CHANGES IN CHLOROPHYLL CONTENT DURING PATHOGENESIS IN COMMON BEAN – RUST PATHOSYSTEM

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Abstract

Common bean rust is a disease of economic importance in bean production areas. When disease intensity is high the pathogen Uromyces appendiculatus can cause up to 100% yield loss. The aim of present study is to investigate the changes in chlorophyll content during pathogenesis in common bean after inoculation with U. appendiculatus. The investigation is carried out in greenhouse conditions. Plants of cultivar "Skitiya" were inoculated with spore suspension of the pathogen. Starting from the day after inoculation (DAI), daily for 15 days, leaf samples of inoculated and non-inoculated plants were used to determine chlorophyll content spectrophotometrically. The results show that no differences between non-inoculated and inoculated plants were observed in term of chlorophyll 'a' (chl a) and chlorophyll 'b' (chl b) content during first three DAI. In fourth and fifth DAI the values of the indexes decreases but still not significantly. White small spots appear on abaxial and adaxial leaf surface in 6th DAI which indicate that latent period is six days. At the same time chl a is 174,03 mg/m^3 and chl b is 40,43 mg/m^3 in inoculated leaves which is significantly lower than non-inoculated leaves. First pustules with infection type (IT)=5,6 (0.5-0.8 mm and >0.8mm in diameter) occur 11 DAI and chl a is 114,23 mg/m^3 and chl b is 34,63 mg/m^3. The differences between inoculated and non-inoculated leaves are statistically significant. The pustules are fully developed 13 DAI. The results demonstrate that infection of U. appendiculatus cause reduction of chlorophyll content in leaves of common bean during pathogenesis process, and more specifically during incubation period.

Key words: chlorophyll, common bean, P. vulgaris, rust, U. appendiculatus

INTRODUCTION

Bean rust is one of the key diseases in common bean in many countries around the world (Stavely et al., 1983; Makhumbila et al., 2025). Moderate temperatures between 16-22°C, rainfalls, leaf wetness and high air humidity favored disease development (Araya and Steadman, 1994). In such conditions from 25 to 100% yield losses can be reached in critical growth stages (Stavely and Pastor-Corrales, 1989; Steadman et al., 1996; Leitão et al., 2023).

The causal agent of bean rust is phytopathogenic biotroph fungus *Uromyces appendiculatus* (Pers.:Pers.) Unger (*Basidiomycota*, *Pucciniales*). The pathogen is macrocyclic, autoaecial, and forms five types of spores: teliospores, basidiospores, pycniospores, aeciospores and urediniospores (Stavely, 1991; Kolmer et al., 2009).

The urediniospores are spread by air during the growing season. They germinate at an optimal temperature of 16-25°C, with a latent period varying from five days at 20-25˚C daytime and 16-18˚C nighttime temperatures to ten days at an average daily temperature of 15.4-17.8°C (Beleva, 2010). Depending on the environmental conditions and the host, the incubation period is 12-15 days (Imhoff et al., 1982; Stavely, 1991; Beleva, 2010). Infection with urediniospores is favored by

high relative humidity and retention of dew drops for 10-18 hours (Harter et al., 1935; Stavely, 1991).

The pathogenesis process in rust diseases starts with recognition between pathogen and host plant when urediniospores land on the plant tissue surface (Osuna-Caballero et al., 2024). The spores are stabilized by hydrophobic interactions and adheres to the cuticular surface (Hahn, 2000). When favorable conditions are present, the spore metabolism is activated and the spore form germ tube which moves on the plant tissue surface (More et al. 2018; Osuna-Caballero et al., 2024). If it successfully locates a stoma, cell morphogenesis occurs in the apical tip of the germ tube to form an appressorium over it, serving to penetrate the host (Osuna-Caballero et al., 2024). Hydrophobins, cell wall proteins, form amphipathic layers in fungal cell walls to ensure effective penetration and as a result the appressorium is adhered to the stomata. The process continues with formation of a penetration peg from the appresorium that pushes through the stoma to enter the intercellular space within the host leaf in which a substomatal vesicle is formed (Kolmer et al., 2009). An initial infection hypha emerges and, upon contacting a mesophyll cell, can differentiate at its tip into a haustorial mother cell (HMC). Then the pathogen can enter the mesophyll cell via a neckband and forms a haustorium which is the feeding structure of the pathogen (Osuna-Caballero et al., 2024). In the host tissue the rust pathogen continues to develop secondary hyphae which infects more cells and continue extracting nutrients to expand the colony within the host. At 7–10 days after inoculation, mycelia in the tissue forms sporogenous cells in the intercellular space under the epidermis (Kolmer et al., 2009) and spores emerge through an opening on the tissue surface created by pressure exerted from within, leading to what we know as a pustule (Osuna-Caballero et al., 2024).

Many physiological processes in plants are disrupted because of interaction between the host plant and pathogen like photosynthesis (light reaction and carbon assimilation), respiration, translocation of metabolites and nutrients (Mandal et al., 2009).

According to Lindenthal et al. (2005) rust pathogens reduce chlorophyll substantially, resulting in severe damage to photosynthesis in different crops. Reduction in chlorophyll content in plant-rust pathosystems is reported by Mishra et al. (2015) (wheat - *Puccinia striformis* Westend. f. sp. *tritici* Eriks.) and Koleva&Yankova (2026) (common bean – *U. appendiculatus* and alfalfa - *Uromyces striatus* J. Schröt).

The aim of present study is to investigate the changes in chlorophyll content during pathogenesis in common bean after artificial inoculation with spore suspension of *U. appendiculatus*.

MATERIALS AND METHODS

The investigation was carried out in greenhouse conditions in Technical university of Varna in 2024.

Susceptible to common bean rust cultivar Skitiya was sown in plastic pots with a peat-perlite mixture (3:1). The plants are grown at 20-25℃ daytime and 16-18℃ nighttime temperature.

Primary leaves of plants were inoculated with spore suspension of *U. appendiculatus* (2.0 x 10^4 uredospores/ml), and 0.1% Tween 20 was added (Stavely, 1983). After inoculation the plants were placed in humid chamber

(20℃, relative humidity >95%) in dark for 18h, after which they were grown under the conditions described above. Non-inoculated plants were used as control.

Starting from the day after inoculation (DAI) for 15 days leaf samples were collected and used for pigment extraction. Leaf tissue (0.1 g) in three replications is cut into small pieces with scissors. Quartz sand, calcium carbonate and 10 ml 80 % acetone (in small portions gradually) were added. The extract is filtered with filter paper and diluted five times (0.5 ml filtrate + 2.5 ml acetone (Tzvetkova and Anev, 2017; Koleva and Yankova, 2026).

The pigment extract was used to estimate chlorophyll ‘a’ (chl ‘a’) and chlorophyll ‘b’ (chl ‘b’) content in inoculated and non-inoculated plants using spectrophotometer Pharo.

Duncan’s multiple range was used to analyze the data (Duncan, 1955).

RESULTS AND DISCUSSIONS

The results show that no differences between non-inoculated and inoculated plants were observed in term of chl ‘a’ and chl ‘b’ content during first three DAI (Table 1). The values of chl ‘a’ are between 262.6 / 258.5 mg/m^3 and chl ‘b’ are between 86.3 / 86.5 mg/m^3 for non-inoculated and inoculated with *U. appendiculatus* plants, respectively.

In fourth and fifth DAI the values of the indexes in inoculated plants decreases but still not significantly (Table 1).

White small spots, first symptoms of bean rust, appear on abaxial and adaxial leaf surface in 6th DAI which indicate that latent period is six days (Figure 1) at 20-25℃ / 16-18℃ (day/night) in cultivar Skitiya.

Figure 1. First symptom of common bean rust six days after inoculation with *U. appendiculatus*

At the same time chl ‘a’ is 174.03 mg/m^3 and chl ‘b’ is 40.43 mg/m^3 in inoculated leaves which is significantly lower than in non-inoculated (260.37 for chl ‘a’ and 83.10 for chl ‘b’). During next four days the tendency for decreasing chl ‘a’ and chl ‘b’ content in inoculated plants continues.

Table 1. Chlorophyll content in leaves of common bean non-inoculated and inoculated with *U. appendiculatus*

DAI*	Non-inoculated		Inoculated	
	Chl 'a'	Chl 'b'	Chl 'a'	Chl 'b'
1	262.60** a	86.3 a	258.6 a	86.2 a
2	262.34 a	85.68 a	259.36 a	86.5 a
3	261.8 a	85.65 a	258.5 a	86.4 a
4	259.33 a	85.07 a	227.73 a	83.00 a
5	260.60 a	91.00 a	216.67 a	74.00 a
6	260.37 a	83.10 a	174.03 b	40.43 b
7	253.37 a	84.63 a	174.07 b	35.60 b
8	248.53 a	82.77 a	158.93 b	31.73b
9	251.68 a	82.64 a	134.36 b	31.65 b
10	253.86 a	82.58 a	125.86 b	31.56 b
11	249.87 a	88.80 a	114.23 b	34.63 b
12	250.68 a	85.16 a	152.36 b	54.52 b
13	251.60 a	84.83 a	172.87 b	67.40 b
14	253.54 a	83.95 a	174.32 b	70.02 b
15	254.86 a	84.62 a	181.75 b	78.36 b

* days after inoculation; ** mg/m^3;
a, b– Duncan`s multiple range test (p<0,05)

First pustules with infection type (IT) = 5,6 (0.5-0.8 mm and >0.8mm in diameter) occur 11 DAI (Figure 2). Chl ‘a’ is 114,23 mg/m^3 and chl ‘b’ is 34,63 mg/m^3 in inoculated plants (Table 1). The

differences between inoculated and non-inoculated leaves in terms of chlorophyll content are still statistically significant.

Figure 2. Occurring of first pustules of common bean rust 11 days after inoculation with *U. appendiculatus*

The pustules are fully developed 13 DAI so that is the duration of the incubation period. IT is 6,5 and DI (disease intensity) according to modified Cobb scale (Staveky, 1985) is 50 %.

During last four days (from 12 to 15 DAI) chl ‘a’ and chl ‘b’ in inoculated plants increase and in case of chl ’b’ no differences are found between inoculated and non-inoculated plants 15 DAI. According to Kolmer et al. (2009) the host cells are likely manipulated by signals from the haustoria to maintain viability to allow the transport of sugars and aminoacids across the extrahaustorial membrane into the haustoria.

In our previous investigation was found that *U. appendiculatus*, significantly reduce chlorophyll content (chlolophyll “a” and chlorophyll “b”) in leaves with disease intensity 10% and above in field conditions (Koleva and Yankova, 2026).

Harter et al. (1935) determined that urediniospores of *U. appendiculatus* germinate in 16-25°C for 6-8 hours. Peterson and Aylor (1995) found that inoculation phase in this pathosystem continue 3 days. It cannot be determined when exactly the inoculation phase of pathogenesis finish and incubation period begin but it is obvious that in our investigation latent period in cultivar Skitiya after inoculation with *U. appendiculatus* is 6 days and incubation period is 13 days. Studying carbon uptake and utilization. Livne (1964) found that a loss of photosynthetic capacity can occur with onset of sporulation, because of loss of chlorophyll. Our result demonstrate that loss of chlorophyll starts 4 DAI, exactly during incubation period.

Madden et al. (2007) formulated so called ‘infectious period’ as the length of time between the start of production of infectious units and the end of production of infectious units. Following that in our study infectious period is 3 days. During these days chlorophyll content in inoculated plants is still significantly lower than non-inoculated plants but with higher values.

CONCLUSIONS

The results of our investigation about changes in chlorophyll content during pathogenesis in common bean – *U. appendiculatus* pathosystem give us the basis to draw the following conclusions:

During the first tree days after inoculation with *U. appendiculatus* chlorophyll content does not change.

Next two days chlorophyll ‘a’ and chlorophyll ‘b’ decrease but the reduction is statistically significant from sixth to fifteen days after inoculation (except for chlorophyll ‘b’ in 15 DAI).

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