



# The Influence of Salicylic Acid Treatment on Photosynthetic Pigments, Bioactive Compounds and Antioxidant Capacity in Drought-Stressed *Petunia grandiflora*

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## RESEARCH ARTICLE

### Abstract

Drought is widely recognized as the most severe environmental danger to horticulture in almost all regions across the globe. The objective of this study was to determine if applying a foliar treatment of salicylic acid (SA) at 400 ppm could mitigate the negative effects of drought on *Petunia grandiflora*. Four experimental variants were implemented for this purpose, with regular watering or moderate drought conditions. These variants were observed for duration of 14 days. The level of photosynthetic pigments was markedly greater in the samples exposed to drought but treated with foliar SA, in comparison to the samples exposed to drought without any treatment ( $128.28 \pm 8.78$  and  $38.01 \pm 1.15$   $\mu\text{g/g}$  total chlorophyll (a+b), respectively). The total polyphenol content and antioxidant capacity of both the leaves and flowers of *Petunia* were assessed. The polyphenols content was significantly reduced by drought. However, following the application of SA, anthocyanin levels increased in comparison to the sample that had been subjected to drought. The results demonstrate that applying SA to the leaves mitigates the adverse impacts of drought, providing a solution to the issue of water scarcity in ornamental plants, particularly *Petunia grandiflora*.

**Keywords:** drought, *Petunia grandiflora*, chlorophylls, total phenols, FRAP

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## INTRODUCTION

The *Petunia* genus was established as a genus by Jussieu in 1803 (Wandenbussche et al., 2016), phylogenetically belonging to the Order Solanales, family Solanaceae (Olmstead et al., 2013). *Petunias* are some of the most widespread ornamental plants grown in gardens, pots or window boxes, considered endemic to South America (Souza et al., 2023; Olmstead et al., 2013 and Stehmann et al., 2009). The Solanaceae family, considered to be a medium-sized family according to Solanaceae Source, comprises over 100 genera and 3000 species spread across all ecosystem types (Morris and Taylor, 2017), with the highest diversity found in the tropical regions of America, Australia, Africa and Indo-Pacific (Olmstead et al., 2013; Dupin et al. 2017 and Echeverría-Londono et al., 2020). The common garden *Petunia*, *Petunia hybrida*, is derived from *Petunia integrifolia* and *Petunia axillaris*, which are two of the many species of *Petunias* endemic to South America. Its geographic distribution includes the temperate and subtropical regions of Argentina, Uruguay, Paraguay, Bolivia and Brazil, with a center of diversity in southern Brazil. Species diversity is in danger of diminishing significantly owing

to human intervention, especially in the form of grassland destruction (Gerats and Strommer, 2009). *Petunias* are particularly notable for a variety of flower colors and sizes, and most varieties grow easily and are maintained. In Romania, the *Petunia* genus is highly appreciated and cultivated as a decorative plant in the summer season. Cantor et al. 2015, morpho-decorative characterized seven different varieties of *P. hybrida*, imported from Hungary, of different colors beyond to *Petunia grandiflora* type: White, Hot Pink, Patio Red, Famous Blue, Lila White, Purple and Hot Red recommending the use of the varieties in commercial culture and landscaping in our country.

Salicylic acid (SA) is a naturally occurring phenolic compound and an important signalling molecule involved in specific biotic and abiotic stress responses as it regulates metabolic and physiological processes in plants (Zhang and Li 2019; Arif et al. 2020; Bortolin et al. 2020, Prakash et al. 2021). SA is shown to be involved in physiological processes such as seedling growth, root growth, leaf photosynthesis, ion homeostasis, secondary metabolite production and fruit ripening (Hernández et al., 2017; Rajeshwari and Bhuvaneshwari, 2017). Other studies have highlighted the benefit of SA in nitrogen and proline metabolism and on the antioxidant system of plants (Jahan et al. 2019; Ignatenko et al. 2019; Madany et al. 2020).

Plants face a variety of environmental stresses during the growing season, and these stresses can limit the chances of plants growing and surviving (Soroori et al., 2021). The most prevalent environmental stress is drought which affects the growth of many plants. When plants do not receive enough water, they are subjected to a stress called water deficit, which is recognized as a limiting factor in plant growth in most parts of the world (Pei et al., 2013). Under drought conditions, the water content of the soil decreases, which prevents water from circulating inside the root cells. As a defence against water loss, plants reduce transpiration (Bray, 1997; Hatamifar et al., 2017). In addition to reduced growth rate, stress caused by excessive drought disrupts plant physiology, causing accelerated production of reactive oxygen species, resulting in oxidative stress in plants (Pal et al., 2015) inducing oxidative damage to plant cellular components (Rebi et al., 2021), protein denaturation (Sajjad et al., 2019) and nucleic acid damage (Debnath et al., 2021).

Mechanisms of stress tolerance vary among plant species and varieties and involve multiple biological processes (Chen et al., 2019). Plants use different strategies to cope with drought stress, they often respond to a lack of water by accumulating dissolved substances in cells or altering their internal osmotic pressure, which has led to increased ecological tolerance (Al-Yasi et al., 2020). The use of osmotic substances such as proline, glycine betaine, trehalose, etc. by foliar application significantly reduces the destructive effects of stress on plants (Soroori et al., 2021).

Due to global warming, drought is likely to become an acute problem (Mahmood et al., 2021). Water scarcity is an extreme environmental problem that causes dramatic economic and sociological problems by reducing plant yield (Soltani et al., 2021). In this regard, there is a need to adopt horticultural practices to address anticipated plant water stress. Interest in ornamental plants with low water requirements will steadily expand (Kapoor et al., 2020). Like other ornamentals, *Petunia* has become a challenge for growers seeking to increase the quality of their production with smaller and more compact plants (Hatamifar et al., 2017).

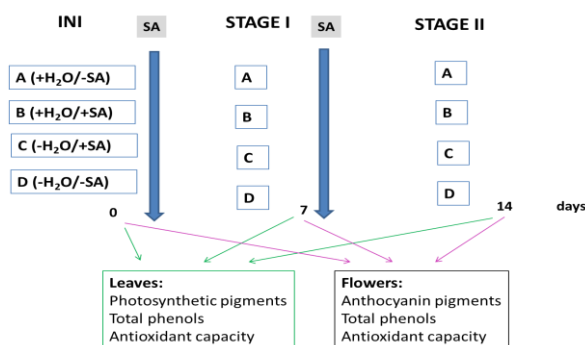
The aim of the study was to determine whether foliar application of SA to *Petunia* plants undergoing drought mitigates the negative effects of this abiotic factor. Therefore, we monitored certain morphological traits such as the number of buds and flowers, as well as the levels of photosynthetic pigments, total polyphenol content, and antioxidant capacity.

## MATERIALS AND METHODS

### Plant materials and experimental design

The *Petunia* plants were purchased from a commercial source when they were in the blooming phase. The plants were housed in a closed solar-type environment with a day time maximum temperature of 22°C, a night time minimum of 19 °C, and a humidity level of around 60%. The growing medium was produced and supplied by Gramoflor GmbH & Co. KG, located at Diepholzer Straße 173, D49377 Vechta, Germany.

The substrate consisted of a blend of peat and wood fibers. The experimental plan is illustrated in Figure 1.



**Figure 1.** Experimental design. The codification of samples is explained in Table 1

Four experimental variants were designed (made in triplicate, for a total of 39 *Petunia* plants, in the same stage of development) and the labelled are presented in Table 1.

**Table 1.** Experimental variants of *P. grandiflora*

| Variants                               | Labelled    | Treatment  |
|--|-------------|--|
| <b>Variant 0</b>                       | INI         | <i>P. grandiflora</i> from the beginning of the experiment   |
| <b>Variant 1 (+H<sub>2</sub>O/+SA)</b> | A I and AII | During the 14-day experiment, the <i>Petunia</i> plants were regularly watered, resulting in a soil water content of 75.66 ± 5.13%.  |
| <b>Variant 2 (+H<sub>2</sub>O/+SA)</b> | BI and BII  | The <i>Petunia</i> plants were watered throughout the 14-day experiment and received foliar application of salicylic acid (SA) at a concentration of 400 ppm. The first application was done at the beginning of the experiment (0 day), and the second application was done after 7 days, prior to the start of the experiment (Stage I). |
| <b>Variant 3 (-H<sub>2</sub>O/+SA)</b> | CI and CII  | The <i>Petunia</i> plants were exposed to a moderate drought condition, with a soil water content of 37.33 ± 2.51%, for a period of 14 days. However, they were also treated with SA (400 ppm) through the leaves, both at the beginning of the experiment and again after 7 days.   |
| <b>Variant 4 (-H<sub>2</sub>O/-SA)</b> | DI and DII  | The <i>Petunia</i> plants were exposed to moderate drought conditions and were not treated with salicylic acid (SA) throughout the experiment.   |

The duration of the treatment was 14 days. Two applications of spraying with salicylic acid (SA) at a concentration of 400 ppm were conducted, initially (at day 0) and after 7 days (Figure 1). The treatment period was divided into two stages (I and II), during which the flowers and leaves were collected for analysis. The leaves and flowers of *Petunias* were collected at three different stages: the initial stage (INI) before the experiment started, stage I after 7 days of treatment with SA, and stage II after 14 days from the start of the experiment. The *Petunia* plants were also assessed using biometric measurements, including color, number of buds, and number of flowers. Additionally, biochemical analyses were conducted, which will be described in detail below.

#### Determination of moisture from *Petunias* leaves and flowers

Samples of fresh *Petunia* leaves and flowers were collected at the initial stage, after 7 days of treatment (stage I), and 14 days after the beginning of the experiment (stage II). Both the leaves and flowers were dried in the oven, at 105°C up to a constant weight. The moisture (%) of the leaves and flowers was determined using the formula (1):

$$\text{Moisture \%} = \frac{\text{fresh weight} - \text{dry weight}}{\text{fresh weight}} \times 100 \quad (1)$$

#### Determination of photosynthetic pigments from *Petunia* leaves

For the extraction of photosynthetic pigments (chlorophyll a and b, carotenoids), 0.5 g of fresh leaves were macerated with 10 mL of cold 95% ethanol following the protocol described by Nayek et al., 2014. Afterwards, the samples were centrifuged at 12,000 rpm for 10 min at 4 °C. A volume of 0.5 ml of the supernatant was mixed with 4.5 ml of cold 95% ethanol and used for spectrophotometric quantification of pigments at wavelengths of 664 nm, 649 nm and 470 nm (Shimadzu 1240 mini UV-Vis, Kyoto, Japan). The concentration of photosynthetic pigments (µg/mL) was calculated based on the equations presented by Nayek S. et al., 2014. All determinations were made in triplicate.

#### Determination of total phenols from *Petunias* leave and flowers

The total phenolic content in the leaves and flowers of *Petunia* was measured using the Folin-Ciocalteu method with some adjustments (Singleton et al. 1999; Memete et al. 2022). The 0.1 mL extract was mixed with 1.7 ml of distilled water, 0.2 mL of freshly prepared Folin-Ciocalteu reagent (diluted 1:10, v/v), and 1ml of 7.5% Na<sub>2</sub>CO<sub>3</sub> solution. The mixture was then incubated in the dark, at room temperature for 2 hours. The absorbance was recorded at 765 nm using a Shimadzu mini UV-Vis.

### Fingerprinter of anthocyanin pigments from *Petunia* flowers

Anthocyanins were extracted from *Petunia* flowers using ethanol acidified with 0.1% trifluoroacetic acid. The visible spectrum of each sample was then measured using a Shimadzu UV-Vis spectrophotometer.

### Determination of antioxidant capacity

Antioxidant capacity was determined using FRAP (Ferric-Reducing Antioxidant Power) assay according with Benzie and Strain with some modification (Memete et al., 2022). A volume of 0.1 mL was allowed to react with 0.5 mL of FRAP working solution (freshly prepared by mixing 300 mM acetate buffer (pH 3.6) with 20 mM FeCl<sub>3</sub> and 10 mM TPTZ solution in the ratio 10:1:1 (v/v/v) and 2 mL distilled water for 1 h in the dark. The absorbance was measured at 595 nm and the results were reported as mmol TE/g.

### Statistical analysis

The values were expressed as the mean ± SD (standard deviation) (n=5). The data were analyzed using GraphPadPrism (GraphPad Software, Inc., La Jolla, CA, USA) and subjected to one-way analysis of variance. To determine statistically significant differences, Tukey's multiple comparison test was applied at a significance level of  $p < 0.05$ . Different lowercase letters indicate significant differences between samples ( $p < 0.05$ ).

## RESULTS AND DISCUSSIONS

The potential of SA in alleviating the adverse impacts of drought stress on *Petunia* plants was evaluated. During the experiment, the number of flowers and buds in the first and second stages was monitored, and the results are presented in Table 2.

**Table 2.** The number of buds and flowers among the moisture content of leaves and flowers of *P. grandiflora* at initial stage and during the treatment (stage I and II)

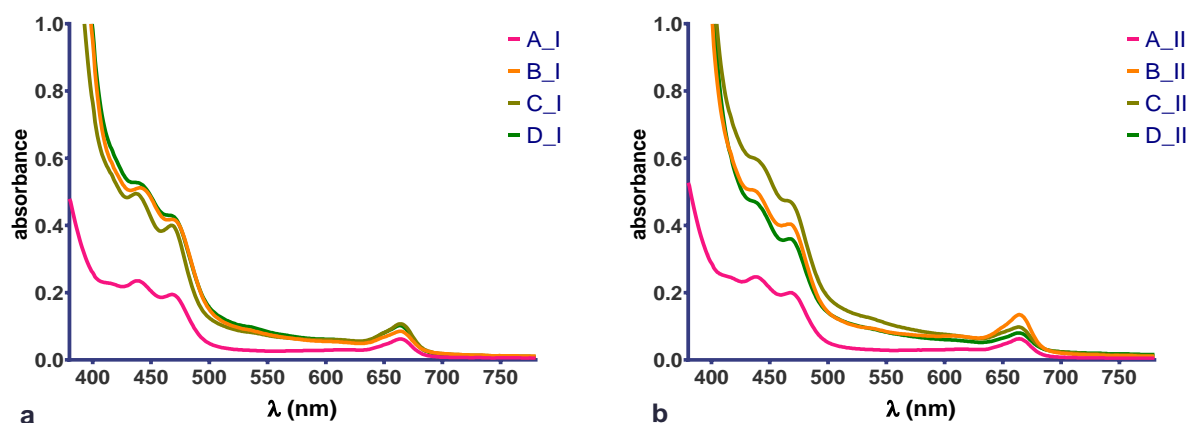
| Stage   | Samples               | No. buds               | No. flowers            | Moisture of leaves (%)    | Moisture of flowers (%) |
|---------|-----------------------|------------------------|------------------------|---------------------------|-------------------------|
| Initial | <i>P. grandiflora</i> | 3.00±0.00 <sup>b</sup> | 2.33±0.58 <sup>a</sup> | 89.33±1.53 <sup>b</sup>   | 90.00±1.00 <sup>a</sup> |
| I       | AI                    | 3.00±1.00 <sup>b</sup> | 4.33±0.58 <sup>a</sup> | 87.78±0.63 <sup>b</sup>   | 90.88±0.31 <sup>a</sup> |
|         | BI                    | 4.67±0.00 <sup>a</sup> | 5.00±2.65 <sup>a</sup> | 85.75±0.59 <sup>b</sup>   | 90.03±0.20 <sup>a</sup> |
|         | CI                    | 3.17±2.08 <sup>b</sup> | 3.66±0.58 <sup>a</sup> | 84.23±0.45 <sup>a</sup>   | 89.60±0.51 <sup>b</sup> |
|         | DI                    | 2.33±1.54 <sup>c</sup> | 2.00±1.00 <sup>a</sup> | 78.74±2.01 <sup>e</sup>   | 87.87±1.02 <sup>c</sup> |
| II      | AII                   | 3.00±1.00 <sup>b</sup> | 4.00±1.00 <sup>a</sup> | 95.19±0.45 <sup>f</sup>   | 90.57±0.23 <sup>a</sup> |
|         | BII                   | 5.00±1.00 <sup>a</sup> | 5.33±0.58 <sup>b</sup> | 91.19±0.63 <sup>a,b</sup> | 90.08±0.69 <sup>a</sup> |
|         | CII                   | 2.60±1.00 <sup>c</sup> | 4.00±1.00 <sup>a</sup> | 79.18±1.16 <sup>c</sup>   | 76.78±0.52 <sup>d</sup> |
|         | DII                   | 0.00±0.00 <sup>d</sup> | 0.66±0.58 <sup>c</sup> | 54.67±9.29 <sup>d</sup>   | 48.33±2.33 <sup>e</sup> |

Note: Results represent the mean ± SD of three replicates. Small letters represent a different significance ( $p < 0.5$ ) between samples, per column

Table 2 indicates that the number of buds and flowers is increasing in the experimental variants variants A and B that receive regular irrigation, indicating a normal development of the plant. In samples subjected to drought Stage I, there was a notable decrease in both the number of buds and flowers. This decrease was further accentuated after 14 days of treatment Stage II, when there was a complete disappearance of buds and a drastic reduction in the number of flowers Variant DII. Applying SA under regular watering conditions leads to a significant increase in the quantity of buds and flowers when compared to the control sample in both stages of the experiment variants BI and BII). Additionally, during periods of drought, it is evident that the application of SA does not result in a significant decrease in the number of buds and flowers, when compared to the control sample. In contrast, there were notable reductions observed in the samples subjected to stress conditions and not treated with SA.

Arshad et al. 2023, conducted a study for 60 days. The researchers administered SA foliar treatments to *Gladiolus grandiflorus* L. plants under drought conditions. The treatments involved concentrations of 40, 80, and 120 ppm SA,

applied at 15-day intervals, beginning on the 30th day after planting. The results indicated that the application of SA at a concentration of 120 ppm had a beneficial impact on various plant features, including plant height, number of leaves per plant, number of florets spike, flower diameter, spike length, rachis length, spike diameter, leaf area and leaf length. Ethanol extracts from *Petunia* leaves were subjected to both qualitative analyses using VIS spectra in the range of 350-750 nm Figure 2 and quantitative analysis of photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids), Table 3.



**Figure 2.** Visible spectra (400-700 nm) of *Petunia* leaf extracts harvested in stages I (a) and II (b).

Chlorophylls have two major light absorption bands, as shown in Figure 2: one in the visible blue band (less than 460 nm) and another in the red band (630-670 nm). The peak absorbance wavelengths for chlorophyll a were 666 nm and 434 nm, while for chlorophyll b they were 653 nm and 453 nm, respectively. Table 3 shows the amount of photosynthetic pigments ( $\mu\text{g/g}$ ) obtained in the two stages of experiment.

**Table 3.** Green pigments (Chlorophyll a and Chlorophyll b) and carotenoids of *P. grandiflora* leaves submitted to different treatment

| Stage   | Samples | Chlorophyll a             | Chlorophyll b              | Carotenoids             | Total Chlorophyll a+b     | Ratio of chlorophyll a/b |
|---------|---------|---------------------------|----------------------------|-------------------------|---------------------------|--------------------------|
| Initial | INI     | 135.20±12.30 <sup>b</sup> | 50.32±6.23 <sup>a</sup>    | 9.36±2.65 <sup>b</sup>  | 185.52±10.36 <sup>b</sup> | 2.69±0.18 <sup>a</sup>   |
|         | AI      | 130.00±7.56 <sup>b</sup>  | 54.94±3.68 <sup>a</sup>    | 8.44±1.12 <sup>b</sup>  | 184.94±10.33 <sup>b</sup> | 2.36±0.12 <sup>b</sup>   |
| I       | BI      | 174.64±5.36 <sup>a</sup>  | 65.48±7.25 <sup>a</sup>    | 10.52±3.20 <sup>b</sup> | 240.12±8.69 <sup>a</sup>  | 2.66±0.06 <sup>a</sup>   |
|         | CI      | 128.25±9.25 <sup>b</sup>  | 57.19±9.11 <sup>a</sup>    | 7.71±6.11 <sup>b</sup>  | 185.44±5.66 <sup>b</sup>  | 2.24±0.02 <sup>b</sup>   |
|         | DI      | 62.69±2.36 <sup>e</sup>   | 29.76±4.12 <sup>c</sup>    | 5.22±2.66 <sup>c</sup>  | 92.46±11.69 <sup>d</sup>  | 2.11±0.07 <sup>b</sup>   |
|         | AII     | 129.00±6.33 <sup>c</sup>  | 51.92±1.66 <sup>a,c</sup>  | 11.21±1.11 <sup>b</sup> | 143.92±9.33 <sup>c</sup>  | 2.43±0.05 <sup>a,b</sup> |
| II      | BII     | 186.75±4.69 <sup>c</sup>  | 54.99±11.20 <sup>a,c</sup> | 12.79±3.65 <sup>a</sup> | 151.74±15.32 <sup>c</sup> | 2.37±0.04 <sup>b</sup>   |
|         | CII     | 87.96±1.03 <sup>c,d</sup> | 40.32±4.99 <sup>a,c</sup>  | 5.67±2.89 <sup>d</sup>  | 128.28±8.78 <sup>c</sup>  | 2.18±0.09 <sup>b</sup>   |
|         | DII     | 20.69±0.65 <sup>f</sup>   | 17.32±0.98 <sup>c</sup>    | 2.06±0.21 <sup>e</sup>  | 38.01±1.15 <sup>e</sup>   | 0.63±0.05 <sup>c</sup>   |

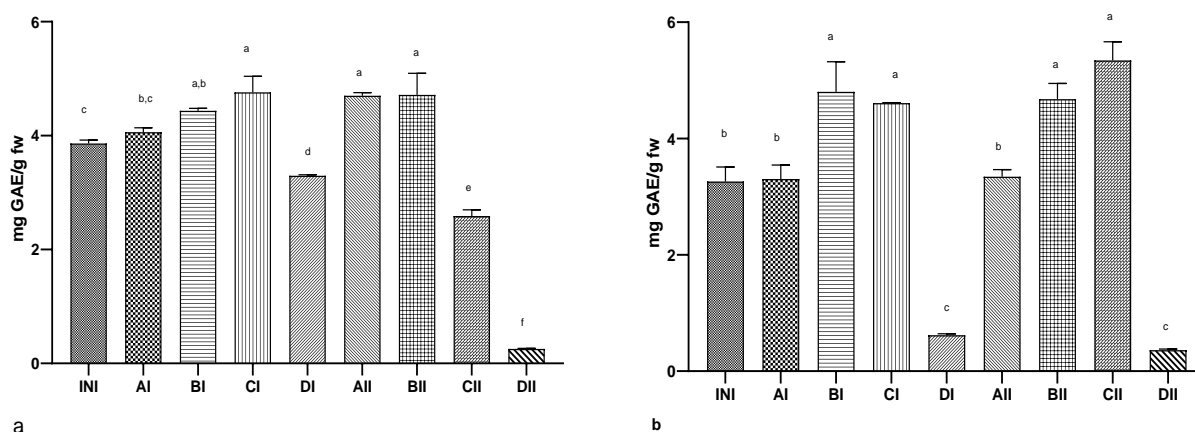
Note: Results represent the mean ± SD of three replicates. Small letters represent a different significance ( $p < 0.5$ ) between samples, per column.

The BI variant exhibited the highest concentration of chlorophyll a, whereas no significant differences in statistics were observed between the AI and CI variants. In comparison to the samples from the initial phase of the experiment, a notable decrease is seen under stressful circumstances and in the absence of SA treatment. After a

duration of 14 days from the start of the treatment, a notable reduction in the concentration of chlorophyll a in D II variant is observed Table 3. When SA was applied to drought-exposed *Petunias*, it did not result in significant reductions compared to the AII and BII variants. In the case of chlorophyll b, a similar pattern is observed as with chlorophyll a, with the lowest values being recorded in the variants subjected to drought without treatment with SA (DI and DII). The amount of carotenoids is affected by the drought conditions, resulting in significant reductions compared to the other samples. The application of SA during drought conditions led to the alleviation of the drought-induced effects Table 3. Significant differences in total chlorophyll content (a+b) were observed only between the drought-treated samples (DI and DII) and the other experimental groups, at both stages of the experiment.

Plants show various reactions to drought stress, with photosynthesis being particularly susceptible to its effects. Plants employ the strategy of completely or partially closing their stomata in order to restrict transpiration and minimize water loss and carbon fixation in leaves during periods of drought stress. Drought stress decreases the process of photosynthesis, inhibits the movement of photoelectrons and the production of ATP through light (Fang et al., 2023). Drought induces a decrease in chlorophyll content, which can cause chloroplasts to break down and destabilize the chlorophyll protein complex, resulting in minimal flower clusters (Arshad et al., 2023). Application of SA treatments to plants improves the photosystem assimilation capacity, induces carbohydrate accumulation and increases mineral uptake positively influencing the number of flowers (Karlidag et al., 2009). In Khalvandi et al.'s 2021 study, a concentration of 0.5mM of SA was applied to the leaves of six autumn wheat varieties that are susceptible to drought. This application resulted in significantly increased levels of chlorophyll a and b in the plants, with a 7.05% increase in chlorophyll a and a 4.08% increase in chlorophyll b, compared to the control samples. Phenolic compounds, which are secondary metabolites, do not have a direct impact on the growth and development of plants. These compounds provide protection to plants against diseases or specific damages, while improving the plant's color, flavor and aroma (Kumar et al., 2023).

Our study focused on analyzing the levels of total phenolic compounds in *Petunia* leaves and flowers when subjected to drought conditions and treated with foliar application of SA. The results obtained are shown in Figure 3.



**Figure 3.** Total phenols content from leaves (a) and flowers (b) of *P. grandiflora*. Results represent the mean  $\pm$  SD of three replicates. Small letters represent a different significance ( $p < 0.5$ ) between samples.

The results demonstrated a significant impact of drought on the concentration of total polyphenols, with a stronger impact observed as the duration of this abiotic stress increased. Polyphenols compounds are a secondary metabolite with varied and important functions in the plant world, representing an extensive group of phytochemicals (Bei et al., 2024).

The leaves sample which went through a 7-day drought and received treatment with foliar SA (variant CI) showed an increase in the total polyphenol content. Following an additional 7-day period of drought (CII and DII), the polyphenol content exhibited a significant decrease in comparison to the samples that were regularly watered Figure 3a. Similar to the result that is seen in the case of leaves, there is a higher total polyphenol content in the *Petunia* flower samples (BI, BII, CI, and CII) where SA was applied as compared to the control sample (AI and AII). Regarding flowers, the drought led to a significant and dramatic reduction in the levels of polyphenols Figure 3b. Research on numerous plant species has demonstrated an enhanced synthesis of flavonoids in response to drought conditions, resulting in increased resistance for these plants (Kumar et al., 2023).

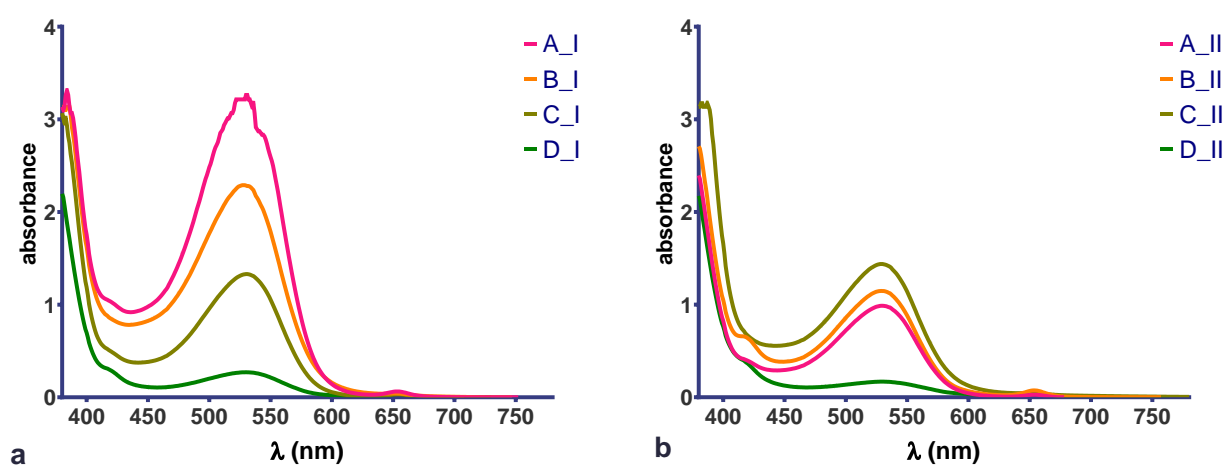
When 1.0 mM SA was applied to *Ocimum sanctum* L. through the leaves, it resulted in the highest levels of eugenol ( $17,829.53 \pm 243.11 \mu\text{g/g}$  dry extract), total phenolics ( $444.10 \pm 2.80 \text{ mg GAE/g}$  dry extract), and total flavonoids

( $382.69 \pm 6.49$  mg QE/g dry extract). These levels were 282.96, 1.76, and 2.14 times higher, respectively, compared to the control group (Panumart et al, 2024). Preciado-Rangel 2019, found that applying SA at a concentration of 0.15mM to *Cucumis sativus* L. plants resulted in the highest concentrations of bioactive compounds. Another study also supported the idea that stimulation with SA would enhance the content of bioactive compounds. Hence, the external administration of SA in plant cultivation may serve as a viable option for enhancing both the economic and nutritional worth, as well as establishing a basis for the standardization of certain techniques (Ramos-Sotelo et al., 2023).

Anthocyanins, a specific group of flavonoids, play a crucial role in determining the intense red and blue colors found in flowers. The anthocyanins, are the primary molecules responsible for coloration of petals, for example pelargonidin derivatives create orange-red hues, cyanidin derivatives produce red hues, and delphinidin derivatives result in blue hues (Vankar et al., 2010).

The flowers of *Petunia* contain acylated delphinidin-type anthocyanins and flavonols. These flowers have a reddish-purple color at low pH and a violet color at high pH. The flower color increases when the flowers produce cyanidin-type anthocyanins and/or non-acylated anthocyanins (Tsuda et al., 2004).

The UV-Vis spectra in the range of 350-750 nm were plotted against the acidulated methanolic extracts of *Petunia* flowers Figure 4.



**Figure 4.** Visible spectra of *Petunia* flowers extracts harvested in stages I (a) and II (b)

Figure 4 shows that anthocyanin pigments exhibit an absorption maximum around 520-530 nm and the intensity of peak is directly proportional to the concentration. The highest amount of anthocyanins in the first stage of the experiment was observed in the watered samples (AI and BII), while drought caused a decrease in their concentration pigments. However, when SA is applied to *Petunias* through foliar application during a moderate drought (CI), the peak intensity of anthocyanins is significantly higher compared to the DI sample. This indicates a beneficial impact of SA application. However, during a prolonged period of drought (stage II), there is a change in the intensity of the peak specific to anthocyanins. In sample CII, a higher intensity is observed Figure 4b, followed by sample BII and AII. In the case of sample DII, the intensity of peak is very low.

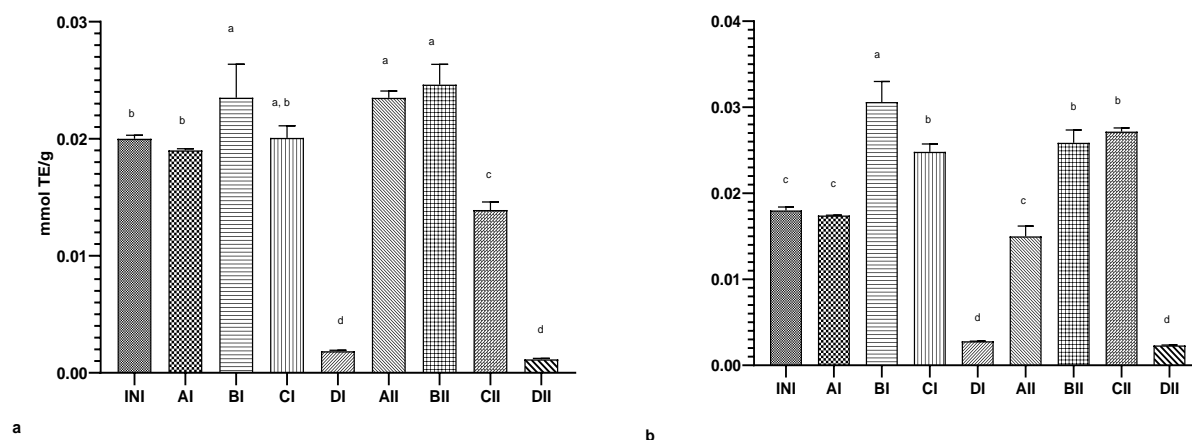
Phenolic acids and flavonoids exhibit specific antioxidant properties that protect plants from the negative consequences of water deficit conditions. Drought stress increases the production of phenolics and flavonoids, which ultimately enhances plant productivity (Kumar et al, 2023). According to research by Naing et al. (2021), drought induces plants to accumulate anthocyanins, which then act as a significant antioxidant to get removal of ROS produced on by drought stress. González-Villagra et al. (2022) applied SA (0 and 0.5 mM) to *Aristotelia chilensis* plants under medium drought stress for 14 days. The results showed that the action of SA reduced the rate of CO<sub>2</sub> assimilation by 41.9%, stomatal conductance by 40.7%, increased plant growth rate by 13.5%, increased superoxide dismutase and ascorbate peroxidase activity by 65%, decreased total phenol content by 30%, and increased antioxidant capacity by 60% in *A. chilensis* plants. This highlights the significance of SA in the regulation of plant mechanisms. Salicylic acid potentially influences plants by enhancing enzymatic activity, particularly chalcone synthase, in the pathway that regulates anthocyanin synthesis (Ghasemzadeh et al., 2012).

In our experiment, the antioxidant capacity of *Petunias* leaves and petals was determined by FRAP assay and the results are presented in Figure 5.

The sample that received the SA treatment BI and BII, Figure 5, and was regularly watered had the highest antioxidant capacity for both leaves and flowers. In this case, which is similar to the situation with the total

polyphenol content, drought leads to a significant reduction in antioxidant capacity. However, the use of SA treatment reduces the impact of drought on bioactive compounds and antioxidant capacity.

When *Thevetia peruviana* suspension plant cultures were treated with 300 $\mu$ M SA, the antioxidant capacity, determined by ABTS assay, increased by 1.66 times compared to the control culture (Mendoza et al., 2018).



**Figure 5.** Antioxidant capacity determined by FRAP assay from leaves (a) and flowers (b) of *P. grandiflora*

## CONCLUSIONS

This study investigates the impact of applying salicylic acid (at a concentration of 400 ppm) to the leaves of *P. grandiflora* plants that have undergone drought. The study focuses on examining various physiological and biochemical factors. The results showed that applying SA to the leaves had a positive effect on the growth of *Petunia* plants. This was observed by an increase in the concentration of photosynthetic pigments (chlorophyll a and b) compared to plants that were not treated with SA. Furthermore, the samples that underwent drought and received the SA treatment exhibited a significant presence of bioactive compounds belonging to the polyphenol class. In addition, anthocyanin levels increased after SA was applied as compared to the sample that had been subjected to drought. Comprehensive research is required to identify the mechanism through which the application of SA to leaves reduces the impact of drought. According to the obtained results, we conclude that treating *Petunia* with SA is a viable option to help the plant resist water stress, such as short-term drought.

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## Conflicts of Interest

The authors declare that they do not have any conflict of interest.

## REFERENCES

- Al-Yasi H, Attia H, Alamer K, Hassan F, Ali E et al. Impact of drought on growth, photosynthesis, osmotic adjustment, and cell wall elasticity in Damask rose. *Plant Physiol Biochem.* 2020 May; 150:133-139. doi: 10.1016/j.plaphy.2020.02.038.
- Arif Y, Sami F, Siddiqui H, Bajguz A and Hayat S. Salicylic acid in relation to other phytohormones in plant: A study towards physiology and signal transduction under challenging environment. 2024; *Environ. Exp. Bot.* 175, 104040. doi: 10.1016/j.envexpbot.2020.104040
- Arshad M, Ibadullah J, Shahid N, Asma A, Saira S et al. Influence of drought and foliar application of salicylic acid on growth and development of *Gladiolus*, *Eur. Chem. Bull.* 2023; 12 (Regular Issue 12), 3320-3329.
- Bei MF, Apahidean AI, Budau R, Rosan CA, Popovici R et al. An Overview of the Phytochemical Composition of Different Organs of *Prunus spinosa* L., Their Health Benefits and Application in Food Industry. *Horticulturae* 2024; 10, 29. <https://doi.org/10.3390/horticulturae10010029>



5. Benzie IFF, Strain JJ. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of “Antioxidant Power”: The FRAP Assay. *Anal. Biochem.* 1996; 239, 70–76. <https://doi.org/10.1006/abio.1996.0292>
6. Bortolin GS, Teixeira SB, Mesquita Pinheiro R et al. Seed Priming with Salicylic Acid Minimizes Oxidative Effects of *Aluminum* on *Trifolium* Seedlings. 2020; *J Soil Sci Plant Nutr* 20, 2502–2511. <https://doi.org/10.1007/s42729-020-00316-9>
7. Bray AE. Plant responses to water deficit. *Trends in Plant Science.* 1997; 2 (2): 54 - 45.
8. Cantor M, Krizbai E, Buta E. The Behavior of Some *Petunia* Varieties for Improvement the Romanian Assortment, 2015; *Bulletin UASVM Horticulture* 72(1), Doi:10.15835/buasvmcn-hort:10664
9. Chen Z, Xu J, Wang F et al. Morpho-physiological and proteomic responses to water stress in two contrasting tobacco varieties. 2019; *Sci Rep* 9, 18523. <https://doi.org/10.1038/s41598-019-54995-1>
10. Debnath S, Chandel RK, Devi K, Khan Z. Mechanism and Molecular Response of Induced Genotoxicity and Oxidative Stress in Plants. 2021; Springer, Singapore. [https://doi.org/10.1007/978-981-16-2074-4\\_8](https://doi.org/10.1007/978-981-16-2074-4_8). DOI: 10.48047/ecb/2023.12.12.226
11. Dupin J, Matzke NJ, Sarkinen T et al. Bayesian estimation of the global biogeographical history of the *Solanaceae*. 2017;. *Journal of Biogeography* 44: 887-899. <https://doi.org/10.1111/jbi.12898>
12. Echeverría-Londono S, Särkinen T, Fenton IS, Purvis A, Knapp S. Dynamism and context-dependency in diversification of the megadiverse plant genus *Solanum* (*Solanaceae*). 2020; *J. Syst. Evol.* 58 (6), 767–782. <https://doi.org/10.1111/jse.12638>.
13. Fang Hu, Yunxiang Zhang & Jinping Guo. Effects of drought stress on photosynthetic physiological characteristics, leaf microstructure, and related gene expression of yellow horn. 2023; *Plant Signaling & Behavior*, 18:1. <https://doi.org/10.1080/15592324.2023.2215025>
14. Gerats T. and Strommer S. *Petunia*: Evolutionary, Developmental and Physiological Genetics. 2009. DOI 10.1007/978-0-387-84796-2 2.
15. Ghasemzadeh A, Jaafar HZ, Karimi E., Involvement of salicylic acid on antioxidant and anticancer properties, anthocyanin production and chalcone synthase activity in ginger (*Zingiber officinale* Roscoe) varieties. *Int. J. Mol. Sci.* 2012; 13: 14828-14844. <https://doi.org/10.3390/ijms131114828>.
16. González-Villagra J, Reyes-Díaz MM, Tighe-Neira R, Inostroza-Blancheteau C, Escobar AL, Bravo LA. Salicylic Acid Improves Antioxidant Defense System and Photosynthetic Performance in *Aristotelia chilensis* Plants Subjected to Moderate Drought Stress. *Plants* 2022; 11, 639. <https://doi.org/10.3390/plants11050639>
17. Hatamifar N, Babadaei Samani R. Effect of Paclobutrazol on Some Morphological and Physiological Characteristics of *Petunia* under Drought Stress. 2017; *Journal of Ornamental Plants.*, 2017. 7(2): 125-136.
18. Hernández JA, Diaz-Vivancos P, Barba-Espín G, Clemente-Moreno MJ. On the Role of Salicylic Acid in Plant Responses to Environmental Stresses. 2017; In: Nazar, R., Iqbal, N., Khan, N. (eds) *Salicylic Acid: A Multifaceted Hormone*. Springer, Singapore. [https://doi.org/10.1007/978-981-10-6068-7\\_2](https://doi.org/10.1007/978-981-10-6068-7_2)
19. Humberto Ramos-Sotelo, Marely G. Figueroa-Pérez. Use of salicylic acid during cultivation of plants as a strategy to improve its metabolite profile and beneficial health effects. 2023; *Italian Journal of Food Science*; 35 (1): 79–90. <https://doi.org/10.15586/ijfs.v35i1.2332>
20. Ignatenko A, Talanova V, Repkina N et al. Exogenous salicylic acid treatment induces cold tolerance in wheat through promotion of antioxidant enzyme activity and proline accumulation. 2019; *Acta Physiol Plant* 41, 80. (2019). <https://doi.org/10.1007/s11738-019-2872-3>
21. Jahan MS, Wang Y, Shu S, Zhong M, Chen Z, Wu J, Sun J, Guo S. Exogenous salicylic acid increases the heat tolerance in tomato (*Solanum lycopersicum* L) by enhancing photosynthesis efficiency and improving antioxidant defense system through scavenging of reactive oxygen species. 2019; *Sci Hortic-Amsterdam* 247:421–429. <https://doi.org/10.1016/j.scienta.2018.12.047>
22. Javed I, Awan SI, Ahmad HM and Rao A. Assesment of genetic diversity in wheat synthetic double haploids for yield and drought related traits through factor and cluster analyses. 2016; *Plant Gene and Trait*, 7. DOI: 10.5376/pgt.2016.07.0003
23. Kapoor D, Bhardwaj S, Landi M, Sharma A, Ramakrishnan M, Sharma A. The Impact of Drought in Plant Metabolism: How to Exploit Tolerance Mechanisms to Increase Crop Production. 2020; *Appl. Sci.* 10, 5692. <https://doi.org/10.3390/app10165692>
24. Karlidag H, Yildirim E, Turan M. Salicylic acid ameliorates the adverse effect of salt stress on strawberry. 2009; *Scientia Agricola.* 66(2), 180-187. <https://doi.org/10.1590/S0103-90162009000200006>

25. Khalvandi M, Siosemardeh A, Roohi E, Keramati S. Salicylic acid alleviated the effect of drought stress on photosynthetic characteristics and leaf protein pattern in winter wheat. 2021, *Heliyon* 7 (1), e05908. <https://doi.org/10.1016/j.heliyon.2021.e05908>
26. Kumar K, Debnath P, Singh S and Kumar. An Overview of Plant Phenolics and Their Involvement in Abiotic Stress Tolerance, 2023; *Stresses* 3, no. 3: 570-585. <https://doi.org/10.3390/stresses3030040>
27. Leonardo da Silveira de Souza, Bianca Ott Andrade, João Renato Stehmann. An overview on studies of species complexes in Solanaceae. 2023; *Acta Botanica Brasilica*, 37, e20230032. <https://doi.org/10.1590/1677-941X-ABB-2023-0032>
28. Madany MM, Obaid WA, Hozien W, AbdElgawad H, Hamed BA, Saleh AM. Salicylic acid confers resistance against broomrape in tomato through modulation of C and N metabolism. 2020; *Plant Physiol Biochem* 147:322–335. <https://doi.org/10.1016/j.plaphy.2019.12.028>
29. Mahmood A, Rafique MA, Yaseen G, Zaib M, Arif M. et al. Effect of global change and possible ways to reduce its adverse impact on agriculture in the overall world: A review, 2021; *NVEO*, 16252–16278
30. Memete AR, Teusdea AC, Timar AV, Vuscan AN, Mintaş OS et al. Effects of Different Edible Coatings on the Shelf Life of Fresh Black Mulberry Fruits (*Morus nigra* L.). *Agriculture* 2022; 12, 1068. <https://doi.org/10.3390/agriculture12071068>
31. Mendoza D, Cuaspuđ O, Arias JP, Ruiz O, Arias M. Effect of salicylic acid and methyl jasmonate in the production of phenolic compounds in plant cell suspension cultures of *Thevetia peruviana*. 2018; *Biotechnology Reports*, Volume 19., 2018. <https://doi.org/10.1016/j.btre.2018.e00273>.
32. Morris W, Taylor M, 2017. The Solanaceous Vegetable Crops: potato, Tomato, Pepper and Eggplant. 2017; *Encyclop. Appl. Plant Sci.* 55–58. <https://doi.org/10.1016/b978-0-12-394807-6.00129-5>.
33. Naing AH; Kim CK. Abiotic stress-induced anthocyanins in plants: Their role in tolerance to abiotic stresses. *Physiol. Plant.* 2021; 172, 1711–1723. <https://doi.org/10.1111/ppl.13373>
34. Nayek S, Choudhury I, Haque J, Nishika J, Roy S. Spectrophotometric Analysis of Chlorophylls and Carotenoids from Commonly Grown Fern Species by Using Various Extracting Solvents. 2014; *Res. J. Chem. Sci.* 2014, 4, 2231–2606. DOI:10.1055/s-0033-1340072
35. Olmstead RG. Phylogeny and biogeography in *Solanaceae*, *Verbenaceae* and *Bignoniaceae*: A comparison of continental and intercontinental diversification patterns. 2013; *Botanical Journal of the Linnean Society* 171: 80-102. <https://doi.org/10.1111/j.1095-8339.2012.01306.x>
36. Pál M., Szalai G., Janda T. Speculation: Polyamines are important in abiotic stress signaling. *Plant Sci.* 2015 Aug; 237:16-23. doi: 10.1016/j.plantsci.2015.05.003. Epub 2015 May 14. PMID: 26089148.
37. Panumart Rithichai, Yaowapha Jirakiattikul, Ratchaneekon Nambuddee, Arunporn Itharat. Effect of Salicylic Acid Foliar Application on Bioactive Compounds and Antioxidant Activity in Holy Basil (*Ocimum sanctum* L.)". *International Journal of Agronomy*. <https://doi.org/10.1155/2024/8159886>Pei F., Li X., Liu X. and Lao C. Assessing the impacts of droughts on net primary productivity in China. 2013; *Journal of Environmental Management*, 2024;114: 362–371.
38. Prakash V, Singh VP, Tripathi DK, Sharma S, Corpas FJ. Nitric oxide (NO) and salicylic acid (SA): A framework for their relationship in plant development under abiotic stress. 2021; *Plant Bio.* 23, 39–49. <https://doi.org/10.1111/plb.13246>
39. Preciado-Rangel P, Reyes-Pérez JJ, Ramírez-Rodríguez SC, Salas-Pérez L, Fortis-Hernández M et al. Foliar Aspersión of Salicylic Acid Improves Phenolic and Flavonoid Compounds, and Also the Fruit Yield in Cucumber (*Cucumis sativus* L.). 2019; *Plants* 8, no. 2: 44. <https://doi.org/10.3390/plants8020044>
40. Rajeshwari V, Bhuvaneshwari V. Salicylic acid induced salt stress tolerance in plants. 2017; *Int. J. Plant Biol. Res.* 5, 1067.
41. Rebi A, Ashfaq S, Raza A. Effect of drought on morpho-physiological responses of plant and select the best cultivar with maximum drought tolerance potential; 2021, *NVEO*. 2021, 13445–13456.
42. Sajjad H, Muhammad JR, Muhammad AA, Shaghef E, Iqra Z et al. Oxidative Stress and Antioxidant Defense in Plants Under Drought Conditions. 2019; In: Hasanuzzaman, M., Hakeem, K., Nahar, K., Alharby, H. (eds) *Plant Abiotic Stress Tolerance*. Springer, Cham. [https://doi.org/10.1007/978-3-030-06118-0\\_9](https://doi.org/10.1007/978-3-030-06118-0_9)
43. Singleton VL, Orthofer R, Lamuela-Raventos RM, Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. 1999; *Methods in Enzymology*, 299, 152-178. [http://dx.doi.org/10.1016/S0076-6879\(99\)99017-1](http://dx.doi.org/10.1016/S0076-6879(99)99017-1)

44. Soltani M, Farshad Moradi Kashkooli, Mohammad Souri, Behnam Rafiei, Mohammad Jabarifar et al. Environmental, economic, and social impacts of geothermal energy systems. 2021; *Renewable and Sustainable Energy Reviews*, Elsevier, vol. 140(C). <https://doi.org/10.1016/j.rser.2021.110750>
45. Soroori S., Danaee E., Hemmati K., Ladan Moghadam A. Effect of Foliar Application of Proline on Morphological and Physiological Traits of *Calendula officinalis* L. under Drought Stress. 2021; *Journal of Ornamental Plants*, 2021; 11(1): 13-30.
46. Stehmann JO, Lorenz-Lemke A., Freitas L, Semir JO. "The genus *Petunia*," in *Petunia: Evolutionary, Developmental and Physiological Genetics*. 2009; eds T.Gerats and J. Strommer (New York, NY: Springer), 1–28.
47. Tsuda S, Fukui Y, Nakamura N, Katsumoto Y et al. Flower color modification of *Petunia hybrida* commercial varieties by metabolic engineering. 2004; *Plant Biotechnology*, 21,377-386
48. Vankar Padma S and Srivastava Jyoti. Evaluation of Anthocyanin Content in Red and Blue Flowers. *International Journal of Food Engineering*, vol. 6, no. 4.2010; <https://doi.org/10.2202/1556-3758.1907>
49. Wandenbussche M, Chambrier P, Rodrigues Bento S and Morel P. *Petunia*, Your Next Supermodel? 201;6, *Front. Plant Sci*. 7:72. <https://doi.org/10.3389/fpls.2016.00072>
50. Zhang Y, Li X. Salicylic acid: biosynthesis, perception, and contributions to plant immunity. 2019; *Curr Opin Plant Biol* 50:29–36. <https://doi.org/10.1016/j.pbi.2019.02.004>
51. \*\*\*Solanaceae Source-A global taxonomic resource for the nightshade family. <https://solanaceaesource.myspecies.info>