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Impact of groundnut bud necrosis virus on photosynthetic pigments in cowpea (*Vigna unguiculata* l. walp)

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Abstract
Plant pigments, particularly chlorophyll, are crucial for photosynthesis and overall plant health, impacting not only the plants themselves but also other organisms within the food chain. This study investigates the effects of Groundnut Bud Necrosis Virus (GBNV) infection on the chlorophyll content of cowpea leaves (*Vigna unguiculata* L. Walp). Field samples of healthy and GBNV-infected cowpea leaves were collected from the Jhunjhunu district of Rajasthan, India, and subjected to chlorophyll pigment analysis. The results revealed a significant reduction in chlorophyll a, chlorophyll b, and total chlorophyll in virus-infected leaves compared to healthy ones. Specifically, chlorophyll a content decreased from 15.23±1.70 mg/g to 8.09±2.19 mg/g, chlorophyll b from 9.40±1.23 mg/g to 5.91±0.66 mg/g, and total chlorophyll from 24.62±3.16 mg/g to 13.99±1.65 mg/g. The findings align with previous studies on the chlorophyll-suppressing activities of plant viruses, highlighting the profound and detrimental effects of GBNV on photosynthetic efficiency and plant health.

Keywords: Plant pigments, Chlorophyll, Groundnut Bud Necrosis Virus, *Vigna unguiculata* L. Walp etc.

Introduction
Plant pigments are essential components that provide plants with their diverse colors and facilitate various biological functions. Chlorophyll, the green pigment located in chloroplasts, plays a central role in photosynthesis by converting light energy into chemical energy. This process involves absorbing light, especially in the red and blue wavelengths, to drive the conversion of water and carbon dioxide into oxygen and glucose. As the foundation of the food chain, plants rely on this process as primary producers. Chlorophyll also regulates plant growth, development, and responses to environmental factors like light intensity and temperature.

These pigments are crucial not only for plants but for all living organisms. When consumed, they provide essential nutrients, antioxidants, and protective compounds, contributing to overall health and well-being. For approximately 70 years, researchers have recognized that viral infections significantly affect chloroplasts, the organelles responsible for photosynthesis. A common symptom of viral infection is leaf chlorosis, where chloroplasts undergo pigment and structural changes, leading to reduced photosynthetic activity. Numerous studies since the early 20th century have shown that virus-infected plants often exhibit decreased photosynthesis, a symptom commonly associated with viral presence [1].

Groundnut bud necrosis virus (GBNV) is a notable orthotospovirus that significantly impacts agricultural productivity, particularly in regions cultivating groundnuts and legumes. Transmitted primarily by thrips, small insects acting as vectors, GBNV affects various hosts, including groundnuts, tomatoes, peppers, and many other plants. This virus has negative-sense RNA genome which is segmented into three parts, encoding essential proteins for replication and movement. Plants infected with GBNV display symptoms like bud necrosis, chlorosis, stunted growth, and occasionally death, resulting in significant yield losses and economic consequences [2,3]. This study discusses the impact of field infection of GBNV on photosynthetic pigments in cowpea.

Material and methods
1. Plant Material Collection and Sample Preparation
Healthy and virus-infected (diseased) cowpea leaves were freshly collected from a selected field in the Jhunjhunu district of Rajasthan. These leaves were placed into separate zip lock bags with a few blotting papers in between, then stored in an ice bag and transported to the Department of Botany at the University of Rajasthan for further biochemical analysis.

In the laboratory, the leaves were thoroughly washed first with normal water and then with purified water. Following this, 1 gram of both healthy (HL) and diseased (DL) leaves were weighed out in triplicate and immediately processed for chlorophyll pigment extraction.

2. Extraction of chlorophyll pigments
One gram from each sample (HL &DL) was taken, crushed, and homogenized initially with 5 millilitres of extraction solvent (80% acetone) using a prechilled pestle and mortar. The resulting homogenate was centrifuged, and this process was repeated until no chlorophyll remained in the residue. The final extract was then diluted to a total volume of 50 ml.

3. Estimation of chlorophyll pigments
The optical density of the extracted pigments was measured using a spectrophotometer. Readings were taken at 645 nm and 663 nm wavelengths with a 3 ml capacity cuvette and a 1.0 cm light path. An 80% acetone solution served as the blank. The pigment concentrations were then calculated using as per standard equation [4].

Total Chlorophyll (a+b) = $20.2A_{645}+8.02A_{663}$ mg/liter
Chlorophyll a = $12.7A_{663}-2.69A_{645}$ mg/liter
Chlorophyll b = $22.9A_{645}-4.68A_{663}$ mg/liter
and to calculate chlorophyll content on a fresh weight basis.

Chlorophyll a = $\frac{12.7\ A663 - 2.69\ A645}{a \times 1000 \times W} \times V$ mg/g

Chlorophyll b = $\frac{22.9A645 - 4.68A663}{a \times 1000 \times W} \times V$ mg/g

Total chlorophyll = $\frac{20.2\ A645 + 8.02\ A663}{a \times 1000 \times W}$ x V mg/g

Where,
a= path length of light in the cell (1 cm)
V = extract volume in ml
W = Fresh weight of sample
A_{645}= Absorbance at wavelength 645nm
A_{663} = Absorbance at wavelength 645nm

Data analysis

Statistical analysis of data was done by two-way ANOVA using GraphPad Prism software (8.0.2) and each data set was repeated three times.

Results

Natural infection of GBNV in cowpea plants produces chlorosis symptoms, including chlorotic spots and rings, leading to a significant reduction in green pigments. In this study, chlorophyll a content in healthy cowpea leaves was 15.23±1.70 mg/g fresh weight, which decreased to 8.09±2.19 mg/g in GBNV-infected leaves. Similarly, chlorophyll b content in healthy leaves was 9.40±1.23 mg/g, reducing to 5.91±0.66 mg/g in infected leaves. The total chlorophyll content in healthy leaves was 24.62±3.16 mg/g fresh weight, which dropped to 13.99±1.65 mg/g in infected leaves (Fig 1). The reduction in chlorophyll content was statistically significant, with a p-value <0.02 at a 95% confidence interval

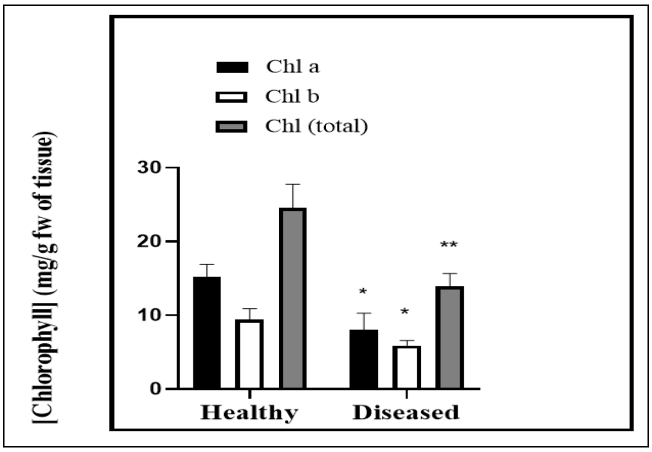

Fig 1: Bar graph showing the mean concentration of chlorophyll a, chlorophyll b and total chlorophyll in healthy and GBNV-infected cowpea leaves. Error bars represent the standard deviation. Significant differences in both groups are indicated by an asterisk (Two-way ANOVA, p< 0.02)

Discussion

Photosynthetic pigments are essential for primary producers, capturing solar energy and converting it into forms usable by plants and other trophic levels, both directly (herbivores) and indirectly. The findings of this study revealed a significant decrease in chlorophyll content in GBNV-infested cowpea leaves compared to healthy ones. Specifically, chlorophyll a, chlorophyll b, and total chlorophyll concentrations were markedly lower in the infected leaves, indicating a profound detrimental effect of Groundnut Bud Necrosis Virus (GBNV) on cowpea (*Vigna unguiculata* L. Walp). This decline in chlorophyll content likely contributes to the symptoms of chlorosis and mottling observed in infected plants, underscoring the importance of chlorophyll in maintaining photosynthetic efficiency and overall plant health.

Several other studies support these findings, highlighting the chlorophyll-suppressing activities of plant viruses. For instance, Arias *et al*. (2005) [5] reported that Sunflower chlorotic mottle virus (SUCMOV) caused chlorotic mottling, stunted growth, and reduced yields in sunflowers. They noted a decrease in the rate of CO_2 fixation as symptoms emerged, despite an increase in soluble sugars and starch. Muqit *et al*. (2007) [6] observed reduced levels of chlorophyll a, b, and total chlorophyll in ash gourd infected with three different viruses. Patel *et al*. (2013) [7] found a significant increase in chlorophyllase enzyme activity in Vigna radiata infected with Mungbean yellow mosaic virus, leading to chlorophyll degradation in infected leaves. Similarly, Shakeel *et al*
. (2016) [8] documented a substantial reduction in chlorophyll components in cucumber plants susceptible to cucumber mosaic virus. Zanini *et al*. (2021) [9] demonstrated that Cassava common mosaic virus (CsCMV) induced systemic infections in cassava plants, causing mosaic symptoms with chlorosis and reducing relative chlorophyll content by up to 35%.

These observations are consistent across various plants, such as cucumber infected by CMV [8,10], Capsicum affected by yellow vein mosaic virus [11], mesta plants [12], and papaya infected by Papaya leaf curl virus [13]. Zhao *et al*. (2016) [1] also found that viral infections commonly disrupt chloroplast structure and function, leading to photosynthesis-related symptoms like chlorosis and mosaic. This reduction in plant pigments ultimately inhibits growth, as supported by studies on viral infections in tomatoes [14] and cucumbers [15].

Furthermore, Liu *et al*. (2014) [16] suggested that plant viruses upregulate chlorophyll-degrading transcript genes, leading to chlorophyll degradation. This mechanism may also explain the reduced chlorophyll content in cowpea leaves following GBNV infection observed in the present study, potentially due to the modulatory effect this virus on the molecular machinery of the cowpea plants.

Conclusion

The findings of this study demonstrate a significantly negative impact on chlorophyll content in virus-infected leaves compared to healthy leaves. Specifically, Chlorophyll a, b, and total chlorophyll concentrations were markedly lower in virus-infected leaves, indicating a detrimental effect of viral infection on chlorophyll levels. This decline in chlorophyll content may contribute to the observed symptoms of chlorosis and mottling in infected plants, highlighting the importance of chlorophyll in maintaining photosynthetic efficiency and overall plant health.

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