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INVESTIGATION OF ANTHOCYANIN LOCALIZATION IN VARIOUS PARTS OF *IMPATIENS BALSAMINA* L.

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ABSTRACT
Impatiens balsamina L. is an annual herbaceous plant of Balsamineaceae family and is cultured as a decorative plant in parks and gardens. Since it contains a large amount of minerals, calcium oxalate crystals and secondary metabolites (coumarin derivatives, scopoletin, isofraxidin), it is examined as both dangerous and medicinal plant. The aim of this study is to determine in which parts (node, internode, petiole, leaf, pedicel, petal) of I. balsamina L. the pigment anthocyanin accumulates and to measure its amount in the tissues by spectrophotometer. It was seen that the pigment accumulates more in the upper parts of the nodes on the stem, and that the amount of anthocyanin was significantly less in the internodes than in the nodes. The anthocyanin is responsible for the red color of flower petals was found in high amount in almost all vacuoles of the papillate epidermal cells.

Keywords: Impatiens balsamina, anthocyanin localization

Introduction
There are several reports on relationships between flower coloration and petal pigmentation (40). The pigments that give different colors to the vegetative organs such as stems and leaves and to the generative organs such as flower, fruit and seed are generally found in plastids localized in the cytoplasm. These compounds are found in chromoplasts as carotenoids and are made up of caroten (orange) and xantophyll (yellow) (11, 12). Besides, anthocyanins are the most significant among the pigments that provide color to flowers in higher plants (1, 7, 22, 24, 33).

The anthocyanins form one of the major groups of pigments belonging to the secondary metabolite group of flavonoids. They are often responsible for the orange, red, and blue colors in fruits, vegetables, flowers, and other storage tissues in plants (3, 13, 34, 38, 40). The anthocyanins, generally found in the vacuoles of epidermal cells, come in different color depending on the pH of vacuole sap (13, 35, 43). As pigments of flowers and fruits, they attract insects for pollination and protect against UV-B irradiation (15, 19, 23, 29).

The biosynthesis of the anthocyanins in plants is known to be regulated by more than 20 genes (7, 18, 37, 39, 40). Although the flowers usually have the same color like the plant, in some cases of dominant deficiency, different phenotypes (white, pink and red plants) could be found (9, 36).

I. balsamina L., is annual herbaceous plant, one of the 500 species that belong to the family, Balsamineaceae. *I. balsamina* L is often cultured as a decorative plant in parks and gardens. This plant can be grown vegetatively or from seeds and needs suitable soil and water conditions. It should be protected from long exposure to sunlight, but it can tolerate excessive temperature if sufficient water and humidity is provided. The plant starts flowering in early April, and keep its flowers until autumn. The petals of *Impatience balsamina* L. may have 15 different colors (white, pink, violet, red, etc.), succulent and translucent stems which are considered as the main factors of the sunlight tolerance.

Since now, there are few studies which have described the localization of anthocyanins. The aim of this study is to determine in which parts (node, internode, petiole, leaf, pedicel, petal) of *I. balsamina* the pigment anthocyanin accumulates and to measure its amount in the tissues by spectrophotometer.

Materials and Methods
Following flowering, cross and surface sections from different parts (node, internode, petiole, leaf, pedicel, and petal) of *I. balsamina* L. were examined under light microscope (Leica binocular), and the photos were taken (with Canon A520) and documented. Further, Mancinelli's (27) method was employed for the determination of anthocyanin content in these parts. According to this method, plant samples of 200 mg of fresh weight were extracted in 3 ml methanol-HCl (1% HCl, v/v) and kept under refrigerator at 3-5º C for 2 days with occasional shakings. Then the extract was filtered and the anthocyanin content in the filtrate was measured at 530 nm, while the chlorophyll content was measured at 657 nm of wavelengths and the results were placed in A_{530}-A_{657} formula. As a result, upon subtraction of chlorophyll absorption, the anthocyanin content was expressed as O.D. 530/g fresh weight.

Results and Discussion
In this study, it was determined during observations that anthocyanin pigment, accumulated especially in the upper parts of nodes in the stem of *I. balsamina*. In the internodes the anthocyanin content was apparently less. This is shown in cross sections in **Fig. 1**.

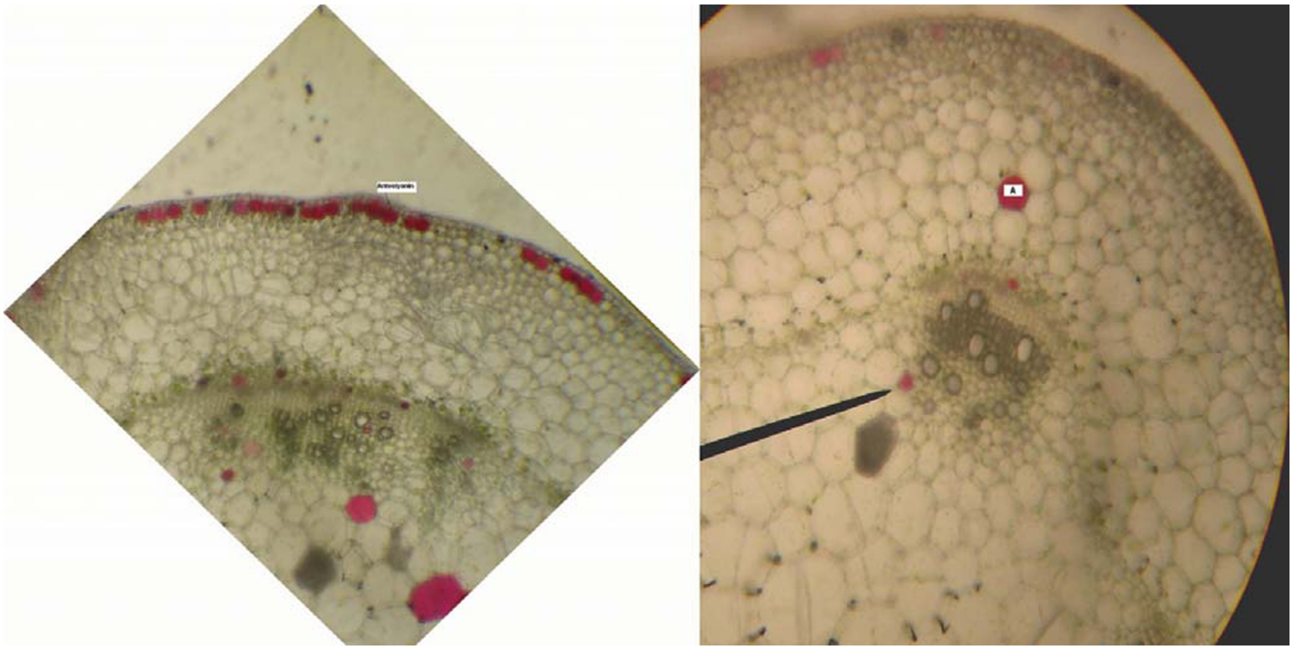

Fig. 1. The localization of anthocyanin in nodes (A) and internodes (B)

As can be seen from **Fig. 1**, anthocyanin was accumulated in the nodes, especially in the vacuoles of parenchymatous cells in the subepidermal layer under epidermis. These parenchymatous cells that contain anthocyanins were located under the epidermis as individual cells or in groups of 2 and 3 cells, in a row and sometimes in two rows. In the pith 2 or 3 cells were found to contain anthocyanin. Above and under the vascular bundles, one or two cells accommodated anthocyanin. As compared with the nodes, anthocyanin localization in the internodes was rarely distributed and only one or two parenchymatous cells had anthocyanin in their vacuoles.

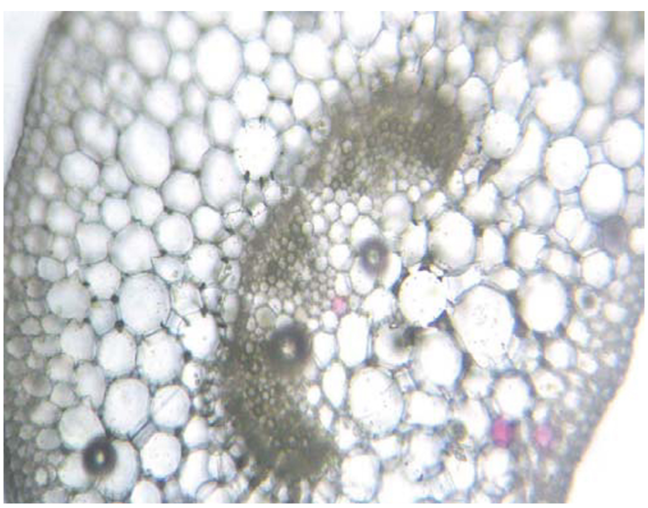

Fig. 2. Anthocyanin localization in the cross-section taken from the petiole

In the cross-section taken from the petiole, it was seen that epidermal cells do not synthesize and store anthocyanin, and anthocyanin was synthesized and stored in only 2 or 3 cells from the parenchymatous cells, in the vacuole of one cell under xylem rays and in the 8-rowed cortex layer under epidermis (**Fig. 2**).

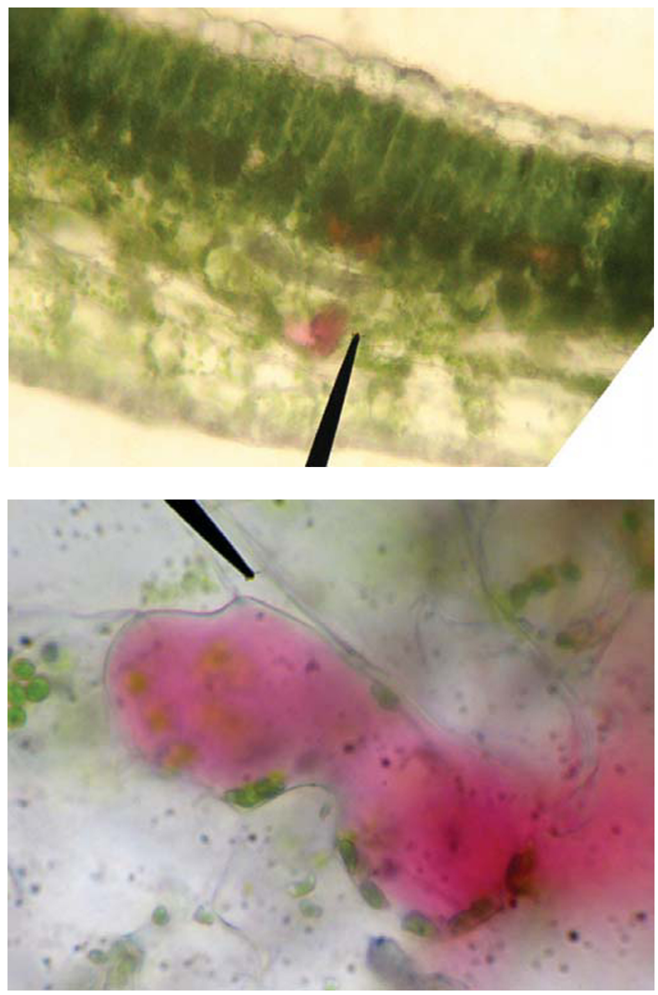

Fig. 3. Appearance of cells with anthocyanin in cross-section (A) and surface section (B) from the leaf

Upon investigation of the cross and surface section taken from the leaf, it was determined that anthocyanin was synthesized and stored in 3 or 4 cells in the lower epidermis in the successive spongy parenchyma just under the palisade cells (**Fig. 3**).

In the cross-section taken from the pedicel, it was determined that anthocyanin was present in the petiole and not in the epidermis, but in the pith anthocyanin was accumulated mostly in the vacuoles of parenchymatous cells (15-20) than in the petiole (**Fig. 4**).

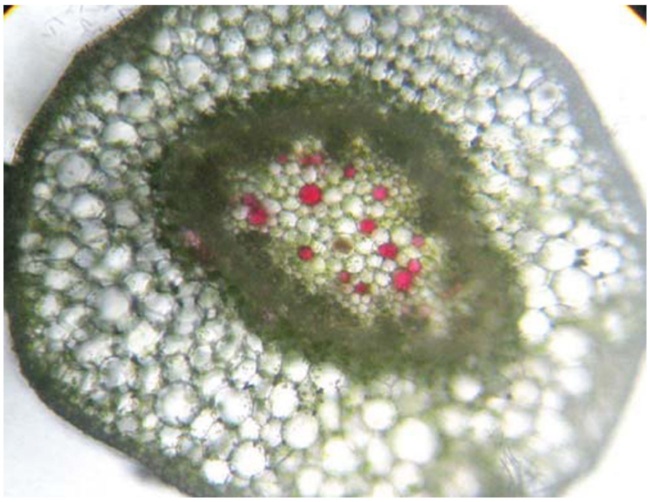

Fig. 4. Distribution of anthocyanin in the cross-section taken from the pedicel

In the cross-section taken from the petal of the red flower, anthocyanin filled the vacuole in almost all papillate upper epidermis cells. No anthocyanin was encountered in the parenchymatous tissue between the two epidermises, and almost all cells in the lower epidermis were filled with the pigment, anthocyanin (**Fig. 5**). This dense accumulation of anthocyanin in the petal epidermises gives the red color of the petal.

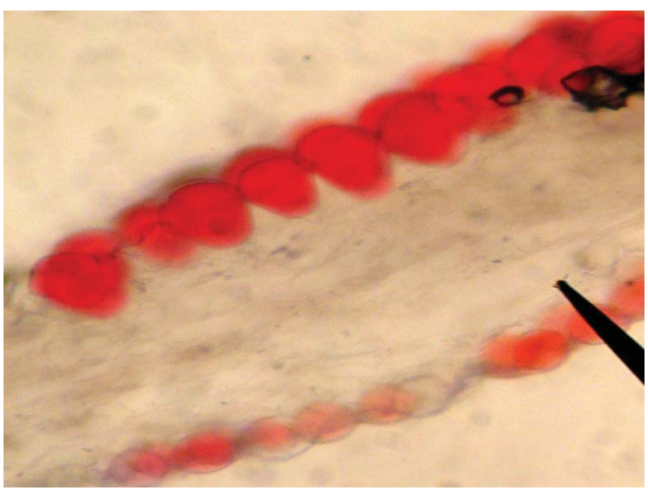

Fig. 5. Anthocyanin localization in the cross-section from the petal

During the examination of surface sections from the petal, it was seen that some cells were red and some were violet (**Fig. 6**). In these violet cells, differently from the red ones, dark violet spherical particles were found. In the literature, such particles are described as anthocyanin bearing structures in the petal epidermis of the rose, spherical structures and crystals in *Mathiola* petals. However, more detailed study is required to prove this matter.

Results showed anthocyanin content in different parts (in some vegetative and reproductive organs) of the plant. **Fig. 7**.

I. balsamina is a decorative plant which is often used in studies on anthocyanin pigmentation (2, 17, 24, 32). In higher plants, accumulation of anthocyanins generally occurs in specific tissues or cells during a specific stage (14, 21). The accumulation of anthocyanins seen in various tissues and organs in the vegetative stage is considered as an indicator against biotic and abiotic stress (4, 6, 7, 10, 20, 41, 42). Anthocyanin biosynthesis in plants also appears to be under manifold controls (18, 19, 25, 30). Genetic capacity for pigment production must be complemented by a sequence of rather definite developmental events and proper environmental and nutritional factors (16, 19, 24).

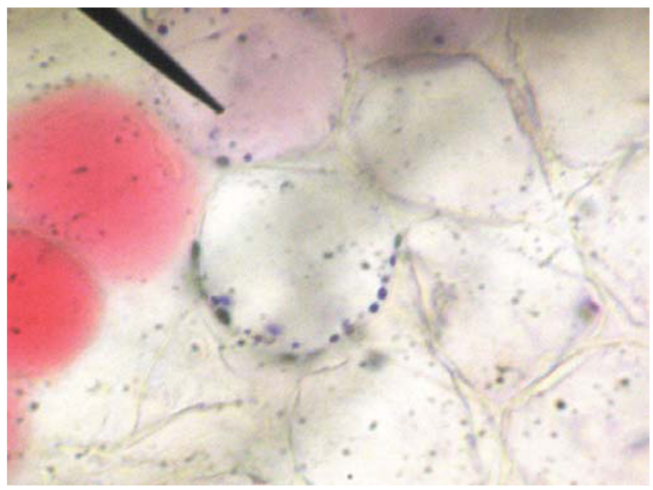

Fig. 6. Violet inclusion particles in the surface section

According to the obtained results, in the stem, anthocyanins accumulate especially in the nodes. (**Fig. 1**). Anthocyanin can be found in the subepidermal layers of the nodes, in the vascular tissue and in the parenchymatous cells in the pith. The accumulation of anthocyanins in the nodes was found to be 72% more than in the internodes (**Fig. 7**). In the petiole, anthocyanin was found only in the parenchymatous cells of the cortex layer, and in small numbers in xylem tissue (**Fig. 2**). In the leaves anthocyanin accumulates in the vacuoles of cells in the spongy parenchyma (**Fig. 3**). It was observed that in the reproductive stage, anthocyanins accumulated in upper epidermal papillae (**Fig. 5**), and as vacuolar inclusions in some of the lower epidermal cells (**Fig. 6**). Several plant species store anthocyanins within vacuolar inclusions that have been loosely termed anthocyanoplasts which have been observed to start as vesicles in the cytoplasm and appear to be membrane bound. More recently, the intravacuolar structures observed in the flower petals of various plants, including carnation and

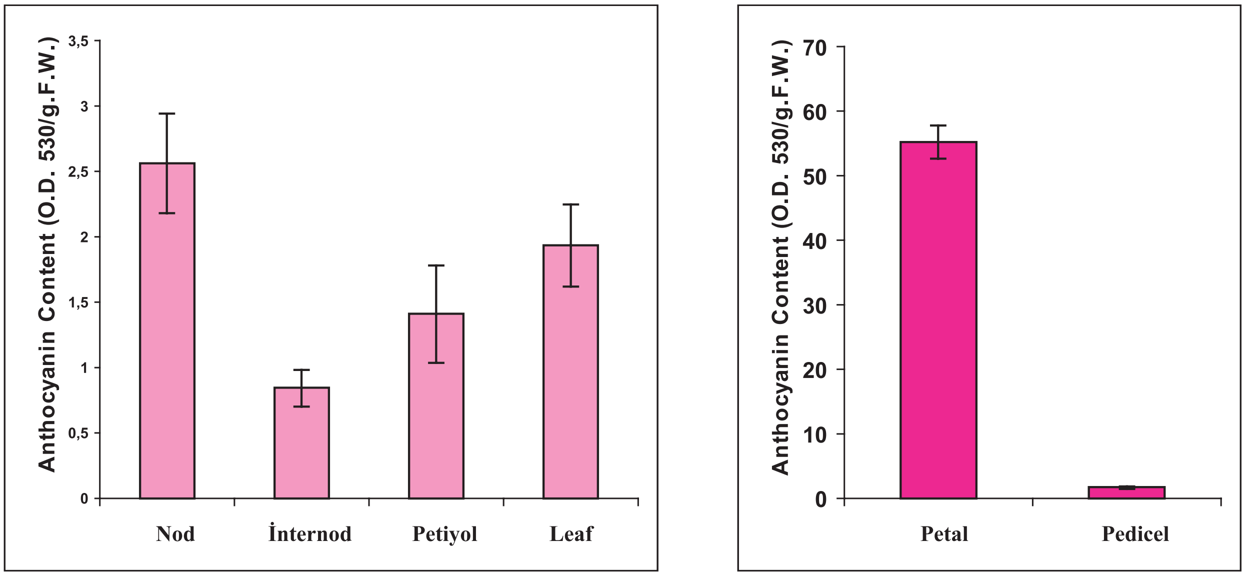

Fig. 7. Anthocyanin content in the vegetative (node, internode, petiole, leaf) and reproductive (pedicel, petal) parts of *I. balsamina*

lisianthus, were termed anthocyanic vacuolar inclusions, or AVIs (8, 28, 31). In the pedicel, anthocyanin was found to accumulate only in the vacuoles of main storage parenchyma cells (**Fig. 4**). In other studies on anthocyanin pigmentation, anthocyanin was reported to accumulate especially in epidermal cells (26). In this study, it was determined that the pigment accumulated in sub-epidermal layers rather than in the epidermis (**Figs. 1-4**). As a result, the presence of anthocyanin determined in the xylem and the pith brings up the question whether this pigment is stored only in the cell where it is synthesized or circulated to other cells through the vascular system.

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