

SABRAO Journal of Breeding and Genetics  
 58 (3) 1151-1160, 2026  
<http://doi.org/10.54910/sabrao2026.58.3.19>  
<http://sabraojournal.org/>  
 pISSN 1029-7073; eISSN 2224-8978



## PHYSIOLOGICAL ADAPTATION OF DYE-YIELDING PLANT SPECIES BASED ON CELL SAP AND PHOTOSYNTHETIC PIGMENTS IN THE SURKHANDARYA REGION, UZBEKISTAN

**Kh. ASLANOVA<sup>1</sup>, B. AMANOV<sup>2</sup>, Kh. MUMINOV<sup>2\*</sup>, O. OMONOV<sup>2</sup>, O. HAZRATKULOVA<sup>3</sup>,  
 O. SHODIYEVA<sup>4</sup>, F. UMIRKULOVA<sup>5</sup>, and M. QURBONOV<sup>6</sup>**

<sup>1</sup>Denov Institute of Entrepreneurship and Pedagogy, Denov, Uzbekistan

<sup>2</sup>Department of Natural Sciences, Chirchik State Pedagogical University, Tashkent, Uzbekistan

<sup>3</sup>Department of English Philology, Termez University of Economics and Service, Termez, Uzbekistan

<sup>4</sup>Department of Natural Sciences and Medicine, Navoi State University, Navoi, Uzbekistan

<sup>5</sup>Department of Natural Sciences, Termez University of Economics and Service, Termiz, Uzbekistan

<sup>6</sup>Termez State Pedagogical Institute, Termiz, Uzbekistan

\*Corresponding author's email: mxa8215@mail.ru

Email addresses of co-authors: xolidaaslanova08@gmail.com, amanov.81@bk.ru, omonovorif1982@gmail.com, ozoda\_hazratkulova@tues.uz, ozoda1971@bk.ru, feruza\_umirqolova@tues.uz, murodjonkurbonoff550@gmail.com

### SUMMARY

The concerned research aimed to investigate the peculiarities of adaptation to water regime and photosynthetic activity in four dye-yielding plant species (*Indigofera tinctoria* L., *Rubia tinctorum* L., *Isatis tinctoria* L., and *Lawsonia inermis* L.) cultivated in the Surkhandarya Region, Uzbekistan. During the study, the leaf cell sap (LCS) concentration and content of the key photosynthetic pigments, such as chlorophylls a and b, total pigment concentration, and carotenoids, succeeded in their determination at the budding stage under normal and water-deficit conditions. The obtained results allowed for identifying the degree of osmotic adaptation and photoadaptation mechanisms of the plants under water-deficit conditions. The results lay down a scientific base for assessing the ecological stability of dye-yielding plants and determining cultivars resistant to water stress conditions with increasing photosynthetic efficiency. Detecting the chlorophylls a and b and carotenoid contents in leaf samples used the spectrophotometric method. The results revealed a considerable decline in photosynthetic pigments' concentration under water-deficit conditions; however, these also showed robust adaptive processes in certain species (especially the species *Indigofera tinctoria* L.). Biochemical analysis disclosed an increase in the anthocyanin and flavonoid contents, ensuring stress tolerance in dye-yielding plants. This approach helps in distinguishing the degree of plant adaptability with enhanced efficiency in natural pigments' production.

Communicating Editor: Prof. Naqib Ullah Khan

Manuscript received: November 25, 2025; Accepted: January 11, 2026.

© Society for the Advancement of Breeding Research in Asia and Oceania (SABRAO) 2026

**Citation:** Aslanova KH, Amanov B, Muminov KH, Omonov O, Hazratkulova O, Shodiyeva O, Umirkulova F, Qurbonov M (2026). Physiological adaptation of dye-yielding plant species based on cell sap and photosynthetic pigments in the Surkhandarya Region, Uzbekistan. *SABRAO J. Breed. Genet.* 58 (3) 1151-1160. <http://doi.org/10.54910/sabrao2026.58.3.19>.

**Keywords:** Dye-yielding plants, natural dye, species, physiology, photosynthetic pigments, chlorophyll a and b, carotenoid, cell sap, water-deficit conditions

**Key findings:** The dye-yielding plant species (*Indigofera tinctoria* L., *Rubia tinctorum* L., *Isatis tinctoria* L., and *Lawsonia inermis* L.) exhibited distinct physiological responses to water-deficit conditions. The species *Rubia tinctorum* L. and *Lawsonia inermis* L. showed higher cell sap concentrations, suggesting a considerable capacity for drought adaptation.

## INTRODUCTION

In the present era, interest has been growing in restoring natural dye sources and applying them in various industries based on their ecological safety. Natural dyes are distinct with their chemical stability, biological activity, and lack of harm to human health. Therefore, determining physiological and biochemical characteristics of dye-yielding plants and their association with ecological conditions has become one of the viable strategies (Kodirova et al., 2024; Khodjayeva et al., 2025).

The Surkhandarya oasis shows characteristics of unique climatic features, such as the highest temperatures, water-deficit conditions, intense sunlight, low humidity, and soils with varying mechanical compositions. These environmental factors considerably affect the physiological processes of crop plants, pigment formation, synthesis of metabolites, and the activity of biochemical reactions. Therefore, analyzing the ecological adaptability of dye-yielding plants cultivated in this region, the process of colorant formation, and their quantitative variations were of significant practical importance (Normurodov et al., 2025).

With the increasing demand for environmentally friendly and renewable resources, interest in natural dyes gradually grows. The Surkhandarya Region, with its unique climate, soil, and flora, also hosts various plants beneficial for obtaining natural dyes. A thorough study of the physiological and biochemical properties of such plants enables their industrial-scale processing. Currently, an enhanced need for ecologically clean and natural pigments is amplifying the demand for natural colorants, particularly for use as an alternative to chemical dyes in the textile industry. Therefore, a thorough study of the

physiological and biochemical traits of dye-yielding plants, analyzing their photosynthetic activities, water regimes, and adaptation mechanisms, is of considerable practical necessity (Amanov et al., 2020).

One of the important dyes currently receiving attention is indigo. Indigo belongs to the group of carbonyl compounds and is one of the oldest natural blue dyes, known since antiquity, derived from the *Indigofera tinctoria* plant. The indigo utilization, implemented as a natural food colorant, has the aim of reducing carcinogenic effects of synthetic dyes (Wahyuningsih et al., 2017; Ariyatun et al., 2022; Hartl et al., 2024). *Indigofera tinctoria* contains both indigo and indirubin and other bioactive compounds, such as flavonoids, steroids, and alkaloids, which possess antioxidants and other biological activities (Ariyatun et al., 2022; Archana et al., 2025).

Primarily, indigo has wide uses in dyeing textiles, especially denim, silk, cotton, and other wool products. However, in previous years, the application area of indigo has also expanded into different food industries. This is because indigo served as a natural food colorant, particularly in beverages, sweets, dairy products, candies, and decorative products (Ariyatun et al., 2022). The *Rubia tinctorum* L. pigments are primarily anthraquinones, which have been traditionally useful as natural dyes possessing various biological activities. *Rubia tinctorum* L. pigments mainly accumulate in roots, with alizarin being the principal red dye widely used for coloring textiles, in addition to its antioxidant properties. Generally, the plant pigments' composition mainly depends on the extraction methods, plant cultivars, and cultivation conditions (Henderson et al., 2013; Ford et al., 2018).

The pigments of the plant *Isatis tinctoria* are mainly natural indigo and its derivatives. These dyes have historically been widely applicable for dyeing textiles, in medicine, as well as for coloring woods and plastics (Mocquard *et al.*, 2022; Sharif *et al.*, 2024). Furthermore, natural indigo pigments obtained from *Isatis tinctoria* ensure stable color retention by using wood and polylactic acid-based polymers (Jordan and Laaksonen, 2023; Vauquelin *et al.*, 2024). Moreover, the pigments and other secondary metabolites (flavonoids and phenolic acids) of *Isatis tinctoria* possess antioxidant, anti-inflammatory, antimicrobial, and antiviral properties (Nguyen *et al.*, 2019; Speranza *et al.*, 2020; Miceli *et al.*, 2023). Indigo pigment has indirect formation in the leaves of *Isatis tinctoria*; rather, it becomes stored in the form of several indigo precursors (*indicin*, *isatan A*, *isatan B*, and *isatan C*). When the leaves entail damage and exposure to air, these precursors undergo conversion into indoxyl through enzymatic hydrolysis and oxidation, and subsequently into indigo (blue pigment) and indirubin (red pigment) (Speranza *et al.*, 2020).

The *Lawsonia inermis* L. (henna) pigments primarily contain the lawsone (2-hydroxy-1,4-naphthoquinone), a substance renowned for its reddish-orange color. Lawsone was most abundant in the leaves, which also contain other bioactive compounds, such as flavonoids, phenolic compounds, tannins, terpenoids, and antioxidants (Al-Snafi, 2019; Elansary *et al.*, 2020; Othman *et al.*, 2020; Batiha *et al.*, 2023). Lawsone is the main coloring pigment in the plant, being recorded with the highest concentration in plant leaves (Oda *et al.*, 2018; Elansary *et al.*, 2020; Batiha *et al.*, 2023).

The lawsone content and most other pigments in the leaves were higher in natural conditions, whereas lawsone decreases under laboratory conditions, although the antioxidants and flavonoids increased (Pistelli *et al.*, 2023). Environmental conditions, such as light, salinity, and growing site, significantly affect the quantity and composition of pigments. Therefore, proper selection of growing conditions is essential for obtaining

optimal pigment yield (Marzec and Szadkowski, 2019; Abba *et al.*, 2020; Ahmad and Ansari, 2020; Akilandaeswari and Muthu, 2021).

Water-deficit condition is one of the most crucial abiotic stress factors affecting vital activities of plants. It sharply alters not only the processes of growth and development but also interrupts the physiological processes, such as photosynthesis, transpiration, metabolism, and intracellular balance (Maisura *et al.*, 2014; Maghsoudi *et al.*, 2019). With global climate change, the precipitation regime has gained disruptions, intensifying the problem of water-deficit conditions in dye-yielding plants in Surkhandarya, Uzbekistan. In *Isatis tinctoria* L., the water stress directly influences its leaf biomass, pigment concentration, and indigo content (Khadka *et al.*, 2020). The *Lawsonia inermis* L., conversely, exhibited a decrease in photosynthetic pigments, dry matter in leaves, and photosynthetic productivity under drought-stress conditions (Kamalabadi *et al.*, 2024). These variations considerably revealed the sensitivity of the pigments' system to water stress conditions.

Past research enunciated that in response to water-deficit conditions, plants enhance the concentration of cell fluid through osmotic protection mechanisms. Consequently, retaining intracellular water protects the cells from damage (Mamedov, 2015; Khadka *et al.*, 2020). Parallel to these physiological processes, the photosynthetic pigment system shifts to an active defense state: carotenoids protect the chlorophyll from oxidation, while anthocyanins mitigate the light stress conditions. The photosynthetic process is vital in living organisms, as it involves the synthesis of organic compounds along with the release of oxygen (Beknazarov, 2009). Photosynthesis efficiency depends on the concentration of chlorophylls a and b, while carotenoids protect this physiological system from oxidative stress damage (Maisura *et al.*, 2014). Thus, water deficiency not only enhances the leaf cell sap (LCS) index but also causes variations in the pigment ratio.

In the analysis of photosynthetic pigments, solvent systems based on ethanol (C<sub>2</sub>H<sub>5</sub>OH), acetone (C<sub>3</sub>H<sub>6</sub>O), and diethyl ether

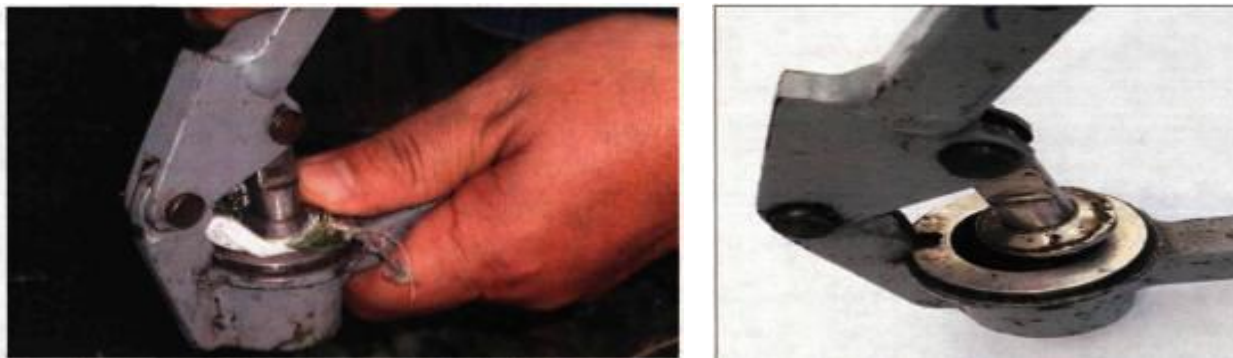
are widely used solutions as the most effective methods. These pigments appear within chloroplasts, and, in addition to absorbing light, transferring energy, and facilitating photochemical reactions, they also perform protective functions during abiotic stress conditions (Maghsoudi *et al.*, 2019; Khadka *et al.*, 2020). The presented study aimed to evaluate the physiological processes, cell sap concentration, and variations in photosynthetic pigments in relation to water stress and ecological factors in specific dye-yielding plants grown in the Surkhandarya Region, Uzbekistan.

## MATERIALS AND METHODS

The following research conducted during 2025 was under field conditions at the Scientific Research Institute of Fine-Fiber Cotton Breeding, Surkhandarya Region, Uzbekistan.

Dye-producing plants included *Indigofera tinctoria* L., *Rubia tinctorum* L., *Isatis tinctoria* L., and *Lawsonia inermis* L., which entailed scrutiny in field experiments carried out in a subplot size of 24 m<sup>2</sup>, using a randomized complete block design with four replications. The conduct of experiments proceeded on two plots with differing soil moisture levels comprising a) soil moisture before irrigation maintained at 56%–58% and b) soil moisture before irrigation not falling below 78%–80%.

Plants' cell sap concentration determination used the refractometric method on samples collected in triplicate from each plot. A hand press (Figure 1) and a Ruoshui 2GHS refractometer, China (Figure 2), were pieces of equipment used for cell sap determination. For analysis, the selection of six replicate samples came from the third morphologically developed leaf at the growth point. Measurements continued before irrigation, along with soil moisture assessment



**Figure 1.** The hand press and the procedure for extracting cell sap using device (Sharif *et al.*, 2024).



**Figure 2.** Ruoshui 2GHS refractometer (Sánchez-Pérez *et al.*, 2008).

beneath the plants (Ford *et al.*, 2018). The soil moisture, as measured, employed a soil moisture meter (Hangzhou Quality Lab Scientific Instrument Co., Ltd., China). Additionally, air temperature and humidity measurements utilized an Assmann psychrometer.

In both experiments, for the compilation of recorded data, the equations used in all practical procedures, were as follows:

$$\bar{x} = A + bxi \quad G = i\sqrt{\frac{\sum fxa^2}{n} - b^2}$$

$$m\% = \frac{m}{\bar{x}}$$

$$V = \frac{Gx100}{\bar{x}} \quad m = \frac{G}{\sqrt{n}}$$

The central value of class A; b is a correction factor calculated using the following formula:

$$b = \frac{\sum fxa}{n};$$

Where i = class interval, f = deviation of frequencies, M = arithmetic mean, G = standard deviation, X = the sum of observations, m = the standard error of the arithmetic mean, n = sample size, V = coefficient of variation, and m% = error percentage.

$$\bar{x} = \frac{\sum X}{n} \quad G = \sqrt{\frac{\sum (X - \bar{x})^2}{n-1}}$$

$$V = \frac{Gx100}{\bar{x}} \quad m = \frac{G}{\sqrt{n}}$$

Where x = arithmetic mean, G = average deviation, X = the sum of observations, m = the standard error of the arithmetic mean, n = sample size, and V = coefficient of variation.

In both field experiments, the concentrations of chlorophylls a and b and carotenoids were successful as determined in the leaves of pigment-producing plants. Under field conditions, the samples collected came from the 3rd to 4th leaves, counted from the plant's growth point. Each leaf sample of 50 mg entailed placement into a test tube, with the leaf samples homogenized in 5 mL of 95% ethanol solution (Ford *et al.*, 2018).

The homogenate received centrifugation at 5000 rpm for 12 minutes. The absorbance values of the resulting extract, corresponding to chlorophyll a, chlorophyll b, and carotenoids, underwent measurement at wavelengths of 664, 649, and 470 nm using an Agilent Cary 60 UV-Vis spectrophotometer. Based on these absorbance readings, the concentrations of chlorophyll a, chlorophyll b, and carotenoids in plant leaves incurred calculations using the following equations (Nayek *et al.*, 2014).

$$\text{Chlorophyll a (mg/g)} = 13.36A_{664} - 5.19 \times A_{649}$$

$$\text{Chlorophyll b (mg/g)} = 27.43A_{649} - 8.12 \times A_{664}$$

$$\text{Carotenoids (mg/g)} = (1000A_{470} - 2.13 \times \text{Chl a} - 97.63 \text{ Chl b}) / 209$$

$$F \text{ (Mg/g)} = (V \times S) / P$$

Based on the values obtained using these formulas, determining the average pigment content for each plant species, their relative ratios, and physiological variations induced by water stress was successful. The reliability of these findings received assessment through statistical analysis.

## RESULTS AND DISCUSSION

Cell sap concentration (CSC) emerged as one of the most sensitive physiological indicators for evaluating the water regime status of crop plants. The results revealed CSC reflects the ecological adaptability of plants in interaction with environmental conditions, having a direct

association with their drought tolerance. Observations conducted under the conditions of the Surkhandarya Region, Uzbekistan, disclosed that leaf cell sap concentration decreased as and when soil moisture increased, whereas it rose under soil dehydration. These variations were ascribable to the plant's ability to conserve more water, regulate osmotic pressure, and maintain intracellular homeostasis. Therefore, CSC dynamics sustained evaluation as a reliable diagnostic criterion for determining the physiological status of pigment-producing plants under water stress conditions (Maghsoudi et al., 2019; Speranza et al., 2020). In quantifying the photosynthetic pigment system of the plant's chlorophylls a and b and carotenoids, past findings indicated pigment concentration is a key factor determining photosynthetic activity and ecological stability in crop plants (Maisura et al., 2014). Under water-deficit conditions, pigment levels declined, which corresponds to a reduction in photosynthetic efficiency. However, the relative stability of carotenoid content enhanced their photoprotective function (Maisura et al., 2014).

In general, the relationship between cell sap concentration and variations in photosynthetic pigments reflects the complex physiological response of plants to water stress conditions. These results provide a sound basis for assessing the drought adaptability of dye-producing plants, identifying drought-tolerant genotypes, and enhancing their pigment productivity (Khodjayeva et al., 2025). Based on the above information, the cell sap concentration entailed detection in dye-producing plants, namely *Indigofera tinctoria*

L., *Rubia tinctorum* L., *Isatis tinctoria* L., and *Lawsonia inermis* L. species. It continued by examining the dynamics of variations in relation to soil moisture levels during the flowering stage (Table 1).

During the flowering stage of the studied dye-producing plants, cell sap concentration exhibited significant variations under different soil moisture levels (Khadka et al., 2020). According to results, under optimal moisture conditions (78%–80%), all the plants demonstrated lower cell sap concentrations, indicating sufficient water availability within the vacuoles. In contrast, the limited moisture conditions (56%–58%) and water-deficit conditions lead to reduced vacuolar water content and a relative increase in the concentration of solutes (sugars, amino acids, proline, and other osmotic compounds) dissolved in the sap. The said process represents the plants' osmotic adjustment mechanism, whereby osmotic pressure increases to retain cellular water. However, this mechanism manifests differently across the plant species (Elansary et al., 2020; Batiha et al., 2023).

For instance, in *Indigofera tinctoria* L., the recorded cell sap concentration was 8.1% under optimal conditions and increased to 10.3% under limited moisture conditions, reflecting a 27% rise. This species originates from tropical regions and exhibits moderate drought tolerance. Although its deep roots facilitate water uptake, the cell sap concentration increases rapidly in its leaves. Such a type of plant's adaptation helps delay the leaf tissue wilting (Ahmad and Ansari, 2020; Akilandaeswari and Muthu, 2021).

**Table 1.** Effect of soil moisture levels on cell sap concentration during the flowering stage of dye-producing plants (%).

Species	Soil moisture	
	Optimal moisture (78%–80%)	Limited moisture (56%–58%)
<i>Indigofera tinctoria</i> L.	8.1±0.5	11.3±0.4
<i>Rubia tinctorum</i> L.	11.5±0.4	15.8±0.5
<i>Isatis tinctoria</i> L.	7.1±0.3	9.9±0.3
<i>Lawsonia inermis</i> L.	10.5±0.2	14.2±0.4

In *Rubia tinctorum* L. (dyer’s madder), the cell sap concentration was 11.5% under optimal conditions and rose to 14.8% under limited moisture, representing a 28%–30% increase. The primary reason for this process is the accumulation of anthocyanins and phenolic compounds in plant roots. Under water-deficit conditions, the levels of osmotic substances (such as proline and sugars) in both roots and leaves increased sharply, resulting in a significant rise in cell sap concentration. The madder plant is considerably moderately tolerant, responding actively to stress conditions; however, it requires substantial water (Oda *et al.*, 2018; Batiha *et al.*, 2023).

In *Isatis tinctoria* L., the cell sap concentration was 7.1% under optimal conditions and increased to 8.9% under limited moisture, representing a 25% rise. The said species seemed highly drought-tolerant due to its deep-growing taproot system and the presence of trichomes on leaf surfaces. Consequently, even under water stress conditions, the increase in cell sap concentration was relatively lower than other species, revealing its adaptation to arid conditions (Elansary *et al.*, 2020).

In *Lawsonia inermis* L. (henna), the cell sap concentration was 10.5% under optimal conditions and rose to 13.2% under limited moisture, reflecting a 25%–27% increase. Although this species is naturally adapted to semi-arid regions, its large leaf surface area contributes to high transpiration rates. As a result, under water-deficit conditions, the concentration of solutes increases rapidly in leaf vacuoles. The results indicated that while the said plant is

moderately tolerant, its high transpiration makes it more susceptible to drought stress conditions (Elansary *et al.*, 2020; Batiha *et al.*, 2023).

In this research conducted under the agroclimatic conditions of the Surkhandarya Region, Uzbekistan, scientists analyzed the levels of key photosynthetic pigments. These are chlorophyll a, chlorophyll b, total pigment concentration, and carotenoids during the budding stage of the selected dye-producing plant species. A comparative analysis across the different species revealed the pigment concentrations for various plant physiological traits (Table 2). The analysis of the pigment system of the dye-producing plants expressed that under water stress conditions, the quantity of photosynthetic pigments significantly varies among the four species. Notably, *Rubia tinctorum* L. and *Lawsonia inermis* L. exhibited a higher pigment richness, whereas the lower pigment levels in *Isatis tinctoria* L. suggest its sensitivity to drought stress conditions (Ahmad and Ansari, 2020; Akilandaeaswari and Muthu, 2021).

The results displayed that overall, the chlorophyll a content ranged from 2.35±0.31 to 5.74±0.27 mg/g across the different species. However, the highest concentration of chlorophyll a was evident in *Rubia tinctorum* L. (5.74±0.27 mg/g), indicating a highly active photosynthetic apparatus. The lowest value of chlorophyll a resulted in *Isatis tinctoria* L. (2.35±0.31 mg/g), suggesting reduced pigment synthesis under water stress conditions. The species *Indigofera tinctoria* L. (3.32±0.24 mg/g) and *Lawsonia inermis* L. (3.23±0.26 mg/g) maintained moderate levels

**Table 2.** Photosynthetic pigment content of dye-producing plants during the budding stage under agro-climatic conditions of Surkhandarya, Uzbekistan during 2025.

Species	Pigment composition per fresh weight (mg/g)			
	Chlorophyll a	b	Chlorophyll Total	Carotenoid
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	concentration $\bar{x} \pm S\bar{x}$	rate $\bar{x} \pm S\bar{x}$
<i>Indigofera tinctoria</i> L.	3.32±0.24	2.84±0.15	4.05±0.13	0.35±0.04
<i>Rubia tinctorum</i> L.	5.74±0.27	3.97±0.16	4.4±0.16	0.48±0.05
<i>Isatis tinctoria</i> L.	2.35±0.31	1.25±0.18	3.2±0.29	0.28±0.03
<i>Lawsonia inermis</i> L.	3.23±0.26	2.35±0.21	2.94±0.24	0.42±0.06

of chlorophyll a concentrations. The spectrophotometric analysis of chlorophylls a and b, total chlorophyll, and carotenoid contents in the leaves of grass pea (*Lathyrus sativus* L.) revealed varied values (Khodjayeva et al. 2025).

Overall, the chlorophyll b content ranged from  $1.25 \pm 0.18$  to  $3.97 \pm 0.16$  mg/g among the four different dye-producing species. *Rubia tinctorum* L. again showed the highest value of chlorophyll b ( $3.97 \pm 0.16$  mg/g), indicating stable chlorophyll synthesis in its leaves. The lowest chlorophyll b value appeared in *Isatis tinctoria* L. ( $1.25 \pm 0.18$  mg/g), further confirming its sensitivity to water stress conditions. The species *Indigofera tinctoria* L. ( $2.84 \pm 0.15$  mg/g) and *Lawsonia inermis* L. ( $2.35 \pm 0.21$  mg/g) maintained intermediate levels of chlorophyll b, suggesting partial preservation of pigment synthesis. Past studies revealed that at the flowering phase, the highest values of chlorophyll b emerged in the leaves of broad bean (*Vicia faba* L.) (Omonov et al. 2023; Khodjayeva et al. 2025).

Thus, although total pigment concentrations, particularly chlorophylls a and b, decreased under water-deficit conditions, carotenoids remained relatively stable. This suggests the plants activate protective mechanisms to defend against oxidative stress conditions caused by increased light and to preserve the photosynthetic apparatus. The results based on the analysis of the photosynthetic pigment system also demonstrated the direct physiological impact of water stress on plants.

Carotenoid content also varied among the four species, ranging from  $0.35 \pm 0.04$  to  $0.48 \pm 0.05$  mg/g. The highest levels of carotenoid content were evident in *Rubia tinctorum* L. ( $0.48 \pm 0.05$  mg/g) and *Lawsonia inermis* L. ( $0.42 \pm 0.06$  mg/g), indicating that these species maintained the highest carotenoid synthesis, which reflects the active functioning of their photoprotective mechanisms. In contrast, the species *Indigofera tinctoria* L. ( $0.35 \pm 0.04$  mg/g) and *Isatis tinctoria* L. ( $0.28 \pm 0.03$  mg/g) showed relatively lower carotenoid levels.

Overall, the reduction in chlorophyll a and b levels leads to a decline in photosynthetic activity, while the relative stability of carotenoid content reveals the activation of photoprotective responses. Notably, the highest carotenoid levels in *Rubia tinctorum* L. ( $0.48 \pm 0.05$  mg/g) and *Lawsonia inermis* L. ( $0.42 \pm 0.06$  mg/g) reflect the effective functioning of their photoprotective systems, suggesting these species have developed stable physiological responses to oxidative stress conditions (Marzec and Szadkowski, 2019; Abba et al., 2020). In conclusion, the plant's responses to water-deficit conditions are emergent through two primary mechanisms: maintaining osmotic balance via increased cell sap concentration and protecting the photosynthetic apparatus through stable carotenoid synthesis (Maghsoudi et al., 2019; Khodjayeva et al., 2025).

Cell sap concentration and pigment systems serve as complementary indicators, enabling a comprehensive assessment of plant physiological tolerance to water stress conditions. These results provide a valuable basis for the selection of dye-producing plants and the planning of water-saving agronomic practices under arid conditions, such as those found in the Surkhandarya Region, Uzbekistan. The results obtained are of great value in determining the dynamics of pigment formation in plants under the ecological conditions of Surkhandarya, Uzbekistan, for the efficient use of natural dye sources and the production of environmentally friendly products.

## CONCLUSIONS

The results revealed four pigment-producing plant species (*Indigofera tinctoria* L., *Rubia tinctorum* L., *Isatis tinctoria* L., and *Lawsonia inermis* L.) exhibited distinct physiological responses to water deficit conditions. Throughout the study, a considerable correlation was evident between the cell sap concentration (CSC) and the photosynthetic pigment system. As soil moisture decreased

from 78%–80% to 56%–58%, the CSC levels increased across all plant species, indicating activation of osmotic adjustment mechanisms. Notably, the species *Rubia tinctorum* L. ( $11.5 \pm 0.4\% \rightarrow 15.8 \pm 0.5\%$ ) and *Lawsonia inermis* L. ( $10.5 \pm 0.2\% \rightarrow 14.2 \pm 0.4\%$ ) showed the higher cell sap concentrations, suggesting a robust capacity for drought adaptation.

## REFERENCES

- Abba Z, Gumel SM, Idris AA, Ibrahim MA (2020). Formulation of paint using natural pigment from *Lawsonia inermis* leaves. *Int. J. Adv. Chem.* 8(1): 155–159.
- Ahmad R, Ansari K (2020). Chemically treated *Lawsonia inermis* seeds powder (CTLISP): An eco-friendly adsorbent for the removal of brilliant green dye from aqueous solution. *Ground Sust. Dev.* 11(3): 100417.
- Akilandaeaswari B, Muthu K (2021). One-pot green synthesis of Au-Ag bimetallic nanoparticles from *Lawsonia inermis* seed extract and its catalytic reduction of environmental pollutants. *J. Taiwan Instt. Chem. Eng.* 127: 292–301.
- Al-Snafi AE (2019). Medicinal value of *Lagerstroemia speciosa*: An updated review. *Int. J. Current Pharm. Res.* 11(5): 18–26.
- Amanov B, Abdiev F, Muminov Kh, Shavkiev J, Mamedova F (2020). Valuable economic indicators among hybrids of Peruvian cotton genotypes. *Plant Cell Biotechnol. Mol. Biol.* 21(67–68): 35–46.
- Archana B, Fatima S, Akhila B, Poojitha B, Fatima M (2025). Examinations of *Indigofera tinctoria* Linn's pharmacognostic and preliminary phytochemical analyses. *Int. J. Exp. Biomed. Res.* 4(1): 9–15.
- Ariyatun A, Marwoto P, Sudarmin S, Wardani S, Saptono S (2022). Identification of active compounds of tantrum leaves (*Indigofera tinctoria*) natural textile dyes through maceration extraction method. *Biosaintifika: J. Biol. Biol. Edu.* 14(3): 435–443.
- Batiha G, Teibo J, Shaheen H, Babalola B, Teibo T, Al-Kuraishy H, Al-Garbeeb A, Alexiou A, Papadakis M (2023). Therapeutic potential of *Lawsonia inermis* Linn: A comprehensive overview. *Naunyn-Schmied. Arch. Pharm.* 397: 3525–3540.
- Beknazarov BO (2009). *Plant Physiology. Textbook.* Publishing House Alokachi. Tashkent.
- Elansary H, Szopa A, Kubica P, Ekiert H, Al-Mana F, Al-Yafsi M (2020). Antioxidant and biological activities of *Acacia saligna* and *Lawsonia inermis* natural populations. *Plants* 9.
- Omonov O, Amanov B, Muminov K, Buronov A, Tursunova N (2023). Physiological and biochemical composition of sunflower (*Helianthus annuus* L.) *SABRAO J. Breed. Genet.* 55(6): 2159–2167.
- Ford L, Rayner C, Blackburn R (2018). Degradation of lucidin: New insights into the fate of this natural pigment present in Dyer's madder (*Rubia tinctorum* L.) during the extraction of textile artefacts. *Dyes and Pig.* 154: 260–294.
- Hartl A, Polleichtner A, Novak J (2024). Purplish Blue or Greenish Grey? Indigo qualities and extraction yields from six species. *Plants* 13(7): 918.
- Henderson R, Rayner C, Blackburn R (2013). Isolation and extraction of lucidin primeveroside from *Rubia tinctorum* L. and crystal structure elucidation. *Phytochemistry* 95: 105–108.
- Jordan J, Laaksonen P (2023). Color stability of polylactic acid pigmented with natural indigo of *Isatis tinctoria* in artificial weathering. *Acta Hort.* 1361(18): 163–168.
- Kamalabadi N, Sarhadi H, Shirzadi M (2024). Assessment enhancing drought tolerance in henna (*Lawsonia inermis* L.) ecotypes through sodium nitroprusside foliar application. *Open Agric.* 9(1): 20220346.
- Khadka K, Earl HJ, Raizada MN (2020). A Physio-morphological trait-based approach for breeding drought tolerant wheat. *Front. Plant Sci.* 11: 715.
- Khodjayeva N, Fayziyev V, Amanov B, Muminov Kh, Buronov A, Omonov O, Tursunova N, Usmanova M (2025). Physiological and biochemical characteristics of the broad bean (*Vicia faba* L.). *SABRAO J. Breed. Genet.* 57(4): 1644–1651.
- Kodirova S, Amanov B, Muminov Kh, Abdiyev F, Buronov A, Tursunova N, Kurbanbayev I (2024). Physiological and biochemical parameters of the exotic species of grass pea (*Lathyrus sativus* L.). *SABRAO J. Breed. Genet.* 56(4): 1513–1523.
- Maghsoudi K, Emam Y, Ashraf M, Arvin MJ (2019). Alleviation of field water stress in wheat cultivars using silicon and salicylic acid applied separately or in combination. *Crop Past. Sci.* 70: 36–43.
- Maisura M, Chozin A, Lubis I, Junaedi A, Ehara H (2014). Some physiological character responses of rice under drought conditions

- in a paddy system. *J. Int. Soc. South. Asian Agric. Sci.* 20(1): 104–114.
- Mamedov DSh (2015). Concentration of leaf sap as an indicator of the need for irrigation in the garden. *Bull. Mich. State Agrar. Univ.* 2: 27–30.
- Marzec A, Szadkowski B (2019). Improved aging stability of ethylene-norbornene composites filled with lawsone-based hybrid pigment. *Polymers* 11(4): 723.
- Miceli N, Kwiecień I, Nicosia N, Speranza J, Ragusa S, Cavò E, Davì F, Taviano M, Ekiert H (2023). Improvement in the biosynthesis of antioxidant-active metabolites in *Isatis tinctoria* by elicitation and precursor feeding. *Antioxidants* 12(5): 1111.
- Mocquard J, Lamer A, Fabre P, Mathieu C, Chastrette C, Vitrai A, Vandenbossche V (2022). Indigo dyeing from *Isatis tinctoria* L.: From medieval to modern use. *Dyes and Pig.* 207: 110675.
- Nayek S, Choudhury IH, Jaishee N, Roy S (2014). Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents. *Res. J. Chem. Sci.* 4(9): 63–69.
- Nguyen T, Marcelo P, Gontier E, Dauwe R (2019). Metabolic markers for the yield of lipophilic indole alkaloids in dried woad leaves (*Isatis tinctoria* L.). *Phytochemistry* 163: 89–98.
- Normurodov Sh, Muminov Kh, Amanov B, Abdukadirov M, Nurmetov Kh, Bektaeva Kh, Shodiyeva O, Pulatov S, Alikulov H, Pardayev O (2025). The genus *Gossypium* L.: Comparative analysis of its physiological traits in some species and introgressive lines. *SABRAO J. Breed. Genet.* 57(5): 1919–1926.
- Oda Y, Nakashima S, Kondo E, Nakamura S, Yano M, Kubota C, Masumoto Y, Hirao M, Ogawa Y, Matsuda H (2018). Comparison of lawsone contents among *Lawsonia inermis* plant parts and neurite outgrowth accelerators from branches. *J. Nat. Med.* 72: 890–896.
- Othman M, Othman R, Ismail A, Hazni H, Ahmad K, Razzak M, Yusoff Z, Awang K (2020). HPLC-QTOFMS analysis on the ethanol: water (80:20) extract of *Lawsonia inermis* leaves. *Sains Malay.* 49: 1597–1613.
- Pistelli L, Najjar B, Di Renzo G, Curadi M, Muscatello B, De Leo M, Scartazza A (2023). Production of bioactive and aroma volatile compounds of *Lawsonia inermis* L. cultivated under different growth conditions. *Nat. Prod. Res.* 38: 3998–4008.
- Sánchez-Pérez C, Leyva-García V, García-Valenzuela A, Soto-Astorga R (2008). Spectroscopic refractometer using a double prism scheme for optical characterization of liquid mixtures. *Rev. Sci. Instr.* 79(4): 046107.
- Sharif M, Lashari MH, Farooq U, Idris M, Afzal MA (2024). Diagnostic efficacy of hand-held digital refractometer for determining total serum protein in indigenous sheep of Pakistan. *Plos One* 19(3): e0295107.
- Speranza J, Miceli N, Taviano M, Ragusa S, Kwiecień I, Szopa A, Ekiert H (2020). *Isatis tinctoria* L. (Woad): A review of its botany, ethnobotanical uses, phytochemistry, biological activities, and biotechnological studies. *Plants* 9(3): 298.
- Vauquelin R, Juillard-Condât L, Joly N, Jullian N, Choque É, Martin P (2024). Study of woad (*Isatis tinctoria* L.) extracted indoxyl precursors conversion into dyes: Influence of the oxidative media on indigo recovery yields and indigotin/indirubin ratio measured by HPLC-DAD method. *Molecules* 29(20): 4804.
- Wahyuningsih S, Wulandari L, Wartono MW, Munawaroh H, Ramelan AH (2017). The effect of pH and colour stability of anthocyanin on food colourant. *IOP Conf. Ser.: Mater. Sci. Eng.* 193: 012047.