorophyll content, pH, and light microscopy). These leaves were actively growing and still expanding but not old enough to be shaded by new foliage.

Anthocyanin, chlorophyll and pH determination

For anthocyanin determination, eight leaf discs (1cm^2) were collected from fresh leaf tissues and immediately frozen in liquid nitrogen. Samples were ground in liquid nitrogen and anthocyanins were extracted in 1ml 1% HCL in iso-propanol and left in the dark at 4^oC overnight (Singh et al., 1999). Samples were centrifuged at 10000Xg for 10min, diluted 5X with deionized water, and 500ml of the supernatant were used for the determination of absorbance by spectrophotometry (BioRad SmartSpec 3000, California) at 535nm and 650nm. Anthocyanin content was calculated according to the following formula A= (A535-(2*A650))/leaf area used (8 cm2) as previously described by Murray and Hackett (1991). The absorbance at 650nm was deducted from the absorbance at 535nm to correct the value for the chlorophyll content. Each mean is represented as the absorbance value of eight independent determinations per treatment.

For chlorophyll determination, eight leaf discs (1cm^2) were collected from fresh leaf tissues and immediately frozen in liquid nitrogen. Samples were ground in liquid nitrogen and chlorophyll was extracted in 1ml 96% ethanol and left in the dark at 4^oC overnight. Samples were centrifuged at 10000Xg for 10min and 500ml of the supernatant were used for the determination of absorbance at 649nm and 665nm. The total chlorophyll A/B concentration was calculated by summing the individual calculations for chlorophyll A and B according to the formula Chl A = (9.78*A665) - (0.99 *A649)/ per leaf area used (8 cm2) and Chl B = (21.4*A649) – (4.65*A665)/ per leaf area used (cm^2) as previously described (Goncalves, et al. 2001). Each mean is represented as the absorbance value of eight independent determinations per collection.

For pH determination, one 3-cm^2 piece was collected from fresh leaf tissues and ground in 5000µl deionized water with a mortar and pestle and measured with a pH meter as previously

described by Spelt (2002). The pH measurements were recorded as the mean of eight replicate samples per collection time point in E2.

Samples for anthocyanin and chlorophyll analysis and pH level were collected for RG and UF for both treatment conditions at all weekly time points throughout E1 and E2. Analysis of variance (ANOVA) and a Tukey's test were performed to determine treatment effects ($p=0.05$).

Fresh Weight and Light Microscopy

Fresh weight was determined by excising the entire plant at the soil line and weighing it on a balance (PS7S01-S, Mettler Toledo). Fresh weight was recorded as the total weight of eight plants (g) per treatment at each weekly collection. After fresh weight was determined, leaf tissues were used for microscopy. Fresh leaf tissues were hand-sectioned along the longitudinal axis of the leaves and immediately viewed under a compound light microscope (Nikon – Labophot-2). Microscopy photos were taken at a magnification of 200X for plants at each weekly time point in E1.

RNA isolation and cDNA synthesis

Total RNA was extracted from coleus leaves using the TriZOL TM – LS reagent method according to the manufacturer's manual (Gibco BRL, Invitrogen, USA) with modifications as previously described by Verdonk (2006). Total RNA samples were further purified with a phenol-chloroform treatment and precipitated with 3M sodium acetate and iso-propanol according to Sambrook et al. (1989). Samples were treated with DNase, with modifications as previously described (Dal Cin et al., 2005) and single stranded cDNA was synthesized in a final volume of 20µl, with modifications as previously described (Dal Cin et al., 2007).

Isolation of Partial Clones Involved in Coloration

At the beginning of the experiments, no genomic or EST (Expressed Sequence Tag) sequences for coleus were present in the database (http://www.ncbi.nlm.nih.gov/BLAST/). The

identification of specific clones encoding the elements involved in anthocyanin biosynthesis in coleus was pursued by the amplification of partial clones from genomic DNA or from cDNA with degenerate primers. The design of the degenerate primers was performed by the identification of conserved region of the genes of several species in which the elements involved in the anthocyanin pathway have been extensively characterized. The degenerate primers were specifically designed in this study (ACT, PAL, CHS, DFR, UFGT, AN1, and AN2) or were designed as previously sequenced from *Perilla frutescens* (ANS and F3H) (Gong, 1997) (Table 3-1). The amplification profile for all clones was as follows: an initial hold at 94^{o}C for 5min, 40 cycles and a final hold at 72^{o}C for 5min. Each cycle was composed of an initial denaturation for 60s at 94^{o}C, 60s at an annealing temperature (Table 3-1) specific for the primer couple and an extension at 72^{o}C for 60s. Reactions were carried out with the GeneAmp PCR System 2700 (Applied Biosystems, California) using 0.025U/ µl Taq-polymerase (Amersham Biosciences, Piscataway, NJ), 0.5µl of cDNA, 2µM primers, 1.5mM MgCl2, buffer 1X and 1mM dNTPs in a final volume of 25 µl. Products were electrophoresed in a 1.5% agarose gel and stained with ethidium bromide. Bands were purified with a Qiagen (USA) gel extraction kit, eluted fragments were ligated into pGEM T-easy vector (Promega, USA), and transformed into chemically competent cells (XL1-Blue genotype: rec A1 end A1 gyr A96 thi-1 hsdR17 sup E44 relA1 lac[F' proAB lacl9ΔM15 Tn10 (Tetr)] Stragene, La Jolla, CA. USA). Colonies were checked with a colony PCR using SP6 and T7 plasmid specific primers. Positive colonies were grown in LB media and plasmids extracted with the TEG-sodium hydroxide method (Sambrook et al. 1989) and plasmids digested with ECORI restriction enzyme to determine clone size. Positive plasmids were sequenced with SP6 and T7 primers and sequences were aligned with the on-line blast-x

program (http://www.ncbi.nlm.nih.gov/BLAST/) and (http://www.sgn.cornell.edu/tools/blast/) to confirm their identity.

Expression Analysis using Semi-Quantitative RT-PCR

RT-PCR expression analysis on genes isolated in this study and encoding PAL1, CHS, DFR, F3H, ANS, UFGT, AN1, and AN2 was performed on coleus cDNA using specific primers (Table 3-2) designed with the aid of the on-line GeneFisher program (http://bibiserv.techfak.unibielefeld.de/genefisher/-). PCR reaction consisted of 1µl of cDNA, 0.75µM primers, 1.5mM MgCl2, buffer 1X and 1mM dNTPs in a final volume of 20µl. The amplification with specific 18S and actin primers (constitutive genes) was performed to verify similar quantities of cDNA used for expression analysis. The choice of two internal controls was used to overcome the limitations due to the use of only the 18S or only the housekeeping gene (Volkov et al., 2003). The amplification profile was as follows: an initial hold at 94^{o}C for 5min, a variable number of cycles (each cycle composed of an initial denaturation at 94^{o}C for 30s, 30s at an annealing temperature specific of the primer couple (Table 3-2) and an extension at 72^{o}C for 15s) and a final hold at 72^{o}C for 5min. Products were electrophoresed in a 1.5% agarose gel stained with ethidium bromide and UV images were acquired with the Gel Logic 100 Imaging System (Kodak, CT). In order to verify primer specificity, amplification products were gel purified and sequenced with the specific primers. PCR reactions were repeated at a different number of cycles to verify expression pattern and reproducibility.

The isolation of SsAN1 and actin (SsACT) genomic clones was performed on 500ng of genomic DNA extracted from coleus leaves with the DNeasy Plant mini kit (Qiagen, USA). The amplification was reported as described above for the isolation of partial clones from cDNA. The isolation of a genomic clone was performed in order to design a couple of specific primers spanning an intron. These primers were also used to verify that genomic DNA was not present in

the cDNA. The presence of genomic DNA may interfere with the analysis by altering the template amount and thus the expression results.

Results and Discussion

Physiological Mechanisms Involved in Foliage Color Change

Light intensity affects foliage color change in RG and UF

Red coloration in coleus is primarily produced by anthocyanin pigments and the amount of anthocyanin present in leaf tissues has been shown to be a function of light intensity (Lebowitz, 1985). To determine if light affected foliage coloration in the coleus varieties RG and UF in this study, leaves representative of the color changes for both varieties at each weekly time point were photographed (Figure 3-1). The results indicated that leaves of both RG and UF had a gradual transition from green to red within the three week period when grown in the direct light treatments. The observation that light affects coloration in coleus foliage is supported by studies in other plant species. In leaves of *Quintinia serrata*, the occurrences of red areas on the lamina were more prevalent in leaves experiencing high light conditions (Gould et al., 2000). Similarly in *Galax urceolata*, leaves exposed to high light turn a deep shade of red whereas in leaves that remain shaded stay green (Hughes et al., 2005).

Furthermore, light intensity was observed to affect how fast the plants transitioned in color. Plants grown under direct light in both E1 and E2 had a more rapid change from green to red than plants grown in the shade. When the four light intensity levels in E1 and E2 were compared, RG and UF plants grown under the lowest light intensity (150 $\mu mol^{-2}s^{-1}$) had the slowest color transition whereas plants grown under the highest light intensity (450 $\mu mol^{-2}s^{-1}$) had the fastest color transition. In addition, RG and UF differed in their rate of color transition. At the start of the experiment, UF leaves were less green in color and throughout the experiments, UF leaves reddened earlier than RG in both direct light and shade treatments. A higher sensitivity to light

may explain the more rapid transition from green to red in UF than in RG (Mancinelli, 1990). Similar results were observed in a study conducted by Mancinelli (1990) in which tomato and cabbage seedlings growing under the same light conditions were compared. It was observed that maximum anthocyanin production in tomato was only 20% of that in cabbage. Thus, plant differences were observed in which cabbage produced more anthocyanins than tomato when grown under the same light condition.

Red coloration in RG and UF foliage is due to anthocyanin accumulation

It has been reported that anthocyanins are the major pigments contributing to the red coloration in coleus plants (Lebowitz, 1985). Anthocyanins are water soluble pigments (Grotewold, 2006) that are synthesized in the cytoplasm, actively transported across the tonoplast, and accumulated in the vacuoles of epidermal or mesophyll cells (Delpech, 2000). To determine where the red coloration occurred in RG and UF, and if it was due to the accumulation of anthocyanins, light microscopy photos of coleus leaf epidermal layers were taken in E1 (Figure 3-2) and anthocyanin concentration measured in absorbance (A535) (Figure 3-3) in E1 and E2. Anthocyanins were found to accumulate on both the upper and lower epidermal layers throughout the experiments. In addition, coloration of the upper epidermal layers for both varieties was darker in color in plants grown under direct light conditions compared to shaded conditions. Similar observations to these have been reported for maize. In several maize varieties, exposure to light induced the accumulation of anthocyanin in the vegetative tissues and resulted in ‘sun-red’ phenotypes (Singh et al., 1999). When the upper epidermal layers of both RG and UF were compared, UF was observed to accumulate more anthocyanins in the upper epidermal layer than RG. This observation further supports the visual observation of the foliage as seen in Figure 3-1 in which the reddening in UF leaves occurred earlier than in RG.

When anthocyanin concentrations were measured in RG and UF (Figure 3-3), the results further supported the visual observations and light microscopy photos. In both E1 and E2, RG and UF plants grown under direct light at week 3 had higher concentrations of anthocyanins than plants grown in the shade. RG and UF had the lowest anthocyanin concentrations at the lowest light intensity ($150\mu mol^{-2}s^{-1}$) and the highest concentrations at the highest light intensity ($450\mu mol^{-2}s^{-1}$). Depending on plant species, light exposure has been shown to be a prerequisite for significant anthocyanin synthesis in vegetative tissues and anthocyanin levels vary in relation to light exposure levels (Mancinelli, 1983; Krol et al., 1995). Comparing RG and UF, at week 0 UF was observed to have higher anthocyanin concentrations than RG. At week 3 for E1 and E2, both varieties had similar anthocyanin concentrations in the sun, whereas in the shade, UF accumulated higher amounts than RG. These observations further parallel the results as observed in the redder foliage color of UF at week 0 and week 3 in E1 and E2.

pH is not a factor affecting foliage coloration in RG and UF

Changes in pH level have been proposed to affect plant coloration (Mol et al., 1998). In *Petunia hybrida* for example, the acidification of the vacuole results in a red color of the flower and mutants have a shift of flower color towards blue (Grotewold, 2006). In order to observe whether color changes in coleus leaves were the result of changes in leaf pH, the pH was measured on a weekly basis for E1 and E2 (Figure 3-4). No significant changes were observed for RG and UF plants grown under direct light and shade treatments and no differences were observed between the varieties. It can be concluded that pH was not a factor contributing to the red coloration in RG and UF.

Light intensity affects chlorophyll concentration and foliage coloration

To determine if light intensity affected chlorophyll concentrations and thus coloration, pigment content were measured for RG and UF in both experiments (Figures 3-6 and 3-7). At

week 3, RG and UF plants grown in the shade accumulated higher amounts of chlorophyll than plants grown in direct light, with the exception of UF in the shade in E1. Both varieties behaved similarly, as they had the lowest chlorophyll concentrations at the highest light intensity ($450\mu mol^{-2}s^{-1}$) and the highest chlorophyll concentrations at the moderate light intensity ($225\mu mol^{-2}s^{-1}$). Similar results have been shown in studies comparing plants grown in the sun and shade. Under low light, plants produce more chlorophyll to maximize photosynthesis whereas at high light less chlorophyll is made to avoid photodamage (Chen and Xu, 2005).

A balance between chlorophyll and anthocyanins appeared to affect plant coloration in RG and UF. Foliage and light microscopy photos showed that throughout the experiment UF accumulated anthocyanins on the upper epidermal layer earlier than RG. Furthermore, green coloration disappeared earlier in UF than in RG in both experiments. This suggests that the balance between chlorophyll and anthocyanin concentrations may be major factors affecting color change in coleus. The results regarding either chlorophyll or anthocyanin concentrations alone were not sufficient to explain the color difference in UF and RG. At $150\mu mol^{-2}s^{-1}$ (shade, E1), the difference in color between UF and RG appeared to be predominantly due to chlorophyll content, whereas at $225\mu mol^{-2}s^{-1}$ and $450\mu mol^{-2}s^{-1}$ (shade and direct light, E2), color was mainly due to anthocyanin accumulation since only a slight difference in chlorophyll quantity was recorded. At $350\mu mol^{-2}s^{-1}$ (direct light, E1), the brighter red color observed in UF was likely due to both a low chlorophyll content and high level of anthocyanin accumulation.

Light intensity affects plant growth in RG and UF

Light is necessary for photosynthesis (Chen and Xu, 2005) and the amount of light is critical for plant growth (Lefsrud, et al., 2006). Light intensity had an effect on plant growth for both RG and UF (Figure 3-7). In E1, light intensity for shade and direct light was $150\mu mol^{-2}s^{-1}$and $350\mu mol^{-2}s^{-1}$, respectively whereas in E2, light intensity for shade and direct light was

225μmol^{-2}s^{-1}and 450μmol^{-2}s^{-1}, respectively. It must be noted here that the day-length in E1, which was conducted in March, was also two hours shorter on average than in E2 conducted in May. Fresh weights of approximately 450g for RG and 300g for UF were recorded at week 3 when they were grown at 350μmol^{-2}s^{-1} (direct light, E1). With an increase in light intensity to 450μmol^{-2}s^{-1}a reduction in fresh weight to 232g for RG and 271g for UF was observed at week 3. It can be reasoned that RG and UF should have higher fresh weights under higher light intensities due to higher photosynthetic activity (Huner et al., 1998). However, a light intensity at 450μmol^{-2}s^{-1}and the addition of a longer day-length may have resulted in photoinhibition. It has been shown that photoinhibition can lead to significant decreases in plant productivity (Huner, et al. 1998). At 150μmol^{-2}s^{-1} lowest fresh weight was recorded for both varieties, at 150g (RG) and 87g (UF). The low fresh weight in UF at 150μmol^{-2}s^{-1}may help to explain the irregularity observed in which UF had the lowest chlorophyll concentrations when grown in the shade in E1(Figure 3-6). Thus light intensities at 150μmol^{-2}s^{-1}and 450μmol^{-2}s^{-1}were suboptimal for maximum plant growth while a range between 350μmol^{-2}s^{-1}for direct light and 225μmol^{-2}s^{-1} for shade appeared to be optimal for maximum plant growth.

Molecular Mechanisms Affecting Foliage Color Change in RG and UF

Isolation and expression of clones related to anthocyanin synthesis

The structural and regulatory genes involved in the anthocyanin biosynthetic pathway have been extensively characterized in other model species such as maize, snapdragon, and petunia (Winkel-Shirley, 2001; Mol et al., 1998; Holton and Cornish, 1995). In *Petunia hybrida*, at least thirty-five genes are known to influence anthocyanin biosynthesis in flowers (Wiering and de Vlaming, 1984; Koes, 1988). In this study, eight genes (PAL1, CHS, F3H, DFR, ANS, UFGT, AN1 and AN2) involved in the anthocyanin synthesis pathway were chosen for expression analysis in coleus. The six structural genes chosen are located at the beginning (PAL and CHS),

middle (F3H and DFR), and end (ANS and UFGT) of the pathway. At the start of the study, these genes were not characterized in coleus. The use of degenerate primers allowed for the identification of several partial clones encoding the proteins involved in anthocyanin biosynthesis and transcription factors that are known to regulate those genes in coleus. The fragments isolated after cloning and sequencing were aligned with other major plant species using the NCBI GenBank BLAST-X program. A list of the isolated partial clones, their sequence identities compared to other published sequences, the Gen Bank accession numbers and blast e-values (measure of the similarity of the queries) are shown in Table 3-5.

Enzymes involved in anthocyanin synthesis in coleus

The number of cycles required to generate a non-saturating optimal level of expression indicated that transcript amounts of DFR and UFGT (24 cycles) were expressed in the most abundance, followed by PAL1 and CHS (27 cycles), F3H (29 cycles) and ANS (30 cycles). Transcript levels of the regulatory enzymes AN1and AN2 were the least expressed as at least 32 and 37 cycles were required to observe an optimal level of expression (Figure 3-8). A low number of 24 cycles required to see expression of DFR and UFGT were to be expected as both enzymes are responsible for the synthesis of specific anthocyanins and thus plant coloration in coleus. DFR catalyzes the reduction of three colorless dihydroflavonols to leucoanthocyanidins (Schijlen, 2004). With three different substrates to catalyze, a higher level of expression was observed for DFR. UFGT has been regarded as an indispensable enzyme of the anthocyanin pathway as it catalyzes the final step required to stabilize anthocyanidins to accumulate as water soluble pigments in the vacuoles (Schijlen, 2004). A moderate number of 27 cycles required for PAL1 and CHS were also to be expected as they both belong to multi-gene families (Durbin et al., 1995, Goto-Yamamoto et al., 2002) and are involved in numerous other processes besides anthocyanin synthesis (Dixon and Palva, 1995). Furthermore, PAL catalyzes phenylalanine, the

precursor for all flavonoid compounds (Koes, 1988) whereas CHS provides the first committed step in anthocyanin synthesis (Grotewold, 2006). Following PAL and CHS, F3H and ANS required 29 cycles. The activity of F3H may be dependent on the products produced by CHS while ANS catalyzes only one of the three products (leucoanthocyanidins) produced by DFR (Schijlen, 2004). Higher number of cycles was observed for the regulatory elements AN1 and AN2 and this may be due to the fact that they are transcription factors and are transient in their regulation (Quattrocchio et al., 1999).

Light intensity and anthocyanin accumulation and their effects on gene expression

The expression of these eight genes (PAL1, CHS, F3H, DFR, ANS, UFGT, AN1. and AN2) was analyzed for RG and UF when grown in direct light and shade conditions in E1 and E2. Transcript levels for all of the genes were observed to be higher in UF than in RG at week 0, the start of the experiment. Higher expression levels observed in UF supported the results previously stated in which UF was darker in color and had higher anthocyanin concentrations at week 0 than RG. However, when the transcript amounts for the structural genes were compared between treatments (direct light versus shade) and in conjunction with anthocyanin concentrations, no clear pattern of expression was observed throughout the experiments. For both varieties, transcript amounts of PAL1, CHS and ANS had minimal variation in levels between direct light and shade treatments in either E1 or E2. DFR and UFGT expression did not correlate with treatment conditions or with anthocyanin concentrations. Transcript amounts of these genes in plants grown under direct light were not always higher than plants grown under shade. Furthermore, where an increase in anthocyanin concentrations were observed in both varieties, transcript amounts for the genes did not increase. Light was shown to affect coloration in RG and UF, but no evidence was found for a correlation between gene expression and foliage coloration.

Although no correlation was found between gene expression and coloration, a correlation was observed between the expression pattern of UFGT and DFR and AN1.

Regulatory genes controlling the transcription of the structural genes have been identified by mutation in maize, snapdragon, and petunia (reviewed by Dooner et al., 1991). In this study, AN1 may play a role in regulating the expression of DFR and UFGT. In E2, expression of AN1 follows similarly to that of UFGT and DFR, while expression of AN2 does not follow the expression patterns of the other biosynthetic genes. Two hypotheses that may be proposed for the similar expression pattern found between AN1, UFGT and to an extent DFR are: (1) AN1, UFGT, and DFR are similarly regulated, or (2) AN1 controls the expression of UFGT and slightly DFR. The second hypothesis is more likely to be found in coleus because a similar pattern of control of AN1 on UFGT was observed in petunia (Quattrocchio et al., 1993). The expression of AN2 does not appear to be tightly correlated with the expression of the other biosynthetic genes nor does it appear to regulate the expression of AN1, as previously described by Spelt et al. (2000).

Since transcript amounts of the structural genes did not correlate with anthocyanin concentrations, regulation of these genes at the level of transcription is proposed to not occur. Thus, post-transcriptional and/or post-translational regulation is the suggested reason for increased anthocyanin content in RG and UF. Evidence for these two types of regulation has been shown in *Petunia hybrida*. In petunia corollas, accumulation of anthocyanins at the later stages of flower development was hypothesized to result from early accumulation of the CHS transcript. Since CHS expression did not always coincide with its effect on anthocyanin accumulation, post-translational effects were proposed (Shvarts, 1997). The activity of AN2 has

also been shown to be regulated post-transcriptionally by a cytosolic WD40 repeat protein encoded by AN11 in petunia (Shvarts, 1997).

F3H is responsible for imparting the color difference between RG and UF

Interestingly, one clear trend observed in the experiment was a difference in transcript amounts of F3H between RG and UF. UF and RG differed in the expression of F3H as consistently higher transcript amounts were observed in UF than in RG in all treatments and experiments (Figure 3-8). F3H is highly conserved among widely divergent plant species and a mutation resulting in a loss of F3H activity, both in *Petunia* and *Antirrhinum*, prevents the progression along the anthocyanin pathway and results in white flowers (Schijlen et al., 2004; Martin et al., 1991). The rapid and darker coloration of the foliage observed in UF is hypothesized to be due to the overexpression of F3H. The overexpression of F3H may lead to a high formation of the substrate dihydrokaempferol (DHK). Hydroxylation of this product at the 3' position forms dihydroquercitin (DHQ) and ultimately to the production of cyanidins based pigments and hence color formation (Scijlen et al. 2004). Thus, the overexpression of F3H may have resulted in the color differences observed between RG and UF throughout the experiments.

Furthermore, overexpression of F3H may help to explain the low fresh weight observed in UF when grown under the lowest light intensity at 150$\mu mol^{-2}s^{-1}$. Even under the lowest light intensity, UF continued to have a color change from green to red. Light intensity has been shown in this study to affect the production of anthocyanins in RG and UF. However, the accumulation of anthocyanins and maintenance carries an energy cost, as it may reduce light capture and ultimately carbon assimilation (Steyn, 2002). Therefore, the poor growth of UF under the lowest light intensity of 150$\mu mol^{-2}s^{-1}$ may be attributed to the overproduction of anthocyanin pigments and low chlorophyll concentrations required to sustain optimal growth.

Conclusions

Light intensity affected foliage coloration in RG and UF in this study. As the light intensity increased, the transition from green to red color occurred more rapidly in RG and UF. Foliage coloration was due to the accumulation of anthocyanin pigments in both varieties. Anthocyanin synthesis was not observed to be regulated at the level of transcription. AN1 was observed to regulate UFGT and to an extent DFR, but regulation of AN1, AN2, and the other structural genes is proposed to occur via post-transcriptional and/or post-translational regulation. It is also possible that other transcription factors may be involved in the regulation of the structural genes and regulatory genes AN1 and AN2 in this study.

Table 3-1. List of the degenerate primers and the corresponding conserved regions, and the annealing temperatures used during amplification.

Gene	Forward primer and conserved region	Reverse primer and conserved region	Tm
PAL1	5'-ARAGYGCNGAGCARCAYAACC-3'	5'-GGVAGAGGWGCACCRTTCCA-3'	64
PAL1	SAEQHNQ	WNGAPL	
CHS	5'-CTGTCCTCCGCATGGCCAAG-3'	5'-GGCCCGAACCCAAACAGCA-3'	64
CHS	VLRMAKDL	WGVLFGFG	
DFR	5'-TYCAYGCWACYGTTCGTGATCC-3'	5'-CTTGYTTWATGATKSAGTARTGAG-3'	60
DFR	ATVRDPE	HYSIIKQ	
F3H	5'-SCARGAYTGGMGNGARATHGTVAC-3'	5'-GCYTGRTGRTCHGCRTTCTTGAABC-3'	60
F3H	QDWREIV	FKNADHQ	
UFGT	5'-ARAVKKTYCTCAAYRTHGGYCCWT-3'	5'-CCRAARAANGGCCTRCAAATCA-3'	64
UFGT	LNVGPS	LICRPFF	
AN1	5'-CGGTTCAAACCGCTCAGTGGA-3'	5'-GGGTTTGGAGGATTAGAAGTGGAGT-3'	64
AN1	SVQTAQW	FSFPPGVG	
AN2	5'-TATGGDGAAGGDAAGTGGCATC-3'	5'-GGAAKBTTCCGAGGTYGAGG-3'	65
AN2	YGEGKWH	VRPRPR	
ACT	5'-CTCGGCAGTTGTGGTAAACATG-3'	5'-CCGTGTCGCTCCTGAAGAGC-3'	64
ACT	RVAPEEH	GYMFTTTAE	

Table 3-2. List of the specific primers and of the annealing temperature used during RT-PCR expression analysis on coleus cDNA.

Gene	Forward primer	Reverse primer	Tm (^{o}C)
PAL1	5'-GCCAAGCCATTGATCTGAGGCAT-3'	5'-CGGAGCTTCTGCATCAATGGGTA-3'	65
CHS	5'-GGAACTCAGTGTTCTGGATTGC-3'	5'-GGTGGATCTCAGCCCCTCCT-3'	62
DFR	5'-CTGGGACTTTGAATGTTGAGGA-3'	5'-GGAATGTGGGCATGAAGAATGG-3'	64
F3H	5'-TGATATGTCGGGTGGCAAGAAAGG-3'	5'-GCCTTCTCTCCTTCCCTCAACTTC-3'	66
ANS	5'-CGGCCTCCAGCTCTTCTACG-3'	5'-GGTCTCCGGTAGTGGCTGGA-3'	66
UFGT	5'-CTGCTTCCTGCTCTCTCTCTTCAC-3'	5'-CCGCAGTGAGTGACGAACACTC-3'	65
AN1	5'-GAGCGATGGATACTACAATGGA-3'	5'-GGAAACGAGAATGATACGCACA-3'	62
AN2	5'-GACCCGATATCAAAAGGGGCTT-3'	5'-CCGAGGTCGAGGCCTTACGAT-3'	65
ACT	5'-CTCGGCAGTTGTGGTAAACATG-3'	5'-CCGTGTCGCTCCTGAAGAGC-3'	68
18S	5'-TTAGCAGGCTGAGGTCTCGT-3'	5'-AGCGGATGTTGCTTTTAGGA-3'	58

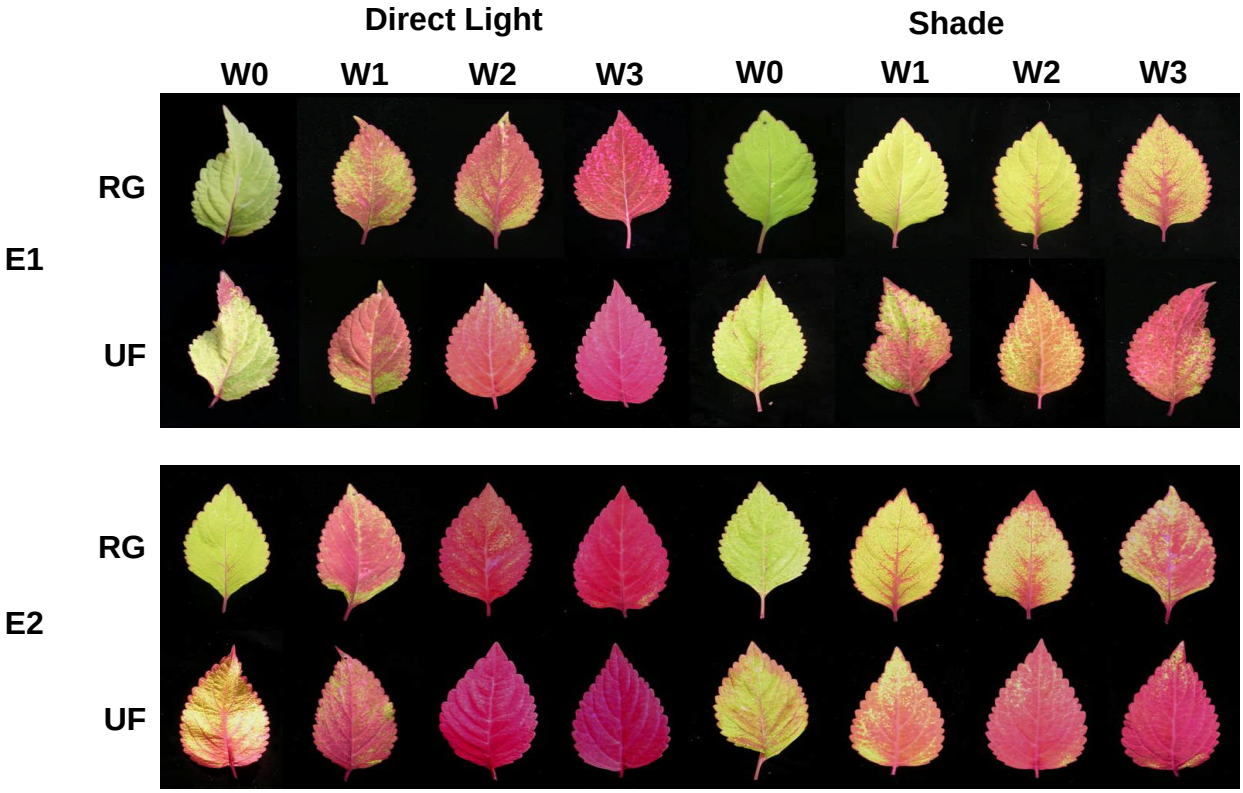

Figure 3-1. Visual foliage color change of coleus leaves throughout the experiment. A) Experiment 1 (E1) conducted 8 March – 29 March, 2007 and light levels for direct sun was 350µmol^{-2}s^{-1} and for shade was 150µmol^{-2}s^{-1}. B) Experiment 2 (E2) conducted 16 May – 6 June, 2007 with light levels for direct sun was 450µmol^{-2}s^{-1} and for shade was 225µmol^{-2}s^{-1}. Photos were taken at each week in E1 and E2 from the start of the experiments (W0).

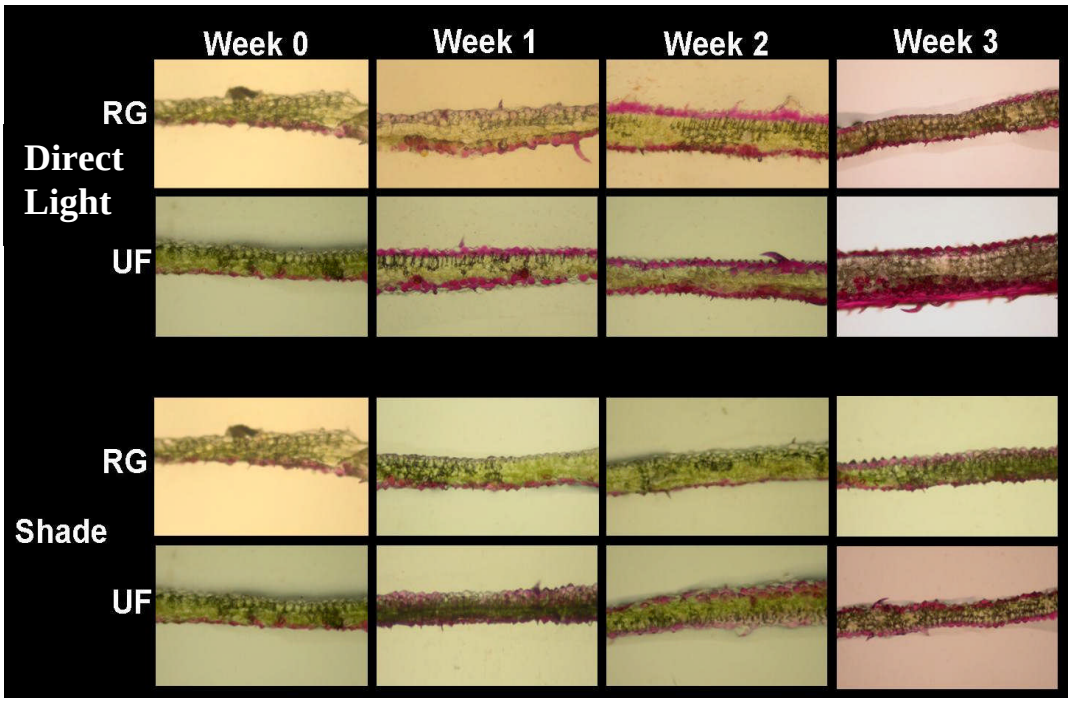

Figure 3-2. Accumulation of anthocyanin on the epidermal leaf tissues of RG and UF grown in the direct light (350µmol^{-2}s^{-1}) and shade (150µmol^{-2}s^{-1}). Fresh leaf tissues were hand-sectioned along the longitudinal axis of the leaves and viewed under a light microscope (Nikon – Labophot-2). Microscopy photos were taken at a magnification of 200X for plants at each weekly time point (W1, W2, and W3) from the start of the experiment (W0) in E1.

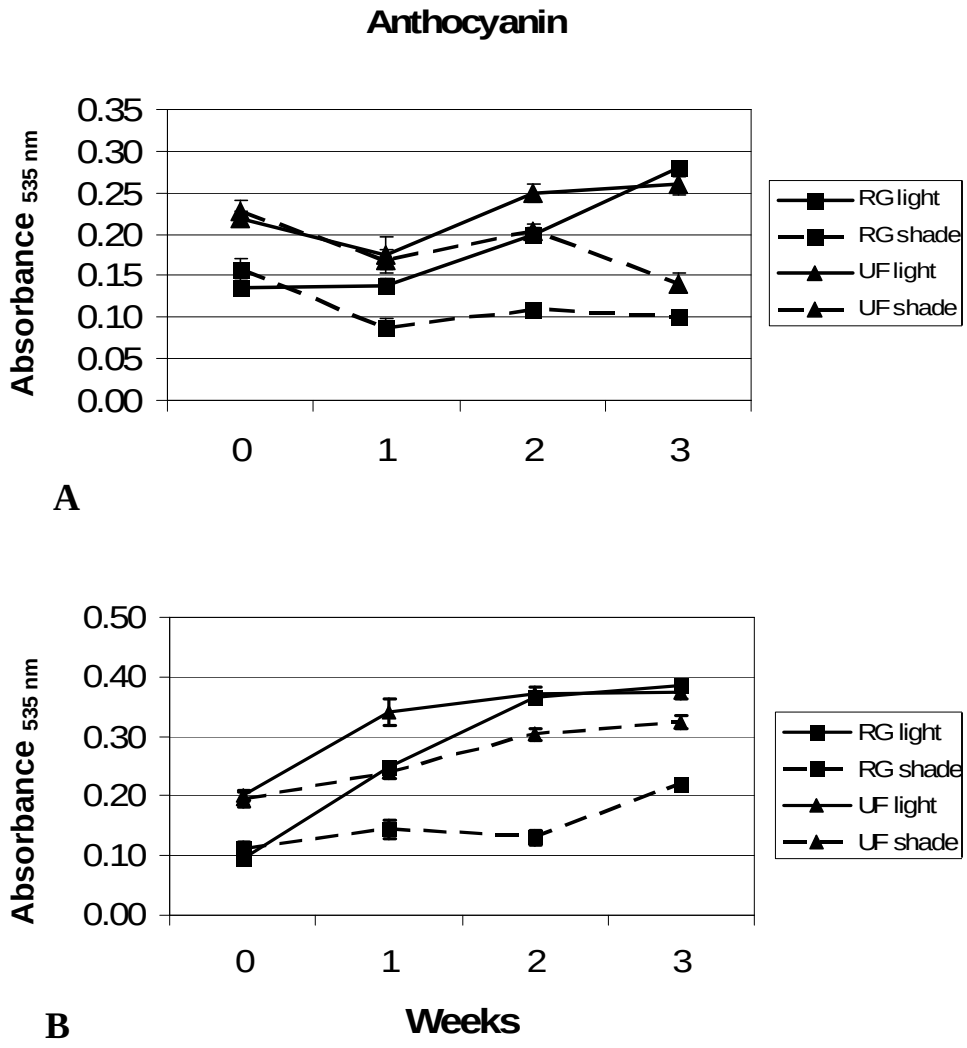

Figure 3-3. Anthocyanin content (A535nm) for RG (solid square) and UF (solid triangle) in the sun (solid line) and in the shade (dashed line). A) Experiment 1 (E1) conducted on 8 March – 29 March, 2007 and light levels for direct light was 350μmol^{-2}s^{-1} and for shade was 150μmol^{-2}s^{-1}. B) Experiment 2 (E2) conducted on 16 May – 6 June, 2007 and the light levels for direct light was 450μmol^{-2}s^{-1} and for shade was 225μmol^{-2}s^{-1}. Anthocyanin content was measured at each week (W1, W2, and W3) from the start of the experiments (W0). Data are means of eight independent determinations per treatment.

Table 3-3. Data analysis for anthocyanin content for RG and UF in direct light and shade conditions.

E1[x]	Week	Sun	Shade	E2[y]	Week	Sun	Shade
RG	0	0.135 cC[z]	0.158 bC	RG	0	0.094 cD	0.112 cD
	1	0.137 cC	0.089 cD		1	0.250 bB	0.144 cD
	2	0.199 bB	0.110 cC		2	0.366 aA	0.131 cD
	3	0.280 aA	0.101 cD		3	0.385 aA	0.222 bC
UF	0	0.219 bB	0.228 aA	UF	0	0.201 bC	0.194 bC
	1	0.179 bB	0.169 bB		1	0.342 aA	0.241 bB
	2	0.249 aA	0.203 aB		2	0.371 aA	0.304 aB
	3	0.261 aA	0.141 bC		3	0.375 aA	0.324 aA

[x] Experiment 1 (E1) was conducted on 8 March – 29 March, 2007 and light levels for direct light was 350μmol^{-2s-1} and for shade was 150μmol^{-2s-1}.

[y] Experiment 2 (E2) was conducted on 16 May – 6 June, 2007 and the light levels for direct light was 450μmol^{-2s-1} and for shade was 225μmol^{-2s-1}.

[z] Anthocyanin content (Absorbance535nm) was measured at each week (W1, W2, and W) from the start of the experiments (W0). Data are means of eight independent determination. Within each experiment, significance within columns are denoted in lowercase letters and significance across rows are denoted in uppercase letters. Variance analysis and Tukey's test was performed to determine significance at the 0.05 level.

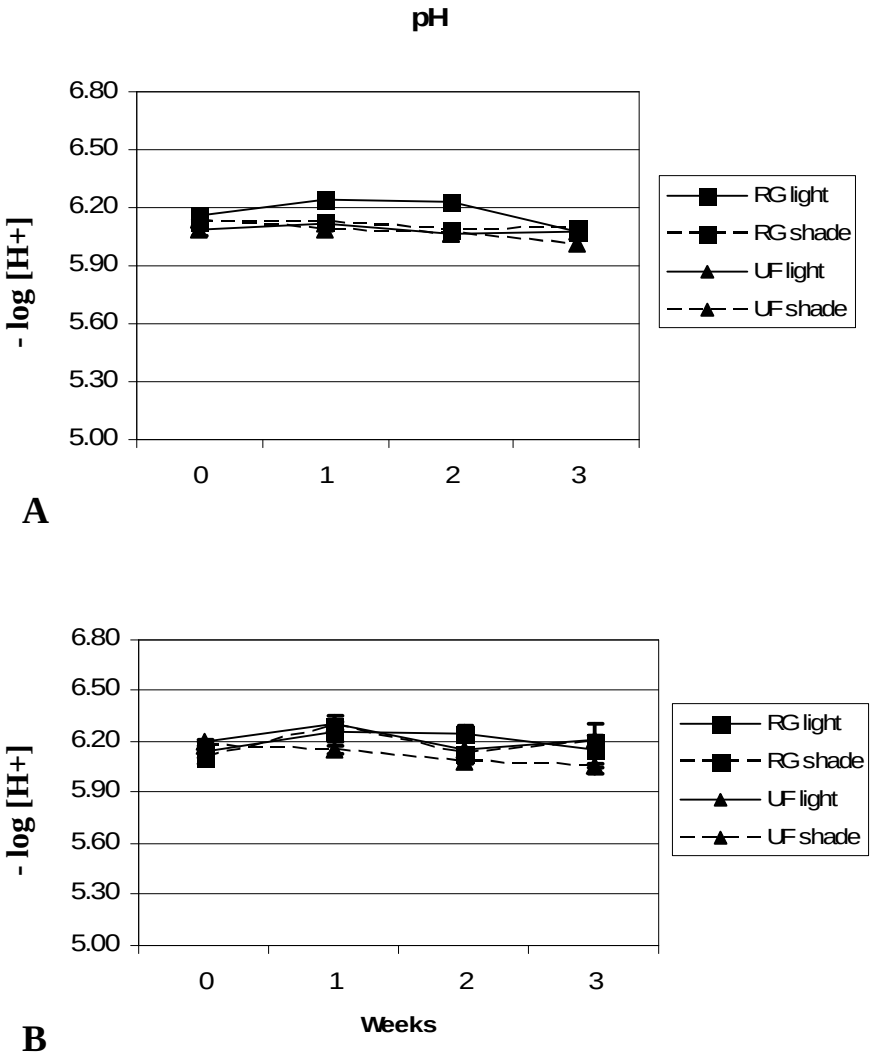

Figure 3-5. pH level for RG (solid square) and UF (solid triangle) in the sun (solid line) and in the shade (dashed line). A) Experiment 1 (E1) was conducted on 8 March- 29 March, 2007 and light levels for direct sun was 350μmol^{-2}s^{-1} and for shade was 150μmol^{-2}s^{-1}. B) Experiment 2 (E2) was conducted on 16 May- 6 June, 2007 and the light levels for direct sun was 450μmol^{-2}s^{-1} and for shade was 225μmol^{-2}s^{-1}. pH data were collected at each week (W1, W2, and W3) from the start of the experiments (W0). Data are means of eight independent determinations per treatment.

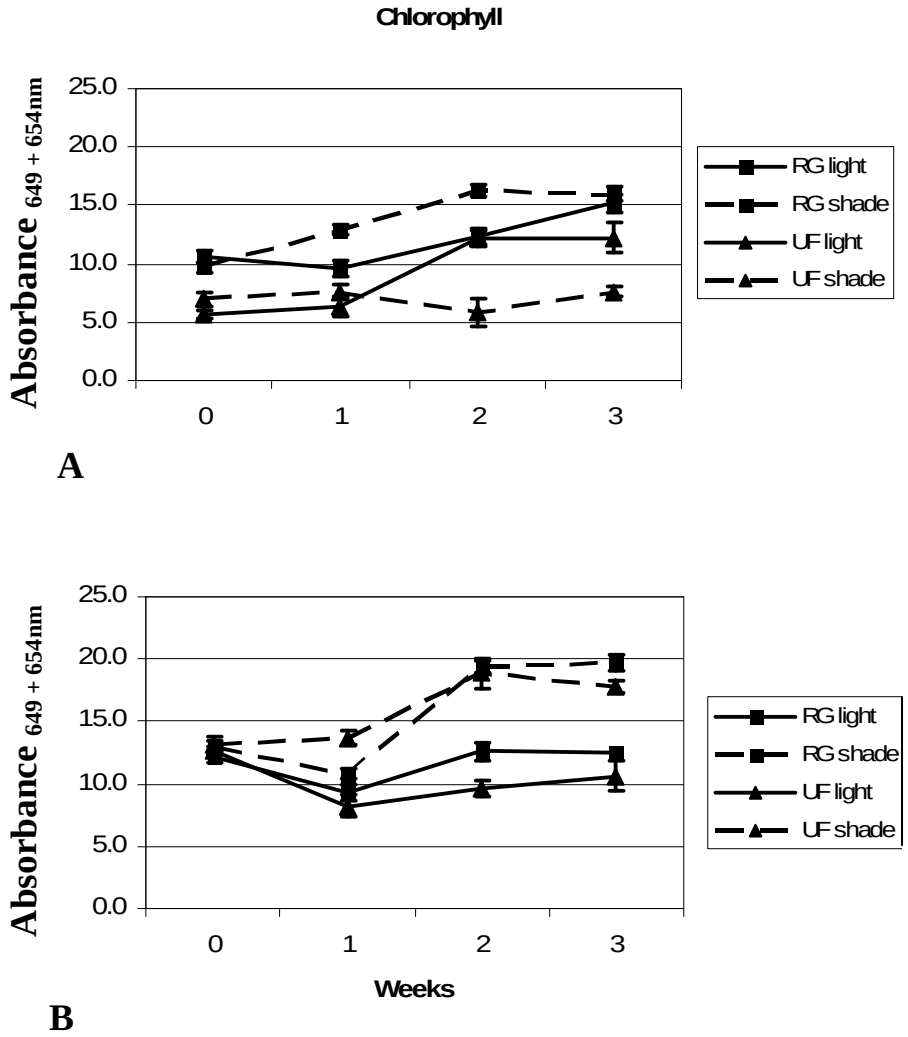

Figure 3-6. Total chlorophyll content for RG (solid square) and UF (solid triangle) in the sun (solid line) and in the shade (dashed line). A) Experiment 1 (E1) was conducted on 8 March - 29 March, 2007 and light levels for direct sun was 350µmol^{-2}s^{-1} and for shade was 150µmol^{-2}s^{-1}. B) Experiment 2 (E2) was conducted on 16 May - 6 June, 2007 and the light levels for direct sun was 450µmol^{-2}s^{-1} and for shade was 225µmol^{-2}s^{-1}. Data for chlorophyll content (Absorbance 649nm+ 654nm) was taken at each week (W1, W2, and W3) from the start of the experiment (W0). Data are means of eight independent determinations per treatment.

Table 3-4. Data analysis measured for chlorophyll content (absorbance values 649nm and 654nm) for RG and UF in direct light and shade conditions.

E1 [x]	Week	Sun		Shade		E2 [y]	Week	Sun		Shade	
RG	0	10.60	bC [z]	9.71	cC	RG	0	12.14	aB	12.97	bB
	1	9.62	bC	12.89	bB		1	9.29	aB	10.81	bB
	2	12.30	aC	16.24	aA		2	12.61	aB	19.41	aA
	3	15.20	aA	15.98	aA		3	12.46	aB	19.66	aA
UF	0	5.69	cD	6.99	cD	UF	0	12.64	aB	13.14	bB
	1	6.80	cD	7.58	cD		1	8.24	aC	13.69	bB
	2	12.12	aC	4.82	dD		2	9.61	aB	18.86	aA
	3	12.22	aC	7.57	cD		3	10.61	aB	17.75	aA

[x] Experiment 1 (E1) was conducted on 8 March – 29 March, 2007 and light levels for direct light was 350μmol^{-2}s^{-1} and for shade was 150μmol^{-2}s^{-1}.

[y] Experiment 2 (E2) was conducted on 16 May - 6 June, 2007 and the light levels for direct light was 450μmol^{-2}s^{-1} and for shade was 225μmol^{-2}s^{-1}.

[z] Chlorophyll content (Absorbance 649nm + 654nm) was measured at each week (W1, W2, and W) from the start of the experiments (W0). Data are means of eight independent determination. Within each experiment, significance within columns is denoted in lowercase letters and significance across rows is denoted in uppercase letters. Variance analysis and Tukey's test was performed to determine significance at the 0.05 level.

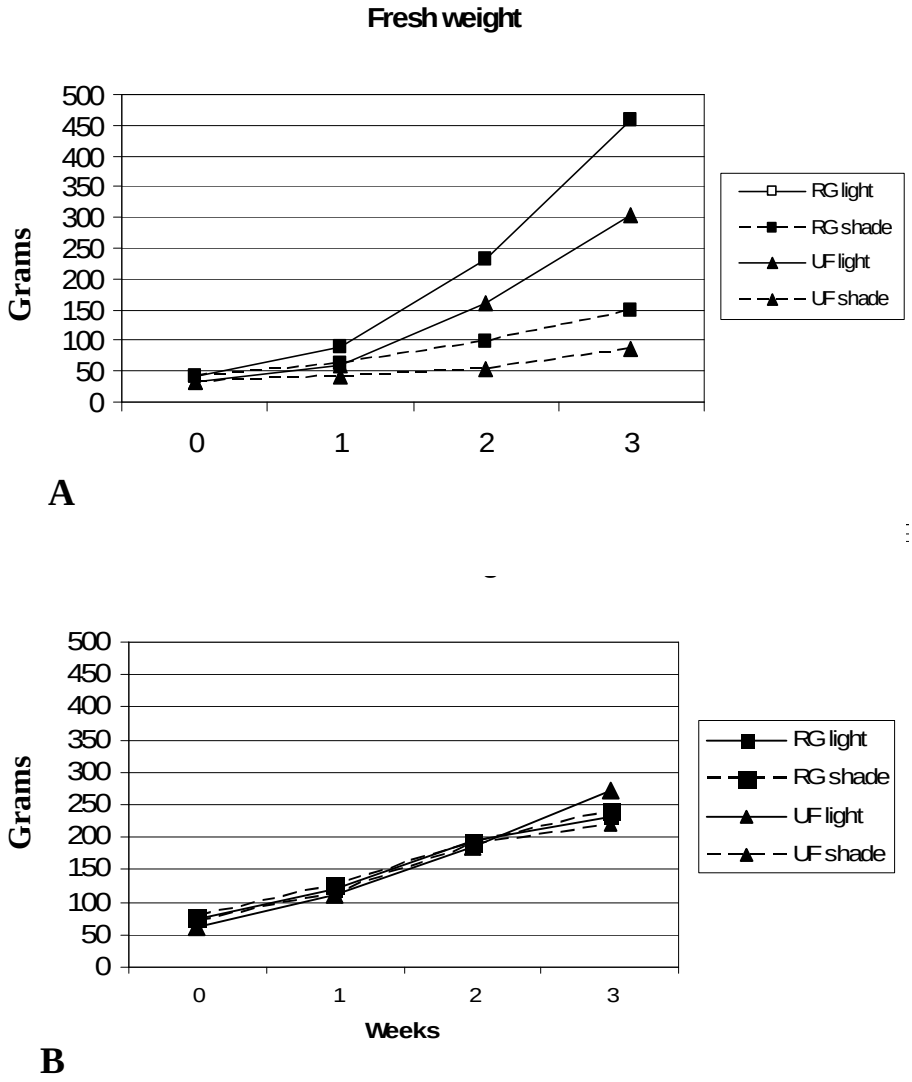

Figure 3-7. Fresh weight for RG (solid square) and UF (solid triangle) in the sun (solid line) and in the shade (dashed line). A) Experiment 1 (E1) was conducted on 8 March – 29 March, 2007 and light levels for direct sun was 350μmol^{-2}s^{-1} and for shade was 150μmol^{-2}s^{-1}. B) Experiment 2 (E2) was conducted on 16 May – 6 June, 2007 and the light levels for direct sun was 450μmol^{-2}s^{-1} and for shade was 225μmol^{-2}s^{-1}. Fresh weight data was collected at each week (W1, W2, and W3) from the start of the experiments (W0). Data were measured as the total fresh weight (in grams) of eight plants per treatment.

Table 3-5. List of the partial clones in anthocyanin biosynthesis in coleus.

Gene	Species	Length	Blast e-values	GenBank accession numbers
PAL1	Ss	698	0	This study (EU019242)
	Vv		5E-103	EF192469
	Ph		1E-109	SGN-E526590
	Nt		8E-107	AB008199
CHS	Ss	619	0	This study (EU019239)
	Vv		1E-99	AB066274
	Ph		7E-100	X14599
	Am		3E-102	X03710
DFR	Ss	583	0	This study (EU019240)
	Vv		3E-78	X75964
	Ph		5E-86	X79723
	Am		2E-87	X15536
F3H	Ss	459	0	This study (EU019241)
	Vv		2E-77	AM430949
	Ph		1E-74	AF022142
	Pa		1E-78	AY817674
UFGT	Ss	412	0	This study (EU019243)
	Vv		3E-31	AB047099
	Ph		2E-32	AF165148
	Ac		3E-33	AB103471
AN1	Ss	558	0	This study (EU019237)
	Vv		1E-35	DQ886417
	Ph		7E-36	EF423846
	It		1E-31	AB154371
AN2	Ss	320	0	This study (EU019238)
	Vv		5E-41	DQ886417
	Ph		7E-43	EF423846
	Ca		3E-43	AJ608992
Actin	Ss	422	0	This study (EU019236)
	Vv		2E-51	AM465189
	Ph		6E-65	SGN-U211816
	Si		5E-52	AB094079

A comparison of the partial clones to published sequences from other plant species. In the table, the length of the partial clones, the accession numbers and blast e-values (similarity of sequences), and the closest homologs to *Vitis vinifera* (Vv), *Petunia hybrida* (Ph), and another relevant species are shown [*Antirrhinum majus* (Am), *Capsicum annuum* (Ca), *Ipomoea tricolor* (It), *Nicotiana tabaccum* (Nt), *Pimpinella anisum* (Pa), and *Silene latifolia* (Sl)]

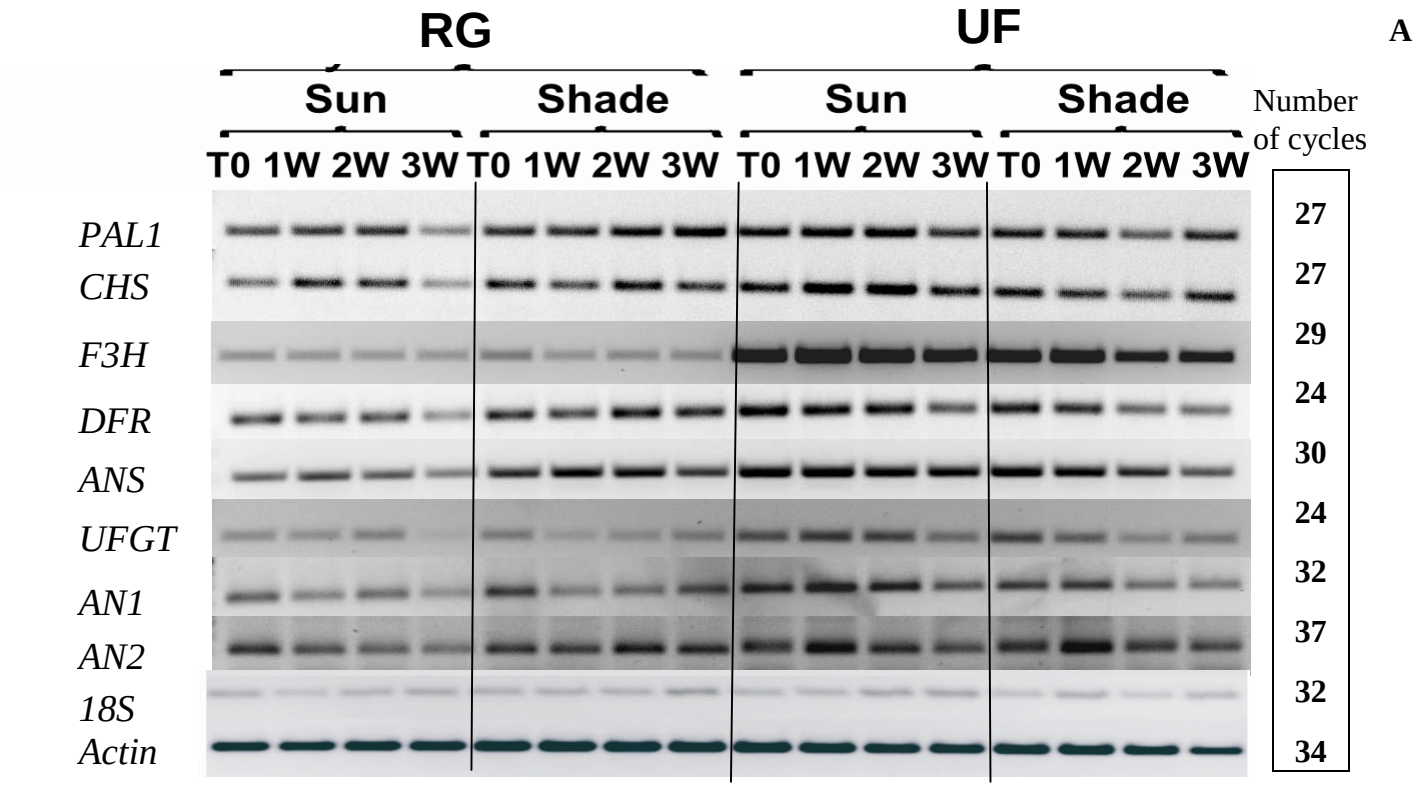

Figure 3-8. Expression analysis of the genes PAL1, CHS, F3H, DFR, ANS, UFGT, AN1, AN2, and controls 18S and ACT for 'Royal Glissade' (RG) and 'UF06-1-06' (UF). A) Experiment 1 (E1) was conducted on 8 March – 29 March, 2007 and light levels for direct sun was 350µmol^{-2}s^{-1} and for shade was 150µmol^{-2}s^{-1}. B) Experiment 2 (E2) was conducted on 16 May – 6 June, 2007 and the light levels for direct sun was 450µmol^{-2}s^{-1} and for shade was 225µmol^{-2}s^{-1}. Samples for expression analysis were collected from leaf tissues at each week (1W, 2W, 3W) from the beginning of the experiment (T0). Representative results of the semi-quantitative PCR as well as the number of cycles are reported.

B

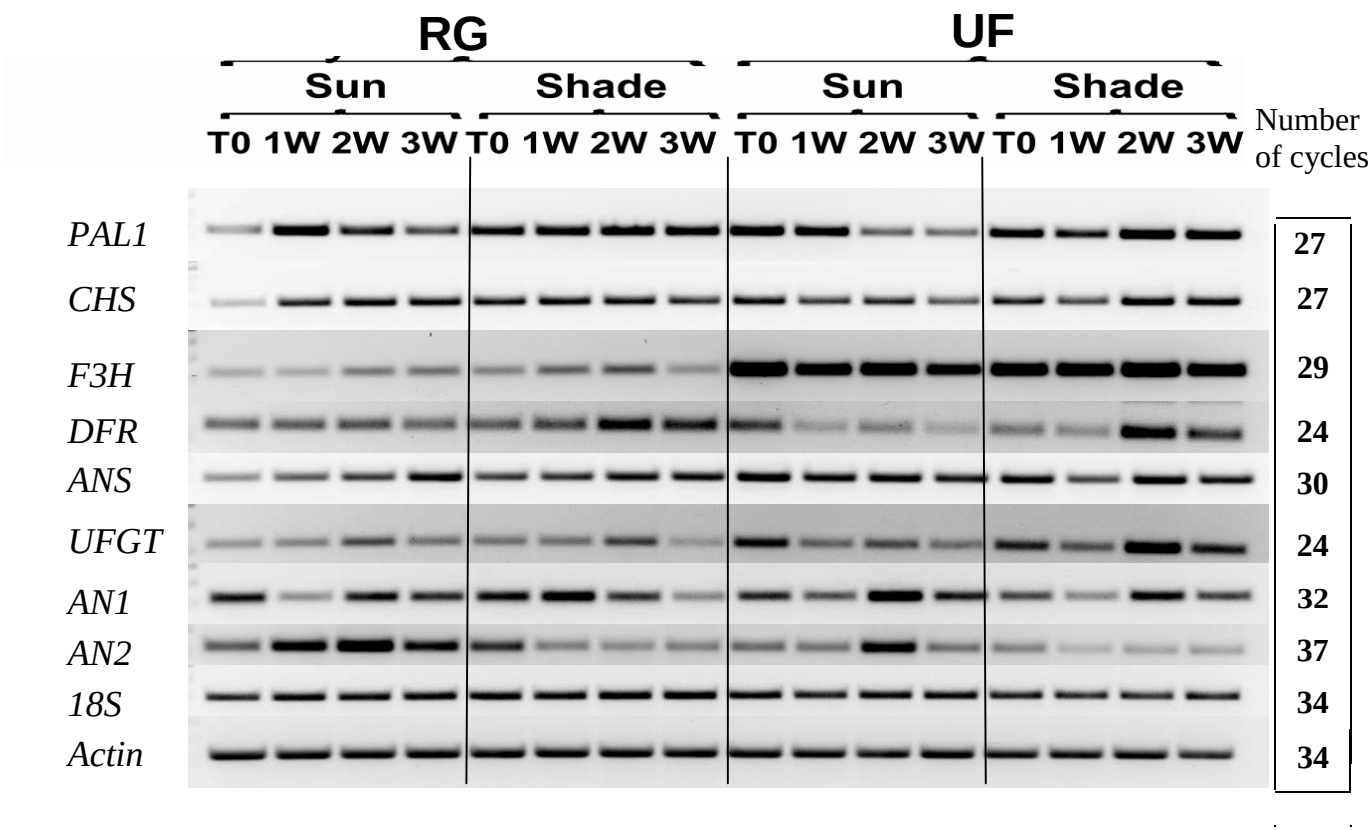

Figure 3-8. Continued.

CHAPTER 4
DEVELOPMENT OF NEW NOVEL COLORED COLEUS CULTIVARS WITH IMPROVED VIGOR, COLOR QUALITY, AND LATE FLOWERING INDUCTION FOR THE COMMERCIAL MARKET

Introduction

Solenostemon scutellarioides (L.) Codd, commonly known as coleus, is unlike many other popular ornamental plants because it is highly prized for its vibrant foliage rather than for its flowers (Lebowitz, 1995). Although seed propagated cultivars exist on the market, many new varieties of coleus are now propagated vegetatively. The success of ornamental plants for the commercial market depends on certain characteristics desired by both production companies and consumers. For coleus, desirable characteristics common to both sides are vigor, color quality, and late flowering induction. Plants with good overall plant vigor have fast growth rates for rapid turnover and the ability to produce maximum cuttings for production (Wilkins, 1988). The basis of all vegetative production requires plants with good branching and the number of apical meristems that are produced ultimately determines the number of cuttings produced (Wilkins, 1988). Lateral branching is important for the attractiveness of a plant to consumers (Hayashi, 2001) and thus varieties with good plant vigor in terms of excellent branching patterns are ideal for the market.

The economic success of ornamentals used for foliage characters often depends on the quality of leaf pigmentation. Flower or foliage color is one of the most important considerations when consumers make plant purchases (Catanzaro, 2005) and faded colors decrease product value (Oren-shamir, 2003). For many ornamental crops, the loss of color quality through color fading is affected by two main environmental factors: light and temperature. Light quantity and quality play a role in pigmentation changes in plants. Small levels of UVB cause leaves of *Dendranthema x grandiflorum* cv ‘Surf’ to become brighter in red color, while in a red-green

coleus cultivar ‘Wizard Velvet Red’, UVB results in the emergence of red pigments (Hoffman, 1999). UVA and UVB induce the synthesis of anthocyanins in leaves of *Kalanchoe x hybrida* ‘Colorado’ (Hoffman, 1999). The production of anthocyanins in plants may serve as a protective mechanism against UV radiation by functioning in the protection of the photosystems (Neill and Gould, 2003). Damages to the photosynthetic apparatus, which results from photoinhibition, are often observed in plants as a bleaching of foliage colors. This bleaching in turn reduces foliage color quality (Krause, 1999). Elevated temperatures also affect color quality by decreasing levels of anthocyanin production in plants (Shaked-Sachray, 2002; Dela, 2003; Oren-shamir, 1999). Low temperature at 17^{o}C induces more accumulation of red pigmentation in leaves of *Cotinus coggygria* cv. ‘Royal Purple’ compared to plants grown at a higher temperature of 29^{o}C (Oren-shamir, 1997). In *Photinia x fraseri* cv. ‘Red Robin’ leaves, red pigmentation decreases with increased temperatures (Oren-shamir, 1999). In regards to coleus, varieties with bright foliage colors that do not fade under high light intensity and high temperature conditions are thus desirable to consumers.

The timing of transition of a plant from a vegetative to a floral state is important for the production of ornamental plants for the market (Chandler, 2005). Early flowering in ornamental crops used for foliage characteristics is often an undesirable characteristic. Early flowering increases landscape maintenance costs due to the need for ‘dead heading’ and decreases foliage color quality as the plant spends more metabolic energy to produce seeds. *Gynura aurantiaca* has the commercial potential for use as a potted plant for its attractive foliage, but flowering induction that leads to production of malodorous flowers is difficult to avoid (Pallez, 2001). Manipulating the timing of floral transition has been investigated through several methods such as pruning (Gerber, 2001), using shade cloths to change photoperiod or light intensity

(Kobayashi, 2006), and plant growth regulators such as ethephon (Hayashi, 2001) but such methods increase production costs. Therefore, it is important for growers to make efforts to produce inexpensive attractive plants and for breeders to develop plants with delayed flowering to reduce the cost of production (Hayashi, 2001).

A wide range of genetic variation for plant vigor, foliage color quality and flowering induction is available in coleus. We have observed that coleus plants lacking vigor at the seedling stage do not grow fast enough for commercial production. We have also observed that plants with strong apical dominance do not produce enough vegetative cuttings for efficient production. Furthermore, early flowering leads to increased maintenance requirements for pruning in the landscape and lower foliage color quality due to translocation of stored reserves from leaves to seeds.

The objective of the UF breeding program is to develop new cultivars through traditional breeding methods for improved vigor for commercial production, better foliage color quality and delayed flowering for consumers. Throughout the three years of the program, improvements have been made in the coleus population for these three variables, and new cultivars have been released with all of these characteristics improved for the commercial market.

Materials and Methods

Breeding Methodology

At the inception of the breeding program in 2003, fifteen commercial coleus cultivars were grown, and open-pollinated seeds were collected to generate the first seedling population. High levels of variability for many different traits such as foliage color, leaf shape and size, and growth habits were observed, likely due to the highly heterozygous nature of coleus (Lebowitz, 1985). The method of phenotypic recurrent selection with half-sib selection with progeny test (Poehlman and Sleper, 1995) was used for the improvement of the quantitative characteristics of

plant vigor, color quality, and late flowering induction. Using the half-sib selection method, a specific number of individuals with the desirable characteristics were selected from an open-pollinated source population and seeds were harvested from each individual separately. A seedling test was grown from each coleus line at the end of the crop cycle in August-October. Using a 72-cell pack tray filled with soilless media (Fafard 2-mix media), 100 seeds from each individual were planted and the remaining seeds were retained at room temperature. Based on the tests, the next crop cycle was reconstituted by planting the remaining seeds from the lines with seedlings displaying high levels of seedling vigor and variability in foliage colors. As each crop cycle was reconstituted with the retained seeds, individuals with superior performances from each yearly crop cycle were isolated from the population and were re-tested every subsequent year for replicated performance. Individuals receiving high rating scores for vigor, color quality, and late flowering induction in addition to novel foliage color had the potential to be released as new cultivars.

Crop Cycle and Yearly Trials

A crop cycle consisted of six major parts: (1) sowing of the seeds (2) three selections of seedlings at eight, ten and twelve weeks after germination in a greenhouse (3) propagation of cuttings in April for the summer year trials starting in May (4) summer field trials, (5) seed collection at end of the field trials in August, and (6) progeny tests in October-November. The summer year trials began when cuttings propagated in April were planted for evaluation. Data were collected once a month at the end of June, July, and August and ended with selections made for individuals that were superior.

Sowing of seeds. The start of the crop cycle began when the seeds were sown in the first week of January. After sowing the seeds using a hand seeder (Seed E-Z Seeder Company), the flats of soil (Fafard 2-mix media) were moved to a polycarbonate greenhouse with temperatures

maintained at 21-27^{o}C for the first week of sowing. During the germination period (ten-twenty) days, soil temperatures were kept above 20^{o}C, as previously described by Lebowitz (1987). The flats were misted by hand every 1 ½ hours starting at 8am and ending at 5pm to maintain humidity until germination occurred (five to seven days). After germination, misting cycles were reduced to three times per day, and once true leaves formed, the seedlings were watered overhead as needed and fertilized with 50-100ppm nitrogen at every other irrigation. Seedlings were then transplanted individually to each cell in a 72-cell pack tray once their first pair of true leaves were present, approximately three weeks after sowing (Figure 4-1).

Selection processes. Three selections were made on seedlings from an initial number of 30,000 at eight, ten and twelve weeks after seed sowing for the variables of vigor, early flowering induction, and novel foliage color. Seedlings were selected visually and those with low vigor or poor branching were discarded from the program. Any seedlings that initiated flowers during this time were also discarded. Remaining seedlings (~2,000) were transplanted into 12.7cm azalea pots, pinched once to promote branching, and grown until the 1st of March. In March, a final selection for seedlings (~ 500) with good vigor, branching, lack of flower induction and novel foliage color was conducted. The remaining seedlings were transplanted into 20.3cm pots and grown under the same conditions for subsequent cutting propagation in April.

Propagation of cuttings. After the three selections, nine cuttings were taken from each of the remaining seedlings and propagated in individual cells of 72-cell pack trays containing soilless media (Fafard 2-mix media). The lengths of the cuttings were at least 7.6cm long and had at least two nodes with four pairs of leaves. The cuttings were placed in a mist house, with misting cycles set for twenty seconds every thirty minutes. After the cuttings had rooted (~ten to fourteen days), they were moved to a 30% shade glass greenhouse and grown under standard

greenhouse conditions (temperature 21-25^{o}C). If a variety did not root well, it was discarded. After two weeks of growth in the greenhouse, the cuttings were subsequently used for evaluation in the greenhouse and field trials starting in April as described below.

Field trials: 2004, 2005, and 2006. Yearly field trials began in April after cuttings were propagated from individuals that remained in the program after the third selection process. These remaining individuals exhibited high vigor, novel foliage color, and had not initiated flowering. Two evaluation locations, a glass greenhouse and full sun outdoors, were used to evaluate coleus plants for the characteristics of vigor, color quality and flowering induction. Of the nine cuttings previously propagated from the remaining individuals, three were used in the greenhouse trial, three were planted in the field trial, and the remainder was discarded.

The 30% shade greenhouse trial location constituted a 'no-pinch' trial, in which cuttings were not pinched so that their natural growth habit could be observed (Figure 4-2). Three replicate plants of each individual were planted in 15.2cm azalea pots, spaced 30cm apart, and placed in numerical order based on their experimental line numbers. After data for vigor (height + branching) were collected in the greenhouse, the same individuals were evaluated in the field trial in June, July, and August.

The outdoor full sun field trial was conducted at the Plant Science Research and Education Unit in Citra, Florida. Individuals were planted on raised ground beds covered with silver reflective mulch to increase the levels of light stress for evaluation of sun tolerance (Figure 4-3). This field location was used only for the 2005 and 2006 trials, while an outdoor location on black tarp in Gainesville, Florida was used for the 2004 trial. For each yearly trial, three replicate plants per line were pinched once before planting in the field, spaced 30cm apart, and irrigated as needed. Slow release fertilizer (Osmocote 8-16-12, N-P_2O_5-K_2O) was utilized at planting. The

plants were planted in numerical order based on their experimental line numbers to facilitate data acquisition. The individuals were evaluated on a 1-5 scale for color quality and flowering induction. Data were collected at three points at the end of each month (June, July, and August) for each yearly trial.

Variables evaluated and data analysis. Variables that were evaluated for all coleus individuals in the program were plant vigor, foliage color quality and flowering induction. Since vigor encompassed a plant's three-dimensional form and took into account both height and width, a vigor index score was derived by summing the individual scores for height and branching. Foliage color quality was rated on the brightness of color pigmentation, with excellent ratings for plants that did not bleach or fade in color. Flowering induction was rated on the appearance of floral initiation.

For the greenhouse trial location, the variables of height and branching were rated on a 1-5 rating scale. For height (measurements taken from soil line), 1 = short (< 10cm), 2 = moderately short, 3 = average, 4 = moderately tall, and 5 = tall (>30cm) and for branching, 1 = poor, 2 = fair, 3 = average, 4 = good, and 5 = excellent. After the individuals were rated on height and branching, a vigor index score was calculated by summing the height and branching scores for each individual.

For the field trial location, color quality and flowering induction were also rated on a 1-5 rating scale. For color quality, 1 = poor, 2 = fair, 3 = average, 4 = good, and 5 = excellent and for flowering induction 1 = full bloom, 2 = flower buds visible, 3 = inflorescence visible, 4 = inflorescence bud forming, and 5 = no flowering. At the end of the crop cycle, the scores received in the greenhouse and field were summed and a final overall score was calculated for each individual. Thus, an individual could achieve a maximum score of ten points for vigor

index (height + branching) in the greenhouse and ten points for each month of June, July, and August in the field (color quality + flowering induction), thus a total overall score of forty points was achievable.

For the variables of plant vigor, color quality, and flowering induction, a percentage was calculated for the number of individuals within each rating score. Percentages were calculated by dividing the frequency of individuals receiving the rating score by the total population number and multiplying by 100. By calculating these percentages, the number of individuals that received high rating scores of four and five's could be compared across the three year trials to determine if improvements in the three variables could be observed across yearly populations.

Seed collection and progeny tests. Seeds were harvested at the end of each yearly field trial in August-December from individuals that had superior performance based on their total rating scores. Mature flower spikes (when calyx were dark brown in color) were collected and placed in brown paper bags and air-dried at room temperature for one week. After air-drying, the flower spikes were placed in a round and small wire-mesh sieve container, and dried flower parts were separated from the seeds. The seeds were placed in seed packets and stored at room temperature because it was observed early on that refrigerated storage resulted in low germination percentages. For each superior individual, a progeny test was performed to evaluate the seedling variability. One hundred seeds of each variety were sown on flats of soilless media (Fafard 2-mix media), placed in a mist house with misting set for thirty seconds every twenty minutes, and transferred to a greenhouse after germination. The seedlings were grown and evaluated between October-November and lines with high vigor and variability in foliage colors were noted (data not shown). Only seeds from these lines were used to reconstitute the next crop cycle starting the following January.

Results and Discussion

At the inception of the coleus breeding program, fourteen commercial cultivars were used as parental lines to start the breeding program. These fourteen cultivars differed in foliage colors, growth habits, leaf shapes, and flowering induction times. The fourteen cultivars used were Stained Glassworks, Elfers, Micanopy, Embers Gator Mint, Astatula, Hurricane Louise, Kiwi Fern, Tilt-a-Whirl, Yalaha, Freckles, Indian Frills, Orange Gator Mint, Trailing Nova, and Red Queen. Unknown to us at the time, parental lines Elfers and Stained Glassworks became the predominant sources for excellent genetics in subsequent crop cycles and produced many of the varieties with the desired characteristics of improved vigor, color quality, and delayed flowering induction.

Vigor

A vigor index was used to evaluate individuals for their three-dimensional form. Since vigor comprises both the height and width of a plant, height and branching was rated for each individual and a vigor index (height rating + branching rating) was calculated to evaluate the individuals for both characteristics. Vigor was rated in the greenhouse because it was a characteristic that was easily screened for at the seedling stage. Seedlings that were tall and had good branching at an early stage displayed the same characteristics at maturity when evaluated in the field. Slight improvements were made for vigor in the greenhouse across the three year trials by reducing height and increasing branching in the populations (Figure 4-4). In 2004, 92% of the population was moderately tall or tall but only 45% of the population had good to excellent branching. Many individuals had strong apical dominance, did not produce enough cuttings needed for mass production, and often grew leggy. These individuals with strong apical dominance may have had genotypes AAAA or AAAa in regards to growth habit. Thus selections were made for individuals with genotypes AAaa because individuals with genotypes AAaa,

Aaaa, or aaaa were desirable to decrease apical dominance and to increase branching. In 2005, 90% of the population was moderately tall or tall but an improvement was made for branching in which 65% of the population received scores of four or five. The population in 2005 had a minimal reduction in apical dominance but better branching than the population in 2004. In 2006, many of the individuals were ideal as 59% of the population was moderately tall or tall but 74% of the population had good to excellent branching. In regards to data for vigor index, individuals with scores of four to five points for height and four to five points for branching with an overall vigor index score of eight to ten points desirable. In 2004, 66% of the population achieved scores of eight to ten points for the vigor index but the individuals in this category were mostly tall as seen in the height data. In 2005, an increase in the vigor index was observed as 74% of the population received scores of eight to ten points; however, individuals in this percentage group were similar in height but had higher branching ratings than in 2004. In 2006, although the vigor index rating decreased to 65%, more of the individuals within this percentage group were ideal as they had excellent branching scores and reduced apical dominance. Improvements were made for vigor by selecting for plants with decreased apical dominance and higher lateral branches. It appeared that equal levels of vigor were maintained each subsequent year, but individuals that were being developed were shorter and better branched in 2006. Furthermore, 2005/2006 may appear to be a breaking point in terms of the allocation of biomass between height and branching. A plant can only produce a certain level of biomass – when dedicated to branching, there is only so much biomass it can allocate to height.

Foliage Color Quality

After the varieties were rated in the greenhouse for height, branching, and vigor index, they were transplanted to the field location for evaluation of color quality and flowering induction. Color quality was not a feasible characteristic to rate in the greenhouse because most

plants maintained excellent color quality when grown under optimal glass greenhouse conditions with little UV. For many plants, high light intensity (with UV radiation) (Krause, 1999) and elevated temperatures results in color fading (Shaked-Sachray, 2002; Dela, 2003; Oren-shamir, 1999) and thus plants with high ratings in the field were those that maintained their vibrant foliage color. Improvement in color quality was made in the program at the end of three yearly trials, as demonstrated by a higher percentage of the population receiving scores of four and five points (Figure 4-5). In the month of June for all yearly trials, over 90% of the population received scores of four or five for color quality and minimal color fading and bleaching of the foliage were observed. These plants had been planted in the field for one month and were not yet affected by higher irradiance levels and temperatures. For the month of July, an improvement was made in both 2005 and 2006 compared to 2004. Over 90% of the population maintained their rating scores of four or five for color quality in 2005-2006 compared to 64% in 2004. The month of August was an important month as the plants had been planted in the field for three months and plants that had excellent color quality distinguished themselves from the plants that had poor color quality. In 2004, only 35% of the population maintained color quality rating scores of four or five. A slight decrease in foliage color quality was observed in 2005 with only 29% of the population receiving rating scores of four or five. However, in 2006, after three yearly trials, significant improvements were made for color quality as 53% of the population maintained good to excellent color quality. August also served as an important month to screen plants for excellent foliage color quality because this month may have had the highest accumulated irradiance and temperature levels when compared to the months of June and July (personal observation).

Selections of new varieties based on visual color quality ratings as conducted in this breeding program have been performed for other plant species such as daylilies (Owings, 2005) and *Abelia x grandiflora* (Robacker, 2006). There is still room for improvements for color quality in coleus because half of the population in 2006 still demonstrated color fading/bleaching. By collecting seeds from plants with high color ratings, the percentage of plants receiving rating scores of four or five can be gradually increased in subsequent populations.

Flowering Induction

After the three selection processes were made at the seedling stage in the greenhouse, all seedlings that had early flowering induction were discarded from the program. Flowering induction was easily screened for at the juvenility stage, however, flowering at the maturity stage required evaluation of the varieties in the field. Improvements for delayed flowering induction were made in the breeding program as the percentage of varieties that had just initiated flowering or had not flowered increased significantly in 2006 from 2004 for the months of July and August (Figure 4-6). For the month of July, 33% of the population in 2004 received scores of four or five for flowering while 67% of the population had already flowered. An improvement was made in 2005 as 56% received scores of four or five with only 44% in flower. Another improvement was made in 2006 as 63% received scores of four or five and only 37% of the population was in flower. Within a three year period, a reduction of 30% for early flowering was achieved for July. August was an important month for data collection on flowering induction because by this time a larger percentage of the population had already flowered. In 2004, only 20% of the population had just initiated flowering or had not flowered as of August. In 2005 37% of the population had just initiated flowering or had not flowered as of August, and in 2006, that figure increased to 48%. Taken together, these figures represent a 28% reduction in early flowering over three years

of selection. Further improvements for delayed flowering induction can likely be achieved since half of the population in 2006 was already in flower in August. By collecting seeds from later flowering varieties, the percentage of varieties that flower later may be increased in subsequent populations. A similar program in which coleus varieties were rated for late flowering was conducted in the spring of 2003 and fall of 2004 in Baton Rouge, Louisiana. Forty-five commercial cultivars were evaluated in the landscape for slowness to flower and the coleus cultivar 'Aurora Black Cherry' received highest rating for late flowering induction (Owings, 2005).

August was designated as the last data collection month because ultimately seeds had to be collected off of varieties that received high rating scores for all three characteristics. In Florida, hurricane season runs from June 1st to November 30th with the major occurrences of storms in September. Seed collection was delayed as long as possible to collect seeds only from varieties that were late flowering. If seed collection was delayed until the end of September, the risk of losing plants to environmental conditions was increased. It was observed, however, that varieties with excellent color quality maintained vibrant foliage colors after the last data collection period in August until the first frost. In 2005 several major storms affected the coleus population in September 2004 and 2005, but in 2006 there were no storms and seeds were collected until late November.

Cultivar Releases

At the end of the yearly trials, the maximum score a variety could attain was forty points (ten points in the greenhouse and thirty points in the field). Varieties with high overall rating scores were retained after each yearly trial and had the potential to become releases from the UF breeding program. Fourteen cultivars were released in 2005 and seven were released in 2006. All

of the releases had improved plant vigor, color quality and delayed flowering induction. Cultivar release information for the twenty-one new releases is shown in Appendix 1.

It is interesting to note that many of the cultivars released from the program were traced back to two predominant parental cultivars used at the inception of the breeding program. Elfers and Stained Glassworks produced many varieties with excellent vigor, color quality and late flowering induction. Elfers is a tall variety with bright red/green foliage color, excellent branching, and late flowering induction. Stained Glassworks is a variety with low vigor, but has bright orange/red foliage color and exceptionally late flowering induction. It is also interesting to note that excellent genetics can be traced back to the maternal parent in an open-pollinated situation. Seeds collected from Elfers and Stained Glassworks have consistently produced several cultivars that have been selected as superior every year. Of the twenty-one varieties released from the program, the genetics for thirteen of the varieties were traced back to either Elfers or Stained Glassworks (see Appendix 1). Vigor does not appear to be a problematic characteristic to breed for in coleus, however color quality and late flowering induction may require further improvements.

Figure 4-1. Growth of seedlings at the start of the crop cycles. Seedlings were germinated in a polycarbonate greenhouse under standard conditions (temperature at 21-27^{o}C) with overhead mist. Three weeks after germination, seedlings were transplanted individually to a cell.

Figure 4-2. 30% shade glass greenhouse trial. Three replicate cuttings of each individual were placed in numerical order according to their experimental line numbers. The individuals were rated for height and branching on a 1-5 scale. For height (measurements taken from the soil line), 1 = short (< 10cm), 2 = moderately short, 3 = average, 3 = moderately tall, and 5 = tall (>30cm) and for branching, 1 = poor, 2 = fair, 3 = average, 4 = good, and 5 = excellent. After the individuals were rated on height and branching, a vigor index score was calculated by summing the height and branching scores for each individual.

Figure 4-3. Full sun outdoor location at Citra, Florida. Three replicate cuttings of each individual were planted on raised ground beds covered with silver mulch. Individuals were rated for the variables of sun tolerance and flowering induction on a 1-5 scale. Sun tolerance was rated on a 1-5 scale, with 1 = poor, 2 = fair, 3 = average, 4 = good, and 5 = excellent. Flowering induction was rated on a 1-5 scale where 1 = flowers visible and plants were in full bloom, 2 = flower buds visible/full blooms, 3 = inflorescence visible, 4 = formation of inflorescence bud, and 5 = no flowering induction. The field trial started in May and ended in August.

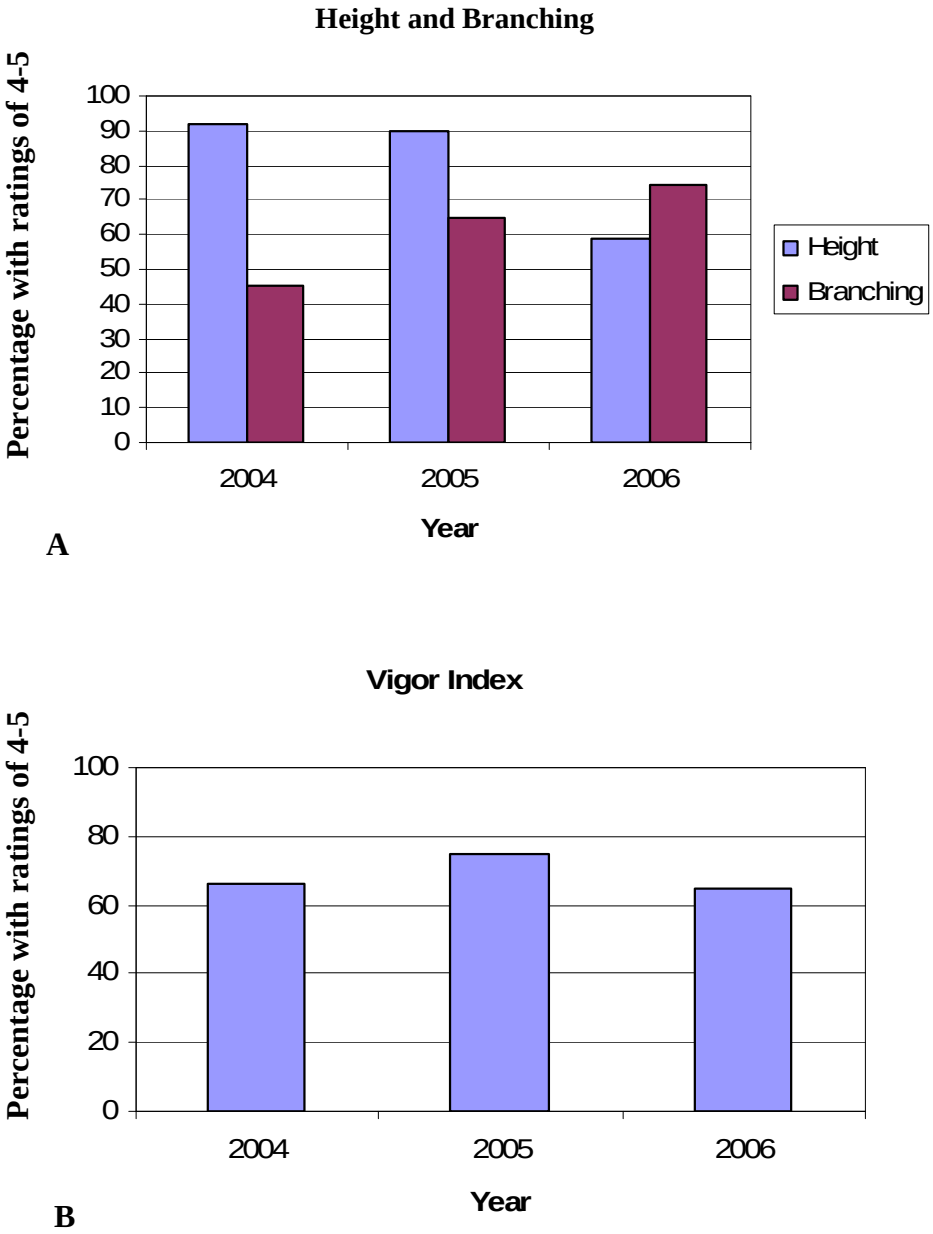

Figure 4-4. Percentages of the population receiving rating scores of four or five for height and branching across the three yearly trials. A) Height and branching were scored on a 1-5 rating scale. For height, 1 = short, 2 = moderately short, 3 = average, 4 = moderately tall, and 5 = tall. For branching, 1 = poor, 2 = fair, 3 = average, 4 = good, and 5 = excellent. B) A vigor index was derived by multiplying the height rating score by the branching rating score.

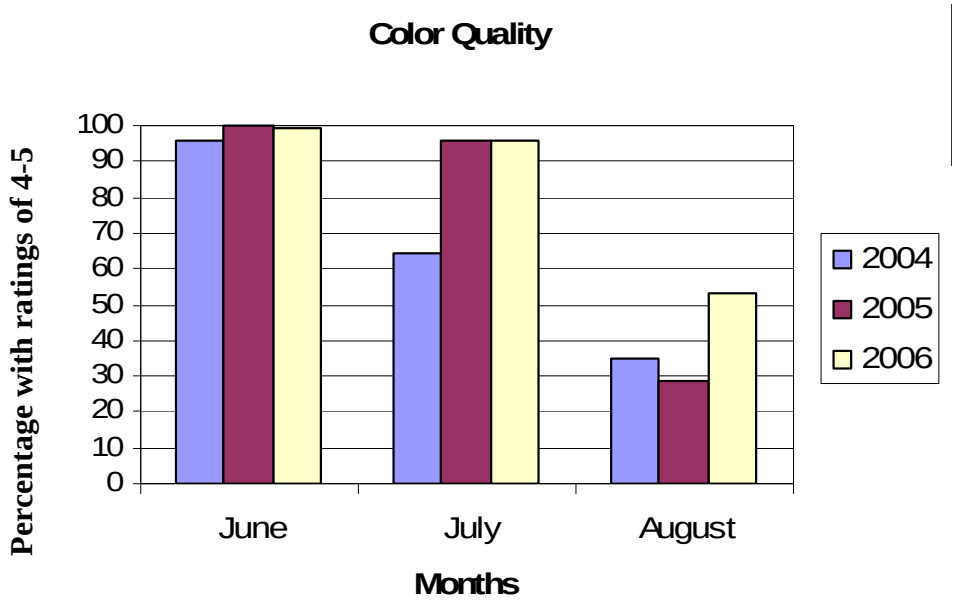

Figure 4-5. Color quality for the months of June, July, and August for years 2004, 2005 and 2006. Percentages of the population receiving rating scores of four or five for color quality across the three yearly trials. Color quality was rated on a 1-5 rating scale, where 1 = poor (color fading/bleaching), 2 = fair, 3 = average, 4 = good, and 5 = excellent (no color fading/bleaching).

CHAPTER 5
FUTURE WORK

The development of several dozen new coleus varieties with more trailing growth habit than RQ and bright orange color similar to S was achieved. In three generations, trailing habit and orange color was combined in one F2 individual (H05-66-5). However, it appeared that all F2 plants with both trailing habit and orange color had early flowering induction as observed in both parental varieties. It appears that early flowering induction and bright orange color are linked; as flowers are undesirable in coleus, improvements are still needed to develop new varieties with trailing growth habit, bright foliage colors, and late flowering induction.

In regards to the coleus breeding program, improvements can still be made for the selection of new varieties with improved color quality and late flowering induction. As the crop cycle did not produce a population receiving rating scores of 5 for all three variables, there is still room for improvements. Such improvements can be made by continually selecting varieties with the desired characteristics and collecting seeds from those selected varieties. Most of the traits in coleus are simply inherited (Rife, 1946); thus a focus on the improvement of coleus for other desirable traits may be of interest. Two traits of interest are cold-tolerance and larger flowers. The development of varieties that can be grown in lower hardiness zones will improve their landscape range and market value. Likewise, developing coleus for larger and unique flower forms will open a new market niche. Intergeneric crosses between coleus and a related genus, Plectranthus, may be feasible as Plectranthus exhibits good cold hardiness and larger, tubular flower forms. As both genus belong to the same family (Lamiaceae) such intergeneric crosses may be performed.

In regards to the molecular study of anthocyanin synthesis in coleus, it has been shown that light influences foliage coloration by increasing production of red pigmentation. However, as only a few genes were identified in this study, the identification of additional genes may be necessary to further understand the physiological and molecular mechanisms involved in the

coloration of coleus foliage. In addition, the biochemistry of anthocyanin and carotenoid accumulation is essential to understand the different pigments that contribute to foliage coloration.

Besides the effect of light influencing coloration in coleus, coloration leading to splotches and variegations has been proposed to be due to the activity of transposons (Lebowitz and Kloth, 1986). As transposons affect the nuclear genome, Mendelian inheritance will be observed for foliar variegation. Coleus will be an ideal candidate to study transposon activity due to its high level of genetic variability for color.

APPENDIX A
NEW CULTIVAR RELEASES WITH IMPROVED VIGOR, BRANCHING, COLOR QUALITY AND LATE FLOWERING INDUCTION

Introduction

The coleus breeding program at the University of Florida, in Gainesville Florida, was initiated in 2003 with a primary emphasis on developing new vegetatively propagated cultivars with improved color quality and late flowering. The secondary emphasis of our program focused on developing new cultivars with novel color, increased vigor, and branching for optimal commercial mass production. The following UF varieties were selected because they have a few or all of these desirable traits. Listed below are the cultivar releases, their origins, foliar description and general performance characteristics. Foliage color was determined using the Royal Horticultural Society Colour Chart (Royal Horticultural Society, 1939) in a glass greenhouse under full sun conditions.

2005 Releases

'Royal Glissade'

'Royal Glissade' (Figure 4-4) is an open-pollinated variety of 'Elfers' (Florida City Series, Lake Brantley Plant Corp). Elfers was chosen for its late-flowering, excellent vigor and optimal branching. Foliage is a speckled pea green (61/1) on a delft rose (o20/1) background on the adaxial leaf surface. Young leaves start as speckled pea green (61/1). Foliage is a garnet lake (828) on the abaxial leaf surface. Leaf shape is cuneate-acute with crenate margins. Mature leaves average 14 cm long and 7 cm wide on plants grown 15 weeks after planting. Royal Glissade is intended for use as a decorative foliage plant for the landscape. This cultivar is exceptional because foliage color remains consistent under all testing locations and has a unique foliage color that reflects gold/bronze in the sunlight and provides a bright contrast among other

ornamentals. With its flowering occurring near October, this variety has all of the desirable traits of sun-tolerance, late flowering, vigor, branching, and color retention.

‘Twist and Twirl’ and its two sports, ‘UF03-6-1B’ and ‘UF03-6-1C’

All three cultivars (Figure 4-5) are open-pollinated varieties of ‘Stained Glassworks Copper’ (Miracle-gro Plants/Metrolina). ‘Stained Glassworks Copper’ was chosen for its bright red foliage color and late flowering characteristics. ‘Twist and Twirl’ foliage is polychromatic canary yellow (2/1), scheeles green (860), jasper red (o18), and aconite violet (937). Young leaves start as a variegated spinach green (o960) and ivy green (ooo1060/2) and other colors appear when leaves mature. ‘UF03-6-1B’ foliage is a vermilion (18) and ivy green (ooo1060/1). ‘UF03-6-1C’ foliage is a canary yellow (2/1) and scheeles green (860). ‘Twist and Twirl’, ‘UF03-6-1B’, and ‘UF03-6-1C’ have different foliage color but identical growth characteristics. Leaf shape is lanceolate and margins are highly lobed. Mature leaves average 12 cm long and 8 cm wide on plants grown 15 weeks after planting. ‘Twist and Twirl’, ‘UF03-6-1B’, and ‘UF03-6-1C’ are intended for use as a decorative foliage plant in the landscape and are unique due to their multi-colored, highly lobed, cut-flower foliage shape and exceptional high vigor. Being nearly isogenic, these cultivars perform well together while providing differences in foliage color. With its multi-colored, red and green foliage, this cultivar series can be easily combined with other ornamental plants.

‘UF03-8-24’

An open-pollinated variety of ‘Elfers’, its foliage is a speckled purple madder (1028) on a lettuce green (861) background on the adaxial surface. Margin edge and abaxial surface are a purple madder (1028). Leaf shape is cuneate-acute and margin slightly serrate. Mature leaves average 12 cm long and 11 cm wide on plants grown 15 weeks after planting. ‘UF03-8-24’

(Figure 4-6) is intended for use as a decorative foliage plant in the landscape. This cultivar works well for commercial cutting production, having excellent vigor and branching. With its green and darker purple markings, this cultivar works exceptionally well in combination with other ornamentals and as a series with ‘Royal Glissade’.

‘Electric Lime’

‘Electric Lime’ (Figure 4-7) is an open-pollinated variety of ‘UF03-1-6.’ ‘UF03-1-6’ is an open-pollinated variety of ‘Astatula’. ‘Astatula’ (Florida City Series, Lake Brantley Plant Corp) was chosen for its late-flowering, excellent vigor, optimal branching, and sun-tolerance. Foliage is a variegated citron green (763) on a scheeles green (860) background on the adaxial leaf surface. As leaves mature, foliage is a variegated chartreuse green (663/1) on a scheeles green (860) background on the adaxial leaf surface. Leaf shape is ovate and cuneate-acute with crenate margins. Mature leaves average 14 cm long and 7 cm wide on plants grown 15 weeks after planting. ‘Electric Lime’ is exceptional because foliage color remained bright green in all testing locations. With its flowering occurring near September, this cultivar has all of the desirable traits. This cultivar is intended for use as a decorative foliage plant for the landscape and in potted containers and will provide a sharp contrast among other ornamentals. ‘Electric Lime’ currently has a plant patent applied for.

‘UF04-5-7’

‘UF04-5-7’ (Figure 4-8) is an open-pollinated variety of UF03-6-1. UF03-6-1 is a selection of ‘Stained Glassworks Copper’. Foliage is a magenta rose (o27/1) diffusing to an ivy green (ooo1060/2), with small spots of scheeles green (860/2) near the margin. Abaxial surface is a magenta rose (o27/1). Leaf shape is rounded-acute with crenate margins. Mature leaves average 6.5 cm long and 5 cm wide on plants grown 15 weeks after planting. ‘UF04-5-7’ is different

from other commercial cultivars because its bright pink foliage remains consistent under partial to full shade conditions and therefore can be used in mixed containers needing a bright color contrast. With its consistent slow growing habit, this cultivar is intended for use as a low maintenance cultivar for potted containers in the landscape.

'UF04-18-3'

'UF04-18-3' (Figure 4-9) is an open-pollinated variety of UF03-13-9. UF03-13-9 is a selection of 'Micanopy' (Florida City Series, Lake Brantley Plant Corp). UF03-13-9 was chosen for its novel orange foliage color. 'Micanopy' was chosen for its excellent vigor and branching. Foliage has interveinal areas and are of a canary yellow ($2/_3$) in the leaf center and diffusing into a carmine ($21/_1$)/purple madder (*10*28) toward the leaf margin. The abaxial surface is jasper red ($o18/_3$). Leaf shape is cordate-acute with highly crenate margins. Mature leaves average 11 cm long and 10 cm wide on plants grown 15 weeks after planting. 'UF04-18-3' is intended for use as a decorative plant for both sun and shade conditions. Although this cultivar has fair ratings for vigor and branching, its combination of highly lobed and crinkly textured foliage, red foliage color, semi-dwarf growth habit, and late flowering makes this cultivar highly unique and different from other commercial cultivars.

'UF04-36-33'

'UF04-36-33' (Figure 4-10) is an open-pollinated variety of 'UF03-6-18'. 'UF03-6-18' is a selection of 'Stained Glassworks Copper'. UF03-6-18 was chosen for its variegated color pattern, excellent vigor and branching. Foliage is a china rose (o24) with ivy green (ooo1060/2) spots throughout leaf blade. Margin is edged with scheeles green (860/2). Leaf shape is cuneate-acute with mild serrate margins. Mature leaves average 10 cm long and 8 cm wide on plants grown 15 weeks after planting. 'UF04-36-33' is unique with its combination of dark-purple

stems and bright red foliage. This cultivar is intended for use as a decorative foliage plant for both sunny and shady landscapes, with red foliage color becoming more intense under partial shade. With its high vigor and branching, this cultivar is excellent for mass propagation and commercial cutting production.

‘UF04-40-26’

‘UF04-40-26’ (Figure 4-11) is an open-pollinated variety of ‘UF03-13-9’. ‘UF03-13-9’ is a selection of ‘Micanopy’. Foliage is sulphur yellow (1/3) in the center, interveinal areas an aureolin (3), and a scheeles green (860). Leaf shape is cordate-acute with margin highly crenate. Mature leaves average 11.5 cm long and 9 cm wide on plants grown 15 weeks after planting. ‘UF04-40-26’ is distinctive with its foliage color pattern and crinkly texture, high vigor, and branching. Despite its high vigor rating, this cultivar is intended for use as a potted plant due to its highly compact and mounded growth habit and would work extremely well as a landscape border plant.

‘UF04-40-125’

‘UF04-40-125’ (Figure 4-12) is an open-pollinated variety of ‘UF03-13-9’. ‘UF03-13-9’ is a selection of ‘Micanopy’. Foliage is a sulphur yellow (1/3) in its center diffusing to a scheeles green (860) near the margin, and the blade speckled with Indian lake (826). Leaf shape is cordate-acute and leaf margin is mildly serrate. Mature leaves average 10 cm long and 8 cm wide on plants grown 15 weeks after planting. ‘UF04-40-125’ is intended for use as a decorative foliage plant in the landscape and as a potted plant in containers. ‘UF04-40-125’ is an excellent sun-tolerant cultivar, with foliage colors remaining consistently bright in all locations tested. It also works well for mass propagation and commercial cutting production. This variety received

an award for best performance at the Floriculture Field Day evaluated at Gainesville, Florida in 2006.

‘UF04-51-33’

‘UF04-51-33’ (Figure 4-13) is an open-pollinated variety of ‘Aurora Orange’ (Ball FloraPlant). ‘Aurora Orange’ was chosen for its bright orange foliage color, leaf shape and growth habit. Foliage is a camellia rose (622/1) diffusing to a dark camellia rose (622) near the margin, the margin speckled with ivy green (ooo1060/3), and the margin edge a lettuce green (861/3) on the adaxial surface. Abaxial surface is porcelain rose (620/3). Leaf shape is oblique-acute and the leaf margin is highly crenate. Mature leaves average 10 cm long and 5 cm wide on plants grown 15 weeks after planting. ‘UF04-51-33’ was chosen for its novel rose-colored foliage and late flowering characteristics. With a lower vigor and branching rating, this cultivar is intended for use in small mixed containers in patio gardens in shady locations.

‘UF04-51-41’

‘UF04-51-41 (Figure 4-14) is an open-pollinated variety of ‘Aurora Orange’. Foliage is a speckled china rose (o24/1) on a purple madder (1028) background. Leaf shape is oblique-acute and leaf margin lobed. Mature leaves average 13 cm long and 5 cm wide on plants grown 15 weeks after planting. ‘UF04-51-41’ is intended for use as a decorative foliage plant in sunny or shady landscapes. ‘UF04-51-41’ is unique for its combination of lobed leaves, rose and dark purple foliage, lobed leaf shape, mounded growth habit, and late flowering. ‘UF04-51-41’ will provide a sharp contrast of color and texture against greens in the landscape. With a high branching rating, ‘UF04-51-41’ is exceptional for rapid commercial cutting production.

‘UF04-51-42’

‘UF04-51-42’ (Figure 4-15) is an open-pollinated variety of ‘Aurora Orange’. Foliage is a rose opal (o22/3) darkening to a dark rose opal (o22/3) near the margin on the adaxial surface. The abaxial surface is magenta rose (o27/1). Young leaves begin as jasper red (o18/1). Leaf shape is oblique-acute and margin medium crenate. Mature leaves average 6.5 cm long and 3 cm wide on plants grown 15 weeks after planting. ‘UF04-51-42’ growth habit and low vigor make this cultivar ideal for 4” container production. ‘UF04-51-42’ was chosen for its exceptional rose-opal foliage color. Despite having a fair rating on vigor and branching, the foliage color was the selection factor and the color is considered novel when compared to other commercial cultivars.

2006 Releases

‘UF04-47-64’

‘UF04-47-64’ (Figure 4-16) is an open-pollinated variety of ‘Elfers’ (Florida City Series, Lake Brantley Plant Corp.). Elfers was chosen for its late-flowering, excellent vigor and optimal branching. Foliage is a tyrian rose (o25) on the adaxial and magenta (27) on the abaxial leaf surface. Leaf shape is cuneate-acute with crenate margins. Mature leaves average 10 cm long and 6 cm wide on plants grown 15 weeks after planting. ‘UF04-47-64’ is intended for use as a decorative foliage plant for the landscape. This cultivar was chosen for its large leaf size, exceptional vigor, and rich deep rose-colored foliage. With a late flowering induction, this cultivar remains bright throughout the summer season.

‘Splish Splash’

‘Splish Splash’ (Figure 4-17) is an open-pollinated variety of ‘Elfers’. Foliage is mottled sap green (62/1), maroon (1030), and dutch vermillion (717/2) on both the adaxial and abaxial leaf surface. Leaf shape is rounded-acute with crenate margins. Mature leaves average 11 am long and 8 cm wide on plants grown 15 weeks after planting. ‘Splish Splash’ is unique for its

spectacular mottled foliage color and works great alone or as an addition for mixed containers. With a full growth habit, high vigor, and excellent branching, it is intended for use a decorative foliage plant and will work well as a hedge plant in the landscape.

'Pineapple Splash'

'Pineapple Splash' (Figure 4-18) is an open-pollinated variety of 'UF03-8-21'. 'UF03-8-21' is a selection of 'Elfers'. 'UF03-8-21' was chosen for its bright color foliage and late-flowering. Foliage is carmine (21/1) along the mid-vein diffusing to a fuchsia purple (28) on a uranium green (63/2) background with splotches of uranium green (63/3) throughout the adaxial leaf surface. Foliage color is a uranium green (63/3) on the abaxial leaf surface. Leaf shape is acute-acuminate with crenate margins. Mature leaves average 8 cm long and 3 cm wide on plants grown 15 weeks after planting. 'Pineapple Splash' was chosen for its unique bright yellow and maroon center foliage. Although this cultivar has a fair to average vigor rating, it became one of the few cultivars that looked fantastic and remained exceptional at the end of the trial. With an excellent sun tolerance and late flowering rating, this cultivar works best in small plantings and/or in mixed containers for sunny locations.

'Velvet Mocha'

'Velvet Mocha' (Figure 4-19) is an open-pollinated variety of 'Elfers'. Foliage is maroon (1030) along the mid-vein on a jasper red (o18) background on the adaxial leaf surface and a maroon (1030/1) on the abaxial leaf surface. Leaf shape is acuminate-acuminate with entire or lobed margins. Mature leaves average 9 cm long and 2.5 cm wide on plants grown 15 weeks after planting. 'Velvet Mocha' has both exceptional vigor and branching and late flowering. This cultivar is intended for use as a foliage plant in shady landscapes. Its unique jasper red foliage color on a lanceolate leaf shape and mounded habit makes this cultivar stand out from others.

‘UF06-04-140’

‘UF06-04-140’ (Figure 4-20) is an open-pollinated variety of ‘Elfers’. Foliage is pea green (61) speckled on a rose red (724) background on the adaxial leaf surface. Young leaves start as speckled pea green (61). Foliage color is a maroon (1030/3) on the abaxial leaf surface. Leaf shape is rounded-acute with crenate margins. Mature leaves average 12 cm long and 7 cm wide on plants grown 15 weeks after planting. ‘UF06-04-140’ is intended for use as a decorative foliage plant in the landscape. This cultivar has excellent color for both sunny and shady locations, but appears more rose-red under high light intensities. With flowering induction occurring in late October and exceptional branching and growth habit, this cultivar is ideal for garden performance.

‘Lemon Sunsation’

‘Lemon Sunsation’ (Figure 4-21) is an open-pollinated variety of ‘UF04-18-3’. “UF04-18-3’ is a selection of ‘UF03-13-9’. ‘UF03-13-9’ is a selection of ‘Micanopy’ (Florida City Series, Lake Brantley Plant Corp). ‘UF04-18-3’ was chosen for its novel and bright foliage color. ‘UF03-13-9’ was chosen for its novel orange foliage color. ‘Micanopy’ was chosen for its excellent vigor and branching. Foliage is a canary yellow (2/1) and sap green (62) on the adaxial leaf surface. Foliage color is a spiraea rose (o25) on the leaf margins and a sap green (62) on the abaxial leaf surface. Leaf shape is cuneate-acute with lacerate margins. Mature leaves average 8 cm long and 5 cm wide on plants grown 15 weeks after planting. ‘Lemon Sunsation’ has one of the brightest yellow foliage seen on a coleus plant for full sun conditions. This cultivar has excellent branching and unique leaf shapes. With a maroon border along the leaf margins set in contrast with the yellow foliage color, this cultivar works well in mixed containers or as a decorative foliage plant in the landscape for gardeners wanting to add a dynamic contrast.

‘Big Red Judy’

‘Big Red Judy’ (Figure 4-22) is an open-pollinated variety of ‘Elfers’. Foliage is a cherry (722) on the adaxial leaf surface and a maroon (1030/1) on the abaxial leaf surface. Leaf shape is rounded-acute and crenate margins. Mature leaves average 12 cm long and 7 cm wide on plants grown 15 weeks after planting. ‘Big Red Judy’ was named for its large red leaves and robust growth habit. This cultivar works well in large potted containers or in the landscape. Its excellent vigor, bright foliage color, late flowering, and overall appearance distinguishes it from other red coleus varieties.

Figure 4-4. ‘Royal Glissade’

Figure 4-5. (A)‘Twist and Twirl’ (B) ‘UF03-6-1B’ (C) ‘UF03-6-1C’

Figure 4-6. ‘UF03-8-24’

Figure 4-7. ‘Electric Lime’

Figure 4-8. ‘UF04-5-7’

Figure 4-9. ‘UF04-18-3’

Figure 4-10. ‘UF04-36-33’

Figure 4-11. ‘UF04-40-26’

Figure 4-12. ‘UF04-40-125’

Figure 4-13. ‘UF04-51-33’

Figure 4-14. ‘UF04-51-41’

Figure 4-15. ‘UF04-51-42’

Figure 4-16. ‘UF04-47-64’

Figure 4-17. ‘Splish Splash’

Figure 4-18. ‘Pineapple Splash’

Figure 4-19. ‘Velvet Mocha’

Figure 4-20. ‘UF06-4-140’

Figure 4-21. ‘Lemon Sunsation’

Figure 4-22. ‘Big Red Judy’

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BIOGRAPHICAL SKETCH

Penny Nguyen was born in Go Gong, Vietnam, and immigrated with her family to the United States at the age of 5. After graduating from Pensacola High School, she completed her Bachelor of Science degree at Jacksonville University, Jacksonville, Florida with a major in biology and minor concentrations in chemistry and marine science. While working on a manatee research program in the summer of 2003, she was accepted into the doctoral program at the University of Florida. Penny was a recipient of the Alumni Fellowship and began her graduate studies in plant breeding. Throughout the 4 years of her research, she focused on the genetics of trailing habit, how light influenced foliage color changes, and developing new coleus cultivars for the commercial market. She and her major advisor successfully implemented the first coleus breeding program at the University of Florida. After her completion, 21 new coleus cultivars have been released, with 8 sold on the commercial market and garnering royalties. Her list of achievements include a patent applied for one coleus cultivar (‘Electric Lime’) and the development of several dozen new coleus varieties with trailing habit and bright orange foliage colors. She is currently a plant breeder at Carolina Nurseries, Moncks Corner, South Carolina.