2009

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Recommended Citation

Liu, Miao, "Development of a rapid and effective screening method for basal stress tolerance of Petunia x hybrida" (2009). *LSU Master's Theses*. 2316.
https://digitalcommons.lsu.edu/gradschool_theses/2316

DEVELOPMENT OF A RAPID AND EFFECTIVE SCREENING METHOD FOR BASAL STRESS TOLERANCE OF *PETUNIA X HYBRIDA*

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of
requirements for the degree of
Master of Science

in

The School of Plant, Environmental, and Soil Sciences

by
Miao Liu
B.S., Beijing Forestry University, 2002
May 2009

ACKNOWLEDGEMENTS

I greatly appreciate the support of committee members, friends, and my family during the completion of this thesis. I would like to acknowledge and thank my advisor, Dr. Jeff S. Kuehny, for his advice and guidance over the past 3 years, for his patience, and for his many revisions to this manuscript; to Dr. Marc Cohn for his suggestion, and direction regarding my research and for his advice in all of his courses I have taken; to Dr. Jeffrey Beasley for serving as a member of my committee and supporting my whole project, for use of his flat-bed scanner, and for his comments on this research.

I would like to thank my husband, Hua Wang, for his support and encouragement; to my uncle and aunt for their continual support and dedication for anything that I do in my life; to my brother, Bo, for being a constant companion throughout our lifetimes. Additionally, I would like to especially thanks to Dr. Patrick Hegwood and the Burden Foundation for providing me the research assistantship and to thank Dr. Kevin McCarter for his help on statistics analysis. I also would like to thank the Ball Horticulture Company for the supply of the petunia seeds and Dr. David G. Clark to supply some of petunia seeds for this research. I also would like to thank my lab members: See Teng Soo, Jennifer Mader, and Ms. Priti Juneja, for their help to set up my experiments, collect data and continually give me advice and keep me doing well.

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ABSTRACT

High temperature and drought stress are two of the greatest impediments to bedding plant growth and development. The objective of this study was to develop a rapid and effective protocol for screening *Petunia x hybrida* for basal heat or drought tolerance. A practical growth system for measuring seedling growth, or seedling growth sensitivity test (SGST), was first established. Based on this protocol, radicle growth rate was chosen over hypocotyl growth as the most reliable and accurate measurement for the SGST. Nineteen petunia cultivars from three plant classes (floribunda, grandiflora, or spreading) were previously evaluated, where cultivars within the same plant class and series were grouped as either best or worst for overall landscape performance, and then subjected to the SGST. Seeds were germinated in Petri dishes at 26°C for 4 days and then subjected to 5-h heat shock at a temperature of 40°C or 6 d drought stress at an osmotic potential of -0.8MPa achieved with PEG 6000 to determine heat or drought tolerance, respectively. The results indicated heat or drought stress significantly affected the relative radicle growth rate of seedlings. However, the imposed stress did not affect all cultivars similarly. While 'Avalanche Lilac' and 'Dreams Burgundy Picotee' had the greatest radicle growth rate, they were considered as more heat susceptible cultivars due to a significant reduction in radicle growth rate under heat stress. 'Avalanche Lilac' was also regarded as the most drought sensitive cultivar. The results from this study indicate that the SGST may be used to determine heat or drought tolerance, but further research should be conducted to determine greenhouse and landscape performance under heat or drought stress.

CHAPTER 1. INTRODUCTION

1.1 BEDDING PLANTS AND *PETUNIA X HYBRIDA*

Bedding plants are one of the primary components of the floriculture industry. In the United States the wholesale value for bedding and garden plants in 2007 was $1.262 billion, which was 32% of total gross sales for floriculture crops (U.S. Department of Agriculture, 2008). Bedding plants can be annual, biennial, or perennial plant species. Annuals are plants that are usually germinated from seeds, then flower and die in one year or season. Biennials flower and subside within two years, and perennials cycle into flowering and dormancy for three years or longer. The majority of bedding plants are annuals, and they are planted in early spring for summer flowering and replanted in the fall for winter flowering.

The name “bedding plant” is derived from their locations and function in the landscape since these plants are suitable for planting in garden beds based on their color and scale. At present, the term has been extended to herbaceous plants, vegetables, some small fruits, and woody plants that can be interspersed in the landscape (Carlson and Johnson, 1985). The largest consumer of bedding plants is the public, and the second largest are landscape contractors (Copes, 2000; Fossler, 1993). This purchasing trend is changing as landscape contractors are providing increased services to homeowners. Bedding plants are generally propagated by seeds in the form of plugs or cuttings. Plugs are grown in greenhouses and produced from seeds in small cells in plug trays (Styer and Koranski, 1997). Plug tray standard sizes are generally 54 cm long, 28 cm wide and 2-5 cm tall. They usually consist of 36 to 800 single cells, which range from 21.7 to 3.5 cm^3 by soil volume (Hamrick, 1989), and the most common plug sizes are 288 and 512 cell plug trays. The total quantity of bedding plants sold as plugs was 60,665 trays in 2007 (U.S. Department of Agriculture, 2008). Due to their high and rapid return on investment, bedding plants are the primary source of income for the floriculture greenhouse industry. Consumer demand for new and improved varieties of bedding plants has continued to increase and this has placed a large demand on ornamental companies to find, breed and introduce new or improved varieties on a yearly basis. The continued development of new or improved bedding plants has placed pressure on growers and landscapers to revisit production and landscape practices based on the ever changing cultural requirements of the new plant material. Changes in weather patterns have caused increased periods of drought and increased minimum air temperatures. Thus, determining which bedding plant varieties can withstand periods of extreme heat and drought has become increasingly important. These two environmental stresses have become the greatest impediments affecting growth and development of these plants in greenhouse production, shipping areas, retail stores and landscapes. Meanwhile, consumers also want to put little effort into maintaining the plants after they are planted in their gardens. There has been little research conducted to further understand the effects of these two stresses on bedding plants since most of this type of research has been conducted on agronomic crops (Adedipe *et al.*, 1971; Freundl *et al*., 1998; Knight and Ackerly, 2003; Kimurto *et al*., 2005; Liu *et al.*, 2002; Sauter *et al*., 2002; Valladares and Pearcy, 1997; Wright *et al*., 2001).

Petunia (*Petunia x hybrida* Hort. ex Vilm.) is native to South America and belongs to Solanaceae family. Petunias are one of the most popular fall, summer and spring bedding plants because of their diversity of brilliant colors and growth habits (Behe and Beckett, 1991). Petunias were hybridized between the white flowering species (*Petunia axillaris* Britton, Stern & Poggenb) and the purple flowering species (*Petunia integrifolia* Schinz & Thell) two centuries ago (Hortorum, 1976). They are usually divided into five classes based on their flowers and plant shapes: grandiflora, floribunda, milliflora, multiflora, and spreading. The total gross sales of petunias were 0.112 billion dollars, which was only less than the sales of geranium in 2007 (U.S.

Department of Agriculture, 2008). Petunias are used either as perennials in warm climates or annuals in temperate places (Armitage, 2001). There has been more genomic research conducted on petunia than any other bedding plant; petunia is used as a model system for comparative genomic research. The genetics of petunia are also being studied at Radboud University (Gerats and Vandenbussche, 2005), University of Florida (Clevenger *et al*., 2004) and Ohio State University (Jones *et al*., 2005). One of the primary links that is missing to complete the chain of understanding for development in genetic improvement of petunia is the response of petunia to environmental stress.

1.2 IMPORTANCE OF SCREENING BASAL STRESS TOLERANCE

Stress is defined as 'impaired function'. For plants, it implies that environmental factors are below or beyond the optimum points so as to harm plant growth and development. Basal stress tolerance is a term that indicates the tolerance of stress without prior acclimation or genetic tolerance (Natarajan, 2005). Global warming has become an increasingly important factor and has already induced numerous effects upon the environment and human life, such as rising temperature, more extreme weather, and increasing evaporation (Peterson *et al.*, 2002). These effects gradually cause high temperature and drought in many areas around the world, defined as global warming. If these weather extremes continue to occur, all plants could be affected adversely to some degree. For example, high temperatures have reduced growth of peanut (*Arachis hypogaea* L.) and wheat (*Triticum aestivum* L.) along with development and yield (Harding *et al.*, 1990; Ketring, 1984). Bedding plants are mostly annual and perennial species with a much smaller scale when compared to woody shrubs and trees; thus they are easily affected by these stresses. The greenhouse is designed to provide optimum conditions for plants to grow and develop. However, high temperature during production has significantly affected greenhouse growers in the southern United States. Most root substrates used for bedding plant production have a high porosity, and the plants grown in these substrates quickly reach mature size; thus, the substrate can become dry very quickly, leading to drought stress. Postproduction longevity of bedding plants is primarily reduced by extremes in moisture or temperature. Plants are frequently placed under stress when moved among a variety of different environments from the retail or wholesale garden centers to the landscape. Currently there exists no protocol for determination of basal stress tolerance of bedding plants. To alleviate drought or heat stress during production, frequent irrigation and specialized cooling are often used if they are considered practical. However, these management practices are often expensive and of limited effectiveness, especially when the plants are being marketed or planted in the landscape. Meanwhile, breeding work continues to provide new or improved varieties, but few of these cultivars are truly heat or drought tolerant even though they are labeled as 'heat lovers' or 'drought tolerant'. Thus, a long term solution to the situation will require development of less heat and drought sensitive cultivars through plant breeding efforts or induction of these tolerances through effective cultural practices. Before this breeding work can be implemented, a method that will accurately and efficiently screen bedding plants for basal stress tolerance must be developed. This method will serve as a reference or check for breeding more stress tolerant bedding plants and would greatly benefit plant producers, landscapers, and home owners.

The objectives of this study were 1) to determine the challenging temperature for heat stress and the challenging osmotic potential for drought stress; 2) to evaluate and develop a method for screening bedding plants for heat or drought stress; and 3) to screen varieties from various classes and determine their basal stress tolerance. During this study, the seedling growth sensitivity (SGS) test, a method used to determine seedling length by using digital imaging prior to and following heat or drought treatment, was developed as a rapid and effective morphological screening tool.

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CHAPTER 2. LITERATURE REVIEW

2.1 BEDDING PLANT PRODUCTION AND POSTPRODUCTION

2.1.1 Bedding Plant Production

Production techniques of bedding plants generally include two methods: the traditional method that is labor intensive and costly, and the plug method. The traditional method for producing bedding plants is by direct seeding in open flats and then transplanting into final containers. Transplanting is conducted when seedlings have two or three true leaves, allowing for separation of seedlings. However, the roots are disturbed and damaged in this process, affecting plant growth rate, and this process is very labor intensive. During the 1970s, a new method called the “plug technique” was developed for more efficient and effective transplanting. “Plug” is a term given to each seedling planted in its own single cell or container, which can be transplanted with minimal damage to the roots, and this process can be more easily mechanized (Styer and Koranski, 1997). Thus, the plug technique reduces root damage and increases seedling quality (McKee, 1981). Since the 1990s, approximately 75% of bedding plants have been produced from plugs (Dill, 1993). Based on the physiological changes of seedlings during production, the process of plug production is divided into four stages: radicle emergence, stem and cotyledon emergence, true leaf appearing, and seedlings ready for shipping with 2-4 true leaves (Koranski and Karlovich, 1989). Temperature and water are important factors that affect the production quality and survival rates of plugs. Edwards (1932) suggested that three temperature points should be assigned according to seed germination rate and uniformity. They were low, optimum, and high temperatures for germination. Following this protocol, different species were categorized into different temperature groups (Atwater, 1980; Bewley and Black, 1985). In the 1960s, Cathey investigated the germination of the most popular bedding plants and found that the optimum temperature for germination usually exists between 24 to 30°C for most seeds of bedding plants species (Cathey, 1969). Water deficiency reduced seed germination, seedling height, and final seedling quality of *Impatiens* L.(Karlovich *et al.*, 1989) and legume plants of field bean (*Vicia faba*), soyabean (*Glycine max*), field pea (*Pisum sativum*) and lupin (*Lupinus albus and L. angustifolius*) (Grzesiak et al., 1996). This research showed that both temperature and water play pivotal roles for the production of plants from seed.

2.1.2 Bedding Plants Postproduction

Production of bedding plants from plug to finished plant takes approximately six weeks, at which time bedding plants are shipped to retail garden centers, wholesalers or landscape contractors. There is a large difference between the production environment in the greenhouse and the sales areas or landscape beds. Production of bedding plants in the greenhouse provides an optimum environment for plant growth and development. However, conditions during postproduction in retail display, shipping areas, or the landscape beds are often not optimum, so plants quickly become stressed primarily due to lack of proper irrigation or temperature control. Temperature is the most significant factor affecting bedding plants during postproduction. It has been estimated that the losses can be as high as 20% from a finished bedding plant through postproduction (Armitage, 1989). Several strategies have been suggested to maintain postproduction quality but are often not implemented. These strategies include toning plants or hardening off plants by withholding water, reducing fertility levels or changing types of fertilizers during the last week of production, reducing night temperature, selecting a suitable growing medium and planting in larger containers (Nelson *et al.*, 1980; Nelson and Carlson, 1987). Most of these strategies are appropriate for acclimating bedding plants for low temperature environments; however, there is no research devoted to drought or high temperature stress.

2.2 HEAT STRESS

Heat stress is defined as the situation in which the air temperature is high enough to cause irreversible damage to plant growth and development (Hall, 2001). Responses of plants to heat stress have been investigated by many investigators. Seeds and seedlings of *Prosopis chilensis* (Mol.) Koch, a thermotolerant leguminous tree, were tested at temperatures of 25, 30, 35, 40, 45, or 50°C to investigate their response to heat tolerance. Results from this study indicated that high temperatures of 30, 35, 40, or 45°C can reduce germination percentage, and no seeds germinated at 50°C. For the seedling test, it revealed that if the higher temperature was used for seeds germination, the seedling can obtain higher optimum temperature for seedling growth. However, seedlings were still injured at 50°C (Medina and Cardemil, 1993). Another study investigated cultivars of flax (*Linum usitatissimum* L.) grown at 40°C for either 3 days or 5 days to determine their responses to heat stress. The results indicated that a high temperature of 40°C for 3 days can delay flowering and reduce seed yield of the cultivar ‘NorMan’, and a temperature of 40°C for 5 days reduced seed yield of most cultivars, excluding ‘McGregor’ and ‘Noralta’. These points suggest that there maybe genetically different heat tolerance among different plant cultivars (Gusta *et al*., 1997).

Tall fescue (*Festuca arundinacea* L.) and perennial ryegrass (*Lolium perenne* L.) sod were subjected to 35 ºC day/30 ºC night and well watered, or subjected to a combined heat and drought stresses in which plants were exposed to 35 ºC day/30 ºC night and not watered. Leaf photochemical efficiency, leaf relative water content, and cell membrane stability of ryegrass were impaired by high temperature more than fescue. Jiang and Huang (2001) indicated that the parameter measured in this study could be used for screening stress tolerance of ryegrass species or cultivars.

Other studies reported that heat stress in the range of 30 to 45°C could deleteriously affect young seedlings. Mutants of *Arabidopsis thaliana* (L.) Heynh were investigated by measuring hypocotyl growth during heat stress to determine their heat tolerance. The germinated seeds were subjected to heat shock (90 min/38°C, 120 min/45°C, or combination of 90 min/38°C, 120 min/22°C and120 min/45°C). The hypocotyls were measured after 2.5 d growth following the treatments. The results showed that heat shock of 45°C reduced the hypocotyl growth of all mutants but heat shock of 38°C did not elicit any significant effects on hypocotyl growth (Hong and Vierling, 2000). Two leguminous plants, *Prosopis chilensis* (Mol.) Stuntz and cultivated *Glycine max* (soybean), were exposed to high temperatures of 35, 40, 45, or 50°C. *Prosopis chilensis*, a heat tolerant species, showed higher relative seedling growth, lower membrane damage, and higher heat shock protein content than *Glycine max* (Ortiz and Cardemil, 2001). Two cultivars of *Salvia splendens,* 'Vista Red' and 'Sizzler Red', were studied for their responses to the high temperatures in a range of 25 to 45ºC. The results revealed that plant height, stem thickness, shoot/root dry weight, net photosynthesis, stomatal conductance and transpiration rate declined as temperature increased (Natarajan, 2005).

The photosynthetic capacity of spring wheat (*Triticum aestivum* L.) was investigated at controlled temperatures of 20 and 35°C. Results indicated that photosynthesis was decreased at 35°C (Harding *et al.*, 1990). Photosynthetic gas exchange, chlorophyll fluorescence, nitrogen level, and lipid peroxidation were investigated in a perennial grass *Leymus chinensis* under heat stress of 23, 29, or 32 ºC. Results showed that high temperature decreased plant biomass, inhibited nutrient uptake, and consequently decreased plant qualities (Xu and Zhou 2006). Six types of bedding plants, which were agastache (*Agastache urticifolia* (Benth) O. Kuntze 'Honeybee Blue'), dusty miller (*Cineraria maritima* L. 'Silverdusty'), petunia (*Petunia x hybrida* 'Wave Purple'), plumbago (*Plumbago auriculata* Lam. 'Escapade'), ornamental pepper

(*Capsicum annuum* L. ‘Black Pearl’), and vinca (*Catharanthus roseus* (L.) G. Don ‘Titan’), were grown in greenhouses. Leaf net photosynthetic rate, stomatal conductance, and transpiration were measured to determine the responses to the drought stress by withholding soil moisture and heat stress by putting the matured leaf in heated cuvettes at 20, 25, 30, 35, or 40°C. Results showed leaf net photosynthetic rate and stomatal conductance linearly or quadratic decreased in all species. However, petunia photosynthesis parameters were the most rapidly reduced among them. For the transpiration rate, all species except petunia represented increased in value when temperature increased, but petunia gave the maximum transpiration rate at 30°C and decreased at 35 and 40°C. They considered that 30ºC could be a peak point of heat stress tolerance for petunia ‘Wave Purple’. Moreover, this study reported that petunia may be more sensitive to high temperature and drought because its leaf net photosynthetic rate and stomatal conductance declined more rapidly compared with the other species as temperature increased or as drought stress became more severe (Niu and Rodriguez, 2006).

Marcum (1998) first suggested that cell membrane thermostability (CMT) could be a method to screen heat tolerance for Kentucky bluegrass (*Poa pratensis* L.) for breeding purposes. Cultivars of ‘BM-3’, ‘Midnight’, ‘Lavang’, ‘Nugget’ and ‘Ryss’ were grown at 41°C for either 47d or 62d in incubators, and CMT was measured to reflect the tolerance of cultivars under heat stress. The CMT method was also used to measure the amount of electrolyte leakage from leaf segments of wheat (*Triticum aestivum* L.) exposed to a heat shock at 39°C (Ibrahim and Quick, 2001). Short duration exposure of *Salvia splendens* Ker Gawl to 35°C, for three hours every third day until flowering, showed a significant difference in characteristics between heat sensitive and heat tolerant cultivars of *Salvia splendens* Ker Gawl in terms of morphological (leaf thick, leaf size, stomata) and physiological (gas exchange and transpiration) responses (Natarajan and Kuehny, 2008). Plant response to heat stress can also be characterized by a set of proteins called heat shock proteins. Heat shock proteins include several protein families, such as hsp100, hsp90, hsp70, hsp60, and small hsps. Heritable differences in high temperature tolerance in sorghum (*Sorghum vulgare*) were correlated to levels of heat shock proteins (Ougham and Stoddart, 1986). Parsell and Lindquist (1993) considered that thermotolerant cells express high levels of heat shock proteins, and these proteins control the development of heat tolerance in plants. The main role of hsps under non-stress situations is to help the synthesis, and transport of the target proteins. Under heat stress, hsps change to prevent aggregation and promote proper refolding of damaged proteins. However, in a study by Natarajan (2005), results identified that hsps did not correlate with heat tolerance of *Salvia splendend* or pansy (*Viola x wittrockiana*). Thus, with these two bedding plants hsps did not increase in response to heat stress and may not be an effective marker for screening bedding plant cultivars.

There are numerous methods to measure the effect of heat stress and the ability of the plant to tolerate varying degrees of stress. The most important measurement of stress in terms of marketability is the visual quality of the plant to the consumer. This can be measured by evaluating plant quality. The determination of heat or drought stress tolerance of bedding plants is based on these quality ratings, which are based on subjective field studies. These studies are easily influenced by other environmental factors and hard to quantify direct effects. In addition, regional field studies require many years of data collection and can be very expensive. Thus, a rapid and effective measurement is needed to screen the heat tolerance of the bedding plants.

2.3 DROUGHT STRESS

Water is central to all physiological processes in plants; for example at the cellular level, it is the major medium for transporting metabolites and nutrients (Crafts *et al*., 1949 and Kozlowski, 1964). Too much water can cause hypoxia, and too little water can cause drought stress. Water stress can affect photosynthesis and other metabolic pathways during production and postproduction of all plants because it may compose up to 95% of the biomass of leaves and roots in herbaceous plants. If the water status is unbalanced because of an insufficiency of water, plants will suffer from water stress, which is often referred to as drought stress. For bedding plants, extremes in substrate moisture content play an important role that may affect photosynthesis and other metabolic pathways during production and postproduction. Plants may have morphological, physiological and molecular responses to drought stress.

Grzesiak *et al.* (1996) measured seed germination, seedling growth (seedling height, shoot dry weight, and root dry weight) as a screening technique to determine different drought tolerance of 18 cultivars of field bean (*Vicia faba*), soyabean (*Glycine max*), field pea (*Pisum sativum*) and lupin (*Lupinus albus and L. angustifolius*). Results indicated that cultivars showed different responses to the water potential at -0.3 and -0.6 MPa induced by mannitol ($C_6H_{14}O_6$). Drought sensitive cultivars (field pea and lupin) had lower germination percentages and slower relative seedling growth rates, compared to drought tolerant cultivars (field bean and soybean). Six-month-old rose plants (*Rose* L. cv. “Madelon”) were used to explore the flowering response to the same level of drought stress simulated by suspending irrigation for 5-7 days. The study showed that flower quality was decreased by reducing the length of flower bud and increased malformation of petals (Chimonidou-Pavlidou, 2004).

Germination and seedling growth of three cultivars (‘Bolero’, ‘Sprinter’, and ‘Utrillo’) of the pea (*Pisum sativum* L.) were investigated under salt and drought stress of -0.2, -0.4, -0.6, or -0.8MPa stimulated by sodium chloride (NaCl) and polyethylene glycol (PEG) 6000. Data indicated that simulated drought stress alone can restrain germination rate, seedling growth, and decrease shoot dry weight (Okcu *et al.*, 2005). Five chaffy-seeded grass cultivars, big bluestem (*Andropogon gerardii* Vitman), sand bluestem (*Andropogon hallii* Hack.), little bluestem

(*Schizachyrium scoparium* (Michx.) Nash), yellow bluestem (*Bothriochloa ischaemum* (L.) Keng. var. *ischaemum* (Hack.) Celarier and Harlan), and indiangrass (*Sorghastrum nutans* (L.) Nash) were investigated for their germination and seedling growth under drought stress of -1.6, -0.8, -0.4, or -0.2 MPa simulated by mannitol at 7, 14, and 21 d after sowing. The data revealed that drought stress resulted in a low germination percentage, slower seedling growth and less shoot/root dry weight during early seedling growth and yellow bluestem showed the least effect under drought stress. This study also suggested that these parameters could be a reliable method to select the more drought tolerance cultivars in different genotypes of chaffy-seeded grasses (Springer, 2005).

Studies have reported that not only seed germination, hypocotyl growth, radicle growth, leaf area and thickness, and flower quality can be affected by drought stress, but some other physiological characteristics can be affected. One-month-old legumes cultivars of *Phaseolus vulgaris* cv. Carioca, *Vigna unguiculata* cv. IT83D and *V. unguiculata* cv. EPACE-1 were investigated for drought tolerance by withholding water and comparing leaf gas exchange and leaf relative water content. Results indicated that *V. unguiculata* cv. EPACE-1, a more tolerant cultivar, incurred less injury to the leaves and decreased stomatal conductance. The authors suggested that the physiological parameters of leaf gas exchange and leaf relative water content can be used as a screening method for the drought tolerance evaluation in legumes (Cruz de Carvalho *et al*., 1998).

Grzesiak *et al*. (2003) screened the drought tolerance of triticale (*Triticale x triticosecale* Wittmack) by testing its physiological response when grown under drought conditions in the field for two years. They evaluated leaf gaseous exchange, leaf water potential, chlorophyll content and fluorescence, and leaf injury for 11 different grain lines (CHD 12, CHD 147, CHD220, CHD247, ‘Gabo’, ‘Kargo’, ‘MAH’, Maja’, ‘Migo’, ‘Mieszko’ and ‘Wanad’). They confirmed that there existed a relationship between the physiological factors measured and drought tolerance for different genotypes. Nine-day-old seedlings of two barley (*Hordeum vulagre*, L.) cultivars 'Houters' and 'Odeski' were investigated for membrane injury and chlorophyll fluorescence under drought stress using polyethylene glycol (PEG 8000. After germination, seedlings were transferred in 25% PEG 8000 solutions by immersing the roots for 6 to 48 h. The data suggested that chlorophyll fluorescence and membrane injury decreased as the duration of drought increased (Kocheva *et al*., 2004).

Six one-year-old ornamental herbaceous perennials, *Echinacea purpurea* (L.) Moench (Purple Coneflower), *Gaillardia aristata* Pursh (Blanketflower), *Lavandula angustifolia* P. Mill. (English Lavender), *Leucanthemum x superbum* (J.W. Ingram) Berg. ex Kent 'Alaska' ('Alaska' Shasta Daisy), *Penstemon barbatus* (Cav.) Roth var. *praecox nanus rondo* ('Rondo Mix' Penstemon), and *Penstemon x mexicali* Mitch. 'Red Rocks' ('Red Rocks' Penstemon) were used to investigate response to drought stress induced by withholding water (1-week irrigation, 2-week irrigation, and 4-week irrigation). Based on visual quality rating, gas exchange and osmotic potential of leaves, leaf areas, and dry weight of roots, leaves, and stems, 'Rondo Mix' penstemon was recommended as a more suitable plant material for droughty areas, and 'Purple Coneflower' was determined to be the worst drought tolerant plant compared to the other five species. This study also confirmed that plant growth and gas exchange were obstructed under drought conditions. The more severe drought stress, the more damage incurred (Zollinger *et al*., 2006). Hassanein and Dorion (2006) investigated the morphological and physiological traits of geraniums (*Pelargonium x hortorun* L.H. Bailey) to develop a better screening method for selecting or breeding drought tolerance under greenhouse conditions. One-month-old rooted cuttings in 12 cm diameter pots were subjected to drought stress at -0.02, -0.04, -0.06 or -0.08 MPa by withholding water, and a tension meter was used to maintain the correct treatment levels. The authors tested the stem height, the number of leaves, leaf area, stomatal density and leaf water content and confirmed there was a correlation between morphological or physiological traits and drought conditions. Based on those five parameters, they separated the eleven different cultivars of geraniums into two groups: drought tolerant and drought sensitive.

Den Berg and Zeng (2006) simulated drought stress at -0.3, -0.6, -0.9, -1.2, -1.5, -1.8 or -2.1MPa by using PEG 6000 and explored the germination percentage and seedling growth (shoot and root length) of three grasses (*Anthephora pubescens* Nees, *Heteropogon contortus* L., and *Themeda triandra* Forssk.) under drought in controlled environmental chambers. They found that the more severe the level of drought stress, the more germination capacity and shoot lengths were reduced. However, no seeds germinated at -2.1MPa, and the root length of *A. pubescens* increased at -0.6MPa and then decreased at -0.9MPa at the fifth day after the treatments were initiated. Results indicated that an adaptive period for plants subjected to drought stress might occur as measured by root elongation.

Various methods of screening for heat or drought tolerance have been determined to correlate with changes in morphological and physiological characteristics of various plants. Most of these studies were conducted at the seedling stage of plant growth for both agronomic and ornamental crops. The use of various methods for screening drought or heat tolerance of breeding lines or cultivars of agronomic crops have been proven to be highly successful. However, the research conducted on ornamentals, such as bedding plants, is based primarily on regional field trials, which are primarily based on subjective quality ratings and are not effective or efficient due to unpredictable heat or drought stress events and interactions with other environmental factors. Therefore, development of a more efficient and effective screening method for bedding plants associated with heat or drought stress at the seedling stage could greatly enhance selection of ornamental plant material for breeding and regional landscape use.

2.6 TREATMENT METHODOLOGY

Heat stress is easily induced and controlled by increasing temperatures in greenhouses or growth chambers; or projects can be conducted in the field during the summer. Most projects have been conducted in greenhouses because it is relatively convenient to control the levels of temperature (Jiang and Huang, 2000; Niu and Rodriguez, 2006; Xu and Zhou, 2006). However, these types of studies are hard to replicate due to the space required for statistical replication. If studies focus on the initial stages of seed germination and seedling growth, growth chambers can be used, and temperatures in these chambers can be more accurately controlled and temperature treatments more easily replicated.

Compared with heat stress, drought is more complicated because it is difficult to quantify the level of stress. One method used to induce drought stress is to reduce water frequency or withhold water from pots or containers. These methods are mainly conducted in greenhouse or field studies, and treatments are usually divided into three levels: well-watered, moderate drought, and severe drought, such as the trials conducted on *Phaseolus vulgaris* and *Vigna unguiculata* (Cruz de Carvalho *et al.*, 1998), *Rose* L. cv. 'Madelon' (Chimonidou-Pavlidou, 2004), *Impatiens capensis* Meerb. (Heschel and Riginos, 2005), tall fescue (*Festuca arundinacea* L.) orperennial ryegrass (*Lolium perenne* L.) (Jiang and Huang, 2001) and six ornamental herbaceous perennials by Zollinger *et al.* (2006).

Drought stress of geraniums (*Pelargonium x hortorun* L.H. Bailey) was investigated by using tensiometers to measure the soil water potential to control levels of drought stress (Hassanein and Dorion. 2006). Xu and Zhou (2006) used the dew-point potential meter to measure soil matrix potential to manage the degree of water stress. Drought stress was divided into four levels: mild drought, moderate drought, severe drought, or extreme drought, according to soil water potentials with a range of -0.3 to -0.15, -0.99 to -0.72, -1.97 to -1.45, or -2.34 to -

2.27MPa to determine the effect of the combination stress of drought and high temperature on perennial grass (*Leymus chinensis*). They found that the combination of heat and drought stress can worsen those effects on physiological traits during plant growth and development.

Drought stress can also be simulated by applying various levels of neutral salts or polymers, such as polyethylene glycol (PEG), mannitol, glucose, or sodium chloride (NaCl). This technique has been used to investigate seed germination, seed vigor, and early seedling growth during drought conditions. As early as the 1960s, Parmar and Moore (1968) reported that three different osmotic substrates (NaCl, PEG6000, and mannitol) had varying effects on corn (*Zea mays* L.) seed germination and seedling growth. Polyethylene glycol 6000 most negatively affected germination and seedling growth, while NaCl was least effective during that study. These results indicated that not all osmotic substrates were suitable for stimulating drought stress because of changes to metabolism in treated plants. Lawler (1970) found that the higher weight molecules of PEG1000 and PEG 4000 were more effective at simulating drought than the relatively smaller molecules of mannitol that could be absorbed by roots, and the larger molecules, such as PEG 20,000, could cause viscosity problems for the solution. After this study, PEG solutions were widely used for simulating drought stress. One such study investigated cucumber (*Cucumis sativus* L. Marketer) hypocotyl growth under PEG 6000 and mannitol solutions. Michel (1971) found that mannitol damaged some guard cells and confounded hypocotyls development. Three types of grass (*Lespedeza stipulacea* Maxim., *Lolium multiflorum*, and *Bouteloua curtipendula* Torr.) were investigated under the combination of heat and drought stress to compare their morphological responses during the seedling stage. Drought stress was simulated by PEG 20,000 (Masiunas and Carpenter, 1984).

Similarly, several studies investigated morphological and physiological characteristics during plant growth and development under drought stress by using PEG solutions for Durum wheat (*Triticum durum* Desf.) (Lutts and Kinet, 2000), barley (*Hordeum vulagre*, L.) (Kocheva *et al*., 2004) and various grass species cultivars (*Anthephora pubescens*, *Heteropogon contortus*, and *Themeda triandra*) (Den Berg and Zeng, 2006).

Okcu *et al*. (2005) compared NaCl and PEG 6000 and their ability to simulate drought stress of plants. Three pea (*Pisum sativum* L.) cultivars ('Bolero', 'Spring' and 'Utrillo') were investigated in the study with drought treatments of -0.2, -0.4, -0.6, or -0.8MPa. The results revealed that root length, shoot length, seedling fresh weight, and seedling dry weight were decreased as the concentration of solutions was increase, and none of cultivars had root growth at -0.8MPa of PEG 6000. It also indicated that PEG 6000 had a greater effect than NaCl, which was similar to the finding of Parmar and Moore (1968).

In conclusion, growth chambers have been proven to be more effective and efficient when conducting heat or drought tolerance research on seedlings because temperatures and other environmental factors can be more accurately controlled and more easily replicated. Induction of drought stress is more effectively applied using higher molecules weight of PEG since they do not appear to affect the morphology or metabolism of the plant.

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CHAPTER 3. DEVELOPMENT OF AN APPROPRIATE METHOD FOR EVALUATING SEEDLING GROWTH

3.1 INTRODUCTION

Several studies have shown that physiological measurements such as cell membrane stability, stomatal conductance, net photosynthesis, transpiration, and components of leaf photosynthesis can be used to screen for heat or drought tolerance (Al-Khatib and Paulsen, 1999; Jiao and Grodzinski, 1996; Medina and Cardemil, 1993; Natarajan and Kuehny, 2008). Researchers have also used morphological characteristics to screen for stress tolerance because these characteristics are easily measured and replicated.

Seedling or radicle length has been adopted as a useful method to test seed vigor for lettuce (*Lactuca sativa* L.) (Smith *et al*., 1973). Radicle growth of three types of grass, *Lespedeza stipulacea* Maxim. (sideoats grama), *Lolium multiflorum* (annual ryegrass), and *Bouteloua curtipendula* Torr. (Korean lespedeza), were measured to determine heat and drought tolerance. Radicle measurements were recorded 48 h after sowing (Masiunas and Carpenter, 1984). Grzesiak *et al*. (1996) determined the drought tolerance of eighteen legume cultivars by measuring germination percentages, seedling lengths, and dry weights. Germination stage was recorded when the radicle length was 5 mm, and drought treatments were applied as cotyledons of seedlings were emerging. Measuring hypocotyl growth was also used to determine heat tolerance of mutants of *Arabidopsis thaliana* (L.) Heynh. (Hong and Vierling, 2000). Ortiz and Cardemil (2001) reported that two leguminous plants, *Prosopis chilensis* (Mol.) Stuntz and cultivated *Glycine max* (soybean), were exposed to high temperatures of 35, 40, 45, or 50°C. *Prosopis chilensis*, a tolerant species, showed higher relative seedling growth than *Glycine max*. Similarly, one study suggested that the parameters of germination and seedling growth could be a reliable method to evaluate drought tolerance for different genotypes of chaffy-seeded grasses (Springer, 2005).

These studies indicate that measuring seedling growth rate after germination could be rapid, quantitative, and an effective technique to screen bedding plants for heat or drought tolerance. Geneve and Kester (2001) used digital images to measure growth parameters of small size seeds to help reduce errors occurring when measured by hand. Seeds of the following plants were included: tomato (*Lycopersicon esculentum* Mill.'New Yorker'), pepper (*Capsicum annuum* L. 'North Star'), vinca (*Catharanthus roseus* (L.) G. Don. 'Little Bright Eye'), marigold (*Tagetes patula* L. 'Little Devil Flame'), and impatiens (*Impatiens walleriana* Hook. f. 'Impact Lavender'). To obtain legible images, germination paper, blue blotters, and clear cellulose film were tested. The results indicated that cellulose film could support seed germination and seedling growth similar to germination paper. The cellulose film also provided a better method for digital imaging and measurement.

Measurement of seedling growth seems to be an effective method for screening for plant stress tolerance for many crops. This screening method could also prove to be very useful for screening bedding plants for drought and heat tolerance. However, a protocol must first be developed that is accurate and efficient for screening bedding plant species and cultivars, which would include an appropriate growing system such as container type, substrates and a technique for measuring seedling growth. The optimum temperature must be determined to prevent seed or seedling damage due to stress prior to treatment application. It is also important to determine the stage of seedling growth to apply the treatments. For example, Grzesiak *et al*. (1996) applied drought stress treatments when cotyledons of legume seedlings began to emerge. Finally, the appropriate heat or drought stress treatments must be determined for providing the most reliable data. Thus, the objectives of this study was to determine a) the appropriate growing system for germination and measurement of seedling growth, b) the optimum temperature for germination, c) the appropriate stage of seedling growth for treatment application, and d) temperature and osmoticum levels that will invoke a stress response.

3.2 MATERIALS AND METHODS

3.2.1 Plant Material

Petunia x hybrida Hort. ex Vilm. was chosen as the model plant for this research. Petunia is one of the most popular fall, winter, and spring bedding plants (Behe and Beckett, 1991). Moreover, it is a widely-cultivated genus of flowering plants of South American origin and a member of the Solanaceae family that is genetically closely related to tomato, potato, and tobacco. Because the genome of all of these crops, including petunia, have been mapped, genomic data can easily be transferred between these species (Gerats and Vandenbussche, 2005), and the effect of environment on growth might be correlated with the genetics of this family.

Seeds of *P. x hybrida* Dreams™ 'Midnight' (Ball Horticultural Company, West Chicago, IL) were evaluated. Seeds were separated into 500 or 1000 seed lots according to the weight (12,500/g or 0.08 g /1000seeds), and then placed in 1.5ml Snap-Cap plastic micro centrifuge tubes (DOT Scientific Inc., Burton, MI). Tubes were put in opaque plastic bags and then kept in a sealed plastic box. All seeds were stored in a refrigerator maintained at a temperature of approximately 0°C and an average relative humidity (RH) of 60% (Fig. 3.1A&B) recorded by a HOBO® Pro Series™ data logger (Onset Computer Corporation, Bourne, MA) which was placed within the plastic box.

3.2.2 Growing System and Measuring the Growth of Radicle and Hypocotyl Growth

Three substrates: white filter paper (Whatman® 1, Ø125mm), black filter paper (Whatman® 551, Ø125mm) (Whatman® International Ltd., Maidstone, England) or transparent

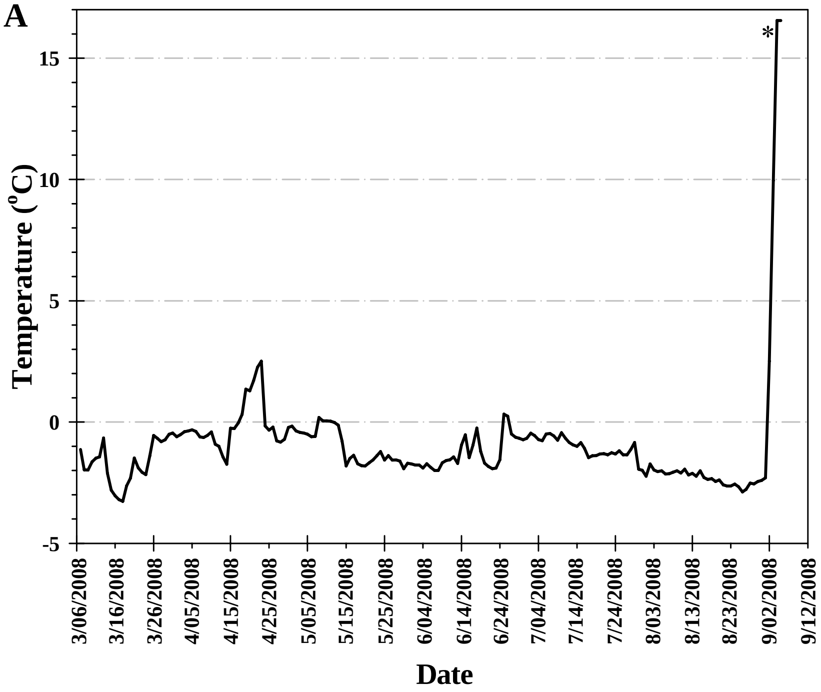

Figure 3.1A. Ten-day average air temperatures during storage of *Petunia x hybrida* 'Dreams Midnight' seeds inside a refrigerator at Louisiana State University, Baton Rouge. *Temperature increased because of power failure.

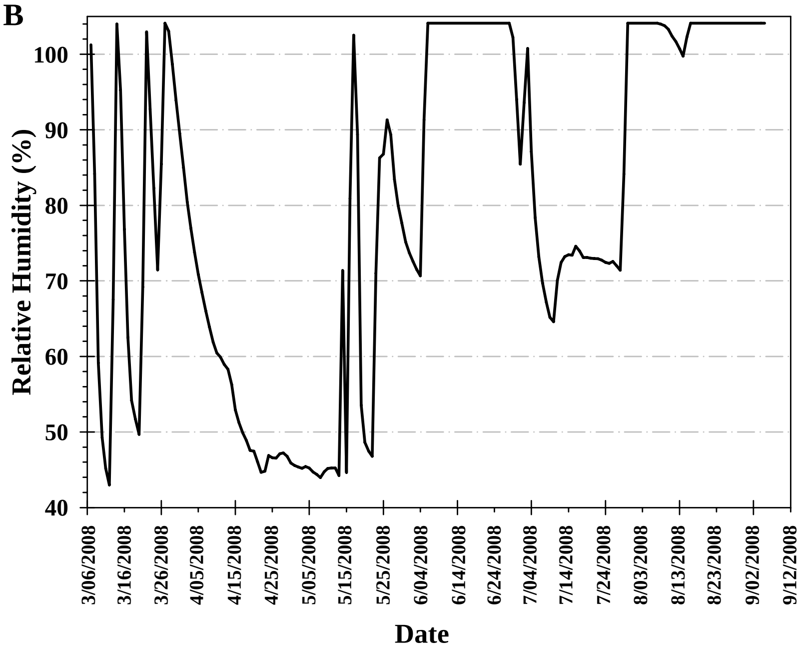

Figure 3.1B. Ten-day average relative humidity during storage of *Petunia x hybrida* 'Dreams Midnight' seeds inside a refrigerator at Louisiana State University, Baton Rouge.

cellulose film (Paper Mart Company, Los Angeles, CA) were presoaked with distilled deionzied (DI) water and tested for their ability to sustain germination, support seedling growth and allow for quantifying growth. Two different Petri dishes were used to determine their practicality for use as a suitable growth system. One was round without a grid (150 x15mm, VWR International, Inc., Suwanee, GA), and the other was square with a grid (100 x 100 x 15mm, Fisher Scientific Company, Houston, TX). Two different positions, horizontal or vertical, were tested for acquiring the most measurable and legible seedling growth parameters. For vertical positioning, seeds were placed on the top of a 0.23mm thin copolymer fishing line (G. Pucci & Sons, Inc., Brisbane, CA), which was attached to the Petri dishes by Scotch® tape (3M Inc., St. Paul, MN) to keep seedlings at the same position in the dish. All the tests were conducted in the growth chamber at 25°C. After the germination, the Petri dishes with seedlings were scanned by a flatbed scanner (Epson Expression® 1680, Epson America, Inc., Long Beach, CA) to evaluate these three substrates.

3.2.3 Determination of Optimum Temperature for Seed Germination

Ten seeds of ‘Midnight’ petunia per Petri dish were germinated at 20, 22, 24, 26, 28, or 30°C to determine optimum germination temperature. The substrate used for this test was the clear cellulose film. The cellulose film was purchased as a roll with a dimension of 30.5 x 254 cm^2 and cut into a 100 x 100 mm^2 piece to fit in the square Petri dishes (100 x 100 x 15mm). Prior to initiation of the experiment, all the cellulose film squares were presoaked with DI water for 24 h. The petunia seeds were placed on one layer of presoaked clear cellulose film in square Petri dishes with lids. To maintain adequate moisture, all Petri dishes were placed in a vertical position in translucent plastic containers of 100 x 100 x 83 mm (Pioneer Plastics, Inc., Dixon, KY). The amount of 50 ml DI water was added into containers to support seedling growth. To minimize evaporative losses, all containers were put into a clear plastic container of 430 x 350 x 150 mm (Really Useful Products Ltd., West Yorkshire, UK). Four layers of paper towel (SCA Tissue North America, Neenah, WI) were placed on the bottom of the container and moistened with 150 ml DI water. Finally, the sealed containers with seeds were placed into growth chambers (EGC, Chagrin Falls, OH). The humidity and light levels in the growth chambers were maintained at 80% RH and a 12-h photoperiod of 3-4 umol.$m^{-2}s^{-1}$ from very high output fluorescent, light bulbs (OERAM Sylvania, Danvers, MA). The arithmetic means of the germination percentages were calculated every 8 h, and germination rates were expressed as the time required achieving 50% germination (T50).

Petri dishes with ten seeds were arranged in a completely randomized design inside each container within growth chambers per treatment. There were three replicate Petri dishes per treatment within each growth chamber. All experiments were performed twice. Data analysis was performed by using the Proc Mixed Procedure of SAS (Statistical Analysis Software, version 9.1, Cary, NC).

3.2.4 Determination of Seedling Stage for Treatment Application

To ascertain the optimum germination stage for initiating treatment application, seedling growth and development was evaluated every 8 h under the optimum germination temperature of 26°C in growth chambers (See section 3.2.3). The percentage of radicle, hypocotyl, and cotyledon emergence were recorded every 8 h using a Carl Zeiss® Stemi DV4 Series Stereo Microscope (Carl Zeiss MicroImaging Inc. Thornwood, NY) in combination with a Canon PowerShot A85 digital camera (Canon U.S.A., Inc., Lake Success, NY). The same experimental design and analysis was used as reported in the previous experiment.

3.2.5 Determination of Challenging Treatment Level for Heat Stress

Six seeds of 'Midnight' petunia per Petri dish were placed on one layer of presoaked cellulose film in 100 x 100 x 15 mm square Petri dishes that were positioned vertically in a square container. Fifty ml DI water was added into the square container to provide the sufficient water for seedling development during the test period. The growing system used was the same as described previously. Seeds were germinated at 26°C with a 12-h photoperiod of 3-4 umol.m-^{2}s^{1} and 80% RH in growth chambers. At the 96th h (4 d) after incubation, three seedlings were randomly selected from the six seedlings and subjected to challenging temperatures of 30, 35, 40, or 45ºC. These temperatures were chosen based on previous studies for the crops of *Prosopis chilensis* (Mol.) Koch (Medina and Cardemil, 1993), leguminous plants (Ortiz and Cardemil, 2001), and bedding plants (Niu and Rodriguez, 2006) with some modifications. The heat stress treatment was applied by gradual increments within 2 h period to increase the temperature from the control level (26°C) to the treatment levels (30, 35, 40, or 45°C). The seedlings were kept at the higher temperatures of 30, 35, 40, or 45°C for 3 h and then maintained at 26°C for an additional 7 d. The control was maintained at 26ºC. The experiment was conducted for 11 d. The hypocotyl length (mm) and radicle length (mm) were recorded by measuring the scanned images prior to and following treatments every day for 7 d.

The experimental design was a completely randomized design with repeated measures. There were twenty-seven seedlings per treatment/chamber. All treatments were replicated twice. Data analysis was performed by using Proc Mixed Procedure of SAS (Statistical Analysis Software, version 9.1, Cary, NC).

3.2.6 Determination of Challenging Treatment Level for Drought Stress

Six seeds were germinated in environmentally controlled growth chambers at 26°C with a 12-h photoperiod of 3-4 umol.m-^{2}s-^{1} and 80% RH (see section 3.2.3). After 96 h, 50 ml of Calbiochem® PEG 6000 (MW 6000, Molecular biology grade, EMD Chemicals Inc., Gibbstown, NJ) solutions replaced the DI water in each container for simulating drought stress. Three seedlings were selected from the six seedlings for data collection. Drought stress was simulated by using solutions of polyethylene glycol (PEG) 6000 according to the equation described by Michel (1983). Drought treatments include -0.2, -0.4, -0.6, or -0.8 MPa osmotic stress, which were measured by a vapor pressure osmometer of VAPRO® 5520 (Wescor, Inc, Logan, UT). The chosen osmotic potentials were based on previous research (Okcu *et al*., 2005; Den Berg and Zeng, 2006) with some modification. The control was DI water. The drought stress trial lasted 11d. Hypocotyl lengths (mm) and radicle lengths (mm) were measured by using the scanned images. Data was recorded every day for 7 d.

The experimental design was a completely randomized block design with repeated measure. Each of the three growth chambers was considered a block. Each growth chamber contained five containers, and each container contained five Petri dishes with three seedlings. The drought treatments of five levels were randomized to five containers. Thus, there were five containers (experimental units) per treatment and twenty-seven seedlings (sample units) per treatment. All treatments were replicated three times. Analysis was performed by using Proc Mixed procedure of SAS (Statistical Analysis Software, version 9.1, Cary, NC).

3.3 RESULTS

3.3.1 Growing System and Measurement for Seedling Growth

The two filter paper substrates allowed for germination; however, neither type provided sufficient contrast for scanning and measurement of seedling growth (Fig. 3.2). The clear cellulose film provided for the best visualization and also uniform germination and growth of seedlings.

Horizontal positioning of Petri dishes produced curved and twisted hypocotyl and radicle growth. Vertical positioning provided for straight, more measurable growth and a more uniform and controlled distribution of water across the substrate (Fig. 3.3). A volume of 50 ml solution was found as the optimum volume to be added in the containers that Petri dishes were in. The distance of the attached lines where seeds were placed from the bottom of Petri dish was 32 mm, and the 50 ml volume solution was 8 mm in height from the bottom of the five Petri dishes when set in the container. The distance not only insured the substrate uniformly absorbed the solution, but also prevented radicle growth into the solution.

When scanning the seedlings, a red color was selected as the scanning background to provide for the best contrast for measurements. The parameter of 400 dots per inch (DPI) resolution was used during the scanning process because this DPI provided for adequate definition for an accurate measurement with the least scanning time (5 minutes per scanning). The scanned images were measured using public software UTHSCSA ImageTool Version 3.0 (Department of Dental Diagnostic Science, University of Texas Health Science Center, San Antonio, TX). The unit of measure was pixels per cm and thus another public software, Pixels to Inches (or Centimeters) Converter (Classical Webdesigns, UK), was used to convert all pixel data to centimeters.

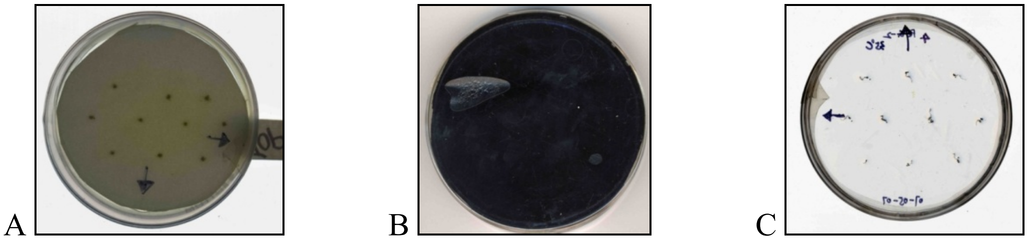

Figure 3.2. *Petunia x hybrida* ‘Dreams Midnight’ seed germinated at a horizontal position A) on white filter paper B) black filter paper or C) transparent cellulose film.

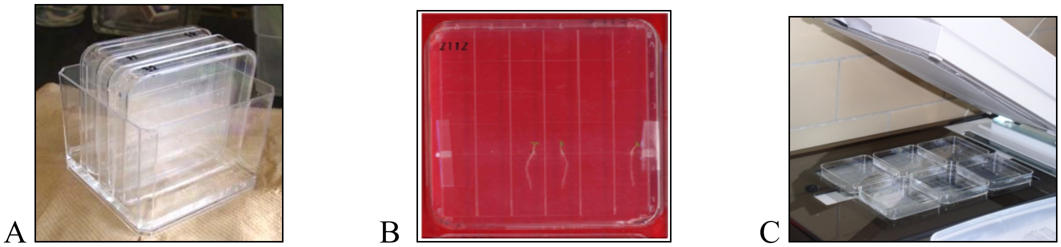

Figure 3.3. *Petunia x hybrida* ‘Dreams Midnight’ seed germinated on a A) vertical position on clear cellulose film within container, B) the scanned image from a scanner, and C) flatbed scanner.

3.3.2 Determination of Optimum Temperature for Seed Germination

There were significant differences in germination percentages between temperatures from 40 to 88 h (Table 3.1). The germination percentages were 100% at 24 and 26°C after 112 and 80 h, respectively. Germination of seeds at 24°C occurred 8 h later than it did at 26°C. The temperature of 26°C gave the faster and more uniform germination, while 30°C gave the lowest final germination percentage (Table 3.1). A temperature of 20°C represented the slowest germination rate and least uniformity at a T50 (number of hours to 50% germination) of 74 h (Fig. 3.4). A temperature of 26°C represented the most rapid germination rate with a T50 at 42 h.

3.3.3 Determination of Seedling Stage for Treatment Application

The results of this study indicated that testa of seeds were broken at 32 h under 26°C; germination (radicle protrusion) began at 40 h; the radicle started to emerge at 40-48 h; the hypocotyl started to emerge at 56-64 h; and the cotyledons emergence began at 64-72 h (Fig. 3.5).

Table 3.1. Percentage germination (radicle emergence) of *Petunia x hybrida* ‘Dreams Midnight’ at 20, 22, 24, 26, 28, or 30°C every 8 h for 120 h.

	Temperature						**Interaction**
Hours	**20°C**	**22°C**	**24°C**	**26°C**	**28°C**	**30°C**	**(Temp. x Hours)**
0h	[Y]0a[X]	0a	0a	0a	0a	0a	NS
8h	0a	0a	0a	0a	0a	0a	NS
16h	0a	0a	0a	0a	0a	0a	NS
24h	0a	0a	0a	0a	0a	0a	NS
32h	0a	0a	0a	0a	0a	0a	NS
40h	0a	0a	0a	37b	37b	33b	NS
48h	0a	0a	20a	73b	60b	57b	*
56h	0a	27b	60c	90d	80d	83d	*
64h	23a	43ab	67b	97c	90bc	90bc	*
72h	47a	60ab	83bc	97c	90c	90c	*
80h	60a	80ab	83ab	100b	90b	90b	*
88h	73a	83ab	90ab	100b	90ab	90ab	*
96h	80a	83a	90a	100a	90a	90a	NS
104h	90a	93a	90a	100a	93a	90a	NS
112h	93a	97a	100a	100a	97a	90a	NS
120h	93a	97a	100a	100a	97a	90a	NS

Values significant (*) or not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey’s correction.

[X] Means of germination percentage within rows followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey’s correction.

[Y] Values in the table are averages (n=60).

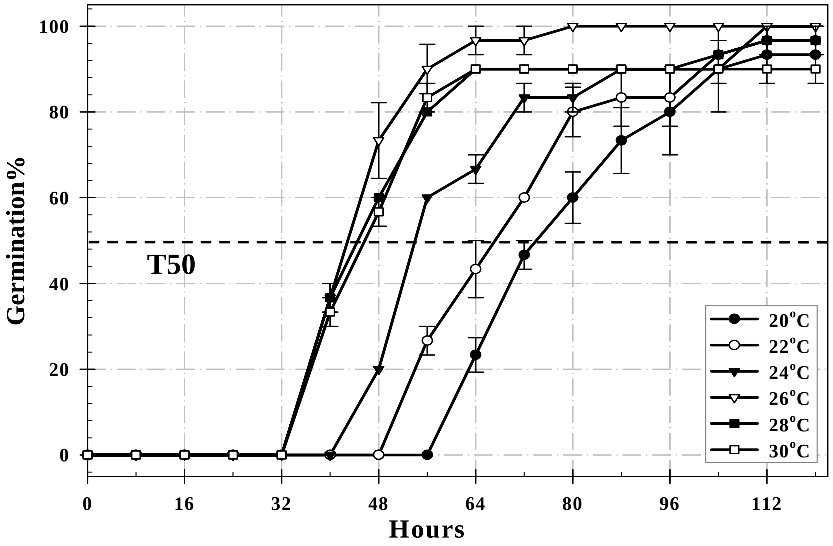

Figure 3.4. Percentage germination of *Petunia x hybrida* ‘Dreams Midnight’ at 20, 22, 24, 26, 28, or 30°C over 120 h. T50 indicates the number of hours to 50 % germination. The vertical lines associated with each mean represents ± SE (n=60).

Radicle emergence was 100% at 80 h; hypocotyl emergence was 100% at 112 h; and cotyledon emergence was 90% at 120 h (Fig. 3.6). The germination process took 32 h to complete development from initial radicle emergence to initial hypocotyl emergence at 26°C. The primary focus of this portion of research was to determine the timeline of radicle and hypocotyl emergence for 'Midnight' petunia for a precise application of heat or drought treatments. The stage for treatment application should be a measurable stage that is easy to distinguish, such as radicle and hypocotyl emergence of germinating seedlings, as shown in Figures 3.5 and 3.6. At 112 h, all seedlings showed 100% hypocotyl growth; however radicle growth was over developed. Thus, 96 h was chosen as the seedling stage for treatment application with greater than 85% hypocotyl emergence and the midpoint of 100% radicle emergence to 100% hypocotyl emergence (80 to 112 h). Based on these results, the seedling stage for initiating treatment application and measurement of hypocotyl and radicle growth was determined to be 96 h after placement on cellulose film for germination.

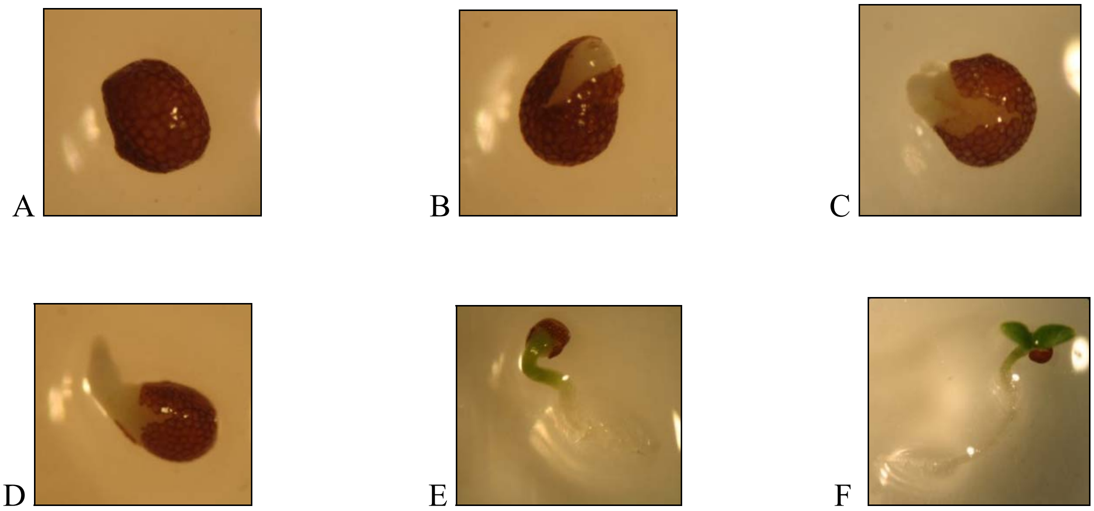

Figure 3.5. Ascertaining germination stages of *Petunia x hybrida* 'Dreams Midnight' at 26°C. Photographs were taken by using a Carl Zeiss® Stemi DV4 Series stereo microscope; A) seed at 0-24 h, B) testa broken at 32 h, C) germination at 32-40 h, D) radicle emerging at 40-48 h, E) hypocotyl emerging at 56-64 h, and F) cotyledon emergence at 64-72 h.

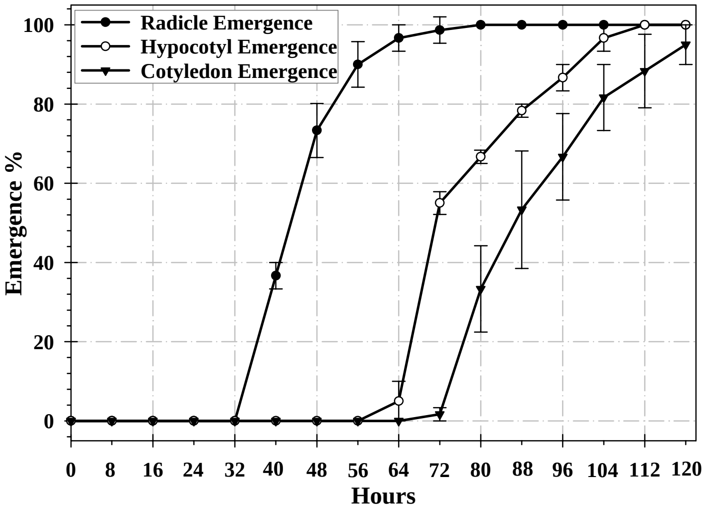

Figure 3.6. Percentage emergence of radicle, hypocotyl and cotyledon of germinating *Petunia x hybrida* ‘Dreams Midnight’ seed at 26°C over 120 h. Data were record every 8 h. The vertical lines associated with each mean represent ± SE (n=60).

3.3.4 Determination of Challenging Treatment Level for Heat Stress

A polynomial quadratic model was found to be the best for seedling growth curves to evaluate the effect of heat stress. The effect of temperature over time (day) was highly significant for hypocotyl and radicle growth (P <0.0001) (Table 3.2), which showed that seedling growth decreased over 7 d regardless of temperature. The effect of temperature alone was also highly significant for hypocotyl and radicle growth, which indicated that seedling growth curves had different intercepts or that the effect of temperature was measurable immediately after the heat shock treatment. The interaction of temperature and time were significantly different for hypocotyl and radicle growth, which showed that seedling growth curves had different slopes and, thus, the rate of growth decreased differently by temperature. To clearly interpret the growth curves, the regression equation for seedlings of each treatment over time are shown in Figs. 3.7 B&D.

Table 3.2. Regression test for hypocotyl or radicle growth of *Petunia x hybrida* 'Dreams Midnight'at 26, 30, 35, 40, or 45°C over seven days.

Effect	Days*Days	Heat	Heat*Days*Days
Hypocotyl length	<0.0001[Y]	<0.001	<0.0022
Radicle length	<0.0001	<0.0001	<0.0001

[Y] Values in table are *P*-values from the regression test.

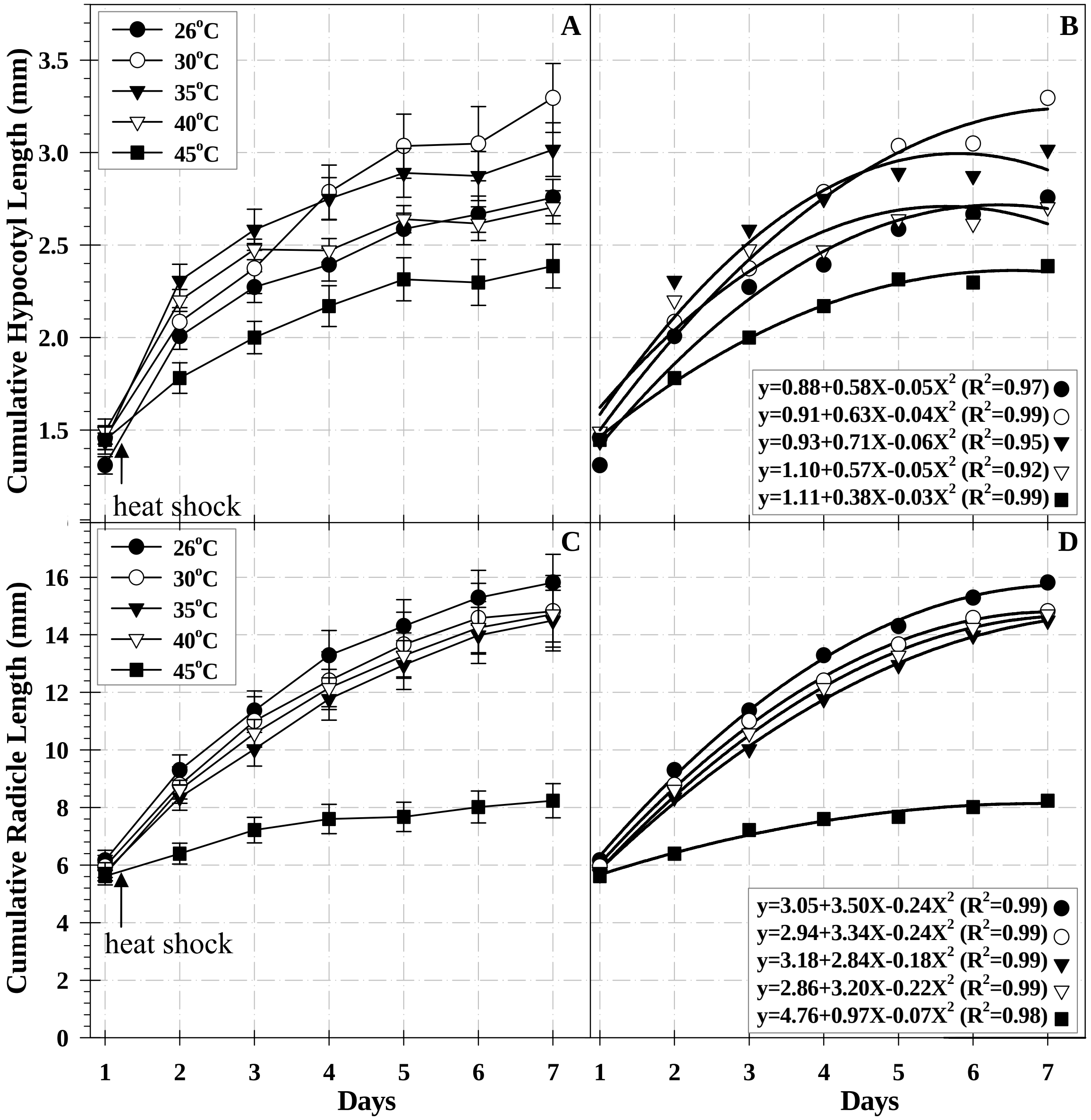

Figure 3.7. A) Cumulative hypocotyl growth; B) best fit model of cumulative hypocotyl growth; C) cumulative radicle growth; and D) best fit model of cumulative radicle growth of *Petunia x hybrida* 'Dreams Midnight' over 7 d after a 3 h heat shock (26, 30, 35, 40, or 45 ºC). The vertical lines associated with each mean represents ± SE (n=27). The polynomial quadratic curves were fitted to the data with an $R^2 \geq 0.92$ for hypocotyl growth and an $R^2 \geq 0.98$ for radicle growth by ProcMixed with $P < 0.01$ at $\alpha = 0.05$.

There was erratic hypocotyl growth during 7 d when plotted as cumulative increase in length or when modeled to fit a quadratic regression ((Table 3.2, Figs. 3.7A&B). The results also indicated that the optimum temperature for hypocotyl growth after 7 d was 30ºC (Fig. 3.7A), in contrast to the 26ºC determined for optimum radicle growth (Fig. 3.7C) and germination (Fig. 3.4). However, cumulative radicle growth increased more uniformly over time for all treatments (Figs. 3.7C&D). Radicle growth steadily decreased while temperature increased based on the growth curves (Fig. 3.7D). Heat shock of 45ºC had the greatest effect after 4 d for hypocotyl and radicle growth. The challenging temperatures of 30, 35, or 40ºC provided for uniform seedling growth without being lethal or deleterious to radicle growth when compared to 45ºC. During the 7 d after a 45ºC heat shock, the increase in radicle growth was 2.6 mm compared to 9.0 mm after a 40ºC heat shock (Table 3.4). Furthermore, heat shock of 45ºC most significantly decreased radicle growth to the extent that growth subsided after 3 d. Although radicle growth at 26, 30, 35 or 40ºC was not statistically different at each day measured (Table 3.4), the interaction between temperature and time was highly significant (Table 3.2) and uniformly decreased (Fig. 3.7D). Thus, measurement of radicle growth was a more accurate measurement of heat shock than hypocotyl growth in this study. Although radicle growth at 26, 30, 35 or 40ºC was not statistically different, the effect of temperature on seedling growth appears to be most critical between 40 and 45ºC for ‘Midnight’ petunia, and therefore 40ºC was chosen as the challenging temperature for determination of effect of heat tolerance because it was the temperature closest temperature to 45ºC that did not inhibit radicle growth.

3.3.5 Determination of Challenging Treatment Level for Drought Stress

Similar to the results found for heat shock treatments, the best fit growth curve was a polynomial quadratic model. The effect of drought stress over time (days) was highly significant

Table 3.3. Cumulative hypocotyl growth of *Petunia x hybrida* 'Dreams Midnight'at 26, 30, 35, 40, or 45°C over seven days.

Hypocotyl Length (mm)							
Temperature (°C)	**Days**						
	1	**2**	**3**	**4**	**5**	**6**	**7**
26	[Y]1.309a[X]	2.007a	2.272a	2.394ab	2.587ab	2.667ab	2.757ab
30	1.457a	2.085a	2.373a	2.786a	3.034a	3.048a	3.295a
35	1.439a	2.307a	2.583a	2.750ab	2.890a	2.873a	3.016a
40	1.491a	2.200a	2.476a	2.471ab	2.640ab	2.615a	2.704a
45	1.447a	1.781a	2.000a	2.170b	2.315b	2.297b	2.386b
Interaction (Temp.x Day)	NS	NS	NS	*	*	*	*

Values significant (*) or not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey's correction.

[X] Means of radicle length within columns followed by the same letters are not significantly different at the 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in table are averages (n=27).

Table 3.4. Cumulative radicle growth of *Petunia x hybrida* 'Dreams Midnight'at 26, 30, 35, 40, or 45°C over seven days.

Radicle Length (mm)							
Temperature (°C)	**Days**						
	1	**2**	**3**	**4**	**5**	**6**	**7**
26	[Y]6.164a[X]	9.294a	11.370a	13.287a	14.304a	15.290a	15.819a
30	5.951a	8.783a	11.002a	12.405a	13.661a	14.583a	14.817a
35	5.764a	8.347a	10.027a	11.770ab	12.952a	13.979a	14.496a
40	5.680a	8.619a	10.577a	12.154a	13.278a	14.242a	14.711a
45	5.612a	6.396a	7.217a	7.599b	7.674b	8.017b	8.235b
Interaction (Temp.x Day)	NS	NS	NS	*	*	*	*

Values significant (*) or not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey's correction.

[X] Means of radicle length within columns followed by the same letters are not significantly different at the 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in table are averages (n=27).

for hypocotyl and radicle growth (P <0.0001), which showed that seedling growth decreased over 7 d regardless of the imposed drought stress (Table 3.5). The effect of drought stress alone was not significant for hypocotyl nor radicle growth, which indicated that seedling growth curves had similar intercepts or that drought stress was not measurable immediately after imposed. The interaction of drought stress and subsequent growing time was highly significant for hypocotyl and radicle growth, which showed that seedling growth curves had a different slopes, and thus the rate of growth decreased differently by imposed drought stress. To clearly interpret the growth curves, the regression equation for seedlings of each treatment over time are presented in Figs. 3.8 B&D.

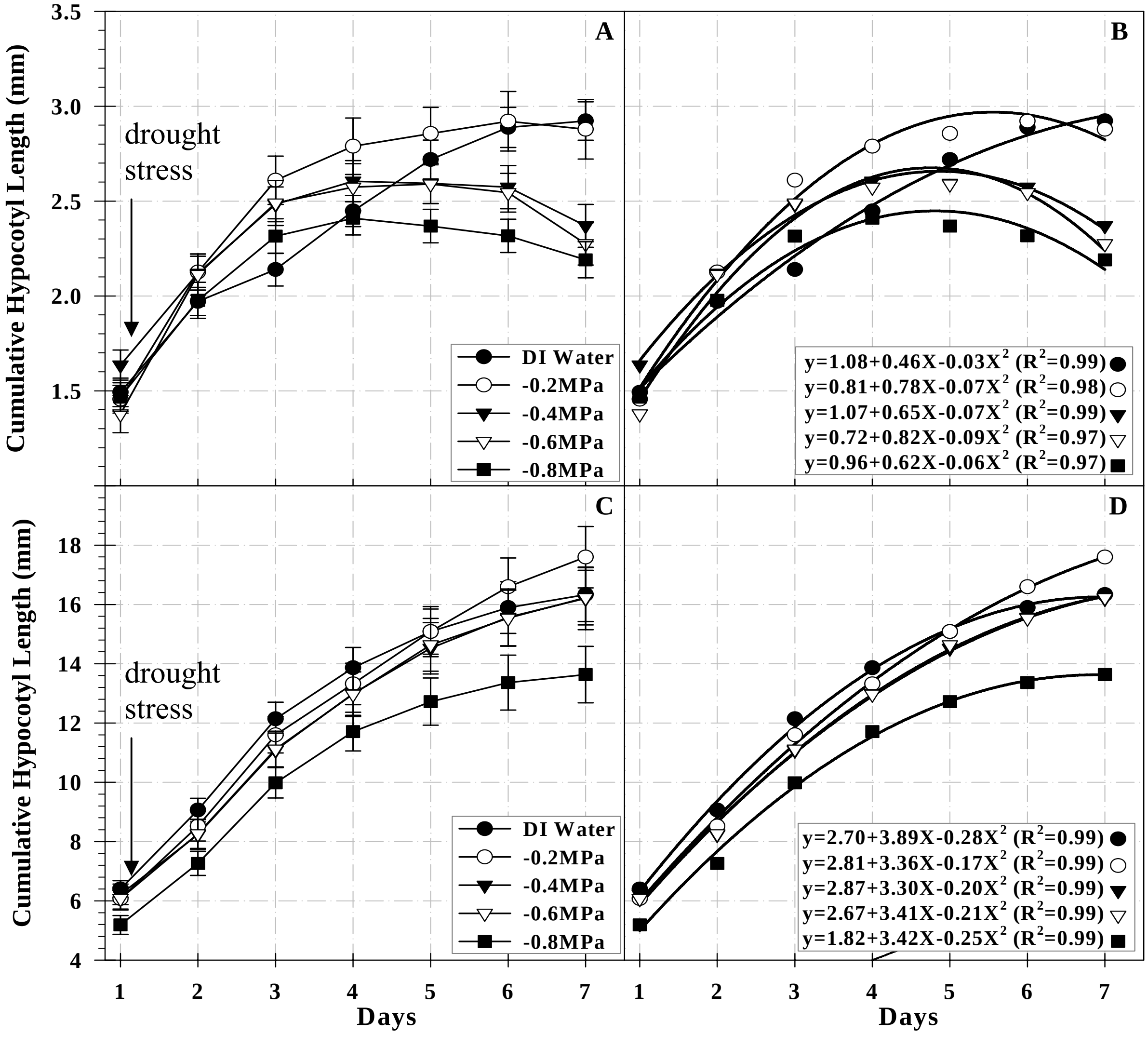

Figure 3.8. A) Cumulative hypocotyl growth B) regression of cumulative hypocotyl growth C) cumulative radicle growth and D) regression of cumulative radicle growth of *Petunia x hybrida* 'Dreams Midnight' under drought stress (0, -0.2, -0.4, -0.6, or -0.8 MPa) over time and the vertical line associated with each mean represents ± SE (n=27). The polynomial quadratic curves were fitted to the data with an $R^2 \geq 0.97$ for hypocotyl growth and an $R^2 \geq 0.99$ for radicle growth by ProcMixed with $P < 0.01$ at $\alpha = 0.05$.

Hypocotyl growth under increasing PEG concentrations showed erratic growth (Fig. 3.8A) as it did after heat shock treatments (Fig. 3.7A). There was no difference between -0.2, -0.4, -0.6 or -0.8MPa per day during 7d of measurement (Table 3.6).

Table 3.6. Cumulative hypocotyl growth of *Petunia x hybrida* 'Dreams Midnight' at drought stress of 0, -0.2, -0.4, -0.6, or -0.8 MPa over seven days.

Hypocotyl Length (mm)							
Osmotic potential (MPa)	**Days**						
	1	**2**	**3**	**4**	**5**	**6**	**7**
0	[Y]1.492a[X]	1.971a	2.139a	2.448a	2.719a	2.888b	2.922b
-0.2	1.456ab	2.126ab	2.610ab	2.789a	2.856a	2.921a	2.879a
-0.4	1.635ab	2.119ab	2.483ab	2.605a	2.593a	2.573ab	2.369ab
-0.6	1.377b	2.113b	2.490b	2.573a	2.590a	2.544ab	2.274ab
-0.8	1.467ab	1.977ab	2.315ab	2.409a	2.368a	2.317ab	2.191ab
Interaction (Drought x Day)	*	*	*	NS	NS	*	*

Values significant (*) or not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey's correction.

[X] Means of radicle length within columns followed by the same letters are not significantly different at the 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in table are averages (n=27).

Table 3.7. Cumulative radicle growth of *Petunia x hybrida* 'Dreams Midnight' at drought stress of 0, -0.2, -0.4, -0.6, or -0.8 MPa over seven days.

Radicle Length (mm)							
Osmotic potential (MPa)	**Days**						
	1	**2**	**3**	**4**	**5**	**6**	**7**
0	[Y]6.399a[X]	9.064a	12.138a	13.864a	15.084ab	15.894ab	16.330a
-0.2	6.080a	8.520a	11.602a	13.318a	15.085a	16.598a	17.596a
-0.4	6.218a	8.235a	11.082a	12.990a	14.521ab	15.567ab	16.203ab
-0.6	6.079a	8.262a	11.121a	12.970a	14.636ab	15.536ab	16.230ab
-0.8	5.190a	7.261a	9.982a	11.709a	12.722b	13.363b	13.633b
Interaction (Drought x Day)	NS	NS	NS	NS	*	*	*

Values significant (*) or not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey's correction.

[X] Means of radicle length within columns followed by the same letters are not significantly different at the 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in table are averages (n=27).

Radicle growth was not significantly affected by any treatment the first four days they were imposed (Table 3.7). However, at day 7, the control and -0.2 MPa treatments had greater radicle growth than at the -0.8 MPa treatment. Thus, results of this study indicated a challenging osmotic stress of -0.8 MPa most significantly affected seedling growth based on a quadratic growth model and change in cumulative growth of the radicle.

3.4 DISCUSSION

The clear cellulose film provided for a more uniform germination and growth of seedlings for ‘Dreams Midnight’ petunia and the highest quality images for measurement by using a flatbed scanner. Geneve and Kester (2001) identified that cellulose film can provide a clear germination medium for small seeds (tomato, impatiens, vinca, and marigold) with equivalent values to standard opaque germination media for the evaluation of seedling growth. Vertical positioning of the Petri dish avoided curved and twisted hypocotyl and radicle growth and provided for a more uniform distribution of solution across the substrate. Mangrich *et al.* (2006) used a vertical unit of two pieces of Plexiglas of (150 x 300mm) sandwiching seeds in wet paper towel with rubber bands to produce vertical radicle growth. Similarly, 10-cm square plates were used to germinate mutants of *Arabidopsis thaliana* (L.) Heynh seeds and were placed in a vertical position to create straight hypocotyl growth (Hong and Vierling, 2000). In this study, the thin copolymer fishing line held seedlings at the same height in the Petri dish when positioned vertically for ease of measurement and caused no deleterious effect on germination. During the process of seedling development, the radicle grew vertically on the cellulose film. However, some hypocotyls did not grow upright due to cotyledon emergence. The cotyledon created a tiny space between the hypocotyl and cellulose film causing the hypocotyl to grow apart from the substrate and in a more irregular fashion. This phenomenon appears to be the cause of a more sporadic measurable growth of hypocotyls found in this study (Figs 3.7 & 3.8).

The Association of Official Seed Analysts (2001) indicated that the optimum temperature range for germination of *Petunia x hybrida* was from 20 to 30°C due to a wide variety of petunia species and cultivars. Results from this study identified 26°C as the optimum germination temperature for petunia ‘Dreams Midnight’, and a temperature of 30°C was the optimum temperature for hypocotyl development. Results from this study also indicated that heat shock temperatures during germination and seedling development could deleteriously affect seed germination and seedling growth rates. These effects were consistent with heat stress studies conducted by Medina and Cardemil (1993), which indicated that high temperatures of 30, 35, 40, or 45°C can reduce germination percentage, and no seeds germinated at 50°C for *Prosopis chilensis* (Mol.) Koch.

My study also indicated that heat shock weakened seedling growth as shown in previous research with a leguminous tree (*Prosopis chilensis* (Mol.) Koch) (Medina and Cardemil, 1993) and two other leguminous plants (Ortiz and Cardemil, 2001). Both studies measured total seedling length. In my study, the hypocotyl and radicle length were measured separately to determine which parameter was more sensitive to the imposed treatment and provided consistent growth for a more precise measurement. Hong and Vierling (2000) measured hypocotyl growth of *Arabidopsis thaliana* (L.) Heynh to screen for heat tolerance among mutants and found that the treatment of 2 h at 45°C damaged all seedling growth. This was similar to the results in this study, in which a heat shock of 3 h at 45°C significantly reduced hypocotyl growth of ‘Dreams Midnight’ petunia.

Increasing concentrations of osmoticum used to simulate drought stress of ‘Midnight’ petunia decreased hypocotyl and radicle growth, most significantly at -0.8 MPa. Okcu *et al*. (2005) found that seed germination percentages and seedling growth of three peas varieties (*Pisum sativum* L.) (‘Bolero’, ‘Sprinter’, and ‘Utrillo’) were restrained under drought stress of -0.2, -0.4, -0.6, or -0.8 MPa simulated by sodium chloride (NaCl) and PEG 6000. Results indicated that all cultivars could germinate under all the drought stress levels, but germination percentage decreased with the increasing concentrations of PEG 6000. The three cultivars 'Bolero', 'Sprinter', and 'Utrillo' all showed no root growth at -0.8 MPa and no shoot growth at -0.6 MPa. Finally, the cultivar of 'Sprinter' was considered to appear more drought tolerance because it produced the higher root length and seedling dry weight at -0.6 MPa.

Seedling growth of five chaffy-seeded grass cultivars of big bluestem (*Andropogon gerardii* Vitman), sand bluestem (*Andropogon hallii* Hack.), little bluestem (*Schizachyrium scoparium* (Michx.) Nash), yellow bluestem (*Bothriochloa ischaemum* (L.) Keng. var. *ischaemum* (Hack.) Celarier and Harlan), and indiangrass (*Sorghastrum nutans* (L.) Nash) were tested under drought stresses of -0.2, -0.4, -0.8, or -1.6 MPa simulated by mannitol. Results indicated that drought stress resulted in slower seedling growth and less shoot/root dry weight during early seedling growth. The cultivar of yellow bluestem showed a slightly greater effect under increasing drought stress, but it was not statistically significant from the other cultivars (Springer, 2005). Den Berg and Zeng (2006) indicated that seed germination percentage and seedling growth of three grasses (*Anthephora pubescens* Nees, *Heteropogon contortus* L., and *Themeda triandra* Forssk.) were inhibited under drought stresses of -0.3, -0.6, -0.9, -1.2, -1.5, -1.8 or -2.1MPa by using PEG 6000. No seeds germinated at -2.1MPa. They observed that root and shoot length decreased with increasing drought stress from -0.6 to -1.2MPa. However, they were unable to determine the level of treatment that was most effective for imposing drought stress based on seedling growth and which species was more drought tolerant among the three grasses.

Previous research has investigated the effect of various heat or drought treatments and treatment levels on seedling growth. Based on the results from this study, the methods used to impose heat shock or drought stress reduced seedling growth of petunia 'Dreams Midnight'. Moreover, the heat shock of 40ºC was chosen as the challenging temperature for determination of effect of heat tolerance, and the osmotic stress of -0.8 MPa most significantly affected seedling growth based on a quadratic growth model and change in cumulative growth of the radicle. In addition, five days after germination was determined to be the most appropriate stage to measure treatment effect on radicle growth. These treatment values and measurements of radicle growth will be used as a basis for efficiently and effectively screening for heat and drought tolerance of petunia.

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CHAPTER 4. SCREENING SEEDLING GROWTH FOR HEAT OR DROUGHT TOLERANCE IN DIFFERENT PETUNIA CULTIVARS

4.1 INTRODUCTION

Bedding plants are one of the primary components of the floriculture industry; the largest consumer is the public and the second largest are landscape contractors (Copes, 2000; Fossler, 1993). However, two of the greatest impediments affecting growth and development of these plants in greenhouse production and landscape are high temperature stress and drought stress. Heat stress is defined as the situation in which the air temperature is high enough to cause irreversible damage to plant growth and development (Hall, 2001). Similarly, drought stress can be defined as an insufficient availability of water that can cause irreversible damage to plant growth and development.

The deleterious effects of these stresses alone or in tandem can occur during production of a finished plant, during postproduction, during sales or after transplanting into the landscape. It had been estimated that the losses could be as high as 20% during processing of a finished bedding plant through postproduction (Armitage, 1989). Heat stress can affect some morphological characteristics, such as reducing plant height, plant yield, leaf area, stem and leaf thickness, root growth, and flower size (Gusta *et al.*, 1997; Harding *et al.*, 1990; Medina and Cardemil, 1993; Natarajan and Kuehny, 2008; Ortiz and Cardemil, 2001). Moreover, it can affect some physiological characteristics, such as cell membrane stability, stomatal conductance, net photosynthesis, transpiration (Medina and Cardemil, 1993; Ortiz and Cardemil, 2001; Natarajan and Kuehny, 2008), damaging components of leaf photosynthesis, and reducing carbon dioxide assimilation (Al-Khatib and Paulsen, 1999; Jiao and Grodzinski, 1996).

Meanwhile, drought stress can also affect some morphological and physiological characteristics. Drought stress reduced seed germination, seeding growth parameters, seedling height, and dry matter of seedlings (Grzesiak *et al*., 1996). Drought stress affected leaf gaseous exchange, leaf water potential, chlorophyll content and fluorescence, and leaf injury (Grzesiak *et al*., 2003). Based on these effects, some morphological characteristics, such as erectness of leaves, stem, leaf thickness, and canopy compactness, and physiological characteristics, such as cell membrane thermostability, stomatal conductance, and single leaf net photosynthesis, have been used as screening tools for heat or drought tolerance in some investigations. However, most of these studies were conducted on agronomic crops (Wright *et al*., 2001, Knight and Ackerly, 2003, Kimurto *et al*., 2005; Valladares and Pearcy 1997). Very little research has been conducted on ornamental bedding plants (Heschel and Riginos, 2005; Natarajan, 2005; Niu and Rodriguez, 2006; Zollinger *et al*., 2006).

Intensive management practices, such as frequent irrigation and specialized cooling, are used to alleviate heat and drought stresses. However, these practices are often expensive and of limited effectiveness, especially when these plants are being marketed or planted in the landscape. Breeding work conducted by bedding plant companies continues to provide new or improved series and varieties every year. The selection of breeding lines is primarily based on morphological characteristics such as flowering, internode length and leaf size. Extensive regional trials are also conducted across the U.S. to help provide guidance to breeders and growers. However, the data collected during these trials is not coordinated nor organized, such that significant conclusions can help determine specific outcomes.

The objective of this study was to screen different petunia series, types and cultivars under heat shock or drought stress with the seedling growth sensitivity test (SGST), a method used to measure radicle lengths of germinated seeds by using digital imaging prior to and following heat or drought treatment.

4.2 MATERIALS AND METHODS

4.2.1 Plant Material

Nineteen cultivars of *Petunia x hybrida* (Table 4.1) were chosen for use in this study. Sixteen cultivars were selected based on their overall performance rating as evaluated by Kelly *et al*. (2007). These cultivars can be grouped into three plant classes; floribunda, grandiflora, and spreading. These classes and cultivars can be further separated into eight different series of petunia; Madness, Dreams, Storm, Ultra, Easy Wave, Ramblin, Avalanche, and Wave. Two cultivars, either the best or worst rated for overall performance were chosen from each series (Kelly *et al*., 2007). Cultivars of 'Mitchell Diploid' and '44568' were provided by the University of Florida. The '44568' is transgenic ethylene-insensitive petunia and 'Mitchell Diploid' is its wild type. Since ethylene is considered as a stress hormone (Hays *et al.*, 2007; Munné-Bosch *et al.*, 2004; Rizhsky *et al.*, 2002), the ethylene insensitive petunia of '44568' was investigated in this study to explore if it would represent different seedling growth sensitivity from 'Mitchell Diploid' under heat or drought. Dreams 'Midnight' was also used in this experiment as the standard for comparison with previous research (Chapter 3) and to validate the accuracy of the technique, SGST, developed in the previous study. Due to the large number of cultivars used in this study, all the cultivars were arranged by numbers as shown in Table 4.1 so that the results could be more conveniently described in the tables or figures. Seeds were stored in the refrigerator from July to September 2008 with the temperature of approximately 0°C and an average relative humidity (RH) of approximately 60% (Figs. 4.1A&B) recorded by a HOBO® Pro Series™ data logger (Onset Computer Corporation, Bourne, MA) which was placed in the same box with the seeds (Figs. 4.1A&B).

Table 4.1 The class, number, series and cultivar, and overall performance of selected *Petunia x hybrida* as evaluated (Kelly *et al*. 2007).

Class	No.	Selected Cultivars	Overall Performance
Floribunda	1	‘Madness Waterfall Mix’	Best
	2	‘Madness Lavender Glow’	Worst
Grandiflora	3	‘Dreams Burgundy Picotee’	Best
	4	‘Dreams Wild Rose Mix’	Worst
	19	‘Dreams Midnight’	Unknown
Grandiflora	5	‘Storm Violet’	Best
	6	‘Storm Red’	Worst
Grandiflora	7	‘Ultra Salmon’	Best
	8	‘Ultra Red’	Worst
Grandiflora	17	‘44568’*	Unknown
	18	‘Mitchell Diploid’*	Unknown
Spreading	9	‘Easy Wave Shell Pink’	Best
	10	‘Easy Wave Red’	Worst
Spreading	11	‘Ramblin Lavender’	Best
	12	‘Ramblin White’	Worst
Spreading	13	‘Avalanche Lilac’	Best
	14	‘Avalanche White’	Worst
Spreading	15	‘Wave Pink’	Best
	16	‘Wave Blue’	Worst

*‘Mitchell Diploid’ and ‘44568’ were provided by Dr. David G. Clark from University of Florida (Gainesville, FL). Other seeds were contributed by Ball Horticultural Company (West Chicago, IL).

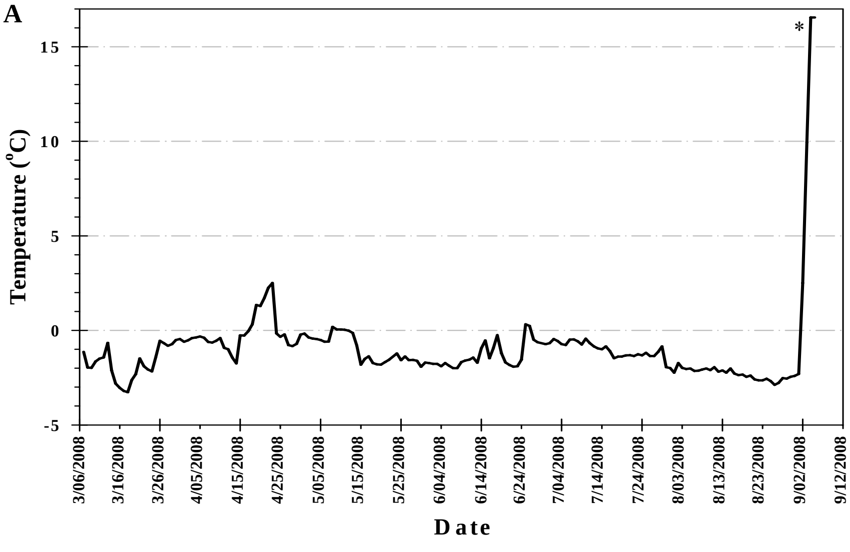

Figure 4.1A. Ten-day average air temperature during storage of all *Petunia x hybrid* seeds inside a refrigerator at Louisiana State University, Baton Rouge.
*Temperature increased because of power failure.

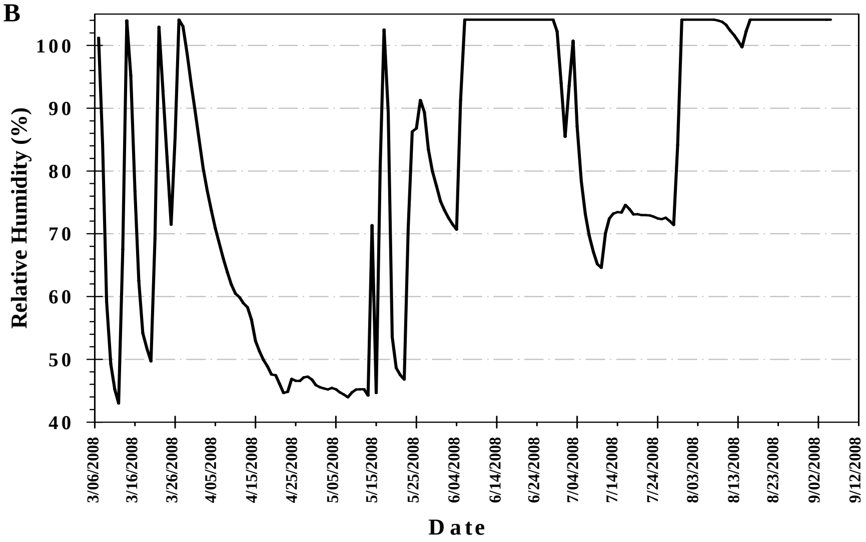

Figure 4.1B. Ten-day average relative humidity during storage of all *Petunia x hybrid* seeds inside a refrigerator at Louisiana State University, Baton Rouge.

4.2.2 Heat Treatment Application

Ten seeds of each cultivar per Petri dish were placed on one layer of presoaked cellulose film in 100 x 100 x 15 mm square Petri dishes that were positioned vertically in a square container of 100 x 100 x 83 mm (Pioneer Plastics, Inc., Dixon, KY). The amount of 50 ml DI water was added into containers. The growth system was similar to that designed in Chapter 3. Seeds were placed on the top of a 0.23mm thin copolymer fishing line (G. Pucci & Sons, Inc., Brisbane, CA) which was attached to the Petri dishes by Scotch® tape (3M Inc., St. Paul, MN) to keep seedling sat the same position in the dish (Fig. 4.2A). To minimize evaporative losses, all containers were put into a clear plastic container of 430 x 350 x 150 mm (Really Useful Products Ltd., West Yorkshire, UK) (Fig. 4.2B). Four layers of paper towel (SCA Tissue North America, Neenah, WI) were placed on the bottom of the container and moistened with 150 ml DI water (Fig. 4.2C). Finally, the sealed containers with seeds were placed into growth chambers (EGC, Chagrin Falls, OH) (Fig. 4.2D). Seeds were germinated at 26°C with a 12-h photoperiod of 3-4 umol.m^{-2}s^{-1}and 80% RH in growth chambers (EGC, Chagrin Falls, OH). After 96 h (4 d) incubation, four seedlings were randomly chosen and kept in the Petri dish as experimental units to be measured for the SGST analysis.

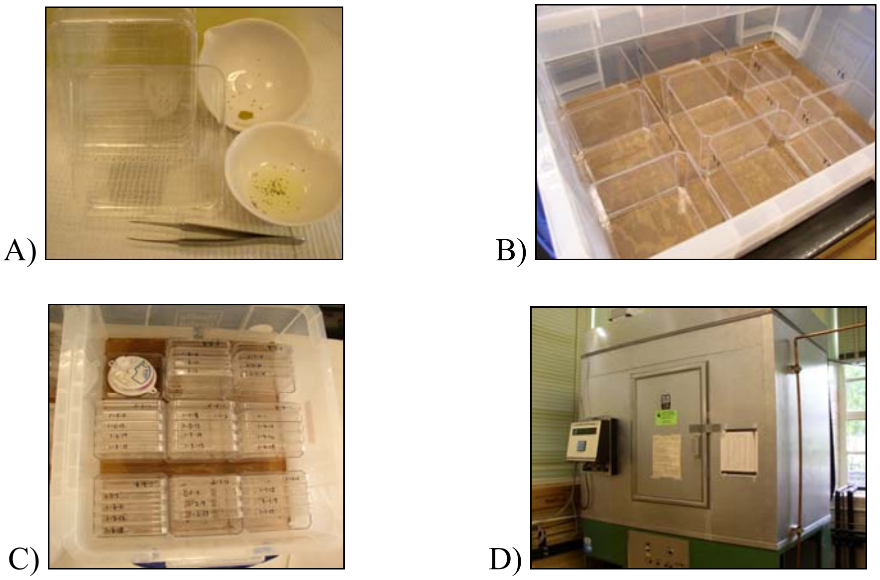

Figure 4.2. Growth system for the SGST analysis: A) seeds placed above the copolymer line within the Petri dish, B) containers within the plastic box with 4 layers moistened paper towel, C) Petri dishes and HOBO® within the plastic box, and D) EGC growth chamber.

Initial radicle lengths for those selected samples were measured at 96 h by using the method described in Chapter 3. The seedlings were subjected to a challenging temperature of 40ºC. The heat stress treatment was applied by gradual increments in temperature over a 2 h period from the control level of 26ºC to the treatments level 40°C. The challenging temperature of 40°C was maintained 3 h and then returned to 26°C for an additional 4 d. The control group was maintained at 26 ºC. Radicle length (mm) was recorded by measuring the scanned images prior to and following treatments after 4 d. The relative growth rate (RGR) of the radicles was calculated for data analysis due to the variation in the initial radicle lengths measured between cultivars. The relative growth rate was expressed as growth in terms of a rate of increase in length per unit of length providing for a more equitable comparison of radicle growth analysis. $\mathrm{RGR} = (1/L)\,(\Delta L/\Delta t) = \Delta\,(\log_e L) / \Delta t = (\log_e L_2 - \log_e L_1) / (t_2 - t_1)$, in which L_2= radicle length at

5 d, L_1= radicle length at 1 d, T_2= 5 d, T_2= 1 d, the unit of RGR is $mm.mm^{-1}d^{-1}$. That is the natural log of the mean length over the interval of days measured (Hunt, 1990).

The factorial experiment was arranged in a completely randomized design. The first factor was temperature and the second was cultivar. There were 16 seedlings per cultivar per treatment per chamber. All treatments were repeated twice. Data analysis was performed by using Proc Mixed Procedure of SAS (Statistical Analysis Software, version 9.1, Cary, NC).

4.2.3 Drought Treatment Application

Ten seeds were germinated in environmentally controlled growth chambers under 26°C with 12-h light of 3-4 $umol.m^{-2}s^{-1}$ and above 80% RH. After 96 h, 50 ml of Calbiochem® PEG 6000 (MW 6000, Molecular biology grade, EMD Chemicals Inc., Gibbstown, NJ) solution was replaced with DI water in each container for simulating drought stress. Four seedlings were selected from the ten seedlings for data analysis. Drought stress was stimulated as reported by Michel (1983). The osmotic potential of -0.8 MPa was chosen as the treatment value based on the results found in Chapter 3. The control was DI water. The parameter of radicle length (mm) was recorded by measuring the scanned images as described previously. Radicle length was recorded prior to and following treatments after 4 d to calculate the relative growth rate for analysis as described previously.

The experiment was a randomized block design. Each growth chamber was considered a block. Eight treatment containers were placed within each growth chamber, and each container had five Petri dishes with four seedlings. Thus, there were four containers (experimental units) per treatment and twelve seedlings (sample units) per treatment. Statistical analysis was performed by using Proc Mixed procedure of SAS (Statistical Analysis Software, version 9.1, Cary, NC).

4.3 RESULTS

4.3.1 Radicle Growth for Different Cultivars under Heat Shock

Based on the radicle RGR of cultivars as categorized by plant type, the main effect of temperature was significantly reduced radicle RGR (*P*=0.0099), and plant classes showed significantly different radicle RGR (*P*=0.0096) (Table 4.2). The class of spreading petunia represented the highest radicle RGR of 0.048 (Table 4.2, Fig. 4.3). However, the interaction between plant type and temperature were not significant at *P*> 0.05 (Table 4.2).

The results from different cultivars indicated that the different genotypes of petunia exhibited significant differences (*P*<0.0001) in radicle RGR (Fig. 4.4) regardless of the heat shock treatment. 'Avalanche Lilac' (0.134 RGR) had a significantly more rapid radicle RGR than all other cultivars (Fig. 4.4). The cultivars of 'Dreams Burgundy Picotee' (0.052 RGR), 'Ultra Salmon' (0.052 RGR), 'Ramblin Lavender' (0.052 RGR), 'Avalanche White' (0.052 RGR), 'Wave Blue' (0.060 RGR), and 'Dreams Midnight' (0.077 RGR) had significantly different radicle RGR from the cultivars of 'Easy Wave Red' (0.021 RGR) and '44568' (0.022 RGR), which had the lowest RGR (Fig. 4.4).

Table 4.2. Radicle relative growth rates (RGR) of three classes of petunia seedlings grown under control (26°C) or heat shock (40°C).

Temperature (°C)	**Plant Class**	**RGR (mm mm^{-1}d^{-1})**
26	Floribunda	^{Y}0.038
	Grandiflora	0.050
	Spreading	0.057
40	Floribunda	0.036
	Grandiflora	0.040
	Spreading	0.048
Temperature		*
Plant Class		*
Interaction		NS

Values significant (*) or not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey's correction.

[Y] Values in table are averages. Each class has different numbers of observation: Floribunda (n=32), Grandiflora (n=144), and Spreading (n=128).

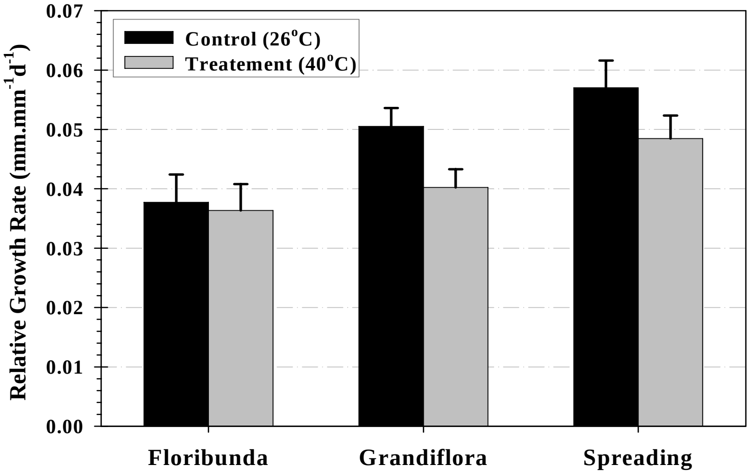

Figure 4.3. Comparison of raicle relative growth rates (RGR) within each of petunia classes under control (26°C) and heat shock (40°C) treatment. The vertical lines associated with each mean represent ± SE.

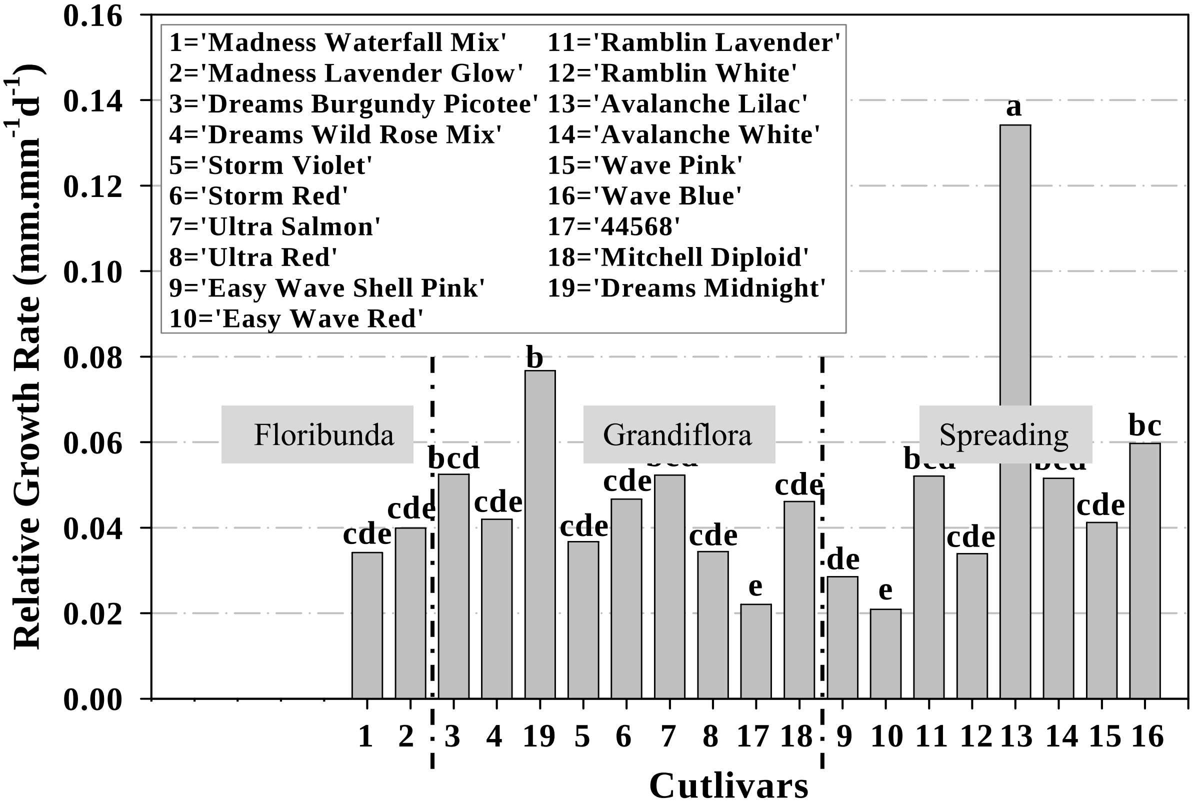

Figure 4.4. Relative growth rates of 19 *Petunia x hybrida* cultivars during 5 d of growth recovery period after heat shock at 40°C for 3 h. The 19 cultivars were categorized into three plant types of floribuna, grandiflora, and spreading. Means of each bar markered by the same letters are not significantly different at the 5% by lsmean procedure in SAS with Tukey's correction (n=32).

Table 4.3. Radicle relative growth rates (RGR) of 19 *Petunia x hybrida* cultivars during 5 d of growth recovery period after heat shock (40°C) for 3 h.

Cultivars	**No.**	**Temperature(°C)**	**RGR(mm.mm^{-1}d^{-1})**
‘Madness Waterfall Mix’	1	26	^{Y}0.029
		40	0.040
‘Madness Lavender Glow’	2	26	0.047
		40	0.033
‘Dreams Burgundy Picotee’	3	26	0.067
		40	0.037
‘Dreams Wild Rose Mix’	4	26	0.043
		40	0.041
‘Storm Violet’	5	26	0.047
		40	0.026
‘Storm Red’	6	26	0.046
		40	0.047
‘Ultra Salmon’	7	26	0.061
		40	0.044
‘Ultra Red’	8	26	0.044
		40	0.025
‘Easy Wave Shell Pink’	9	26	0.033
		40	0.024
‘Easy Wave Red’	10	26	0.025
		40	0.017
‘Ramblin Lavender’	11	26	0.042
		40	0.062
‘Ramblin White’	12	26	0.032
		40	0.035
‘Avalanche Lilac’	13	26	0.158
		40	0.111
‘Avalanche White’	14	26	0.065
		40	0.038
‘Wave Pink’	15	26	0.036
		40	0.047
‘Wave Blue’	16	26	0.066
		40	0.054
‘44568’	17	26	0.026
		40	0.018
‘Mitchell Diploid’	18	26	0.044
		40	0.049
‘Dreams Midnight’	19	26	0.079
		40	0.074
Temperature			*
Cultivar			*
Interaction			*

Values significant (*) or not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey’s correction.

^{Y} Values in table are averages (n=16).

The temperature of 40°C significantly impaired radicle RGR (*P*=0.0011). The interaction between cultivar and heat shock treatment was significant, and thus the high temperature did not impair radicle RGR of these cultivars at the same way (Table 4.3). The results shown in Fig 4.5 indicated some cultivars, for example 'Madness Waterfall Mix' (0.029 vs. 0.040 RGR), 'Ramblin Lavender' (0.042 vs. 0.062 RGR), and 'Wave Pink' (0.036 vs. 0.047 RGR), had an increased radicle growth rate when subjected to the temperature of 40°C. However, cultivars of 'Dreams Wild Rose Mix' (0.043 vs. 0.041 RGR), 'Storm Red' (0.046 vs. 0.047 RGR), 'Ramblin White' (0.032 vs. 0.035 RGR), 'Mitchell Diploid' (0.044 vs. 0.049 RGR), and 'Dreams Midnight' (0.079 vs. 0.074 RGR) had no change in growth rate after heat shock of 40°C. The remaining cultivars had decreased radicle RGR after subseqyent to 3 h high temperature treatment.

However, the 3 h heat shock had no significant effect on radicle relative growth rate of cultivars, except the cultivars of 'Dreams Burgundy Picotee' and 'Avalanche Lilac' (Fig 4.5). Heat shock decreased the RGR of 'Dreams Burgundy Picotee' by 0.03 and 'Avalanche Lilac' by 0.047. This indicated that these two cultivars may be less heat tolerant compared to the remaining cultivars because the heat shock had a significantly more negative effect,, even though 'Avalanche Lilac' had the greatest radicle growth rate. Ornamental plant breeders often select breeding lines that have a more vigorous growth rate as being more stress tolerant in that they might have the ability more rapidly out-grow the effect of the imposed stress (Ball Horticultural, personal communications). However, this does not appear to be the case with 'Avalanche Lilac' and heat tolerance. The remaining cultivars had similar growth rates between the control and heat shock treatment. Thus, those cultivars could be characterized as more heat tolerant than the cultivars 'Dreams Burgundy Picotee' and 'Avalanche Lilac'.

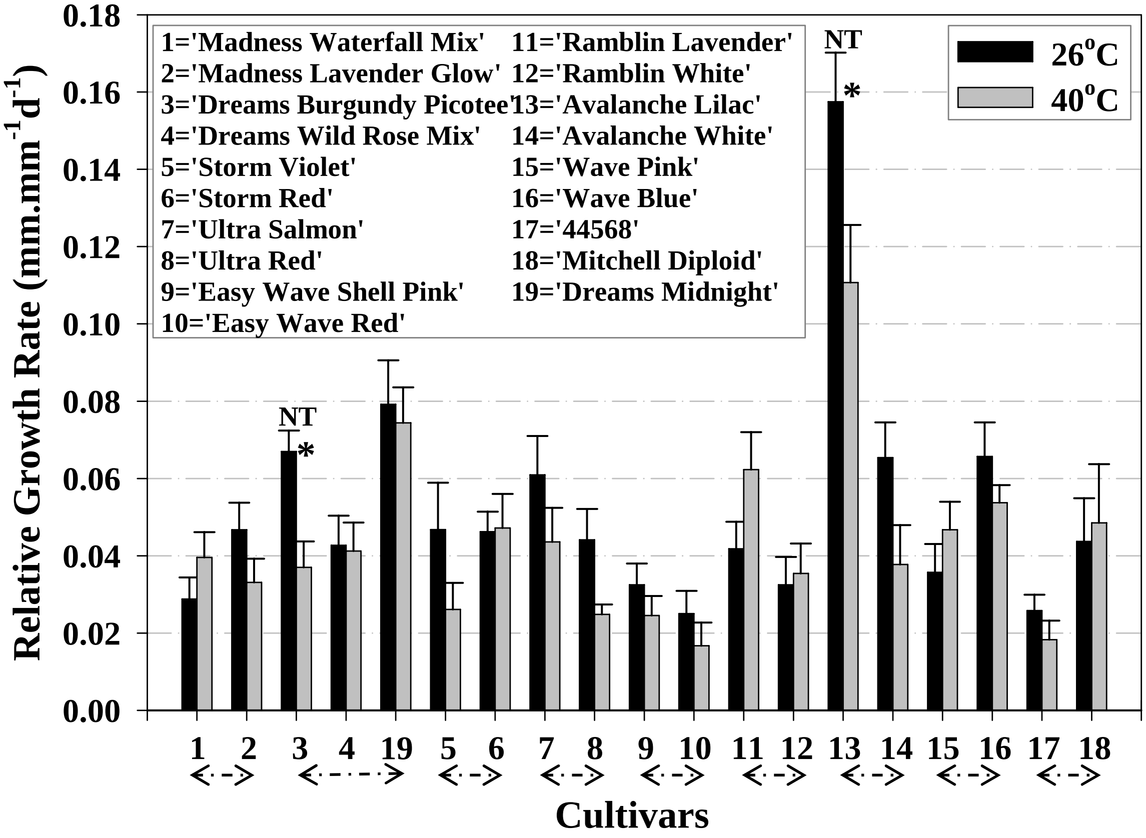

Figure 4.5. Radicle relative growth rates of 19 *Petunia x hybrida* cultivars under control (26°C) and heat shock (40°C) for 3 h. 'NT' represented the non-tolerance. The arrow means that cultivars are from the same series. Values significant (*) at the 5% level by the lsmean procedure in SAS with Tukey's correction. The vertical lines associated with each mean represent ± SE (n=16).

Cotyledon burning or damaging symptoms were noticed for 'Dreams Burgundy Picotee' and 'Avalanche Lilac' after the 3 h heat shock with the temperature of 40°C (Figs 4.6A, B, &C) indicating their greater sensitivity to high temperature. The remaining cultivars represented more normal seedling development, and no necrosis was observed (Figs 4.6D, E, &F).

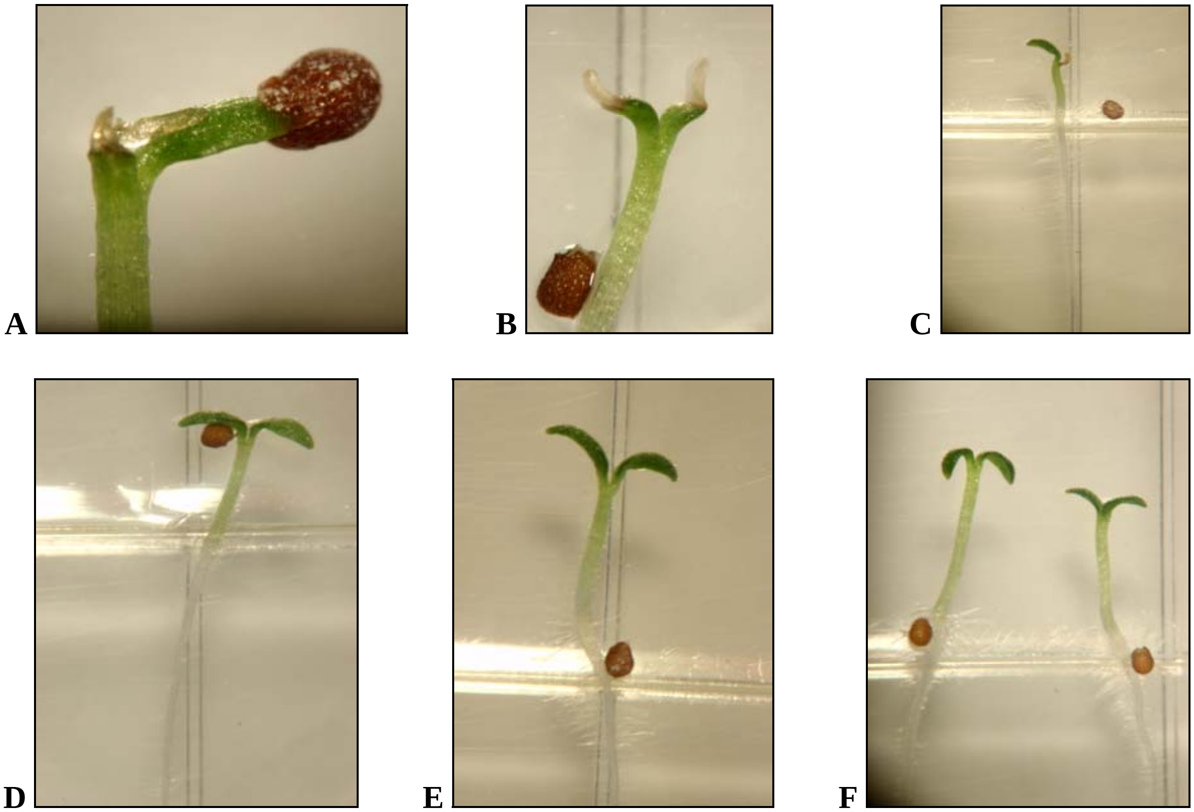

Figure 4.6. Seedling development of petunia cultivars under heat shock (40°C). Photographs were taken by using a Carl Zeiss® Stemi DV4 Series stereo microscope. A) & B) ‘Avalanche Lilac’, C) ‘Dreams Burgundy Picotee’, D) ‘Madness Waterfall Mix’, E) ‘Storm Red’, and F) ‘Easy Wave Red’.

4.3.2 Radicle Growth for Different Cultivars under Drought Stress

The main effects plant class (P=0.0002) and drought stress (P=0.0012) on radicle RGR were significant, but the interaction of plant class and drought stress was not significant at the conventional 0.05 level (Table 4.4). Drought stress significantly reduced the radicle growth rate of the three petunia types. The spreading petunia class showed greater radicle RGR than the other two. Even though the spreading petunia class appeared to be more negatively impacted by drought stress, there was no interaction of plant class and drought stress for all cultivars, and thus no effect of drought stress on petunia type (Fig. 4.7).

Table 4.4. Radicle relative growth rates (RGR) of three plant classes of petunia under control (DI water) or drought stress (-0.8MPa).

Drought stress (MPa)	**Plant Class**	**RGR ($mm.mm^{-1}d^{-1}$)**
	Floribunda	[Y]0.030
0	Grandiflora	0.035
	Spreading	0.051
	Floribunda	0.027
-0.8	Grandiflora	0.027
	Spreading	0.036
Drought stress		*
Plant Class		*
Interaction		NS

Values significant (*) or not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey's correction.

[Y] Values in table are averages. Each class has different numbers of observation: Floribunda (n=32), Grandiflora (n=144), and Spreading (n=128).

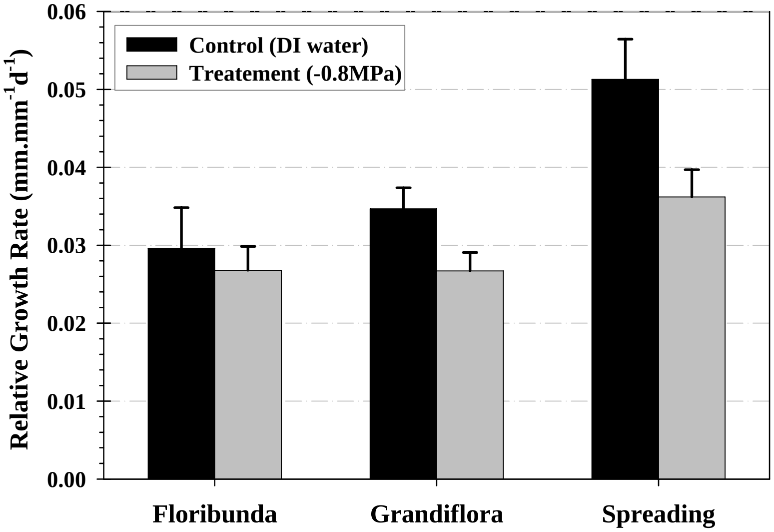

Figure 4.7. Radicle relative growth rates (RGR) of three plant types of petunia under control (DI water) or drought stress (-0.8 MPa). The vertical lines associated with each mean represent ± SE.

Similar to the results from the heat shock study, there was a significant difference in the radicle RGR between petunia cultivars (*P*<0.0001) on (Table 4.5). Drought stress significantly affected the radicel growth (*P*<0.0009) (Table 4.5). 'Avalanche Lilac' (0.122 RGR) had the most rapid radicle growth rate compared to all other cultivars. The cultivars 'Easy Wave Shell Pink'

(0.015 RGR) and ‘Easy Wave Red’ (0.011 RGR) had the lowest raidcle RGR when compared to the others. The cultivars of ‘Avalanche White’ (0.061 RGR) and ‘Wave Blue’ (0.055 RGR) had significantly different radicle RGR from the cultivars of ‘Madness Lavender Glow’ (0.026 RGR), ‘Dreams Wild Rose Mix’ (0.029 RGR), ‘Storm Violet’ (0.027 RGR), ‘Storm Red’ (0.026 RGR), ‘Ultra Red’ (0.018 RGR), ‘Ramblin White’ (0.024 RGR), ‘Wave Pink’ (0.022 RGR), and ‘Mitchell Diploid’ (0.023 RGR) (Fig. 4.8).

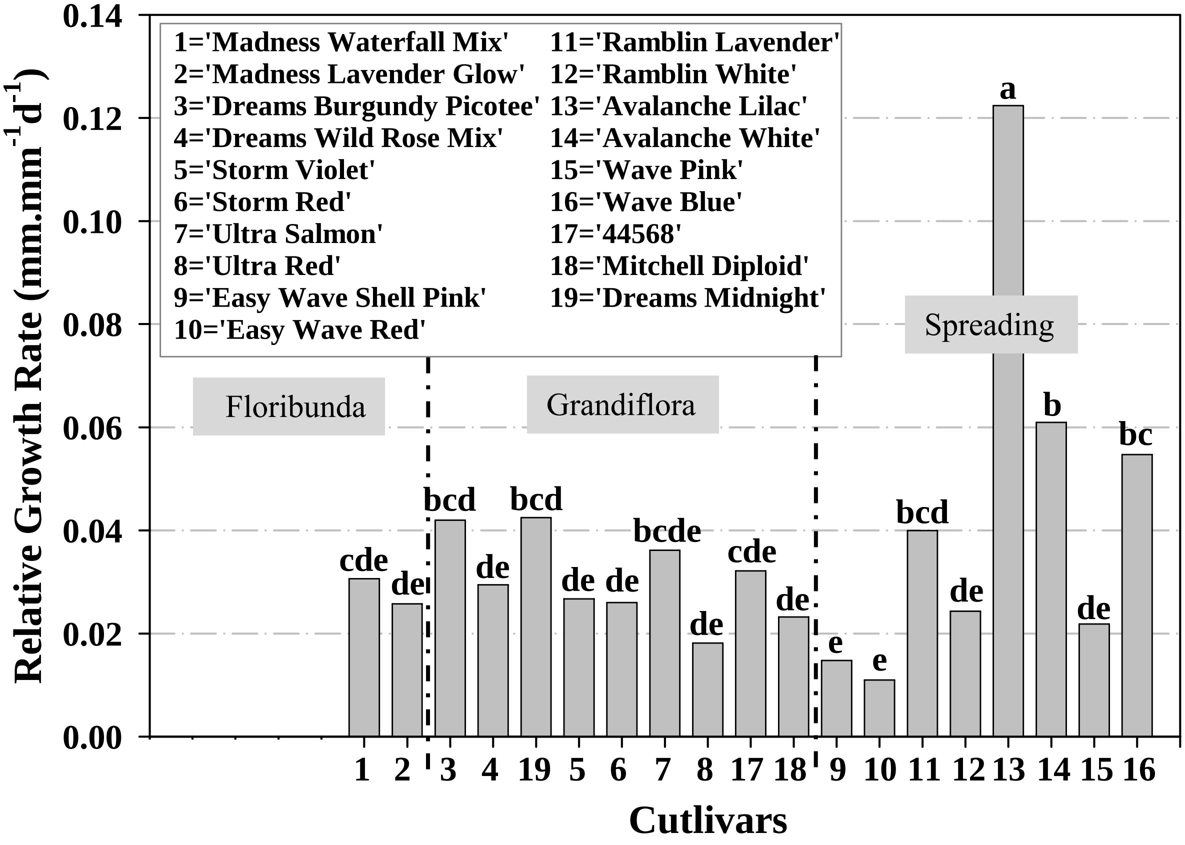

Figure 4.8. Relative growth rates of 19 *Petunia x hybrida* cultivars during drought stress study. 19 cultivars were categorized into three plant types of floribuna, grandiflora, and spreading. Means of each bar markered by the same letters are not significantly different at the 5% by lsmean procedure in SAS with Tukey’s correction (n=24).

Table 4.5. Radicle relative growth rates (RGR) of 19 *Petunia x hybrida* cultivars under control (DI water) or drought stress (-0.8 MPa) during the drought study.

Cultivars	**No.**	**Drought (MPa)**	**RGR (mm.mm^{-1}d^{-1})**
‘Madness Waterfall Mix’	1	0	[Y]0.037
		-0.8	0.024
‘Madness Lavender Glow’	2	0	0.022
		-0.8	0.029
‘Dreams Burgundy Picotee’	3	0	0.046
		-0.8	0.038
‘Dreams Wild Rose Mix’	4	0	0.035
		-0.8	0.024
‘Storm Violet’	5	0	0.022
		-0.8	0.031
‘Storm Red’	6	0	0.031
		-0.8	0.021
‘Ultra Salmon’	7	0	0.042
		-0.8	0.030
‘Ultra Red’	8	0	0.024
		-0.8	0.012
‘Easy Wave Shell Pink’	9	0	0.017
		-0.8	0.013
‘Easy Wave Red’	10	0	0.013
		-0.8	0.009
‘Ramblin Lavender’	11	0	0.044
		-0.8	0.036
‘Ramblin White’	12	0	0.032
		-0.8	0.017
‘Avalanche Lilac’	13	0	0.157
		-0.8	0.088
‘Avalanche White’	14	0	0.074
		-0.8	0.047
‘Wave Pink’	15	0	0.024
		-0.8	0.020
‘Wave Blue’	16	0	0.050
		-0.8	0.059
‘44568’	17	0	0.036
		-0.8	0.028
‘Mitchell Diploid’	18	0	0.027
		-0.8	0.020
‘Dreams Midnight’	19	0	0.049
		-0.8	0.036
Drought			*
Cultivar			*
Interaction			*

Values significant (*) or not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey’s correction.

[Y] Values in table are averages (n=12)

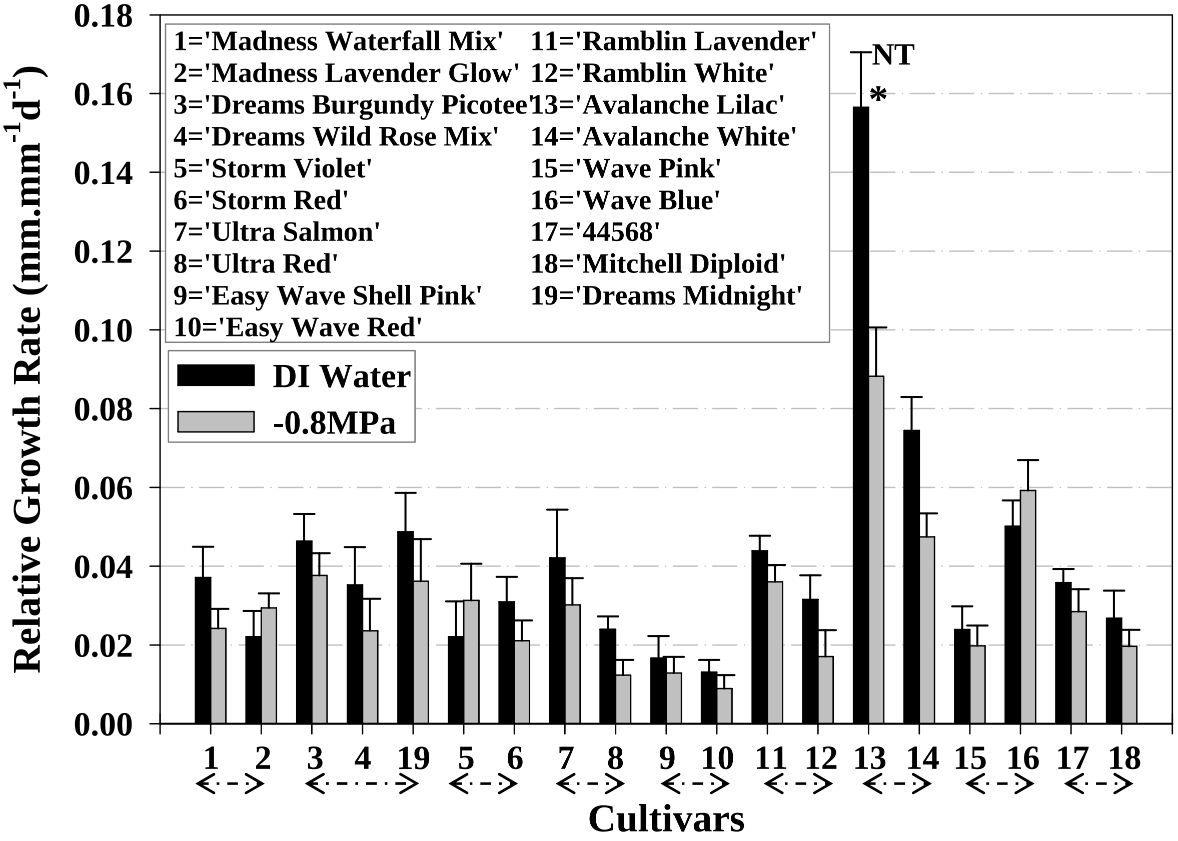

Figure 4.9. Radicle relative growth rates of 19 *Petunia x hybrida* cultivars under control (DI water) and drought stress (-0.8 MPa). ‘NT’ represents non-tolerance. The arrow indicate that cultivars are from the same series. Values significant (*) at the 5% level by the lsmean procedure in SAS with Tukey’s correction. The vertical lines associated with each mean represent ± SE (n=12).

The interaction of individual cultivars and drought stress was significant (*P*=0.0002) (Table 4.5). This indicated that the drought stress affected the cultivars differently. The cultivar ‘Avalanche Lilac’ showed the greatest radicle growth rate, which was consistent with its best overall performance in that series (Kelly et al., 2007). However, drought stress significantly decreased the radicle growth rate of ‘Avalanche Lilac’ (0.157 vs. 0.088 RGR) (Fig 4.8). Thus, the cultivar of ‘Avalanche Lilac’ was considered as the least drought tolerant cultivar for this study. There was no significant effect of drought stress on the radicle RGR of the remaining cultivars. Therefore, based on the screening technique used in this study, these cultivars would appear to be drought tolerant.

4.4 DISCUSSION

The results from the heat shock study showed that radicle RGR was significantly impaired by the temperature of 40°C. Not all cultivars responded the same way to the high temperature. Some cultivars had a significantly reduced relative radicle growth rate, indicating their sensitivity to heat shock. These results were similar to that of previous research with other species. Hong and Vierling (2000) investigated mutants of *Arabidopsis thaliana* (L.) Heynh by measuring hypocotyl growth to determine heat tolerance after heat shock treatments. Results showed that the imposed heat shock (90 min/38^{o}C, 120 min/45^{o}C, or combination of 90 min/38^{o}C, 120 min/22^{o}C and120 min/45^{o}C) reduced hypocotyl growth of all mutants; however, heat shock of 38^{o}C alone did not significantly affect hypocotyl growth, and heat shock of 45^{o}C alone was fatal to all the mutants. Only the combination heat shock treatment of 90 min/38^{o}C, 120 min/22^{o}C and120 min/45^{o}C showed a significant difference in hypocotyl growth. The mutants (sensitivity to high temperature) hot1, hot2, hot3, and hot4 had zero hypocotyl elongation when compared to 6.2 mm hypocotyl elongation of Col-O ecotype. Thus, they reported that the Col-O ecotype (wild type) was more heat tolerant than the mutants.

Seven sorghums genotypes (*Sorghum bicolor* L. Moench)‘290R’, ‘IS20964’, ‘Segaolane’, ‘CK60B’, ‘N250B’, ‘IS1347’, and ‘IS20969’ were screened for heat tolerance by recording coleoptile growth (Setimela *et al.*, 2005). Heat shock of 50^{o}C was applied by using a hot water bath. Coleoptiles growth was recorded at 8, 20, 32, or 44 h after heat shock treatment. Although the interaction of the time of exposure in the hot water bath and genotype was not significant for all cultivars, ‘IS2096’ was considered as more heat tolerant, based on much greater coleoptile growth rate at 50°C.

In the present study, radicle RGR of ‘Dreams Burgundy Picotee’ and ‘Avalanche Lilac’ showed a significant decrease at a heat shock of 40°C. The remaining cultivars showed no difference in radicle RGR, and one explanation could be that all the other cultivars are more heat tolerant. Heat tolerance of these cultivars may have already been induced during the germination process at 26°C. The Association of Official Seed Analysts (2001) indicated that the optimum temperature range for germination of *Petunia x hybrida* was from 20 to 30°C due to a wide variety of petunia species and cultivars. It is not practical to germinate 19 cultivars at different optimum temperatures, and according to the results of ‘Dreams Midnight’ in chapter 3, the temperature of 26°C was chosen the germination temperature for all cultivars.

Drought stress (-0.08 MPa) had a significant effect on radicle growth rate and the interaction of plant type and temperatures was significant. However, only the cultivar ‘Avalanche Lilac’ was considered the least drought tolerant cultivar based on the significant decrease of radicle growth rate compared to all other cultivars. Another study measured radicle growth of three types of grass (*Lespedeza stipulacea* Maxim., *Lolium multiflorum*, and *Bouteloua curtipendula* Torr.) under the combination of heat shock of 22, 27, 32, or 37°C and drought stress of 0, -0.32, -0.77, or -0.98 MPa during the seedling stage in growth chambers. *Lolium multiflorum* was considered as the most sensitivity species at 37°C and -0.98MPa based on the radicle length after a 48-h treatment (Masiunas and Carpenter, 1984). Springer (2005) explored five chaffy-seeded grass cultivars of ‘big bluestem’, ‘sand bluestem’, ‘little bluestem’, ‘yellow bluestem’, and ‘indiangrass’ under drought stress simulated by mannitol at 0, -0.2, -0.4, -0.6, or -0.8 MPa. Drought stress reduced germination percentages and reduced seedling growth rates. ‘Yellow bluestem’ showed the least effect under drought stress, which indicated a more drought tolerant cultivar, but the germination percentages and seedling growth rates of this cultivar were not statistical different from other four cultivars. Grzesiak *et al.* (1996) found that the more tolerant cultivars of field bean (*Vicia faba*), soyabean (*Glycine max*), field pea (*Pisum sativum*) and lupin (*Lupinus albus and L. angustifolius*) had less of an effect on seedling growth rate under drought stress. Both root and shoot growth was impaired, but shoot growth was more inhibited than root growth in pea (*Pisum sativum* L.) under salt and drought stress (Okcu *et al*., 2005) compared to this petunia study using radicle growth rate as the measured parameter. Results from Den Berg and Zeng (2006) indicated that root growth was a better measurement of drought stress of three grasses (*Anthephora pubescens* Nees, *Heteropogon contortus* L., and *Themeda triandra* Forssk.). Niu and Rodriguez (2006) investigated morphological and physiological responses of six bedding plant species agastache, dusty miller, petunia 'Wave Purple', plumbago, ornamental pepper, and vinca during heat and drought stress under greenhouse conditions. The results indicated that petunia could be a more sensitive species to heat and drought stress. The cultivars tested in the petuia study differed in sensitivity to the imposed drought stress of -0.8 MPa. The '44568' transgenic ethylene-insensitive petunia had a lower radicle growth rate, but it was not significantly different from the wild type 'Mitchell Diploid' for both heat and drought stress. Ethylene insensitive cultivars would more affect by stresses than ethylene sensitivity cultivars based on the ethylene trait. This point did not comferm the results from Clark *et al.* (1999). In their study, Clark *et al.* noticed 7-d old seedlings of '*Never ripe* tomato' (*Lycopersicon esculentum* Mill.) (ethylene insensitivity) showed more abnormal roots, longer taproots, and shorter hypocotyls when subjected to adverse soil condition in sand (the favorable soil was commercial potting medium).

Initial radicle lengths of the cultivars used in this study were significantly different. 'Avalanche Lilac' had the highest initial radicle growth rate and longest initial radicle length.

These results agree with Kelly *et al.* (2007) that 'Avalanche Lilac' had the best overall performance in the series. However, other cultivars 'Madness Waterfall Mix', 'Storm Violet', and 'Wave Pink' with best performances in the series according to the study by Kelly *et al.* did not have greater radicle RGRs. While genetic difference may be a possible explanation for these results morphological differences such as seeds size and weight might be responsible for these differences or lack of difference. Larger or heavier seeds may have more storage reserves to support seedling growth and development (Castro, 1999). In addition, the membrane leakage in Petri dishes was not checked during the drought stress study, which may have helped provide insight into the effect of the imposed stress on the seedlings. However, the osmotic potential of solution and relative humidity of 80% were kept constant during this study.

This study is the first to report on the effect of heat shock or drought stress on radicle or hypocotyls growth rates of ornamental bedding plants. While this technique shows great promise as an efficient and effective method for evaluating heat or drought stress on various types of petunia, further research will be required to elucidate a more precise heat shock temperature and duration for determining heat stress tolerance and osmoticum for drought stress tolerance.

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CHAPTER 5. SUMMARY AND CONCLUSIONS

High temperature and drought stress are two of the greatest impediments to bedding plant growth and development. Thus, a method that will accurately and efficiently screen bedding plants for basal stress tolerance is essential. The seedling growth, or seedling growth sensitivity test (SGST), of high temperature or drought stress on petunia (*Petunia x hybrida* Hort. ex Vilm.) was developed to determine the stress tolerance of 19 different petunia cultivars. The initial study confirmed that a clear cellulose film was a best substrate to provide visualization of seedling length by using a flatbed scanner and computer software. Vertical positioning of the seeds on a Petri dish provided for straight, more measurable growth and a more uniform and controlled distribution of solution across the substrate. When scanning the seedlings, a red color was selected as the scanning background to provide for the best contrast for measurements. Based on the protocol developed for growing and measuring seedling growth for SGST, radicle growth rate was chosen over hypocotyl growth as the most reliable and accurate measurement of seedling growth. Although radicle growth at 26, 30, 35 or 40ºC was not statistically different, the effect of temperature on seedling growth appears to be most critical between 40 and 45ºC for ‘Dreams Midnight’ petunia, and therefore 40ºC was chosen as the challenging temperature for determination of the effect of heat tolerance. The osmotic stress of -0.8 MPa was considered the most effective osmoticum for affecting seedling growth, based on change in cumulative growth of the radicle using a quadratic growth model .

The SGST was used to determine heat and drought tolerance of 19 cultivars of petunia. During the screening study, the temperature of 40°C significantly impaired the radicle relative growth rate (RGR). The interaction between cultivar and heat shock treatment was significant, and thus the effect of temperature on radicle RGR of these cultivars was not consistent. The heat shock treatment significantly reduced radicle RGR of ‘Dreams Burgundy Picotee’ and

‘Avalanche Lilac’. This indicated that these two cultivars were less heat tolerant when compared to the remaining 17. Based on the SGST and the imposed heat shock, the remaining 17 cultivars were determined to have a basal tolerance for heat stress.

When drought stress was imposed during the SGST, the interaction of cultivar and drought stress was significant, which indicated that the drought stress did not affect the cultivars similarly. The cultivar of ‘Avalanche Lilac’ showed the greatest radicle growth rate, which was consistent with its best overall landscape performance in that series. However, drought stress, like heat shock, significantly decreased the radicle growth rate of ‘Avalanche Lilac’. Thus, the cultivar of ‘Avalanche Lilac’ was considered as the least drought tolerant cultivar. The same cultivars found to have a basal tolerance to heat stress were also found to have basal tolerance to drought stress.

Although ‘Avalanche Lilac’ had the greatest radicle growth rate compared to all other cultivars during both the heat and drought stress studies, the radicle RGR was most significantly affected by the imposed stress. ‘Dreams Burgundy Picotee’ also had a very high radicle RGR and was significantly affected by heat stress similar to ‘Avalanche Lilac’. The fact that these plants have a very high RGR may have predisposed them to a more significant effect of stress on the radicle growth rate. In the ornamental industry it is sometimes assumed that plants with a more rapid growth rate may be able to outgrow the stress, and therefore may be better able to survive when exposed to heat or drought stress. However, this does not appear to be the case at the seedling stage as measured in the SGST. The heat shock treatment of 40ºC for 3 h that was chosen for this study may not have been the optimum temperature and duration for testing basal thermotolerance. Similarly, the imposed drought stress of -0.8 may not have been optimum for testing basal drought tolerance. Therefore, the heat and drought stress treatments used in this study may need to be further refined so that when using the SGST for determining basal stress tolerance, there is a more accurate method of measurement.

This research was the first to attempt and establish a method for determining basal tolerance of bedding plants and the first to report the effect of heat shock or drought stress on radicle growth rates of ornamental bedding plants. While this technique shows great promise as an efficient and effective method for evaluating heat or drought tolerance of various types of petunia, further research will be required to elucidate a more precise heat shock or drought stress treatment for determining basal stress tolerance. Further research should be also be conducted to determine greenhouse and landscape performance of these plants under heat or drought stress when mature to compare with the results found at the seedling stage.

APPENDIX

Table A.1. Raw data of radicle length of 19 *Petunia x hybrida* cultivars under control (26°C) and heat shock (40°C) for 3 h.

Cultivars	No.	Temperature(°C)	Radicle Length(mm) Day1	Radicle Length(mm) Day5
'Madness Waterfall Mix'	1	26	12.655	16.790
		40	14.482	21.817
'Madness Lavender Glow'	2	26	11.576	18.262
		40	12.213	17.172
'Dreams Burgundy Picotee'	3	26	6.522	12.194
		40	6.294	9.114
'Dreams Wild Rose Mix'	4	26	8.438	13.026
		40	7.862	12.215
'Storm Violet'	5	26	7.904	12.907
		40	11.057	14.779
'Storm Red'	6	26	6.995	11.029
		40	6.539	10.918
'Ultra Salmon'	7	26	5.434	10.881
		40	5.978	9.439
'Ultra Red'	8	26	6.492	9.951
		40	5.940	7.466
'Easy Wave Shell Pink'	9	26	12.037	16.441
		40	10.627	13.537
'Easy Wave Red'	10	26	12.805	16.507
		40	14.082	16.885
'Ramblin Lavender'	11	26	13.777	20.591
		40	9.223	17.110
'Ramblin White'	12	26	11.198	16.128
		40	13.437	19.247
'Avalanche Lilac'	13	26	4.637	19.477
		40	6.187	18.789
'Avalanche White'	14	26	10.837	20.677
		40	9.602	14.654
'Wave Pink'	15	26	11.130	15.816
		40	10.567	16.851
'Wave Blue'	16	26	9.448	17.815
		40	9.770	16.343
'44568'	17	26	12.840	16.538
		40	12.624	15.567
'Mitchell Diploid'	18	26	11.404	16.368
		40	7.776	13.050
'Dreams Midnight'	19	26	4.788	10.310
		40	2.674	5.696

Table A.2. Raw data of radicle length of 19 *Petunia x hybrida* cultivars under control (DI water) and drought stress (-0.8 MPa).

Cultivars	**No.**	**Drought(MPa)**	**Radicle Length(mm)**	
			Day1	**Day5**
'Madness Waterfall Mix'	1	0	12.137	17.238
		-0.8	12.498	15.649
'Madness Lavender Glow'	2	0	11.181	14.242
		-0.8	10.471	13.859
'Dreams Burgundy Picotee'	3	0	6.735	10.576
		-0.8	6.504	9.319
'Dreams Wild Rose Mix'	4	0	8.941	13.101
		-0.8	8.996	11.702
'Storm Violet'	5	0	7.847	9.630
		-0.8	9.130	12.795
'Storm Red'	6	0	6.434	8.748
		-0.8	7.125	8.773
'Ultra Salmon'	7	0	4.199	6.959
		-0.8	5.619	7.683
'Ultra Red'	8	0	6.978	8.694
		-0.8	6.247	6.986
'Easy Wave Shell Pink'	9	0	11.968	14.073
		-0.8	12.635	14.266
'Easy Wave Red'	10	0	12.765	14.508
		-0.8	12.813	14.005
'Ramblin Lavender'	11	0	14.645	21.794
		-0.8	14.082	19.507
'Ramblin White'	12	0	11.693	16.081
		-0.8	11.395	13.716
'Avalanche Lilac'	13	0	4.391	17.671
		-0.8	6.278	14.607
'Avalanche White'	14	0	9.630	19.437
		-0.8	10.891	17.250
'Wave Pink'	15	0	11.460	14.546
		-0.8	11.413	13.789
'Wave Blue'	16	0	10.834	17.776
		-0.8	10.650	17.764
'44568'	17	0	16.337	22.656
		-0.8	15.831	20.074
'Mitchell Diploid'	18	0	13.620	17.612
		-0.8	16.082	19.569
'Dreams Midnight'	19	0	4.796	7.707
		-0.8	4.787	7.594

VITA

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