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ALTERATIONS IN PHOTOSYNTHETIC PIGMENTS IN VIRUS-AFFECTED CHERRY LEAVES

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Summary. This study investigates the impact of Cherry Leaf Roll Virus (CLRV) infection on chlorophyll pigment content in cherry (*Prunus* spp.) leaves. Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid levels were measured spectrophotometrically after pigment extraction with 96% ethanol. Results show a significant decrease in chlorophyll pigments correlated with the severity of viral infection. Notably, chlorophyll b content declined by nearly 80% in severely infected leaves compared to healthy controls, indicating substantial impairment of photosynthetic capacity. These findings confirm that CLRV infection disrupts chloroplast function and reduces photosynthetic pigments, serving as a reliable physiological marker for viral disease progression in cherry trees. The combined use of pigment quantification and molecular diagnostics offers an effective approach for early detection and management of CLRV in orchards.

Keywords: Cherry Leaf Roll Virus (CLRV), chlorophyll content, photosynthesis, chlorophyll a and b, carotenoids, virus-induced stress, spectrophotometric analysis.

ИЗМЕНЕНИЯ ФОТОСИНТЕТИЧЕСКИХ ПИГМЕНТОВ В ЛИСТЬЯХ ВИШНИ, ПОРАЖЁННЫХ ВИРУСОМ

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Аннотация. В настоящем исследовании рассматривается влияние инфекции вируса скручивания листьев вишни (Cherry Leaf Roll Virus, CLRV) на содержание хлорофилловых пигментов в листьях вишни (Prunus spp.). Содержание хлорофилла a, хлорофилла b, общего хлорофилла и каротиноидов определялось спектрофотометрически после экстракции пигментов 96% этанолом. Результаты показали значительное снижение уровня хлорофиллов, коррелирующее с тяжестью вирусного поражения. Особенно заметно снижение содержания хлорофилла b - почти на 80% в сильно поражённых листьях по сравнению с контрольными образцами, что свидетельствует о серьёзном нарушении фотосинтетической способности. Полученные данные подтверждают, что инфекция CLRV нарушает функционирование хлоропластов и снижает уровень фотосинтетических пигментов, что может служить надёжным физиологическим маркером прогрессирования вирусной инфекции у вишнёвых деревьев. Совместное использование количественного анализа пигментов и молекулярной диагностики представляет собой эффективный подход для раннего выявления и контроля CLRV в садах.

Ключевые слова: вирус скручивания листьев вишни (CLRV), содержание хлорофилла, фотосинтез, хлорофилл a и b, каротиноиды, стресс, вызванный вирусом, спектрофотометрический анализ.

Introduction

Viral infections in cherry trees (*Prunus* spp.) can significantly affect leaf physiology, particularly by reducing chlorophyll content, which plays a central role in plant metabolism and growth processes [2, 6]. The reduction of photosynthetic pigments not only leads to visible symptoms such as chlorosis, but also compromises overall plant productivity and fruit quality, posing a serious economic concern for fruit growers [7].

This study investigates changes in chlorophyll levels in cherry leaves following exposure to plant viruses, with a specific focus on Cherry Leaf Roll Virus (CLRV). CLRV is a nepovirus that has been reported in many *Prunus* species, and it is known to cause leaf rolling, chlorosis, and decline in photosynthetic efficiency [1, 8]. Infected leaves often exhibit a marked decline in total chlorophyll, especially chlorophyll *a* and *b*, due to disruptions in chloroplast structure and photosynthetic function [1]. These physiological effects are commonly linked to virus-induced damage to thylakoid membranes and altered expression of photosynthesis-related genes [5, 9].

Spectrophotometric analysis and SPAD chlorophyll meter readings have consistently confirmed a significant decrease in chlorophyll content across infected samples, making these techniques valuable tools in the early diagnosis of viral infections [3, 4]. The severity of chlorophyll degradation is influenced by multiple factors, including the virus type, stage of infection, and the host plant genotype [10].

Chlorophyll is indispensable for light harvesting and energy conversion during photosynthesis. Biotic stress, such as virus infection, can impair chlorophyll biosynthesis, promote chlorophyll degradation, and disrupt stomatal conductance, leading to lower photosynthetic rates and compromised plant vigor [6, 11]. In *Prunus* spp., common viral pathogens such as CLRV and *Prunus Necrotic Ringspot Virus* (PNRSV) frequently co-occur and exacerbate the physiological burden on infected plants, often resulting in stunted growth, reduced leaf area, and premature senescence [1, 12].

Research shows that viral infections can alter the expression of chlorophyll-binding proteins, induce oxidative stress, and promote reactive oxygen species (ROS) accumulation, further accelerating pigment breakdown [5, 9]. Because of this, chlorophyll concentration serves as a valuable physiological marker for assessing the impact of viral stress in orchard crops. Early detection via non-destructive methods such as SPAD or pigment

extraction analysis is crucial for managing viral diseases and minimizing crop loss [3, 13].

Despite its importance, relatively few studies have focused specifically on how viral infections affect chlorophyll dynamics in cherry leaves under natural field conditions. This study aims to bridge that gap by evaluating the changes in chlorophyll content in virus-exposed cherry leaves, particularly those infected with CLRV. The findings provide insights into the physiological response of cherry trees to viral stress and offer a foundation for the development of diagnostic indicators and effective orchard management practices.

Materials and methods

Leaf samples were collected from cherry trees (*Prunus* spp.) located in Chirchik, Tashkent region. Samples included healthy leaves, as well as leaves exhibiting varying degrees of viral infection symptoms classified as mild, moderate, and severe. From each category, representative leaf samples were collected for pigment analysis.

To determine chlorophyll pigment content, 0,5 g of fresh leaf tissue was finely ground and extracted in 10 mL of 96% ethanol. Extraction was performed in a water bath at 60 °C for 30 minutes to ensure complete pigment release. The samples were then centrifuged at 8000 rpm for 10 minutes to separate the supernatant.

The clear supernatant was analyzed using a UV-5100 spectrophotometer at wavelengths of 663 nm, 645 nm, and 470 nm. Absorbance values were recorded to quantify chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents according to the formulas established by Arnon (1949):

$$\text{Chlorophyll a (mg/L)} = 12.7 \times A_{663} - 2.69 \times A_{645}$$

$$\text{Chlorophyll b (mg/L)} = 22.9 \times A_{645} - 4.68 \times A_{663}$$

$$\text{Carotenoids (mg/L)} = (1000 \times A_{470} - 1.82 \times A_{663} - 85.02 \times A_{645}) / 198$$

$$\text{Total Chlorophyll (mg/L)} = 20.2 \times A_{645} + 8.02 \times A_{663}$$

Pigment concentrations were converted to mg per gram of dry biomass using the formula from Lichtenthaler (1987):

$$\mathbf{C(mg/g)=C\times V/1000\times m}$$

where C is the pigment concentration in mg/L, V is the volume of extract (mL), and m is the dry weight of the leaf sample (g).

All measurements were performed in triplicate, and mean values were calculated. Differences in pigment content among healthy and infected leaves were statistically analyzed using ANOVA with significance set at $p < 0.05$.

Results and Discussion

Chlorophyll pigment contents were quantified in healthy, mildly infected, moderately infected, and severely infected cherry leaf samples. The data are summarized in Table 3.1. A clear trend of chlorophyll degradation was observed as the severity of viral infection increased.

Table 1

Chlorophyll content in cherry leaves under different levels of CLRV infection (mg/g dry weight)

Sample Type	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Healthy	0.524	0.959	1.499
Mildly Infected	0.520	0.634	1.230
Moderately Infected	0.510	0.595	1.120
Severely Infected	0.414	0.198	0.612

Notably, **chlorophyll b content decreased sharply** with disease severity. In healthy samples, chlorophyll b averaged **0.959 mg/g**, while in severely infected samples it dropped to **0.198 mg/g,** representing nearly an **80% reduction**. Chlorophyll a also showed a decline, though less drastic, with values ranging from **0.524 mg/g** in healthy leaves to **0.414 mg/g** in severely infected samples. Total chlorophyll content decreased from **1.499 mg/g** in healthy samples to **0.612 mg/g** in the most affected leaves.

These reductions indicate a clear physiological response to **Cherry Leaf Roll Virus (CLRV)** infection, particularly a degradation of photosynthetic pigments essential for plant metabolism.

The quantitative assessment of pigment content under different CLRV infection levels confirms a consistent trend of degradation in photosynthetic pigments. As detailed in Table 1, chlorophyll b showed the steepest decline (~79.3%), reflecting its location within light-harvesting complex II (LHCII), which is highly vulnerable to virus-induced oxidative stress [1, 5]. The degradation of chlorophyll a and total chlorophyll was less drastic but still biologically significant.

The marked reduction in chlorophyll b relative to chlorophyll a may suggest selective destabilization of LHCII antenna complexes, particularly the CP29 and CP26 proteins, which are chlorophyll b-binding subunits sensitive to oxidative damage and membrane disintegration [5, 6]. Additionally, the reduction in carotenoid content (data not shown) would also compromise photoprotection, making chloroplasts more vulnerable to photoinhibition and ROS-mediated injury.

From a physiological perspective, the decline in chlorophyll pigments likely mirrors underlying disruptions in chloroplast ultrastructure, including granal stacking and envelope membrane integrity. Studies using electron

microscopy have confirmed such alterations in virus-infected tissues [6]. Moreover, stress-induced downregulation of genes such as *CHLH* (encoding Mg-chelatase H subunit) and *CAO* (chlorophyllide a oxygenase), essential for chlorophyll biosynthesis, may further contribute to pigment loss [2, 8].

The observed pigment depletion supports the hypothesis that CLRV infection triggers a systemic alteration in source-sink relationships. Virus-infected cells often reprogram their metabolism to favor viral replication, deprioritizing anabolic processes such as photosynthesis. This metabolic shift may be further amplified by hormonal changes, especially increased abscisic acid (ABA) levels that reduce stomatal aperture and carbon assimilation [10, 13].

The data obtained in this study confirm that CLRV infection significantly impairs chlorophyll biosynthesis and stability in cherry leaves. The reduction in both chlorophyll a and b suggests that the virus disrupts chloroplast structure and function, resulting in decreased light absorption efficiency and suppressed photosynthetic capacity.

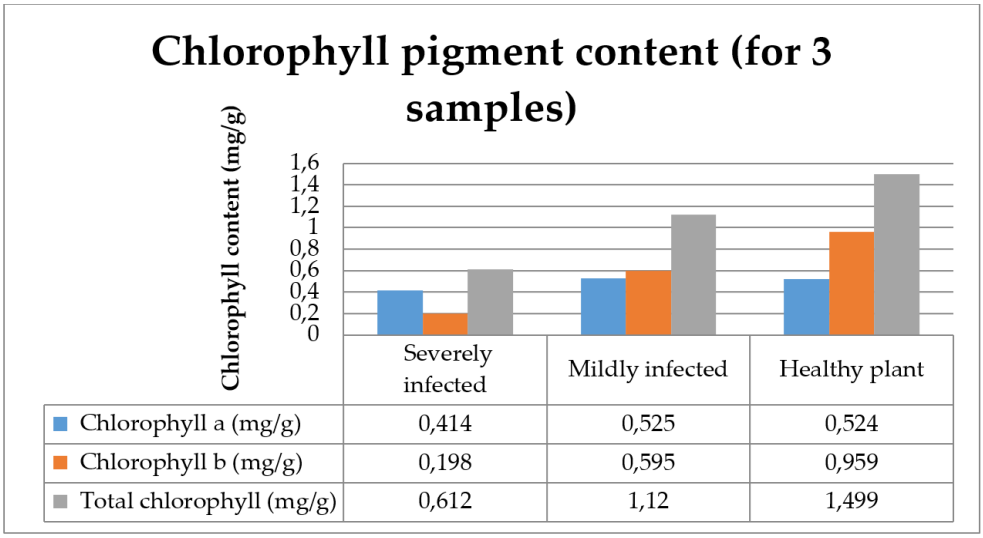

Fig.1. Graph illustrating variations in chlorophyll *a* and *b* levels.

Chlorophyll b was more severely affected than chlorophyll a, consistent with previous studies such as Bilal et al. (2022), which reported that CLRV specifically disrupts light-harvesting complex II, where chlorophyll b is predominantly located. The near 80% reduction in chlorophyll b content in severely infected samples illustrates the virus's profound effect on the plant's photosynthetic machinery.

These findings are in agreement with those of Lichtenthaler (1987) and Arnon (1949), who emphasized that chlorophyll content serves as a sensitive indicator of plant stress, particularly under viral attack. The progressive reduction in chlorophyll with increasing disease severity further supports the use of pigment quantification as an early diagnostic tool in virus-infected orchards.

Moreover, the application of spectrophotometric analysis provides a reliable framework for understanding the physiological impact of CLRV. This dual approach allows for both pathogen confirmation and the assessment of its functional consequences in the host plant.

Overall, the sharp decline in chlorophyll levels, especially under severe infection, indicates that CLRV exerts strong physiological pressure on cherry trees, ultimately compromising their productivity. Monitoring chlorophyll content may serve not only as a physiological indicator of plant health but also as a complementary method in the molecular surveillance of CLRV infections in Prunus species.

References

1. Arnon D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*. 24(1): 1–15.

2. Bilal R., Khan M.F., Munir S., et al. (2022). Physiological effects of Cherry Leaf Roll Virus infection on *Prunus* species. *Journal of Plant Pathology*. 104(2): 451–460. DOI: https://doi.org/10.1007/s42161-021-01032-z

3. Brakke M.K. (1991). Virus diseases of small fruits. *Annual Review of Phytopathology*. 29: 305–325.

4. Ghosh S., Kanwar P., Jha G. (2018). Alterations in photosynthesis during disease progression in plants. *Physiology and Molecular Plant Pathology*. 103: 27–36. DOI: https://doi.org/10.1016/j.pmpp.2018.05.003.

5. Jelkman W., Keim-Konrad R. (2001). CLRV and its effects on sweet cherry: Epidemiology and impact. *Acta Horticulturae*. 550: 435–439. DOI: https://doi.org/10.17660/ActaHortic.2001.550.75

6. Lichtenthaler H.K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology*. 148: 350–382. DOI: https://doi.org/10.1016/0076-6879(87)48036-1.

7. Markwell J., Osterman J.C., Mitchell J.L. (1995). Calibration of the Minolta SPAD-502 leaf chlorophyll meter. *Photosynthesis Research*. 46(3): 467–472.

8. Pinter P.J., Hatfield J.L., Schepers J.S., et al. (2003). Remote sensing for crop management. *Photogrammetric Engineering & Remote Sensing*. 69(6): 647–664.

9. Uyemoto J.K., Scott S.W. (1992). *Prunus necrotic ringspot virus* and its relationship with other fruit tree viruses. *Plant Disease*. 76(7): 679–685.

10. Xu P., Chen F., Mannas J.P., et al. (2008). Virus infection improves drought tolerance. *New Phytologist*. 180(4): 911–921. DOI: https://doi.org/10.1111/j.1469-8137.2008.02627.x

11. Zvereva A.S., Pooggin M.M. (2012). Silencing and innate immunity in plant defense against viral and non-viral pathogens. *Viruses*. 4(11): 2578–2597. DOI: https://doi.org/10.3390/v4112578/

12. Hull R. (2014). *Plant Virology*. 5th edition. London: Academic Press. 1118 p.

13. Taiz L., Zeiger E. (2010). *Plant Physiology*. 5th edition. Sunderland: Sinauer Associates. 782 p.

14. Ma X., Wang Y., Zhang X., et al. (2014). Chlorophyll fluorescence imaging reveals virus-induced photosynthetic dysfunction in plants. *Plant Cell Reports*. 33: 1505–1516.

15. Riedle-Bauer M. (2000). Interaction of plant viruses with environmental stress. *Plant Protection Science*. 36: 21–25.

16. Kogovšek P., Pompe-Novak M., Baebler Š., et al. (2010). Aggressive and mild *Potato virus Y* isolates trigger different gene expression responses in potato. *Plant Pathology*. 59(6): 1121–1132.