Phenotypic and Genotypic Characterization of Foliar Bleaching and Regreening Associated with Heat and Light Stress in *Pelargonium peltatum*

by

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Abstract

Ivy geranium (*Pelargonium peltatum*) develops ivory foliage under high temperature and light stress. Occurrence, severity, and patterns of development and remission of the disorder differ among cultivars. A two-phase, split-plot greenhouse experiment with two environments ["elevated temperature and light stress" (ETL) with high average daily temperature (ADT; 33.8ºC) and daily light integral (DLI; 15.3 moles·day^{-1}) versus Control with 27.6ºC ADT and 9.8 moles·day^{-1} DLI] and sub-plots of 12 *Pelargonium* genotypes (n=6 with 2 pots per e.u.) was conducted during summer 2025. Phenotypic observations and leaf greenness measured with a SPAD meter were taken on mature, juvenile, immature, and newly emerged leaves that developed in the treatment environments. After foliar bleaching occurred in the ETL environment (phase I, 26 d), these plants were moved to the Control environment to observe and measure regreening (phase II, 23 d). Bleaching occurred not only in foliage, but also peduncle and sepals of some cultivars. Leaves near the meristem were most susceptible to foliar bleaching under ETL. The most tolerant cultivars differed in pattern of bleaching and leaf morphology, including lamina venation. Extent of regreening was dependent on leaf developmental stage when ETL occurred, and foliage was unlikely to regreen if the leaf matured during ETL or developed any necrosis. These observations inform further research to identify gene(s) responsible for foliar bleaching under ETL.

Genomic analysis was conducted on 'Ivy League Red' and 'Ivy League Hot Coral', representing cultivars tolerant and sensitive to ETL, respectively. The gene *protochlorophyllide oxidoreductase* (*LPOR*), which is active in chlorophyll biogenesis, was targeted. As the genome of *Pelargonium* is not fully sequenced, comparative sequence analysis from genomically similar

species, including *Theobroma cacao*, *Jatropha curcas*, *Citrus sinensus*, and *Vitis vinifera*, were used to reconstruct a partial sequence of *LPOR* isotype C to examine genetic expression differences between the tolerant and sensitive cultivars. Semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) for the *PORC* transcript showed that expression patterns of *PORC* differed between the two cultivars, suggesting distinct responses to heat stress. In 'Ivy League Red', the transcript of *PORC* was detectable before heat exposure, suggesting a priming state. After heat treatment, its expression decreased. In 'Ivy League Hot Coral,' the *PORC* transcript is initially low and only increases after foliar bleaching begins, a response that is likely insufficient to prevent the bleaching phenotype. These opposite expression patterns contribute to our understanding of the underlying physiology of foliar bleaching.

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Chapter 1 - Phenotypic Characterization of Foliar Bleaching and Regreening Associated with Heat and Light Stress in *Pelargonium peltatum* Cultivars

Introduction

Chlorophyll exhibits highly plastic behavior, particularly under elevated temperature and high light intensity (ETL), adjusting its synthesis, stability, and even shaping how leaves maintain pigmentation in response to these environmental cues (Veen et al. 2025; Nicotra 2011). The effects of ETL on photosynthetic organisms are complex and have become an increasing area of focus as global temperatures rise. Heat and light stress are known to independently trigger a reduction in chlorophyll and impair photosynthetic function (Khan et al. 2025; Ghalami et al. 2026), both producing distinct physiological impacts. More recent studies demonstrate that when these stressors occur simultaneously, their independent effects on photosynthesis interact synergistically (Ferdinand 2026), ultimately having an amplified negative physiological impact on the plant.

These stresses together contribute to a critically understudied pigment loss disorder broadly described as bleaching. Although photosynthetic organisms require their pigmentation to survive, some evidence suggests that bleaching may represent an adaptive defense mechanism (Pompelli et al. 2022; Sullivan et al. 2025; Cordero 2022). This green pigment loss phenomenon, bleaching, integrates processes across chlorophyll biosynthesis, environmental stress perception, and crop adaptive physiology (Bhattacharya et al. 2026; Ferdinand et al. 2026; Alquraish et al. 2026; Veen et al. 2025). Although reductions in chlorophyll concentration are a common

response to environmental stress, complete bleaching of otherwise healthy foliage is uncommon and remains poorly understood in plants.

In 2004, an initial heat stress study conducted with ivy geraniums (*Pelargonium* spp.) in the Southern United States documented the bleaching phenomenon to be associated with annual seasons, occurring in new foliar growth during summer (Harkess 2004). As global temperatures continue to rise, reports of bleaching are increasing (USDA 2026), which makes a more complete understanding of the disorder a pressing need. This seasonal bleaching pattern in new growth has been increasingly reported in diverse plant species, including in species of *Pelargonium* (Harkess 2004), *Ficus* (Hodel et al. 2023), *Malus* (apple) (Chen and Cheng 2010), *Citrus* (Bernardi et al. 2026), and *Persea* (avocado) (Abiola et al. 2025), in addition to otherwise undocumented examples, such as a landscape euonymus (Fig. 1.1). Terminology used to describe the disorder varies from chlorophyll degradation (Woodson 2022), needle blanching (Teskey et al. 2015), photobleaching (Hodel et al. 2023), pigment bleaching (Terán et al. 2024), whitening or foliar bleaching (Dhir et al. 2011, 2013; Horton 2018; Morris 2018), and zonal chlorosis (Chen and Cheng 2009). This wide range of terminology reflects the absence of needed standardized diagnostic criteria as well as limited characterization of the disorder over time, making comparison of reports across species challenging.

Pelargonium spp. are economically important ornamental spring crops in the U.S. greenhouse industry, ranking second in annual wholesale value of floriculture sales ($2.67 billion; USDA 2026); therefore, investigations of the disorder became necessary to understand disorder mitigation, as well as to identify tolerant cultivars to improve selection. The disorder has been reported in many ivy geranium cultivars spanning different phenotypic traits, new interspecific hybrids of *Pelargonium* × *peltatum* crossed with *Pelargonium* × *hortorum* (Harkess

2004; Syngenta Flowers 2023), as well as zonal geraniums (Owen 2017). Studies have revealed that the extent of bleaching is cultivar specific (Dhir et al. 2013) and species specific (Maio et al. 2026). The affected cultivars span diverse leaf morphologies, growth habits, genetic backgrounds, and ploidy levels, suggesting that susceptibility to foliar bleaching is not restricted by these traits.

Pelargonium foliar bleaching studies demonstrated that the disorder consistently occurred when air temperatures exceeded about 31°C for several consecutive days, whereas elevated root-zone temperatures alone failed to induce symptoms (Dhir et al. 2013). Because symptoms develop in the youngest expanding leaves, early investigations considered iron deficiency as a cause; however, tissue analyses revealed no reduction in iron associated with symptomatic tissues (Dhir et al. 2013). These findings led to the hypothesis that heat stress may impair iron metabolism, resulting in physiologically inactive iron accumulated within leaf tissue (Dhir et al. 2013).

Heat stress and high solar irradiance are frequently associated with chlorophyll degradation and impaired chloroplast function, suggesting that environmental stress is central to the development of this disorder (Ghalami et al. 2026) and connects to earlier observations by Dhir et al. (2013) associating the disorder with prolonged elevated air temperature. It has yet to be established how light influences the bleaching disorder in *Pelargonium*.

As the foundation of understanding this disorder has strengthened over time, the physiological basis of foliar bleaching in ivy geranium and other crops remains unresolved. More recent transmission electron microscopy studies have revealed extensive chloroplast degradation, disrupted thylakoid organization, and membrane rupture in bleached tissues, indicating that significant cellular damage accompanies symptom development (Bernardi et al. 2026; Morris

2018). There exists a need to study the temporal progression of symptoms of foliar bleaching followed by regreening because published work only evaluates a node at or near the apical meristem at one point in time, and regreening has not been studied at the whole-plant level.

In addition to the previous studies conducted with foliar bleaching of *Pelargonium*, this genus provides an ideal model system for studying and characterizing this disorder. *Pelargonium* leaves are heteroblastic, providing a basis for studying cellular response in relation to leaf maturation (Jones et al. 2009); genotypes are traced to their South African origins, where diverse microclimates contributed to their leaf and growth habit evolutions (Jones et al. 2013); and spanning at least 280 species, their exceptionally high morphological diversity can be represented as herbaceous annuals, perennials, geophytes, woody shrubs, stem-succulent subshrubs, and evergreen shrubs (Jones et al. 2009; Jones et al. 2013; Nicotra et al. 2011). Additionally, *Pelargonium* species contain the largest chloroplast genome of any terrestrial plant sequenced to date (Chumley et al. 2006), provide foundational evidence for non-Mendelian leaf variegation inheritance (Wehe et al. 2009), and have served as a model for nonlinear specific plastid genome reconfiguration (Weng 2014). Collectively, these features create an ideal model system for studying environmental adaptations to heat and light stress.

The objectives of this experiment were to 1) document the incidence, severity, and temporal progression of bleaching of foliage across developmental stages and a range of ivy geranium cultivars; 2) identify varieties tolerant and sensitive to heat and light stress for future genomics research; and 3) document plant recovery following the abatement of heat stress, as the foliar regreening process has received little attention.

Materials and Methods

Plant material. Twelve cultivars of ivy geranium and related hybrids representing a wide range of heat tolerance across the 'Ivy League,' 'Calliope,' and 'Cascade' series (Syngenta Flowers North America, Gilroy, CA, USA), as well as 'Great Balls of Fire' (Dümmen Orange, Columbus, OH, USA; Table 1), were evaluated (Table 1.1).

On 26 May 2025, all vegetatively-propagated ivy geraniums (except 'Ivy League Red') were transplanted into a 6-inch diameter (770 ml volume) standard green pots (HC Companies, Twinsburg, OH, USA) using a peat-based substrate (Berger BM6, Berger, Saint-Modeste, Quebec, Canada). On 10 Jun, 4-inch pots of vegetatively propagated 'Ivy League Red' obtained from Kaw Valley Greenhouses (Manhattan, KS, USA) were transplanted into the 6-inch pots. All plants were fertigated as needed and uniformly pinched to induce branching and remove inflorescences. Plants were grown in a stock plant greenhouse with temperature set points of 18°C day and 15°C night.

Experimental design and treatments. The experiment was conducted from 13 Jun to 1 Aug 2025 in four glass-glazed greenhouses at Kansas State University's Throckmorton Plant Sciences Center (39.18791° N, 96.58081° W). Four 25-foot x 25-foot greenhouses were arranged as two Environment main plots: Control versus Elevated Temperature & Light (ETL) (Table 1.2). The Control greenhouses had a double layer of white shade compound applied to the glass glazing, and plants were set on the benches closest to the evaporative pads. Elevated Temperature and Light (ETL) greenhouses had no shade compound on the glazing, and plants were set on benches furthest from evaporative cooling pads with water to the pads turned off (Figure 1.2).

Environmental light monitors (HOBO, Onset Computer Corp., Bourne, MA, USA) were set in each greenhouse and logged light intensity every 30 min. Temperature and relative humidity monitors (HOBO, Onset Computer Corp., Bourne, MA, USA) were set in each block on white platforms level with the foliage canopy. Day temperature was determined by averaging readings from the 15 h photoperiod of 06:00 to 20:00, and night temperature was determined by averaging readings from the 9 h noctoperiod of 21:00 to 05:00.

Plot structure. Each greenhouse (two greenhouses of Control, two greenhouses of ETL) had three blocks, or subplots, that all contained two pots of each cultivar (two pots per e.u.). Therefore, each Environment had six replications of each of the 12 cultivars. In total, each greenhouse contained 3 blocks x 12 cultivars x 2 pots per e.u. for a total of 72 pots. Each Environment consisted of 2 greenhouses x 72 pots per greenhouse for a total of 144 pots per Environment, or a total of 288 pots in the experiment.

Phase design. The experiment was structured in two phases. Phase I followed the bleaching process in the ETL environment compared to the Control environment. Phase I occurred from 13 Jun (day 1) to 8 Jul (day 26), 2025. The ETL environment was managed at an average daily temperature (ADT) of 33.8°C and light intensity of 285 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD whereas the Control environment had an ADT of 27.7°C and light intensity of 181 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD (Table 1.2). Phase II followed the regreening process for the ETL plants by moving them into the Control environment after necrosis appeared on the bleached leaf. By 4 Jul (day 22), leaf necrosis began to occur on bleached foliage in the ETL environment, so Phase II was initiated on 9 Jul (day 27). Phase II occurred from 10 Jun (day 28) through 1 Aug (day 50). The Control environment, which now held all of the ivy geranium subplots, was managed at an average daily

temperature (ADT) of 26 to 27°C and light intensity of 97 to 117 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD. Phase II ended when regreening data was collected on day 50 (Table 1) (Fig. 2).

Environment calculations. For temperature, average daily temperature (ADT) was calculated using the standard weighted formula: T = temperature, h = hours. ADT_d = (Tdayh) + (T night h)/24. Day hours were from 6:00 to 20:59 (15 h) and night hours were from 21:00 to 5:59 (9 h). As there were six temperature monitors per environment, each hour was averaged to create one hourly average to use for calculation of average daily temperature for the whole mainplot. Th, i = temperature at hour I from monitor i; n = six monitors.

To calculate average daily temperature (ADT) for each Phase, the ADT values for every day within a phase were averaged together (Table 1.2). Temperature equations:

Hourly Average ($T_h = \frac{1}{n} \sum_{i=1}^{n} T_{h,i}$) **Average Day temperature** ($T_{day} = \frac{1}{15} \sum_{h \in day} T_h$)

Average Night Temperature ($T_{night} = \frac{1}{9} \sum_{h \in night} T_h$)

Average Daily Temperature ($ADT_d = \frac{(Tday \cdot 15) + (T\ night \cdot 9)}{24}$)

Average Phase Temperature ($ADT_{phase} = \frac{1}{D} \sum_{d=1}^{D} ADT_d$

PPFD measurements from 06:00 to 20:59 were averaged between paired environmental monitors within each treatment to calculate a representative hourly illuminance value. Lux values were converted to PPFD using the standard conversion factor for sunlight: (PPFD ($\mu mol \bullet m^{-2} \bullet s^{-1}$) = lux ($mol \bullet m^{-2} \bullet d^{-1}$) x 0.0185. This conversion produced hourly PPFD (HPPFD) measurements. For each day, HPPFD values were averaged to obtain daily PPFD (DPPFD). All DPPFD values within a phase were then averaged to calculate the mean PPFD for that phase (Table 1.2). Daily Light Integral (DLI) was calculated as: DLI ($mol \bullet m^{-2} \bullet d^{-1}$) = (DPPFD x 15h) x 0.0036, where 15h represents the defined photoperiod. DLI values were averaged across all days

within each phase to obtain the mean DLI for that phase (Table 1.2). L_h,1, L_h,2 = lux readings from 2 monitors at hour h. L_h = hourly average lux. Light equations:

Photosynthetic Photon Flux Density

Hourly Average PPFD

$$L_h = \frac{lh,1 + lh,2}{2} \quad HPPFD_h = L_h \times 0.0185$$

Average Daily PPFD $DPPFD_d = \frac{1}{15} \sum_{n=2}^{20} HPPFD_h$

Average Phase PPFD $PPFD_{phase} = \frac{1}{D} \sum_{d=1}^{D} DPPFD_d$

Daily Light Integral

Average Phase DLI

$$DLI_{phase} = \frac{1}{D} \sum_{d=1}^{D} DLI_d$$

DLI = (DPPFD x15h) x0.0036

DLI 1µmol $m^{-2}s^{-1}$ for 1 hour = 0.0036 mol $m^{-2}d$

Conversion Factor for sunlight: 0.0185

Data collected. Relative chlorophyll content, or leaf greenness, was measured using a SPAD-502 meter (Konica Minolta, Tokyo, Japan) on plants in both ETL and Control environments. SPAD measurements were taken at the base of the lamina (Fig. 1.3) on leaves of both plants and averaged to create one data point per cultivar per block. During Phase I, leaves at three nodes were measured at the following developmental stages: Mature (fully unfolded, expanded leaves), Juvenile (unfolded leaves that continued to expand during Environment treatment), and Immature (leaves present pre-treatment on the meristematic node that unfolded and bleached in ETL during Phase I) (Fig. 1.4). Mature growth was marked with a green floral wire and repeated SPAD measures were taken on days 1, 8, and 12. Juvenile growth was marked with a white chenille stem, and repeated SPAD measures were taken on days 12, 15, and 20. Immature growth was marked with a yellow chenille stem, and SPAD was measured on day 22; this leaf was unfolded and fully bleached with necrotic spots in ETL at the time of measurement (Fig. 1.4).

On day 27, Phase II commenced, and the ETL-treated plants were moved into the Control environment for regreening. Bleached, immature foliage with necrotic spots did not show regreening on day 29; however, the emerged ivory leaf that had developed from the meristem

above did regreen; therefore, regreening was measured on this node. SPAD data was collected on the regreening ivory leaves that had emerged during Phase I above a yellow chenille stem used to mark the node on days 29 and 50 (Fig. 1.2)

Photographic documentation & phenotypic ratings. Photos documenting the occurrence of and recovery from foliar bleaching were taken when SPAD readings were collected (Fig 1.5). Leaf phenotypic ratings were assigned on day 50 using a scale of 1 (fully green) to 6 (fully bleached; Fig. 1.5). For the 'Ivy League' series group, foliage at each node was rated individually at the end of Phase II to assess plant resilience after bleaching; new growth that developed above a node with bleached foliage was assessed which measured the variation in developmental recovery rates between cultivars.

Substrate pH and electrical conductivity (EC) and tissue nutrient analyses. To evaluate nutrient availability that may be associated with foliar bleaching, a water-based PourThru substrate extraction (Cavins and Williams 2018) was conducted on 36 pots each of ETL and Control on 1 Aug 2025. Substrate pH and EC were measured on solutions (Bluelab Combo Meter, Tauranga, New Zealand).

To evaluate tissue nutrient concentrations that may be associated with foliar bleaching, leaf tissue from a sensitive and tolerant variety was analyzed for P, K, Ca, Mg, S, Fe, Mn, Zn, and Cu (Table 1.3). About 5 g of fresh weight of the youngest bleached leaves was harvested from the sensitive 'Ivy League Hot Coral' and tolerant 'Ivy League Red' that developed under ETL. A similar amount of green foliage at the same stage of development was harvested from these cultivars that developed under the Control environment. Nitrogen was not determined due to insufficient quantity of foliage for a digest. All samples were dried at 70°C in a forced air oven and ground with a mortar and pestle. Tissue was assayed by Kansas State University's Soil

Testing Lab (Manhattan, KS, USA). All nutrients were determined from a nitric-perchloric digest (Gieseking et al. 1935) and analyzed with ICP-OES (Model 5800, Agilent Technologies, CA, USA).

Statistical analysis. Statistics were performed on all environmental variables (ADT, DLI, PPFD, RH, Dew, and respective Day and Night temperatures) after averages were calculated (see Environment Calculations, Materials and Methods). Each environmental variable was analyzed in JMP Pro using a standard least squares ANOVA. Fixed effects included Treatment (Control vs. ETL), Phase (I vs. II), and the Phase × Treatment interaction. Mean comparisons were conducted using Tukey (Table 1.2).

SPAD measurements were analyzed to compare chlorophyll content between Environments (Control vs. ETL) for each sample date and leaf developmental stage over time. No significant differences were detected between blocks or between the two greenhouses within each Environment; therefore, SPAD values for each cultivar block were pooled within their Environment to generate a single representative SPAD value per cultivar per treatment for each date (Table 1.4). SPAD comparisons between Environments were performed using two-sample *t*-tests for each date within a given leaf stage. Statistical analyses were conducted in JMP Pro using the Fit Model Platform. Results were reported both as a main effect with all cultivars combined (Table 1.5) and as individual cultivar comparisons between ETL and Control (Table 1.4).

Phenotypic rating statistics were performed using the node-level scores generated from the phenotyping scale (Fig. 1.5). Ratings for the four evaluated nodes were averaged for each cultivar within each replication. One-way ANOVA was run on the average node phenotype score, and means were compared with Tukey's HSD (Fig.1.6). Tissue nutrient content of

bleached versus green foliage from two cultivars, 'Ivy League Red' and 'Hot Coral,' was evaluated as a two-way factorial using JMP ver. 16 (SAS, Cary, NC, USA).

Graphics. Graphics and Figures were created utilizing BioRender.com, Microsoft Paint, and Microsoft Excel.

Results and Discussion

Environmental conditions. During Phase I, establishment of two distinct treatment environments (ETL and Control) within four greenhouse rooms using the same equipment was successful. Day and night temperatures, relative humidity, ADT, and light levels (average PPFD and DLI) differed between the ETL and Control environments (Table 1.2). During Phase II, when regreening occurred, and all plants were in the same control-environment greenhouses, only the day temperature differed between the two treatments (29 versus 26.4°C in ETL versus Control, respectively; Table 1.2). Temperature that results in foliar bleaching of ivy geranium was reported by Dhir (2011) and Horton (2018) as reliably occurring above 31°C, and our results support this conclusion. In our ETL treatment, the average day temperature was 36.8°C, 33.8°C ADT, with a DLI of 15.3 mol• m^{-2} •d^{-1} in the ETL environment. In our control treatment, the average day temperature was 27.6°C (27.0°C ADT) with a DLI of 9.8mol• m^{-2} •d^{-1} in the Control environment.

*Description of foliar bleaching and regreening.*The overall pattern of foliar bleaching (Fig. 1.7) begins with the affected leaves becoming lighter green, followed by leaf margins gaining a white-yellow outline. Next, chlorotic to white cells appear at the base of the leaf where the petiole attaches to the lamina. The disorder progresses from the center of the lamina's base upwards and out toward the leaf margins. Next, cells across the leaf blade typically transition

entirely to white, though veins remain green in some cultivars. Symptoms of the disorder are most severe in growth closest to the terminal meristem and are less pronounced or absent on tissue further removed from the growing point. Depending on proximity to the terminal meristem, even peduncles of developing inflorescences will bleach, though petal tissue remains its normal hue (Fig. 1.7). We also provide observations of necrotic lesions forming on bleached foliage and failure to regreen if ETL does not abate for many days (22 days in our study).

Regreening occurs in a similar pattern to bleaching, but in reverse, from the lamina base towards the leaf margins. If juvenile growth was not able to outgrow foliar bleaching, regreening occurred when plants were returned to the control environment. Leaves that were closer to the terminal meristem regreened before juvenile foliage. Juvenile leaves first regreened at their margins, and depending on the severity of bleaching, then the lamina. Juvenile regreening of leaf venation depends on the severity of bleaching, cultivar, and developmental stage of the leaf during ETL stress.

Response to the environment based on cultivar series. Cultivar series were grouped by growth habits. This allowed for grouped morphology characterization of response to ETL. The cultivars used in this study were selections from Syngenta's 'Ivy League', 'Calliope', and 'Cascade' series that represented different growth habits, genetic lineage, and heat tolerance (Table 1.1). The 'Cascade', 'Calliope', and 'Ivy League' series' culture guide recommends an average day range of 22°C to 23°C, average daily temperature (ADT) 21-22mol• m^{-2} •d^{-1}, DLI 16 to 18mols •d^{-1} (Syngenta 2020; 2024). Based on observation, 'Calliope' cultivars were first to show bleaching symptoms, followed by 'Cascade,' then 'Ivy League,' which aligns with the results from Harkess (2004) and Syngenta (2010). In the ETL, we observed that 'Calliope' cultivars' veins bleached initially, followed by the lamina. Flower buds turned slightly pink

before bleaching. During Phase II, veins did not regreen immediately, if at all. The 'Calliope' series did not follow a similar bleaching and regreening leaf maturation pattern as the other series (Fig. 1.8, Fig. 1.9). 'Cascade' cultivars had variegated foliage and developed bleached cells along the lighter green variegation boundaries; in Phase II this cultivar regreened following the variegated boundary patterns. However, new young foliage expanded dark green pigmentation from the lamina center towards the margins, following the variegated boundary less strictly compared to older leaves (Fig. 1.8). Flower pedicels were naturally pink, retained pinkness under initial ETL stress, and eventually bleached.

Cultivars within the 'Calliope' and 'Cascade' series followed similar symptomology respective to their series, whereas cultivars within 'Ivy League' varied significantly (Fig. 1.9). Notably, pink undertones occur naturally in 'Ivy League Red', 'Ivy League Orchid', and less visibly in 'Ivy League Pink.' During ETL stress, these specific cultivars display vibrant pink petioles on affected leaves, as well as pink peduncles and buds. Heat and light stress caused cupping of emerging growth, most severely in 'Ivy League Hot Coral.' 'Ivy League Orchid' lamina emerged circular, whereas other 'Ivy League' cultivars had leaves with fewer sinuses and more expanded lobes.

Environment and leaf stage SPAD main effect. At the start of the study, there was no significant difference between the SPAD in mature growth between the two treatments, and by day 8 there was a significant difference (Table 1.3, Mature). The temperature and DLI in the ETL environment was above 30°C and 15 mol/day, respectively, for 7 days (Fig. 1.10), as SPAD did not significantly change in mature growth by day 8. However, in the Control there was a temperature spike (the only one that occurred until the end of the study) above 30°C at 15 mol/day for 4 days (Fig. 1.11). This spike did not seem to significantly impact Control SPAD, as

the overall SPAD measurement was significantly higher than the ETL. SPAD measurements of the Mature growth ended at day 12, and visual observations did not show any bleaching in this growth regardless of the treatment for the duration of the experiment.

SPAD readings for Juvenile leaves were lower in ETL compared to the Control at every day measured (Table 1.3). Though greenness of Juvenile leaves in ETL was not statistically different from day 12 to 20 (Prob > F = 0.0665), the leaves did show increased bleaching throughout the study (Fig.1.7D, Fig. 1.8, Table 1.3). Some Juvenile leaves in ETL were more bleached than others; if a specific Juvenile leaf was older, it could outgrow the disorder as it enlarged, whereas others that were less mature at the time would succumb to bleaching and ultimately develop necrosis in the sensitive cultivars. We believe that the necrosis occurred because high light intensity was paired with high temperature.

The Immature foliage in the ETL environment was severely bleached by day 22 (2.5 SPAD units in ETL versus 38 SPAD units in Control, <0.0001*). These leaves were completely white (Fig. 1.7), and most of them ultimately became necrotic and died.

During Phase I, new ivory growth emerged while under stress, whereas the Control did not develop this symptomology (Table 1.3, Immature). During Phase II, the closer the leaf was to the apical meristem, the more likely it was to regreen, as long as necrosis had not occurred, as observed in the Immature foliage. In Phase II, the environment had optimal temperatures, and SPAD increased from an average of 2 SPAD units on day 26 to 25 SPAD units on day 50. Even though the temperature had increased above 30°C just before the end of Phase II (Fig. 1.10), higher SPAD was still measured on ETL plants, but SPAD did not change for the Control plants, similar to the ETL Mature leaves in Phase I.

Subplot effect of cultivar: The comparative SPAD results of eleven 'Ivy League' cultivars parse their relative sensitivity to ETL (Table 1.4). 'Ivy League Amethyst' was removed from the dataset due to plant death during ETL and excessive missing data. We expected that cultivar comparison of changes in SPAD for Juvenile leaves would allow for separation of varieties that were more tolerant or sensitive to ETL. However, "resilience", or how quickly varieties recovered from ETL, ultimately became the criterion for distinguishing the tolerant and sensitive categories. In our study, bleached foliage usually died in 'Cascade' cultivars, which provides evidence that the series is not tolerant, or, as discussed next, resilient (n=1 in Table 1.3). The 'Calliope' series and the 'Ivy League' series are reported to be the most tolerant cultivars (Syngenta, 2013). 'Ivy League Red' had the highest SPAD readings of any of the cultivars in both the ETL and Control (Fig. 1.10, Table 1.4). The 'Cascade' series is reported to be among the most sensitive cultivars (Harkess,2004).

Phenotypic ratings. At the end of Phase II, ratings of regreened growth provided an indication of plant resilience, or ability to recover after ETL. General tolerant cultivars restore normal growth and chlorophyll levels in the first node developed post stress, indicating strong developmental endurance. In contrast, slow-recovering cultivars required multiple successive nodes before chloroplast development and leaf expansion returned to normal baselines. 'Ivy League Red' and 'Ivy League Cherry Blossom' had the best ratings, indicating tolerance via ability to recover from ETL (Fig. 1.6). 'Ivy League Hot Coral' and 'Ivy League Orchid' had poorer phenotypic rating means, indicating slower recovery from ETL and, therefore, susceptibility to the disorder (Fig. 1.6). The sensitive cultivar not only had slower pigmentation recovery but also had severely cupped leaves that also slowly recovered. The tolerant cultivar

was not cupped, but compared to normal conditions, the lobes of the new growth were more expanded, not reaching the same severity as the sensitive cultivars.

Substrate and plant tissue nutrients. The PourThru extractions resulted in pH of 5.68 and 5.55 for ETL and Control environments, respectively, which falls within the target range for soilless substrates of 5.5 to 6.3 (Cavins and Williams 2018). EC measured 4.6 and 4.7 $dS \cdot m^{-1}$ for ETL and Control environments, respectively, which falls at the high end of the "normal" range (Cavins and Williams 2018).

Tissue nutrient content. No difference in nutrient content of bleached versus green tissue, or tolerant 'Ivy League Red' versus sensitive 'Ivy League Hot Coral' tissue, occurred for any plant nutrient. Similarly, no interaction between foliage color or cultivar occurred (Table 1.3). Like others (Citations), we found no evidence that the bleaching disorder occurred because of a nutrient disorder and find that it is associated with ETL.

Conclusions

The results from this greenhouse study characterize the temporal progression of foliar bleaching from ETL. Regreening after ETL indicated plasticity of meristematic cells after undergoing ETL stress. Our results are the foundation for a genomics study to evaluate the role of the gene LPorC, protochlorophyllide oxidoreductase C, that is an essential, light-driven enzyme in chlorophyll biosynthesis, as it relates to foliar bleaching under ETL.

The two cultivars that were ultimately selected for use in the genomics study were the more tolerant 'Ivy League Red' and more sensitive 'Ivy League Hot Coral.' These two cultivars represent the range of response to ETL within a series of cultivars that are genetically similar, but not identical. 'Ivy League Red' is known for tolerance (Dhir,et al. 2013, Syngenta 2023), had the highest SPAD readings in the Control and ETL, newly emerged growth during stress did not

show an extreme difference in appearance compared to Control, and was the quickest to recover from stress (best resilience). Whereas the sensitive cultivar is not rated by Syngenta for tolerance to heat stress, the SPAD in the 'Ivy League Hot Coral' was amongst the lowest in the series in Control and ETL; newly-emerged growth during stress was severely cupped compared to the Control; bleached leaves often died, never regreened, and slowly recovered from stress over time as new nodes grew. The other sensitive cultivar with a low recovery statistic, 'Ivy League Orchid,' is discontinued. Despite quick recovery from foliar bleaching, tolerant cultivars do bleach, which ultimately raises questions about the underlying mechanisms responsible for their quick recovery rate.

Figure 1.1 Picture of undocumented foliar bleaching example comparing new growth in full sun (left) vs new growth in shade (right) on the same landscape euonymus plant

Figure 1.2 This figure shows the full experimental design depicting treatment, phases, and targeted leaf stages for a foliar bleaching characterization study in Pelargonium spp.

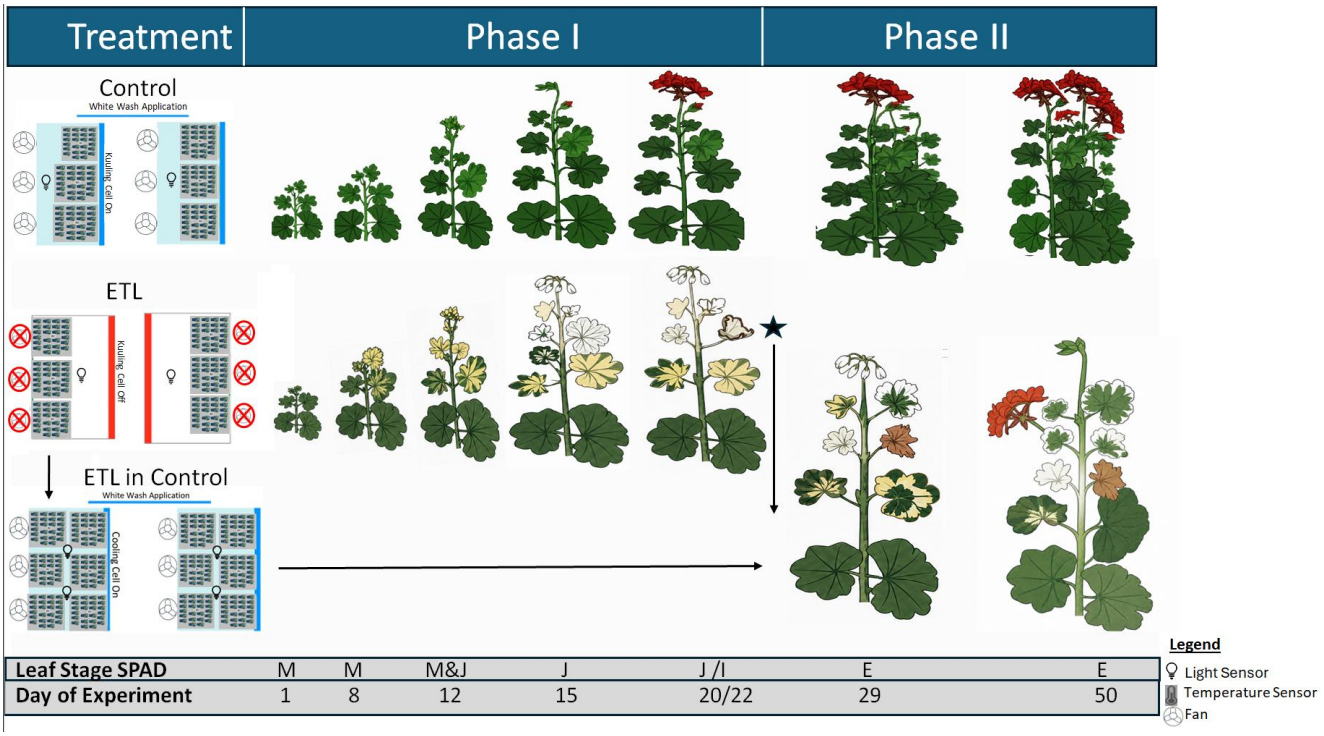


Figure 1.2 Evaluating foliar bleaching and regreening in 11 cultivars under 2 environmental treatments in 4 greenhouses: Control and Elevated Temperature & Light (ETL). The Control plants were placed next to active KÜÜL® Cell Pads, with a whitewash application on the glazed glass ceiling. The ETL plants were placed across from the off KÜÜL® Cell Pads next to the exhaust fans with no whitewash shading applied to the ceiling. Relative chlorophyll content was measured using a SPAD meter across four leaf developmental stages (Mature, Juvenile, Immature, and Emerged) over time. The experiment was conducted in 2 phases: Phase I, during which plants were exposed to ETL conditions and bleaching progression was monitored, and Phase II, initiated once necrosis appeared (Indicated by the star in the figure), where ETL plants were transferred into the Control environment to assess regreening. 2 light sensors were placed in each greenhouse and 1 temperature monitor was placed in every replicate block within a greenhouse (6 per treatment). Created in BioRender (Bowdish 2026).

Figure 1.3 This figure shows the location on the leaf where relative chlorophyll content (SPAD) measurements were measured over time

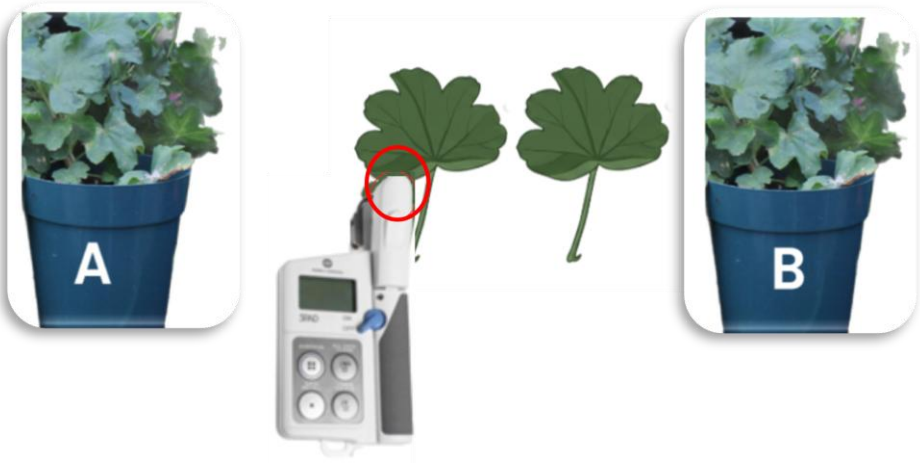


Figure 1.3 This figure shows the location on the leaf where relative chlorophyll content (SPAD) measurements were measured over time to examine how Pelargonium sp. in the Elevated Temperature & Light Stress treatment responded to a prolonged period of stress (Phase I) and were then returned to a Control environment (Phase II) . Measurements were taken on the base of the leaf lamina. Each block contained 2 experimental units per 11 cultivars labeled A and B. SPAD values from units A and B were averaged to generate one SPAD value per cultivar per block. Leaves of selected leaf stages were tagged and measured repeatedly over time to track chlorophyll dynamics across leaf development. Created in BioRender. (Bowdish 2026).

Figure 1.4 Visual of selected leaf stages that were measured over time with SPAD to characterize foliar bleaching leaf maturity parameters

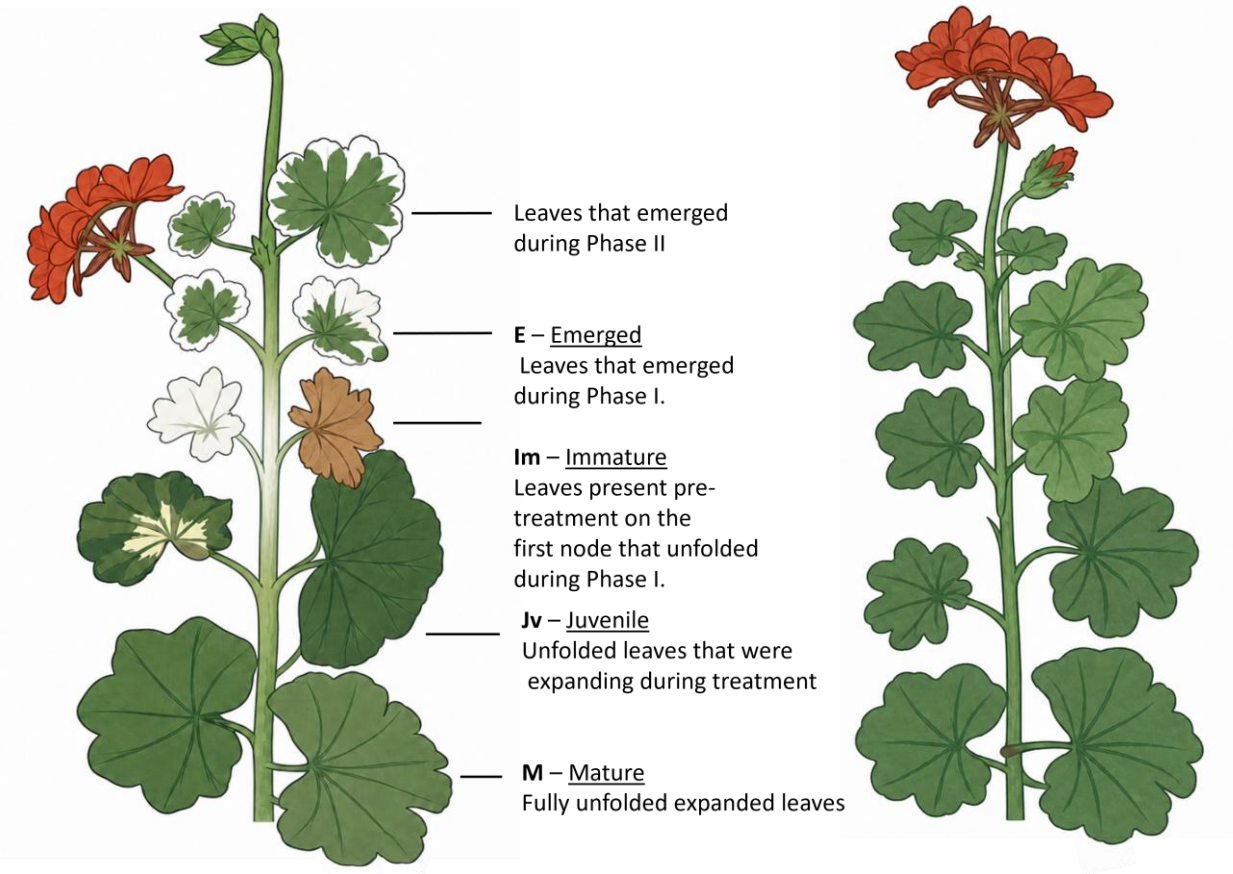


Figure 1.4 This visual depicts how the leaf nodes looked by day 50 in the experiment. The left image shows the leaf stages from the Elevated Temperature & Light Stress treatment, whereas the right image depicts the Control environment that shows how normal leaf morphology appeared. Created in BioRender (Bowdish 2026).

Figure 1.5 Depicts a bleaching and regreening phenotypic rating scale of foliar bleaching severity

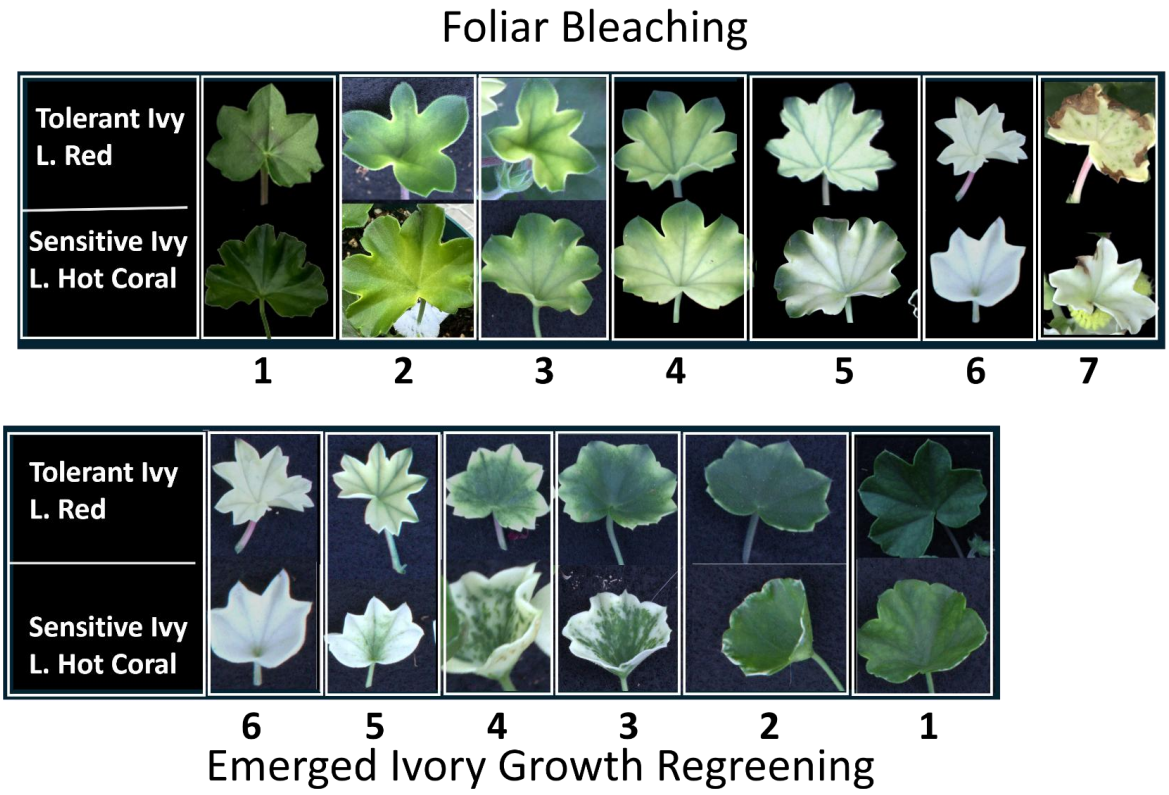
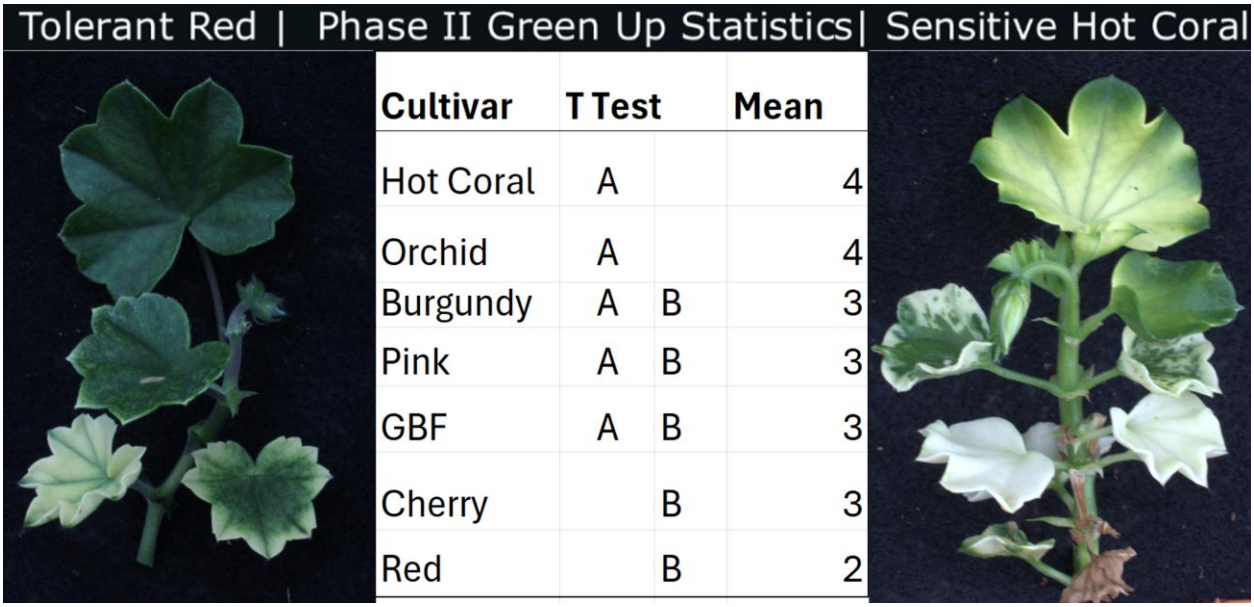


Figure 1.5 Depicts a bleaching and regreening phenotypic rating scale of foliar bleaching severity. The tolerant 'Ivy League Red' and sensitive 'Ivy League Hot Coral' cultivars were used. The scale assembles as follows: 1. No bleaching - fully green. 2. Discoloration and lighter green hue with yellow on margin crevices, or 80% green with white margins. 3. Bleaching leaf base (center of leaf base lamina white) at least half the leaf green and half white margins on the lobe. 4. ⅔ of the lamina is bleached with lobes green OR ⅔ of lamina is green with lobes white. 5. Margine edges of the lobes are green, 80% white OR center green. 6. Fully bleached white. 7. Necrotic. Bleaching and regreening have similar patterns, progressing at the leaf base lamina to the edges, and regreening the same way.

Figure 1.6 To evaluate recovery from bleaching, phenotypic rating statistics were performed using the node-level scores generated from the ratings scale described in Fig. 1.5.



Cultivar	T Test		Mean
Hot Coral	A		4
Orchid	A		4
Burgundy	A	B	3
Pink	A	B	3
GBF	A	B	3
Cherry		B	3
Red		B	2

Figure 1.6 To evaluate recovery from bleaching, phenotypic rating statistics were performed using the node-level scores generated from the ratings scale described in Fig. 5. Ratings for the four evaluated nodes were averaged for each cultivar within each replication. One-way ANOVA was run on the average node phenotype score and means were compared with Tukey's HSD (Fig. 5).

Figure 1.7 Photos showing temporal progression of foliar bleaching and regreening over leaves of varying maturity, consistent in 11 Pelargonium cultivars from 3 cultivar series that had different growth habits, genetic lineages, and heat tolerance

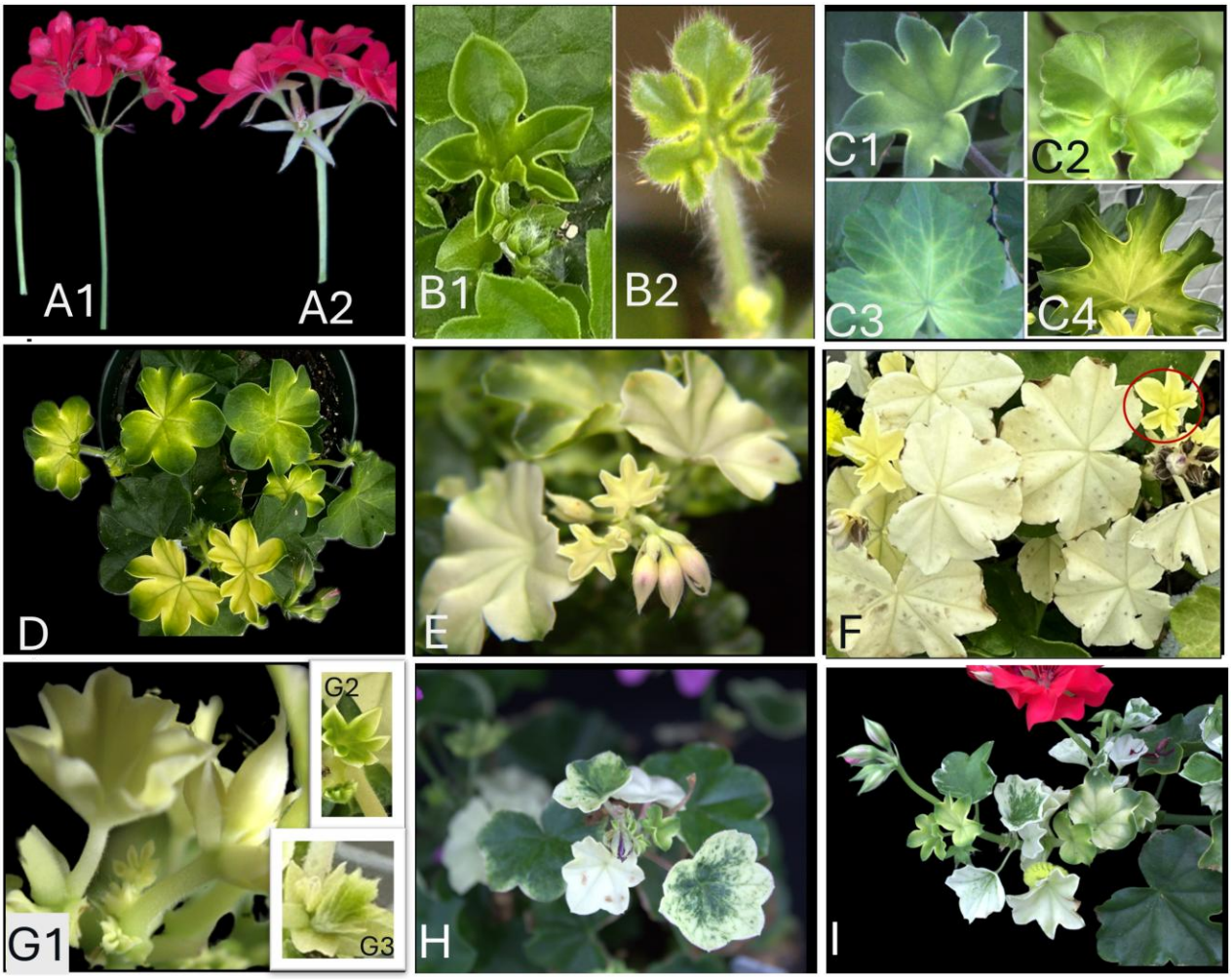


Figure 1.7. Documented in Elevated Temperature & Light (ETL) treatment over 26 days of stress and 24 days of recovery in Control environment conditions. A1: Control flower A2: bleached ETL flower, petals are not impacted; B: Phase I of ETL, Initial symptoms start on leaf edges of immature growth, B1:'Ivy League' series B2: 'Calliope' series, C. Bleaching progressed to leaf base expanding outwards towards leaf edges C1: 'Ivy League' Juvenile lamina yellowing C2: 'Calliope' immature lamina yellowing C3: 'Calliope' Juvenile veins bleaching C4:'Cascade' Juvenile bleaching along variegation boundaries, D: Overview of bleached node variation between Juvenile leaves, E: Bleached Immature growth and Emerged Ivory growth, F: Necrosis on Immature growth, Emerged ivory growth not necrotic circled; G: Emerged ivory growth cupped with Ivory buds G2G3:Phase II regreening Ivory growth, H: Phase II start regreening 'Ivy League Orchid', I: Phase II end regreening 'Ivy League Hot Coral'.

Figure 1.8 Zonal Interspecific Hybrid 'Calliope' and European Style Cascading Series 'Cascade' bleaching and greening patterns over time that are series specific to their leaf morphology

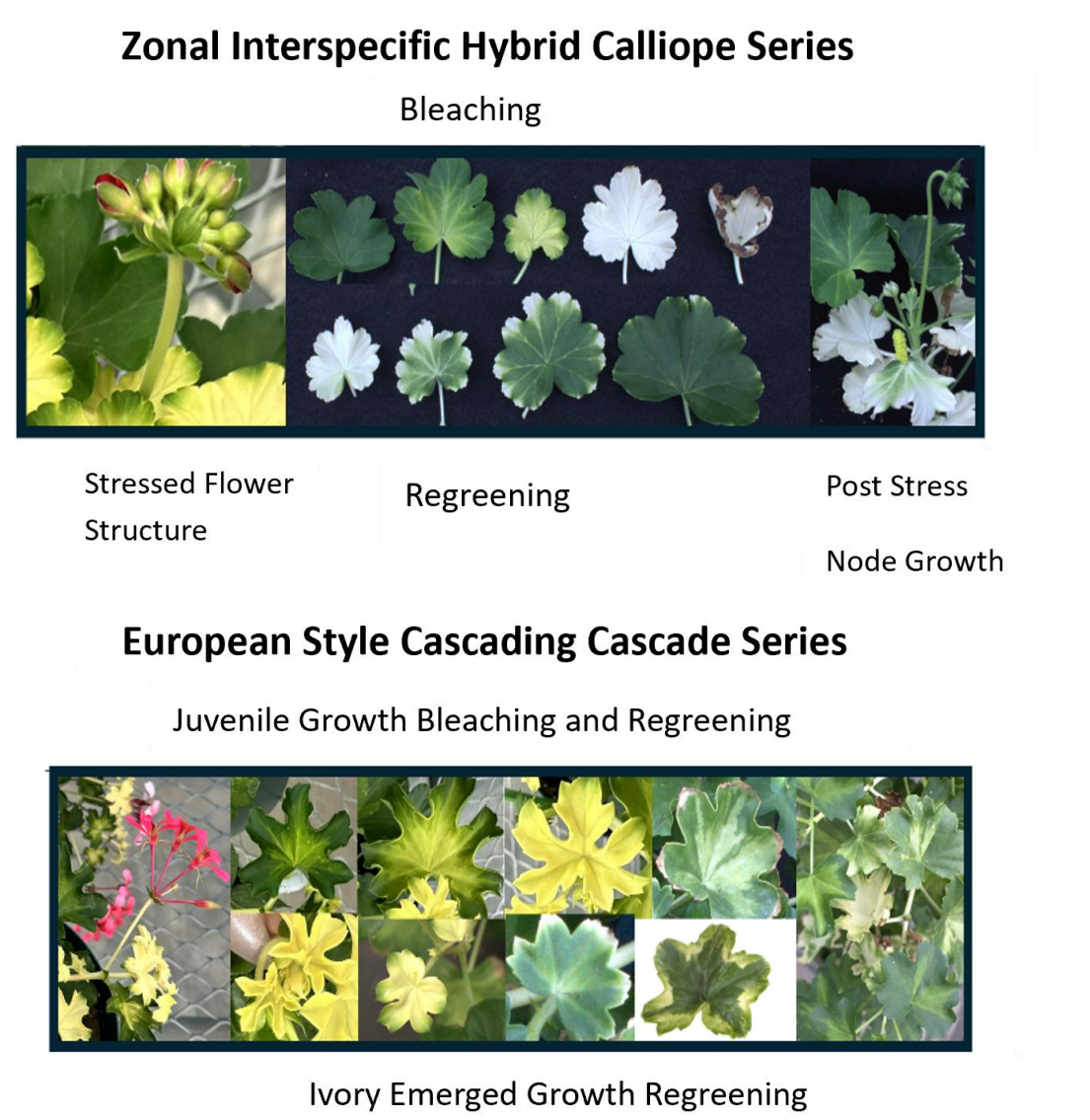
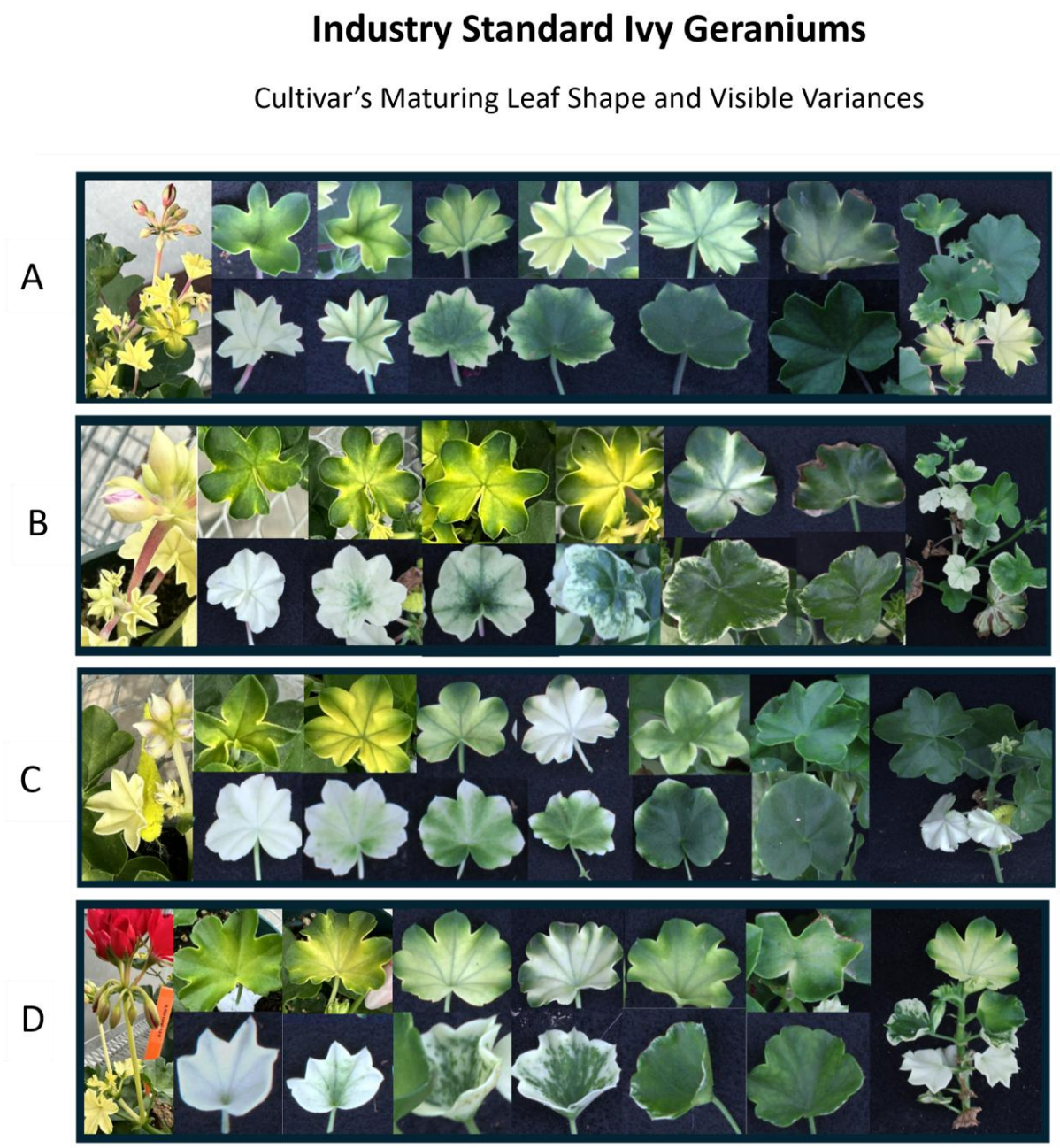


Figure 1.8 Zonal Interspecific Hybrid 'Calliope' and European Style Cascading Series 'Cascade' bleaching and greening patterns over time that are cultivar series specific to their leaf morphology. Bleaching occurs in high temperatures (30oC) and will resume normal pigmentation once temperatures fall below 30oC. 'Calliope' leaves follow the same bleaching and regreening pattern regardless of leaf maturity, as long as the leaf is capable of regreening. The 'Cascade' cultivars will bleach along the green variegated boundary layers. When the leaf regreens, Juvenile leaves will follow this same variegation pattern; however, new Immature growth will attempt to fully regreen before adjusting to a variegated pattern.

Figure 1.9 'Ivy League' Series cultivars exhibit distinct bleaching and greening patterns over time between cultivars and between leaf stage within cultivar



'Ivy League' cultivar series distinct Elevated Temperature and Light stress (ETL) bleaching and optimal temperature regreening differences in leaf morphology pattern between cultivars and between leaf stage within cultivar. Bleaching appearance differed between leaves that had more lobing. Left Image: Flower, top row: Juvenile bleaching to greening, Bottom row: Emerged Ivory growth greening. A: 'Ivy League Red' Deep lobing and distinct pinkness on petioles and flowers, B: 'Ivy League Orchid' Shallow lobing and distinct pinkness on petioles and flower structure, C: 'Ivy League Cherry Blossom' Moderate to deep lobing and no pinkness, D: 'Ivy League Hot Coral' shallow to moderate lobing and no pinkness.

Figure 1.10 This line graph visualizes relative chlorophyll content (SPAD) measurements in specific leaf stages over time in an Elevated Temperature & Light Environment in 11 cultivars representing 3 commercial cultivar series of various growth habits

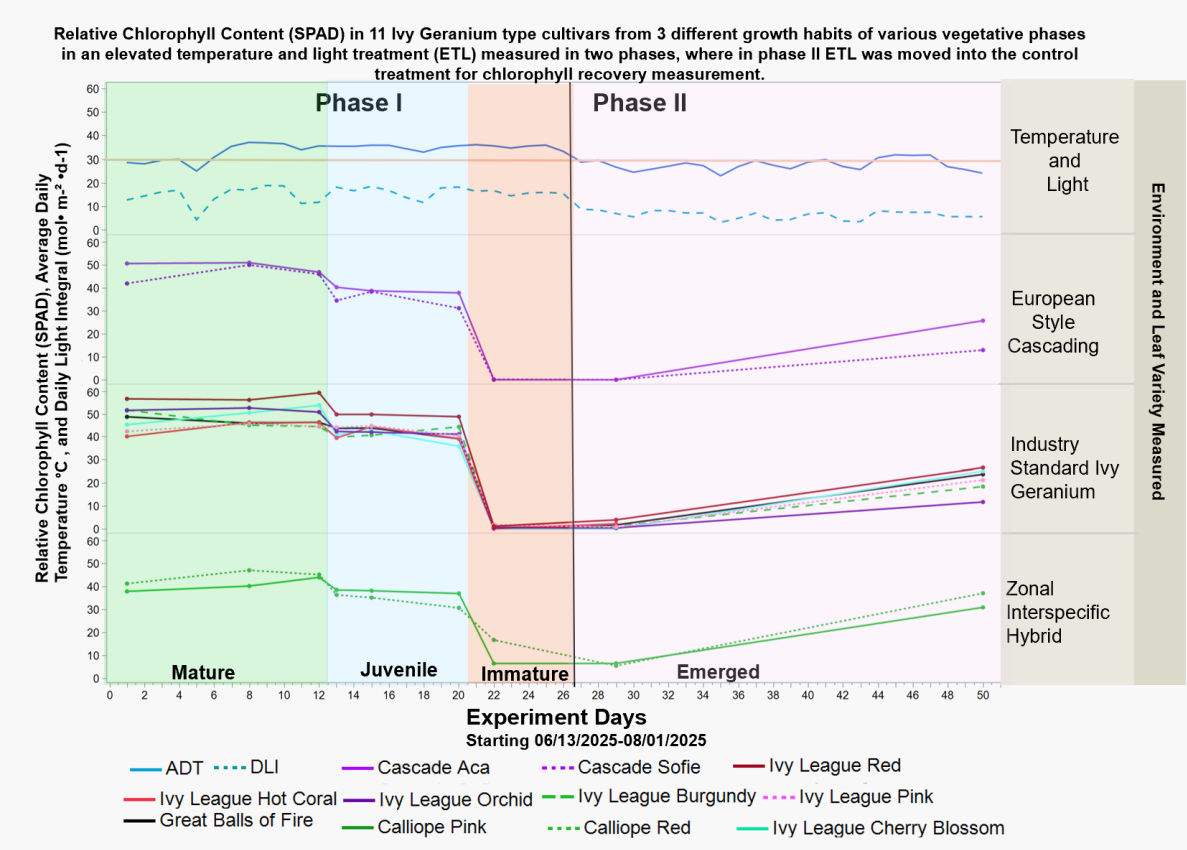


Figure 1.10 This line graph visualizes relative chlorophyll content (SPAD) measurements in specific leaf stages over time in an Elevated Temperature & Light Environment in 11 cultivars representing 3 commercial cultivar series of various growth habits. European-style Cascading (extremely trailing growth, ‘Cascade Aca’, ‘Cascade Sofie’), Industry Standard Ivy Geraniums (semi trailing growth, ‘Ivy League Red’, ‘Orchid’, ‘Hot Coral’, ‘Burgundy’, ‘Pink’, ‘Cherry Blossom’, and ‘Great Balls of Fire’), and Zonal Interspecific Hybrids (upright mounded growth, ‘Calliope Pink’ and ‘Calliope Red’) were monitored throughout the experiment and analyzed for their response to Phase I that had the environmental conditions to induce foliar bleaching and Phase II that placed the plants into the Control Environment for green up. SPAD values are shown for 4 leaf developmental stages over time (Mature, Juvenile, Immature, Emerged) and are plotted alongside the Average Daily Temperature and Daily Light Integral environmental conditions during the experiment. N=6, until day 50 where Cascading = 1, Orchid=4, Cherry Blossom=5, and Hot Coral =0. This figure illustrates how chlorophyll content responded to ETL stress and subsequent recovery across growth habits and developmental stages.

Figure 1.11 This line graph visualizes SPAD measurements of relative chlorophyll content in a Control non-stressed Environment measured over time for 11 cultivars representing 3 commercial cultivar series of various growth habits

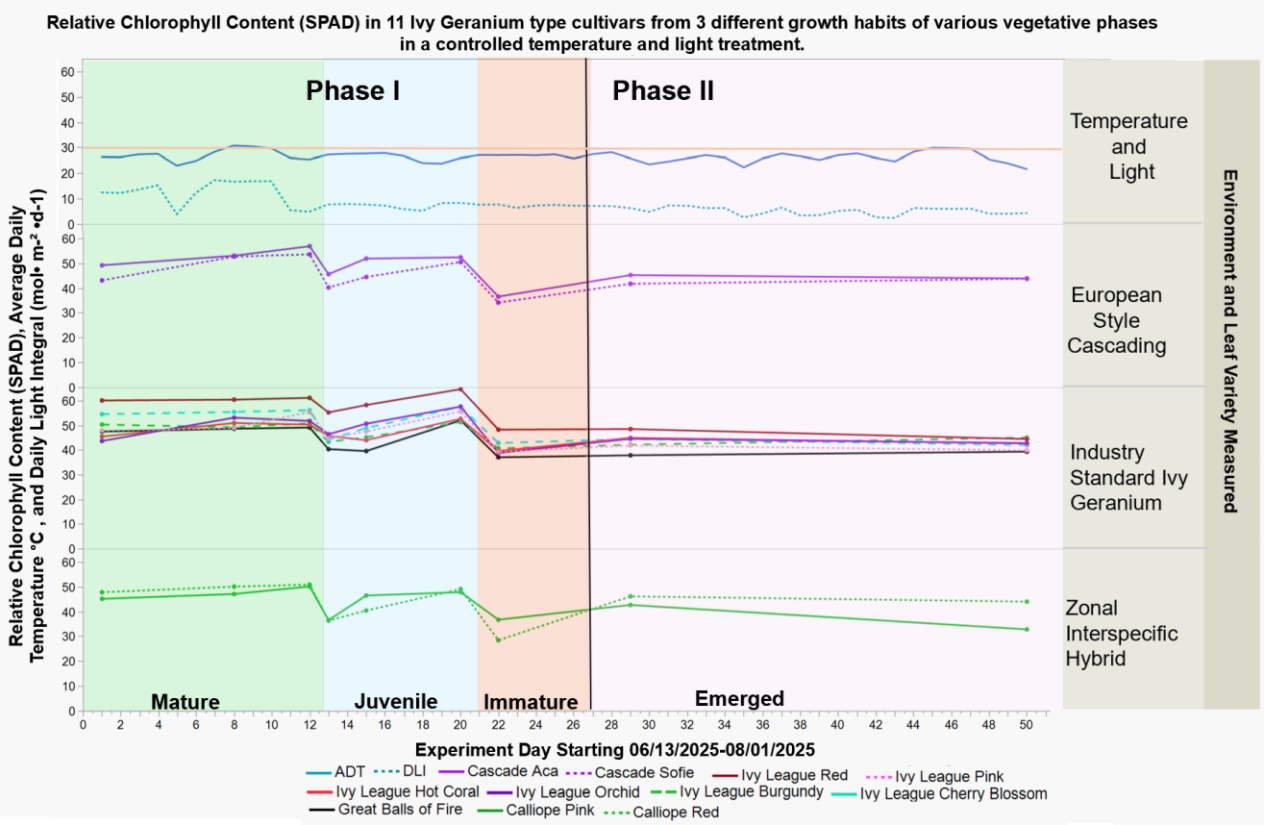


Figure 1.11This line graph visualizes SPAD measurements of relative chlorophyll content in a Control non-stressed Environment measured over time for 11 cultivars representing 3 commercial cultivar series of various growth habits. European-style cascading (extremely trailing growth, ‘Cascade Aca’, ‘Cascade Sofie’), Industry Standard Ivy Geraniums (semi trailing growth, ‘Ivy League Red’, ‘Orchid’, ‘Hot Coral’, ‘Burgundy’, ‘Pink’, ‘Cherry Blossom’, and ‘Great Balls of Fire’), and Zonal Interspecific Hybrids (upright mounded growth, ‘Calliope Pink’ and ‘Calliope Red’) were monitored throughout the experiment and relative leaf greeness was measured (SPAD) to create a normal comparison for the Elevated Temperature & Light Stress (ETL) treatment. SPAD values are shown for 4 leaf developmental stages over time (Mature, Juvenile, Immature, Emerged) and are plotted alongside the Average Daily Temperature and Daily Light Integral environmental conditions during the experiment. The experiment was conducted in 2 phases, Phase I inducing bleaching in the ETL and Phase II placed the ETL in the Control Environment for regreening. N=6. (Created with JMP Pro & Microsoft PowerPoint).

Table 1.1 This table summarizes the 11 cultivars selected for the foliar bleaching experiment, including growth habit, genetic lineage, commercial significance, and reported heat tolerance ratings. Each cultivar is shown with an example Juvenile leaf to highlight

Table 1. Selected cultivars evaluated in the greenhouse experiment that summarize heat tolerance rating, genetic background, and leaf morphology at the juvenile stage of foliage development.			
Cultivar	**Significance/Previous Alliases or lines**	**Lamina Photo**	**Heat Tolerance Rating**
Ivy League Red	Utilized as a heat 'tolerant' cultivar in prior bleaching studies since 2004. Formerly known as Contessa Red or Beach, it has existed on the market since the late 1900s.		Good
Ivy League Amethyst	Evaluated for heat related bleaching in 2004. Formerly known as Contessa Lavender, Amethyst, or Amethyst 96, it has existed on the market since the late 1900s.		Good
Ivy League Burgundy	Formerly known as Contessa Burgundy or Taj Mahal. It has existed on the market since the late 1900s.		Good
Ivy League Deep Pink	Formerly known as Contessa Pink or Pink Spirit, it has existed on the market since the 2000s.	Not depicted	
Ivy League Hot Coral	Formerly known as Temprano Hot Coral it has existed on the market since the 2000s.		
Ivy League Orchid	Introduced in the 2000s, it was permanently removed from production at the end of 2024 due to poor performance.		

Ivy League Cherry Blossom	Introduced in 2020/2021, it is known for retaining petal color despite intense light intensity, unlike previous bicolor cultivars.		Good
Dümmen Orange Great Balls of Fire Pink	Performance is compared to Ivy League Cherry Blossom. In May 2026 Syngenta and Dümmen Orange joined operations, eventually impacting future cultivars.		
Cascade Acapulco	Found to be heat 'intolerant' in 2004 bleaching study. On the market since the late 1900s.		Good
Cascade Sofie	Found to be heat 'intolerant' in 2004 bleaching study. On the market since the late 1900s.		
Calliope Medium Dark Red Dark Leaf	National All-America Selections Winner in 2017		Excellent
Calliope Medium Pink Flame	Often paired with Calliope Medium Dark Red in landscapes.		Good

Table 1.1 This table summarizes the 11 cultivars selected for the foliar bleaching experiment, including growth habit, genetic lineage, commercial significance, and reported heat tolerance ratings. Each cultivar is shown with an example Juvenile leaf to highlight morphological differences relevant to bleaching diagnostics. Together, these cultivars represent the major commercial series of ivy type geraniums used in industry. (Harkess, 2004, Syngenta 2010, Syngenta, 2023)

Table 1.2 Statistical analysis of Environment treatments [Elevated Temperature & Light (ETL) and Control as calculated from environmental monitors (n=6)]. The experiment was conducted in two phases: Phase I Bleaching, Phase II Regreen

Table 2. Environmental conditions between the elevated temperature and light (ETL) versus Control greenhouse environments during Phase I (to induce foliage bleaching of ivy geranium) and Phase II (to allow for regreening of bleached foliage).

Treatment	Environmental Measurements$_z$								
	Temperature °C		RH %		Dew °C		ADT °C	PPFD µmol•m^{-2}•s^{-1}	DLI mol• m^{-2} •d^{-1}
	Day$_y$	Night$_x$	Day$_y$	Night$_x$	Day$_y$	Night$_x$			
Phase I$_w$									
Elevated	36.8a	28.9a	45.8b	65.0b	21.4b	21.1a	33.8a	285.0a	15.3a
Control	27.6b	26.1b	76.0a	84.0a	23.2a	22.0a	27.0b	181.0b	9.8b
Phase II$_v$									
Elevated	29.0a	25.4a	75.0b	84.0a	24.0a	22.4a	27.7b	117.0c	6.3c
Control	26.4b	26.1a	85.0a	85.0a	24.0a	22.4a	26.3b	97.0c	5.2c
Summary Statistics									
Prob>|t| Phase * Environment	<0.0001	0.0005	<0.0001	<0.0001	0.0589	0.5439	<0.0001	0.0003	0.0003
Prob>|t| Phase main effect	<0.0001	0.0005	<0.0001	<0.0001	0.0002	0.0233	<0.0001	<0.0001	<0.0001

Prob>|t| Environment main effect	<0.0001	0.0283	<0.0001	<0.0001	0.0055	0.5316	<0.0001	<0.0001	<0.0001

Table 1.2 Statistical analysis of Environment treatments [Elevated Temperature & Light (ETL) and Control as calculated from environmental monitors (n=6)]. The experiment was conducted in two phases: Phase I, during which ETL conditions induced foliar bleaching allowing for data collection and further documentation of the disorder, and Phase II, during which the ETL plants were moved into the Control Environment and regreening was monitored. Each environmental variable was analyzed using a standard least squares ANOVA with Treatment, Phase, and the Phase x Treatment interaction. Significant differences between treatments in Phase I indicate environmental conditions sufficient to induce bleaching, whereas significant differences in Phase II indicate conditions conducive to regreening post stress. Different letters indicate statistical difference.

Table 1.3 Evaluates tissue nutrient concentration of bleached versus green foliage from the most tolerant and sensitive cultivars.

Treatment	P	K	Ca	Mg	S	Zn	Fe	Mn	Cu
Foliage (n=4)									
Bleached	0.9805	5.785	0.67725	0.46075	0.366	74.45	96.345	182.875	15.025
Green	0.60925	3.0925	0.80225	0.332	0.2965	32.45	55.345	159.675	6.4
Prob>F	ns	ns	ns	ns	ns	ns	ns	ns	0.0143
Cultivar (n=4)									
Red	0.73725	4.3275	0.7885	0.403	0.32725	38.7	50.8575	167.7	9.075
Hot Coral	0.8525	4.55	0.691	0.38975	0.33525	68.2	100.8325	174.85	12.35
Prob >F	ns	ns	ns	ns	ns	ns	ns	ns	ns
Foliage x Cultivar (n=2)									
Bleached x Red	0.887	5.825	0.755	0.4645	0.353	50.05	63.815	193	12.3
Bleached x Hot Coral	1.074	5.745	0.5995	0.457	0.379	98.85	128.875	172.75	17.75
Green x Red	0.5875	2.83	0.822	0.3415	0.3015	27.35	37.9	142.4	5.85
Green x Hot Coral	0.631	3.355	0.7825	0.3225	0.2915	37.55	72.79	176.95	6.95
Prob >F	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 1.3 Evaluates tissue nutrient concentration from a sensitive (‘Ivy League Hot Coral’) and tolerant (‘Ivy League Red’) variety of ivy geranium. Immature leaves of bleached versus green foliage was collected from the cultivars at each developmental stage and was analyzed for P, K, Ca, Mg, S, Fe, Mn, Zn, and Cu. Tissue nutrient content of bleached versus green foliage from two cultivars, ‘Ivy League Red’ and ‘Ivy League Hot Coral,’ was evaluated as a two-way factorial using JMP ver. 16 (SAS, Cary, NC, USA).

Table 1.4 Statistical analyses results of SPAD on leaf stages over time between environments of different Pelargonium sp. cultivars'.

		Phase I									Phase II		
Target Growth Stage		Mature			Mature Change Over Time T Test	Juvenile			Juvenile Change Over Time T Test	Immature	Emerged		Emerged Change Over Time T Test
Day	Mean	1	8	12	1*8*12	12	15	20	12*15*20	22	29	50	29*50
Ivy League Red	ETL	56.67	56.18	59.3	0.5057 (+)	49.87	49.83	48.87	0.8378	1.1	3.92	26.62	0.0013* (+)
	Control	60.1	60.43	61.15	0.8213	55.25	58.27	64.67	0.0276* (+)	48.28	48.52	44.47	0.187 (-)
	ETL*Control T Test	0.5737	0.2293	0.2869		0.2752	0.0981	0.0202*		<.0001*	<.0001*	0.0136*	
Ivy League Pink	ETL	42.45	45.77	44.6	0.3095 (+)	44.2	44.9	40.48	0.283 (-)	1.77	0.72	21.33	0.0044* (+)
	Control	47.88	48.95	55.43	0.0372* (+)	44.8	47.53	55.63	0.0001* (+)	39.3	41.85	39.92	0.6382 (-)
	ETL*Control T Test	0.1496	0.0871	0.0050*		0.8285	0.4231	0.0053*		<.0001*	<.0001*	0.0196*	
Ivy League Hot Coral	ETL	40.22	46.38	46.3	0.0385* (+)	39.58	44.48	39.33	0.6814 (+/-)	0.05	2.23	--- n=0	1
	Control	45.49	50.97	50.3	0.0456* (+)	45.72	44	52.7	0.0434* (+)	39.63	44.97	42.65	0.5227 (-)
	ETL*Control T Test	0.224	0.0706	0.0114*		0.1228	0.8944	0.0095*		<.0001*	<.0001*		
Ivy League Orchid	ETL	51.62	52.7	50.87	0.888	42.43	42.08	41.18	0.8035	0.48	0.4	11.73 n=4	0.0032* (+)
	Control	43.69	53.12	51.82	0.0010* (+)	46.43	50.68	57.6	<.0001* (+)	38.85	44.55	42.85	0.7196 (-)
	ETL*Control T Test	0.0022*	0.9053	0.7522		0.3066	0.1038	0.0059*		<.0001*	<.0001*	0.0002*	
Ivy League Burgundy	ETL	51.78	45.17	44.57	0.0822 (-)	39.95	40.77	44.43	0.1643 (+)	0.08	1.15	18.48	0.0006* (+)
	Control	50.35	49.25	50.83	0.9615	43.38	45.17	51.35	0.0011* (+)	40.7	42.18	45.07	0.3094 (+)
	ETL*Control T Test	0.8044	0.3182	0.1698		0.2548	0.0621	0.0843		<.0001*	<.0001*	0.0001*	
Ivy League Cherry Blossom	ETL	45.38	50.55	53.87	0.0434* (+)	40.97	42.75	35.92	0.2918 (-)	0	0	25.16 n=5	0.0030* (+)
	Control	54.52	55.45	56.15	0.337 (+)	44.27	48.92	57.58	<.0001* (+)	42.87	44.47	42	0.5021 (-)
	ETL*Control T Test	0.0986	0.0096*	0.3345		0.4504	0.2059	0.0033*		<.0001*	<.0001*	0.0724	
Great Balls of Fire	ETL	48.8	46	46.42	0.2731	43.77	43.88	39.22	0.2687 (-)	0.38	1.68	23.8	0.0004* (+)
	Control	47.52	48.65	49.13	0.5735 (+)	40.37	39.6	52.07	0.0012* (+)	37.07	37.88	39.35	0.6635 (+)
	ETL*Control T Test	0.7398	0.1004	0.2936		0.3618	0.3078	0.0230*		<.0001*	<.0001*	0.0100*	
Calliope Pink	ETL	37.77	40.1	43.86	0.0707 (+)	38.4	38.05	39.85	0.541	6.4	6.45	30.82	0.0002*(+)
	Control	45.2	47.15	50.18	0.2637 (+)	36.62	46.53	47.88	0.0424* (+)	36.77	42.73	32.88	0.0542 (-)
	ETL*Control T Test	0.1418	0.0658	0.1101		0.5586	0.1213	0.0012*		<.0001*	<.0001*	0.7155	
Calliope Red	ETL	41.15	46.95	45.06	0.0756 (+)	36.25	35.05	30.6	0.0752 (-)	16.63	5.4	36.97	0.0011* (+)
	Control	47.85	50.12	50.92	0.4787 (+)	36.43	40.48	49.1	0.0011* (+)	28.5	46.2	44.08	0.4436 (-)
	ETL*Control T Test	0.0815	0.431	0.1467		0.9379	0.0775	0.0020*		0.3663	<.0001*	0.305	
Cascade Sofie	ETL	41.88	49.93	45.93	0.3512 (+)	34.47	38.32	31.12	0.3816 (+/-)	0.15	0	12.9 n=1	<.0001* (+)
	Control	43.18	52.75	53.65	0.0047* (+)	40.28	44.53	50.53	0.0216* (+)	34.22	41.78	43.8	0.5754 (+)
	ETL*Control T Test	0.7911	0.4395	0.1612		0.3341	0.2152	0.0004*		<.0001*	<.0001*		
Cascade Aca	ETL	50.57	50.92	46.81	0.2878 (-)	40.23	38.68	37.78	0.5897 (-)	0	0	25.7 n=1	<.0001* (+)
	Control	49.23	53.1	56.91	0.0414* (+)	45.67	51.93	52.45	0.2535 (+)	36.62	45.28	43.93	0.732 (-)
	ETL*Control T Test	0.6885	0.5344	0.0121*		0.2318	0.0286*	0.0069*		<.0001*	<.0001*		

n=6 unless noted , (+)=positive change, (-)=negative change

Table 1.4 Statistical analyses results of 11 different *Pelargonium* sp. cultivars' relative chlorophyll content (SPAD) compared between an Elevated Temperature & Light (ETL) versus a Control environment. Relative leaf greenness (SPAD) was measured on different leaf stages over time to determine how leaf maturity parameters influence bleaching. The Immature leaf on day 22 was necrotic and could no longer regreen, therefore ETL plants were moved into the Control environment for Phase II regreening. SPAD comparisons between treatments were performed using two-sample t-tests for each date within a given leaf stage. Statistical analyses were conducted in JMP Pro using the Fit Model Platform.

Table 1.5 Statistical analysis of main effect of Environment on leaf maturity stages.

Phase I											Phase II		
Leaf Maturity		Mature			Change over time T Test	Juvenile			Change over time T Test	Immature	Emerged		Change over time T Test
Day		1	8	12	1*8*12	12	15	20	12*15*20	22	26	50	26*50
SPAD mean of all cultivars	ETL	46.21	48.24	47.96	0.1293	40.92	41.71	38.71	0.0665 (-)	2.46	2	24.67 n=47	<.0001* (+)
	Control	48.64	51.81	53.32	0.0001* (+)	43.97	47.06	53.78	<.0001* (+)	38.44	43.67	41.91	0.1207 (-)
	ETL *Control T Test	0.1065	0.0011*	<.0001*		0.0464*	<.0002*	<.0001*		<.0001*	<.0001*	<.0001*	
n=66 unless noted , (+)=positive change, (-)=negative change													

Table 1.5 Statistical analysis of main effect of Environment on leaf maturity stages. The Elevated Temperature & Light (ETL) treatment was managed at an average daily temperature (ADT) of 33.8°C and daily light integral (DLI) of 15.3 mol• m^{-2} •d^{-1} to induce foliar bleaching in Phase I, whereas the Control environment had an ADT of 27.7°C and DLI of 9.8 mol• m^{-2} •d. Leaf greenness measured with a SPAD meter was taken on mature leaves measured on days 1, 8, 12; juvenile leaves measured on days 12, 15, and 20; and bleached, immature leaf on day 22 (Phase I); and emerged leaves on days 26 and 50 after ETL ended (Phase II).

Chapter 2 - Genotypic Characterization of Foliar Bleaching Associated with Heat and Light Stress in *Pelargonium peltatum* Cultivars

Foliar bleaching is a physiological disorder affecting many terrestrial plants under elevated heat and light stress, whereby young foliage loses chlorophyll and new foliage emerges ivory. *Pelargonium* species are well documented to demonstrate this disorder which is especially pronounced in ivy geranium, *Pelargonium peltatum* (Miao et al 2026; Harkess 2004) and in several of its intergeneric crosses with zonal geranium (*Pelargonium* x *hortorum*; Dhir, 2013; Owen 2017). In an earlier greenhouse study, we observed that the occurrence and recovery of foliar bleaching is strongly shaped by stage of leaf development, proximity of a leaf to the apical meristem, and cultivar-associated chlorophyll stability. These morphological and anatomical differences raise questions about why ivy geranium cultivars respond differently to elevated temperature and light stress (ETL). Because the pigment chlorophyll is associated with leaf greenness, which decreases under ETL and increases when ETL abates, we sought to investigate a genomic association with the disorder in the chlorophyll biosynthesis pathway using *P. peltatum* cultivars.

Chlorophyll biosynthesis is a central physiological pathway regulating plant development, stress response, and plastid-to-nucleus signaling. A key enzyme in this pathway is light-dependent protochlorophyllide oxidoreductase (LPOR). Before chloroplasts fully develop, LPOR resides within the prolamellar body of etioplasts; upon light exposure, etioplasts differentiate into functional chloroplasts, and LPOR helps with assemblage of new thylakoid membranes. LPOR is encoded in the nucleus and imported into plastids, making the enzyme a

critical component of retrograde signaling between the chloroplast and the nucleus (Li et al. 2025; Gabruk 2015).

LPOR's primary role is the reduction of protochlorophyllide (Pchlide) to chlorophyllide (Chlide). Timely LPOR-mediated reduction is essential for normal green pigmentation and functioning chloroplasts. When this reduction fails, Pchlide accumulates and generates reactive oxygen species (ROS) that damage chloroplast membranes and ultimately cause cell death (Li et al. 2025). Recent studies show that LPOR not only prevents this phototoxification, but can also structurally reorganize itself in response to stress. Under heat stress, LPOR proteins can assemble into protective "shield-like" structures that stabilize membranes and mitigate photodamage (Alquraish 2026).

Temperature and light intensity regulate LPOR activity (Vedalankar 2024). Earlier work with *Pelargonium peltatum* suggested that future studies should examine this gene, specifically the LPOR isotype B, LPORB. These researchers observed that during elevated temperature stress new growth was emerging with not with green pigmentation, but as ivory (Dhir 2013). Similarly, ivory foliage was observed emerging from the apical meristem in Arabidopsis seedlings under 40^{o}C heat stress. Interestingly, this study found that mutants lacking PORC expression did not exhibit bleaching in existing foliage at 23^{o}C, but bleaching occurred under heat stress at 42^{o}C, and bleaching was more significant in the mutant than the wild type (Alquraish 2026). With this background, examination of the gene in stress tolerant and sensitive *Pelargonium peltatum* cultivars may provide insight as to how POR-associated chlorophyll biosynthesis expression contributes to bleaching tolerance. Among the LPOR isoforms, PORC is uniquely responsive to environmental conditions (Li et al. 2025); for these reasons, this study aims to examine PORC expression under ETL.

Pelargonium has long served as a model system for plastid biology, providing foundational evidence for non-Mendelian variegation inheritance (Wehe et al. 2009) and lineage-specific plastid genome reconfiguration (Weng 2014). Although *Pelargonium* possesses the largest chloroplast genome of any plant sequenced to date (Chumley et al. 2006), its nuclear genome remains only partially assembled due to extensive repeat content (Wehe et al. 2009). Nonetheless, several Illumina shotgun datasets and SRA short-read libraries exist for *Pelargonium* and closely related taxa making expression analysis possible. As summarized by Floris Breman (2021), accredited *Pelargonium* phylogenetic studies consistently rely on highly conserved plastid and nuclear genes. Because LPOR is highly conserved across angiosperms, conserved PORC coding regions from related species can be used to reconstruct a partial sequence for *Pelargonium*, providing a practical entry point for expression work.

The objective of this pilot study was to establish an initial foundation for POR expression analysis in *Pelargonium peltatum*. Sequences from related species, including *Jatropha curcas*, *Vitis vinifera*, *Theobroma cacao*, and *Citrus sinensis*, were retrieved from National Center for Biotechnology Information (NCBI,2026) and aligned using T-Coffee (Notredame, 2000). A strongly conserved ~300 bp region was identified and used to design primers for amplification in *Pelargonium peltatum*. This strategy enabled the reconstruction of a presumptive partial PORC sequence from *Pelargonium peltatum* control tissue, forming the basis for subsequent expression analysis.

Materials and Methods

Cultivar selection. Two *Pelargonium peltatum* cultivars were selected based on contrasting responses to heat and light induced foliar bleaching documented in Bowdish (Chapter 1). ‘Ivy League Red’ represents the tolerant and ‘Ivy League Hot Coral’the sensitive varieties,

respectively. These cultivars were selected from the high and low ends of the range that we observed in bleaching tolerance and regreening capacity.

Related taxa as a reference genome. Pelargonium peltatum does not currently have an assembled or annotated nuclear reference genome. However, Illumina shotgun datasets and SRA short-read libraries exist for *Pelargonium* and closely related taxa. Closely related taxa was identified from a published de novo transcriptome analysis of rose-scented geranium that provided insights into distribution of top species hits (Narnoliya et al. 2017).

To reconstruct a partial LPOR (PORC) coding region suitable for expression analysis, PORC sequences from related taxa with validated or predicted LPOR proteins were retrieved from National Center for Biotechnology Information (NCBI 2026).

The following PORC predicted sequences were used for alignment: (Table 2.1).

- *Jatropha curcas* XM_012232922.3 (LOC105646961)
- *Vitis vinifera* XM_002264006.4 (LOC100241491)
- *Theobroma cacao* XM_007043490.2 (LOC18608682)
- *Citrus sinensis* XM_006464654.4 (LOC102614959)

Multiple sequence alignment and consensus identification. PORC coding sequences were aligned using T-Coffee Version 11.00 (Notredame, 2000), to identify conserved regions across species. In NCBI, the conserved region was used to search for similar sequences in the *Pelargonium peltatum* SRA database using BLASTN. A strongly conserved ~300 bp region was selected as the scaffold for reconstructing the *Pelargonium peltatum* PORC fragment (Figure 2.2).

SRA-based reconstruction. The consensus sequence was used as a BLASTN query against *Pelargonium peltatum* SRA datasets. Blast short-read hits were imported into

SnapGene® software (from Dotmatics; available at snapgene.com), and used as directional digital primers on top of the scaffolding consensus sequence to map boundaries and bridge gaps in the partial PORC region Additional reads from a hybrid *Pelargonium* isolate ("Chinese hybrid isolate GKCL01058310.1") were incorporated to strengthen reconstruction.

Primer design. Primers were designed from the *Pelargonium* SRA reconstructed ~300 bp conserved region using SnapGene® software (from Dotmatics; available at snapgene.com). These primers were used for both DNA-level and cDNA-level amplification. PCR products were visualized by agarose gel electrophoresis.

Initial cDNA amplification and sequencing. Tissue was collected from stock greenhouse plants maintained under shade near KÜÜL® Cell Pads with exhaust fans on to manage non-ETL environmental conditions. The average daily temperature remained below 21°C for 14 days prior to sampling. DNA PCR was performed on the *Pelargonium peltatum* cultivars 'Ivy League Red' (tolerant to ETL), 'Ivy League Hot Coral' (sensitive to ETL), and moderate 'Ivy League Pink'. Amplicons were submitted for sequencing, and sequences were analyzed using T-Coffee, Version 2026, (Notredame, 2000).

Experimental design for expression analysis. Two plants each of well established, vegetatively-propagated 'Ivy League Red' and 'Ivy League Hot Coral' cultivars in 6-inch-diameter pots from the stock greenhouse were placed diagonally from each other in a growth chamber on 27 May 2026. The meristematic points of actively growing nodes were soft-pruned. Plants were fertigated as needed.

The growth chamber was set to 24°C day (13 hours) and 21°C night (11 hours). The DLI was 2 $mol \cdot m^{-2} \cdot d^{-1}$. The experiment was conducted in three phases: Phase I, optimal temperature conditions; Phase II, bleaching temperature conditions; and Phase III, regreening in optimal

temperature conditions. Expression analysis was only conducted on tissue in Phase I and Phase II.

In Phase I, the growth chamber was set at a low temperature of 24°C (13 h day and 21°C, 11 h night) for 14 days. Leaf samples that had emerged from the apical meristem under the treatment environment, equal in size and emergence time, were taken on 9 June 2026.

For Phase II, the plants were moved into the greenhouse to allow for foliar bleaching in direct sunlight. The exhaust fans and KÜÜL® Cell Pads were not running in the greenhouse, and there was no whitewash application to reduce light intensity. The average day temperature was above 31°C. Phase II samples were taken on day 7 when bleaching appeared, and then plants were moved back into the growth chambers to examine Phase III regreening. Regreening occurred only 3 days after returning the plants to the growth chambers below 30°C.

PCR procedure. Total RNA was extracted from the collected tissue using the Zymo Research Direct-zol RNA Miniprep Kit; crude extract was treated with DNase I before purification. cDNA was synthesized from 20 ng of total RNA using the Thermo Fisher Scientific RevertAid First Strand cDNA Synthesis Kit with Oligo(dT) primers. Endpoint PCR was performed using Thermo Scientific DreamTaq DNA Polymerase and LPORCFw/Rv primers to amplify an approximately 300 bp product. The thermal cycling program consisted of an initial denaturation at 95°C for 3 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 20 s, and extension at 72°C for 20 s, with a final extension step at 72°C for 2 min. For the endogenous reference gene, Actin, the primer pair PhACTIN7-Fw/Rv was used to obtain an approximately 300 bp amplicon following a similar procedure, except for the thermal cycling parameters. These parameters consisted of an initial denaturation at 95°C for 3 min, followed by 26 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 20 s, and extension at 72°C for

20 s, with a final extension step at 72°C for 2 min. PCR products (5 μL) were resolved on an agarose gel in 1× TBE buffer and run at 80 V for 30 min. The RT-PCR product was fractionated by gel electrophoresis in agarose gels TBE 1%. Gel red was used for visualization.

Initial trials we used random hexamers for cDNA amplification as we used rRNA 16S as the endogenous control. However, detection for PORC was obtained only after 33 cycles, trials of lower cycles did not show any detection. We decided to use Actin 7, from Pelargonium x hortorum cv. Nittany Lion Red (He et al, 2010). Therefore, dT-primers were utilized and let us reduce the number of cycles to 30 to detect PORC.

The amplifications were obtained after 33 cycles, which was proven to be excessive, after trials of lower cycles did not show any detection. This is why a cycle of 30 was ideal, and dT-primers were utilized.

The conserved 300 bp PORC region identified through T-Coffee alignment (Notredame, 2000) was successfully amplified in *Pelargonium peltatum*. Sequencing confirmed similarity to PORC-like sequences from related taxa, supporting its identity as a partial PORC coding region.

Results and Discussion

Reconstructed alignment. Species *Jatropha curcas, Vitis vinifera, Theobroma cacao,* and *Citrus sinensis* provided a conserved region of PORC, acting as excellent scaffolding species for building alignments with *Pelargonium* SRA hits. This reconstructed region provided a reliable scaffold for primer design and enabled targeted amplification in tolerant and sensitive cultivars. While at this time our amplified region cannot be confirmed as PORC, our partial presumptive PORC sequence did significantly amplify. Collectively, these steps established the molecular

foundation necessary for subsequent POR expression analysis and for evaluating the role of LPOR (PORC) in cultivar-specific bleaching tolerance.

DNA PCR and sequencing. Gel results for 'Ivy League Red', 'Ivy League Hot Coral' and 'Ivy League Pink' were successfully amplified (Figure 2.3). This indicates that our presumable PORC sequence is present in all three cultivars. Additionally, Sanger sequencing results showed few nucleotide differences indicating high conservation at nucleotide level within that region (Figure 2.4). BLASTN results of all 3 cultivar sequences demonstrated at least 90% of query cover and 80 to 89% identity in species such as *Malus, Pyrus,* etc.

Semiquantitive reverse transcriptase-polymerase chain reaction (RT-PCR) for the PORC transcript. The expression patterns of the putative PORC transcript differed between the two varieties, suggesting distinct responses to heat stress. In the tolerant variety the putative transcript of PORC is already detectable before heat exposure, suggesting a priming state. After heat treatment its expression decreases. In the susceptible variety, the transcript of the putative PORC is initially low and only increases after bleaching begins, a response that is probably insufficient to prevent the bleaching phenotype. However, these opposite expression patterns do not necessarily imply that PORC is directly involved in avoiding bleaching in members of *Pelargonium*, as they may simply reflect broader differences in how each variety responds to heat stress. The RT-PCR product was fractionated by gel electrophoresis in agarose gels TBE 1%. Gel red was used for visualization.

The results of our RT-PCR are similar to the results of an *Arabidopsis* study that show PORC knock out mutants exhibit heat sensitivity, whereas overexpressing lines are more tolerant (Alquraish 2026). Similar to the results observed in Bowdish (Chapter 1), the leaves of cultivars

that were found to be more sensitive to heat had slow recovery or died under prolonged ETL stress, whereas tolerant cultivars exhibited the opposite effect.

Endogenous reference gene validation. Random hexamers and oligo(dT) primers produced inconsistent results, likely due to low PORC transcript abundance and stress-dependent expression. **Actin7** produced consistent amplification across cultivars and treatments and was validated as a reliable endogenous reference gene.

Conclusion

This study followed a scaffolding methodological framework designed to investigate LPOR (PORC) expression in *Pelargonium peltatum*, given the absence of an assembled nuclear reference genome. By leveraging conserved PORC coding regions from closely related taxa, aligning them to identify a consensus, and validating that consensus against available *Pelargonium* SRA datasets, we were able to reconstruct a presumptive partial PORC fragment suitable for future analysis and further full gene sequencing. Although the reconstructed sequence cannot yet be confirmed as the definitive *Pelargonium* PORC gene, the successful amplification of this fragment and its high scoring BLAST alignment to POR-like and PORC-like sequences support its use as a provisional target for future expression studies. This research therefore provides the necessary groundwork for primer design, conserved region identification, and sequence reconstruction for future informative experiments aimed at verifying PORC identity, quantifying expression across tolerant and sensitive cultivars, and ultimately furthering the knowledge of how LPOR may influence physiological disorders like foliar bleaching.

Figure 2.1 Snip of Jatropha curcas, Vitis vinifera, Theobroma cacao, and Citrus sinensis alignment in TCoffee.

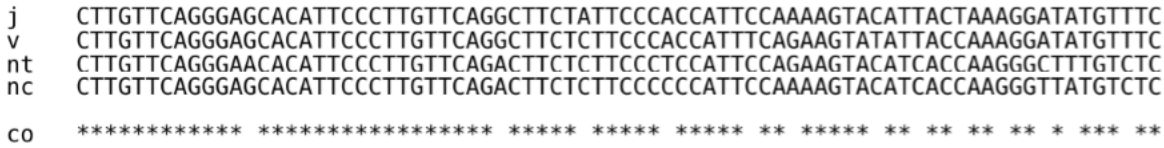


Figure 2.1 To reconstruct a partial LPOR (PORC) coding region suitable for expression analysis, PORC sequences from related taxa (*Jatropha curcas, Vitis vinifera, Theobroma cacao*, and *Citrus sinensis)* with validated or predicted LPOR proteins were retrieved from National Center for Biotechnology Information (NCBI 2026).

Figure 2.2 PORC coding sequences were aligned using T Coffee to identify conserved region of target gene PORC across related species.

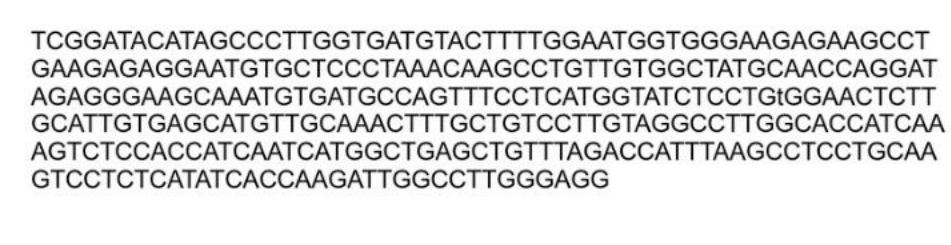


Figure 2.2 PORC coding sequences were aligned using T-Coffee to identify conserved region of target gene PORC across related species. Blasted (BLASTN) against SRA *Pelargonium peltatum*,resulted in a strongly conserved ~300 bp hit. This region was selected as the scaffold for reconstructing the *Pelargonium* PORC fragment.

Figure 2.3 DNA PCR amplification of 'Ivy League Red', ' Ivy League Hot Coral' and 'Ivy League Pink'

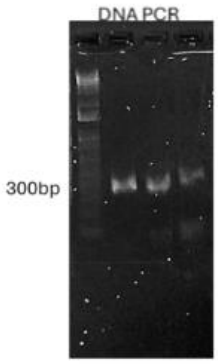


Figure 2.3 DNA PCR amplification of 'Ivy League Red', 'Ivy League Hot Coral' and 'Ivy League Pink' Gel results for 'Ivy League Red', 'Ivy League Hot Coral' and 'Ivy League Pink' were successfully amplified (Figure 2.3). This indicates that our presumable PORC sequence is present in all three cultivars.

Figure 2.4 Sequence alignment of DNA PCR gel amplicons of 'Ivy League Red', 'Ivy League Hot Coral' and 'Ivy League Pink'

```
T-COFFEE, Version_11.00 (Version_11.00)
Cedric Notredame
SCORE=986
*
 BAD AVG GOOD
*
Ivv      :  99
Ivy 1    :  98
Ivy_2    :  99
cons     :  98

Ivy      CTGA-GAGAGGATGNGCTCCCTAAACAAGCCTGTTGTGGCTATGCAACCA-GGATAGAGGGAAGCAAATGTGATG
Ivy_1    CTGAAGANAGGATGNGCTCCCTAAACNAGCCTGTTGTGGCTATGCAACCACGGATAGAGGGAAGCAAATGTGATG
Ivy_2    CTGA-GAGAGGATGTGCTCCCTAAACAAGCCTGTTGTGGCTATGCAACCA-GGATAGAGGGAAGCAAATGTGATG

cons     ****.** ****** *********** ********************** ************************


Ivy      CCAGTTTCCTCATGGTATCTCCTGTGGAACTCTTGCATTGTGAGCATGTTGCACACTTTGCTGTCCTTGTAGGCC
Ivy_1    CCAGTTTCCTCATGGTATCTCCTGTGGAACTCTTGCATTGTGAGCATGTTGCAAACTTTGCTGTCCTTGTAGGCC
Ivy_2    CCAGTTTCCTCATGGTATCTCCTGTGGAACTCTTGCATTGTGAGCATGTTGCACACTTTGCTGTCCTTGTAGGCC

cons     ************************************************** ********************


Ivy      TTGGCACCATCAAAGTCTCCACCATCAATCATGGCTGAGCTGTTTAGACCATTTAAGCCTCCTGCAAGTCCTCTC
Ivy_1    TTGGCACCATCAAAGTCTCCACCATCAATCATGGCTGAGCTGTTTAGACCATTTAAGCCTCCTGCAAGTCCTCTC
Ivy_2    TTGGCACCATCAAAGTCTCCACCATCAATCATGGCTGAGCTGTTTAGACCATTTAAGCCTCCTGCAAGTCCTCTC

cons     ********************************************************************************


Ivy      ATA-TCACCAAGGTTTGGCCTTG--GA
Ivy_1    ATAGTCACCAAGGTTTGGCCTTGGAGG
Ivy_2    ATG-TCACCAAGGT-TGGCCTTGG-AG

cons     **  ********** ********
```

Sequence alignment of DNA PCR gel amplicons of 'Ivy League Red', ' Ivy League Hot Coral' and 'Ivy League Pink.' Sanger sequencing results showed few nucleotide differences (Figure 2.4) indicating high conservation at nucleotide level within that region.

Figure 2.5 Example of meristematic green leaf samples taken for control phase I (24°C) (Left 'Ivy League Red', Right 'Ivy League Hot Coral').

Figure 2.6 Meristematic bleached leaf samples taken for heat stress phase II (average greenhouse temperature above 31C°).

Figure 2.6 Meristematic bleached leaf samples taken for Heat Stress Phase II. (Temp). R=Ivy League Red, S=Ivy League Hot Coral. (1cm)= distance between dotted measurement for size transparency.

Figure 2.7 Gel analysis Semiquantitive reverse transcriptase-polymerase chain reaction (RT-PCR) for the PORC transcript.

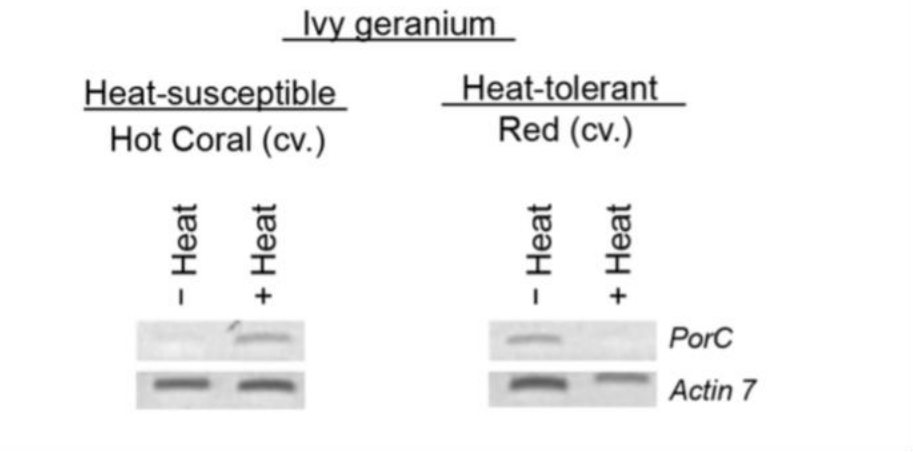


Table 2.1 Target gene PORC sequences, validated or predicted, from Pelargonium related taxa, were retrieved from National Center for Biotechnology Information to align and use as a scaffold to create a conserved sequence for Pelargonium PORC primer design.

Species	Accession #		Gene ID
Jatropha curcas	XM_012232922.3	mRNA	105646961
Vitis vinifera	XM_002264006.4	mRNA	100241491
Theobroma caca	XM_007043490.2	transcript variant X1, mRNA	1859387
Citrus sinensis	XM_006464654.4,	transcript variant X1, mRNA	102614959

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Appendix A - Greenhouse Observations

Phase I, 6 days after ETL began. The first two nodes of ‘Calliope’ varieties had pale yellow to white veins spanning from the pale green to yellow lamina base to its green margins. Flower buds briefly appeared pink prior to whitening. Lamina centers of ‘Cascade’ varieties turned bright yellow, following the lighter green pattern of cells on the lamina to the margins. Flower receptacles retained a natural pink pigmentation early in stress but eventually bleached. Lamina margins of ‘Ivy’ series cultivars were yellow, though the five lobes of the lamina remained green. ‘Cascade’ cultivars bleached along their natural light-green variegation boundaries.

Phase I, 12 days after ETL began. Fully mature leaves on the fourth node from the meristem were not bleached. Significantly, bleaching severity of the third and second node depended entirely on leaf maturity for that cultivar and was not influenced by leaf size. The more mature a leaf was the less progressed bleaching was. In cascade, bleaching occurred in the light green variegated portions first, progressing outwards, however would follow lighter green boundaries less strictly in immature growth. In the other cultivars, the juvenile third node had white veins progressing from the base to the margins, with pale green to yellow lamina; or slightly less mature juvenile leaves on the third node retained dark green lobe margins, and veins that became lighter towards the leaf base. Calliope did not retain green veins in any affected leaves. Leaves on the second node had slightly green margins with fully bleached lobes, with green veins; vein greenness varied (Appendix A Figure 1). In all cultivars the meristematic point was bleaching Flower structures, Petioles and peduncles began bleaching, however sepals and petals never bleached.

Phase I, 18-23 days after ETL began. Juvenile leaves on the third node were able to out-grow bleaching and retained dark green margins. Ivy cultivars displayed the greatest variation. Cultivars (Orchid, Pink, Red) that contained natural pink undertones (anthocyanins) became more visible during bleaching, particularly in petioles, peduncles, and flower buds near affected leaf nodes. As stress prolonged, the pink coloration eventually bleached out, remaining most abundant in Red. Leaves on the second node became entirely bleached, including their petiole. Every cultivar in the ETL except Calliope Red significantly differed from Control, (<.0001). From the fully bleached meristematic point emerged ivory white folded leaves. This new growth continued to unfold and expand despite bleaching.

Phase II, Regreening Day 29. Regreening began on day 27. Severe greenhouse heat caused emerged ivory growth to cup in Hot Coral, become very circular and rounded in Orchid, and caused the lobes to expand in cultivars who have very distinct lobes in their mature leaves. Regreening behaves similarly to bleaching, in that in younger growth chlorophyll will appear first at the leaf base and progressively accumulate towards the margins. During this regreening time four new nodes grew on the cultivars. There appeared to be a period of resilience before the cultivar will resume normal growth and greening again.

Phase II Regreening Day 50: All cultivars had positive significant changes, except for Hot Coral which was found to have poor resiliency. In phase II, 4 leaf nodes were observed and phenotypically rated to examine how the meristem would respond to the Control environment. Significantly, cultivars varied in their ability to produce normally shaped leaves post heat stress, the rate of green up for these leaves, as well as the number of nodes before normal green growth emerged.

Appendix Figure A.1 'Ivy League Red' leaves of varying stages showing green venation under heat and light stress

Appendix B - Growth Chamber Observations

In Bowdish Chapter 1, we established that new growth that had emerged ivory during heat stress would vary in the rate of recovery between cultivars when returned to normal conditions. It was also established that leaf maturity (pre, during, post stress) and cultivar influenced bleaching susceptibility and regreening capability. A pilot study was conducted to design how the environment of the growth chambers should be manipulated to ensure control and stressed conditions that induce bleaching. This study revealed that with a lower daily light integral of less than 2 mol• m^{-2} •d^{-1} (DLI) from white and warm LED bulbs, the temperature to induce bleaching had to be increased from 30 ℃ day temperatures with 27 ℃ night temperatures to 35 ℃ day temperature (13hours) with 32 ℃ night temperature (11 hours).

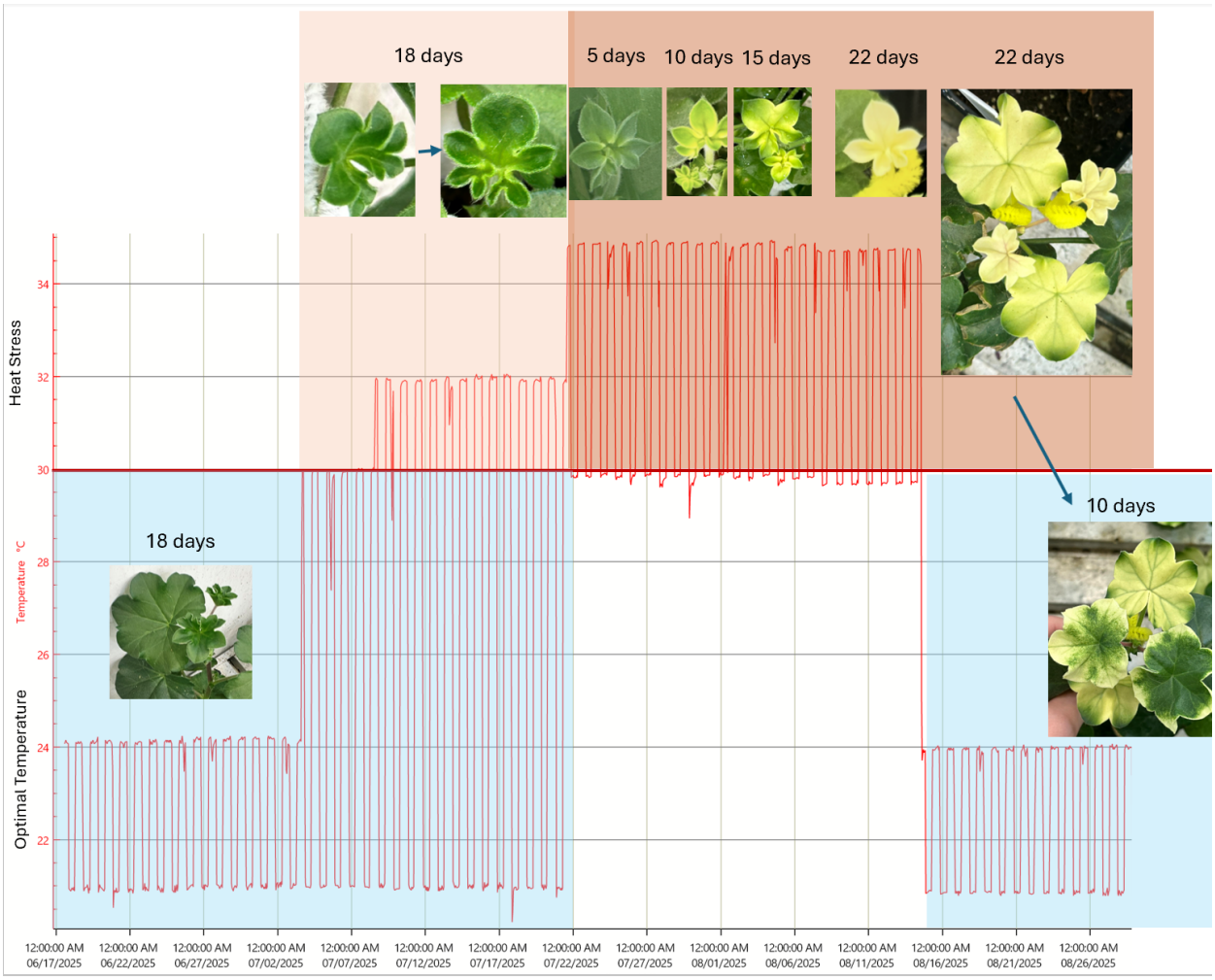
12 vegetatively propagated cuttings, rooted, of 'Ivy League Red', 'Ivy League Cherry Blossom', and 'Ivy League Orchid' cultivars were transplanted into 4-inch diameter standard black pots and placed into the growth chambers on 06/16/2025. To ensure white and warm alternating LED light bulb arrangement would not cause variation in bleaching, the chamber was split into 2 blocks, left and right. Within each block, there were 2 units of each cultivar placed diagonally from one another. The average DLI was below 2 mol• m^{-2} •d^{-1}.

The pilot study was conducted in three phases: Phase I optimal temperature conditions, Phase II bleaching temperature conditions, and Phase III regreening in optimal temperature conditions. The growth chamber was set at a low temperature of 24°C (13 h day and 21°C 11 night for 18 days for Phase I. The Phase II heat stressed environment was induced and set for 30°C day and 27°C night for 18 days; however, bleaching was not observed, so the temperature was increased to 35°C day and 30°C night for 22 days. Phase III regreening lasted until leaves closest to the meristematic point were fully green, which was in 10 days. Tracked hourly with an

environmental monitor (HOBO, Onset Computer Corp., Bourne, MA, USA). DLI measured with a light sensor (HOBO, Onset Computer Corp., Bourne, MA, USA), before the study, at canopy level.)

Our pilot study revealed that despite a DLI of 2 mol• m^{-2} •$d^{-1}$for 18 days, the foliage remained green in temperatures less than 30℃, whereas at 30℃ slight de-greening occurs. When temperatures increased to 35℃, bleaching symptoms appeared 5 days later and progressed till affected leaves were entirely bleached, the meristematic point bleached, and new leaves emerged ivory. In Bowdish Chapter 1, the average DLI was 15 mol• m^{-2} •d^{-1} and the average day temperature exceeded 36 ℃, causing immature growth to progress from yellow to white, with pink undertones in some cultivars, until eventually becoming necrotic by day 22, never regreening in cooler temperatures, and causing cupped emerged growth to staggeringly regreen in newly developed nodes over the course of 20 days. However, in our pilot study, the immature growth immediately whitened, some cultivars had vibrant anthocyanins in leaf lamina and veins, growth did not become necrotic at day 22, was capable of regreening in lowered temperatures, and while the leaf morphology of the emerged growth had less sinuses between the lobes, it did not cup. Additionally, emerged ivory growth only took up to 10 days to partially or fully regreen. (Appendix B Figure 1).

Appendix Figure B.1 Growth chamber pilot study showing how a low DLI requires higher temperatures to bleach



Appendix Figure B.2 Visual demonstrating why pruning is essential for tissue selection due to leaf physiology

Dependent on developmental stage at the onset of stress, strong anthocyanin appearance, maturation during stress, if nodes have one or two leaves, and proximity to meristematic point.

This pilot study revealed that in all cultivars, regardless of tolerance, the specific leaves that are affected by the disorder, as well as their severity, are impacted by entire leaf maturity stage, proximity to the meristematic point, leaf area maturity, leaf expansion during stress, and the development of new leaves during stress.