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# The Chlorophyll and Anthocyanin Content of Potato Mop Top Virus Infected Potato Plants and Their Association with the Foliar Symptoms (Preliminary Study)

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**ABSTRACT**
The purpose of this study was to estimate the effects of Potato Mop Top Virus (PMTV) infections on the level of some foliar pigments content in positive plants (from Carrera, Hermes, Gared, Marvis and Temerar cultivars) with and without symptoms. Performing and monitoring the vegetative state of plants was done under greenhouse conditions, by estimation the chlorophyll (portable device SPAD 502 Chlorophyll Meter) and the anthocyanin (portable device ACM 200 plus, Antocianin Chlorophyll Meter) content of the leaflets. Regarding the content of anthocyanin, it was found small increase of anthocyanin content in case of PMTV infected material without symptoms (but the values were not statistically supported). As opposed the anthocyanin, monitoring the chlorophyll content indicated significant correlations with the type of the tested material.
***Keywords*** **–** Potato Mop Top Virus, symptoms, chlorophyll, anthocyanin

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## I. INTRODUCTION

Considered a common product, cheap food, poor people's food and the plant of poor areas, the potato is actually a product that helps improving the daily diet being rich in carbohydrates, vitamins and minerals. For Romania, the potato is a strategic food, contributing to the national food safety system. Our country is ranked on the third position in Europe in terms of area cultivated with potatoes (after Poland and Germany) [1].Potato is the third most consumed food, after rice and maize [2] and their tubers are recognized as a good source of carbohydrates, essential amino acids. vitamin B1, B3 and B6, potassium, phosphorus and magnesium [3]. Being a good nutrients source, the potato has many damaging enemies (including the viruses) that can reduce the yield and the quality of production.

*Potato mop-top virus* (PMTV) can be transmitted by planting infected seed tubers [4] and by zoospores of the plasmodiophorid *Spongospora subterranea*, the cause of powdery scab [5]. PMTV typically produces slightly raised lines and rings on the tuber surface and/or brown arcs and lines, commonly described as spraing, in the flesh of tubers of sensitive cultivars [6,7]. Plants produces from infected tubers may also produce misshapen or cracked tubers [4], often with reticulate surface cracking, sometimes known as elephant hide blemishing, on the skin [8]. Symptoms may also develop on the foliage of a plant depending on the sensitivity of the cultivar. In summary, there were three types of foliar symptoms: yellow blotches or V shaped chevrons on the leaves, distortion of leaflets accompanied by blotching and a shortening of internodes resulting in a dwarfed appearance (mop-top). In many cases, only a proportion of the stems may bear symptoms of PMTV infection [9]. With insensitive cultivars, PMTV infection can result in no symptoms developing on the foliage [10] but the extent to which symptomless foliar infection might occur in crops containing plants with foliar symptoms has not been examined. Although roguing, i.e. the removal of diseased plants, was suggested by Cooper et al. (1976) [11] as a control measure for improving the health crops infected by PMTV, experimental evidence on its potential impact has not been assessed.

The experimental studies in this work research aimed to evaluate the effects of PMTV infections, on chlorophyll and anthocyanin content in case of plants with and without symptoms.

Another objective of this preliminary study was to verify if the PMTV invisible in non symptomatic plants had effects on the content of photosynthetic pigments, which may affect also plant development and therefore the values of the specific yield parameters (weight and number of tubers) [12].

## II. MATERIAL AND METHODS

*2.1. Potato material*

The potato varieties used in this preliminary study were the following:

- Carrera, Hermes
- Gared, Marvis, Temerar (new Romanian cvs.).

The samples were collected from five commercial potato crops containing relatively high amount of plants with PMTV foliar symptoms (identified in 2019).

The sampling strategy was to identify and to mark with a distinctive cane during Jully 2019 paired replicates, each consisting of a plant with foliar symptoms and a plant with no symptoms neighbore to the symptomatic plant. In some place, two plants with foliar symptoms were selected for each replicate in order to provide sufficient tubers for testing and assessment. When marking the plants, four leaflets were sampled from each plant and tested for PMTV by DAS ELISA. For plants with symptoms, only symptomatic leaflets were taken. On the plants with no symptoms, a leaflet was selected from mid-point on four separate stems. The produce of each plant was harvested separately into bags in October 2019 at least 2 weeks after destruction foliage.

From the material sampled in 2019, for each variety, in year 2020, twenty PMTV infected tubers, (ten collected from symtomatic plants and ten from non symptomatic material) and ten negatives controls were selected and planted in pots under greenhouse conditions.


A. B.

**Fig. 1.** Estimation the chlorophyll content of leaf portable device (SPAD 502 Chlorophyll Meter) (**A**) and the anthocyanin content at leaf (portable device ACM 200 plus, Antocianin Chlorophyll Meter) (**B**).

The evaluation of foliar symptoms of the plants was observed in addition with the evaluation of the photosintetic pigments.

The total number and weight of tubers from each plant were recorded. The tubers were assessed for external and internal symptoms of spraing between February an April after storage at 4-6$^{o}$C and tested for PMTV. From this material, thirty tubers were selected for planting in the following year and the reminder used for assessment.

All plants tested for PMTV infection (with and without symptoms, noninfected) were evaluated for the chlorophyll and anthocyanin content in years 2020 and 2021.

*2.2. Evaluation of the chlorophyll and anthocyanin content using specific contact sensors*

Evaluating the state of plants grown in greenhous was done by:

-evaluation the chlorophyll content of leaf (portable device SPAD 502 Chlorophyll Meter) [13]. The values (determined by the equipment used) indicated the relative content of the chlorophyll quantity present in the plants leaves, measured by the transmittance of leaf at the two wavelengths, 650 nm (red) and 940 (near infrared –NIR) (Fig. 1A).

-estimation the anthocyanin content at leaf (portable device ACM 200 plus, Antocianin Content Meter) [14]. As in the case of determining the chlorophyll content, values indicated by device represents the **relative** sum of anthocyanin quantity present in leaves, estimated by transmittance of plant material, measured at two wavelengths, characteristic for anthocyanin pigment analysis (510nm and 700nm) (Fig. 1B).

Also, the values of the photosynthetic pigments content evaluated using these specific contact sensors are relative.

*2.3. Leaflet and tuber testing by DAS ELISA*

Samples for testing consisted of four leaflets. Leaflets samples placed into the rear of a homogenisation bag (Bioreba, AG, CH) were tested within 24 h of sampling. However, if this was not possible, leaflets were stored at 4$^{o}$C. Five ml of leaf extraction buffer were added to the sample prior to homogenisation, and further 5ml was added to the sample after homogenisation. For tubers, a tissue core, 25-30 mm in length and 5 mm in diameter was taken with a cork borer from the rose and stolon-end of each tuber. The two cores were placed in a homogenisation bag and 5 ml of tuber extraction buffer was added to the sample prior to homogenisation. The samples were tested using DAS-ELISA kits for PMTV and according to the manufacturer's instructions (Bioreba, Swiss). Also, the analysis was performed following the protocol described by Clark and Adams[15] (100 µl per well). Microplates were filled with substrate solution (p-nitro phenyl phosphate) incubated 1 hour and the absorbance values were estimated at 405 nm ($A_{405}$) on TecanSunRise reader (Magellan softwere). The samples having $A_{405}$ values exceeding two times the average of healthy controls were considered virus infected.

*2.4. Statistical analysis*

Data were analysed by ANOVA and Duncan's Multiple Range Test and scored as significant if $P<0.05$.

## III. RESULTS AND DISCUSSION

In this preliminary study, the relative content of the photosynthetic pigments was estimated to be able to interpret in the future the correlations with different parameters of plants growth and with yield specific indicators (especially the PMTV symptoms). The determinations were made from plant material originally infected (specified in material and method).

The results of this research work intend to verify if there are correlations between the content of photosynthetic pigments and the PMTV symptoms), these having repercussions on subsequent parameters of plants growth and then on the specific yield indicators (tubers quality and yield). Also, the paper tried to present a simple method for estimating the relative content of photosynthetic pigments, non-invasive methods, although the values shown are relative.

*3.1. Sampling potato material*

In the selected potato crops, the estimated incidence of plants with foliar symptoms characteristic for PMTV infection varied between 10 % to 35% (table 1).

Table 1. Incidence and symptoms of PMTV infection in five potato crops selected for sampling (in 2019)

| **Cultivar** | **Region** | **Estimated % plants affected by PMTV** | **Symptoms on plants** |
|---|---|---|---|
| Carrera | Covasna | 25 | Faint motting of leafllets, stunting of some stems |
| Hermes | Brasov | 15 | Yellow chevrons on leaflets |
| Gared | Covasna | 15 | Stunted, mopped plants |
| Marvis | Brasov | 10 | Stunting of some leaflets |
| Temerar | Suceava | 35 | Yellow chevrons on leaflets, stunted, mopped plants |

Symptoms ranged from mild (yellow markings on leaflets and no distortion or stunting of the plant or of the leaflet) on plants from cvs. Gared, and Hermes to severe (stunting and mopping of most of stems) on plants of cvs. Carrera and Temerar.

*3.2. Correlations between the level of pigments content and the type of material used (presence/ non presence of symptoms)*

The simple correlation coefficient Pearson revealed significantl higher values regarding chlorophyll content (as compared to the negative control). As seeing in table 2, the are strong correlations between the content of this photosynthetic pigment and the type of material

(visible or not visible PMTV symptoms). Despite the higher values of absorbances in case of infected plants without symptoms, the values of cholophyll content were significantly higher compared with the symptomatic material.

*3.3. Chlorophyll and antocyanin content of non infected material and PMTV infected plants with and without symptoms*

Regarding the content of anthocyanin, there were no significant differences between values recorded in the experimental variants (Fig. 2). Compared to the negative control, however, it was found small increase of the relative anthocyanin content in case of material infected with PMTV without symptoms (but the values were not statistically supported in all cases) (Fig. 2). In year 2, the differences between the values of this parameter and the type of material were higher. In both years, Marvis was the variety with the lowest difference between the anthocyanin content of material with symptoms and without symptoms (Fig. 2).

As opposed the content of anthocyanin, the results remark significantly differences between the variants regarding the relative chlorophyll content of, being observed effects of PMTV incidence.

Table 2. The correlation between content of chlorophyll pigment and anthocyanin at the leaflet and type of material (plants with PMTV symptoms, without PMTV symptoms and noninfected)

| Variables (pigment tested) | Statistical indicators | Material (plants with PMTV symptoms, without PMTV symptoms and noninfected)[a] | Cultivar |
|---|---|---|---|
| Chlorophyll content (units ACI) | Correlation coefficient Pearson | **0.629**** | **0.464**** |
| | Significance threshold | 0.000 | 0.000 |
| Anthocyanin content (units AAI) | Correlation coefficient Pearson | **0.105** | **0.174*** |
| | Significance threshold | 0.472 | 0.002 |
| Absorbances values at 405nm[b] | Correlation coefficient Pearson | **-0.777**** | **0.023** |
| | Significance threshold | 0.000 | 0.696 |

[a]For for each plant was determined mean value for three determinations (in different parts of foliage). Number tests =300 (30 plants x 5 cvs. x 2 years =300)
[b] Values obtained using DAS ELISA test
** Correlation is significant for p<0.01.

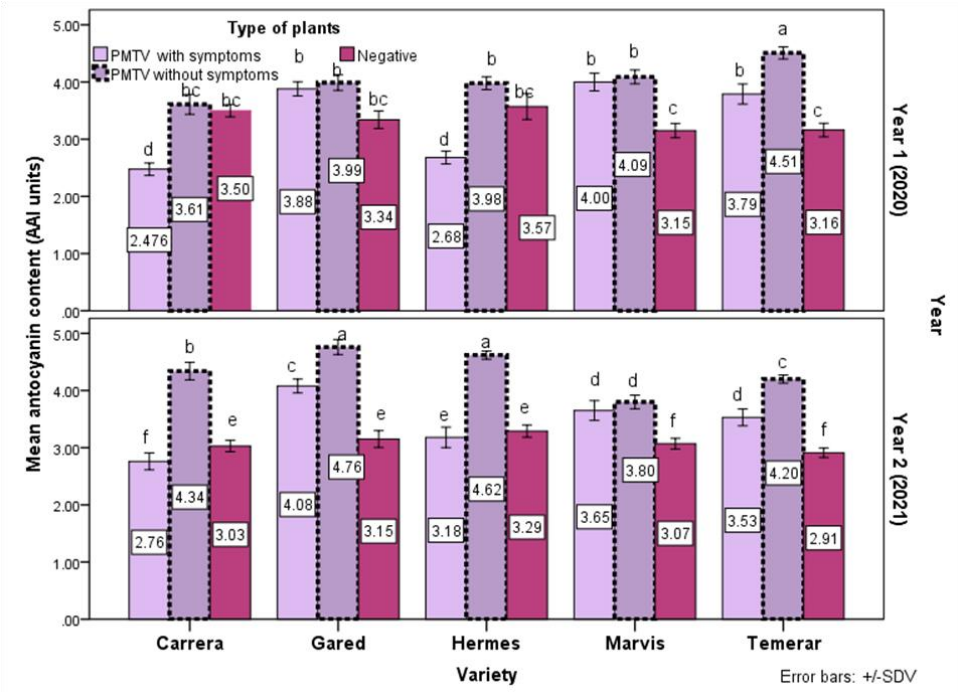


**Fig. 2.** The anthocyanin content (specific contact sensors) of plants from material infected with PMTV (with symptoms, without symptoms) and non infected (under greenhouse conditions). Values not followed by the same letter are significantly different (P=0.05) according to Duncan's test.

In both experimental years, compared with the symptomatic plants, the level of anthocyanin content was higher in the material without symptoms for all varieties tested. Excepting the variety Marvis, all the other genotypes presented significantly higher values of this parameter in non symptomatic material (Fig. 2). The highest level of anthocyanin content was recorded in non symptomatic material from Gared var. (4.76 AAI units in year 2020 and in Temerar var. (4.51 AAI units) in year 2021. The lowest values of this parameter was found in Carrera variety (material with symptoms).

The level of chlorophyll content in the material tested is presented in figure 3. There were find closed values of this parameter in the negative material and in the plants without symptoms (Fig. 3). The chlorophyll relative content of the leaves in all the negative and non symptomatic plants, for all varieties had higher values compared with the other experimental variants. In year 2, the varieties Marvis and Temerar had very close values of this photosyntetic pigment content in non symtomatic plants and negative controls (Fig. 3).

The highest values of chlorophyll content for the material tested was find to Marvis and Temerar varieties.

PMTV infection of potato plants by soil borne *S. subterranean* does not cause symptoms in the foliage regardless of the sensitivity of a variety but can produce spraing in tubers of sensitive varieties [16,17].

Davey (2008) [18] found that high occurrence of tubers infection by PMTV in seed potato crop of some varieties were associated with infection from soil borne inoculums and not with planting PMTV infected seed tubers.

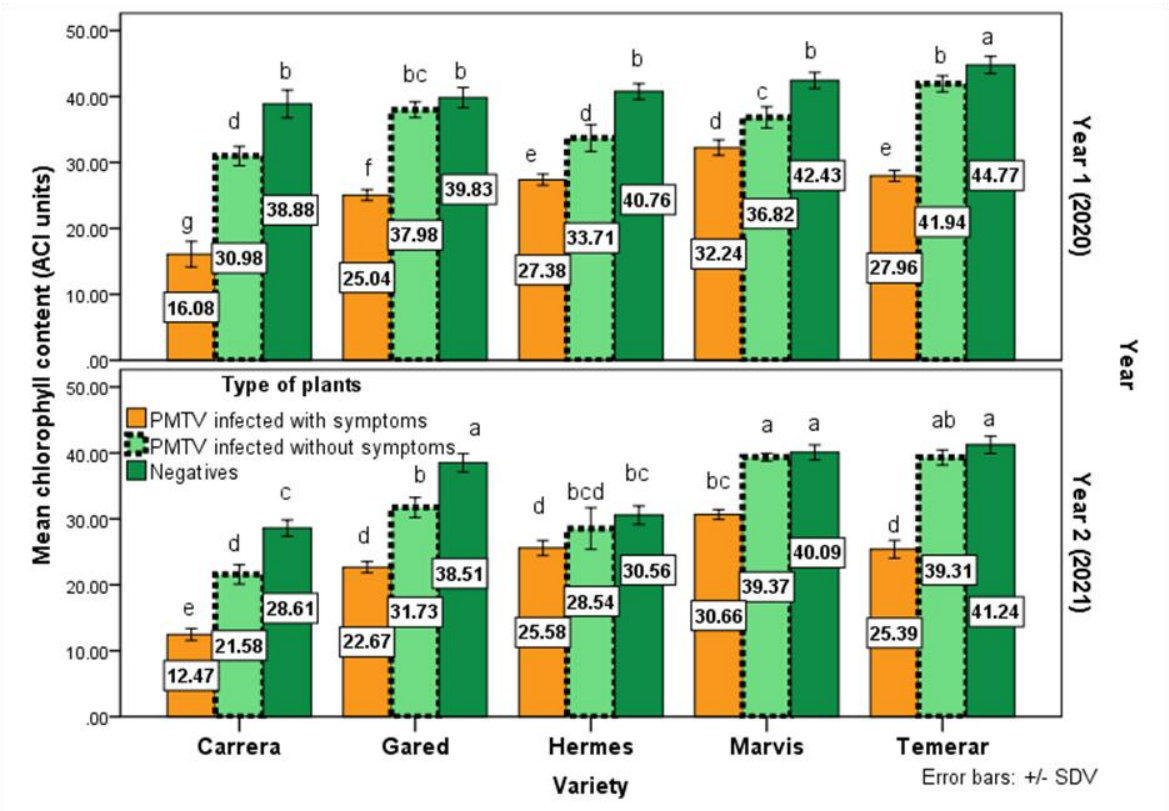


**Fig. 3.** The chlorophyll content (specific contact sensors) of plants from material infected with PMTV (with symptoms, without symptoms) and non infected (under greenhouse conditions). Values not followed by the same letter are significantly different (P=0.05) according to Duncan's test. The statistical analysis was done for each year of the experiments.

All the tubers selected in our study contained high amounts of PMTV infection of plants with a range of foliar symptoms and, thus, it seems like that the foliar symptoms in our tests were derived from primary infection occurring the previous year from soil-borne inoculums.

Symptomless infection by PMTV is known to occur in potato tubers [19] but its occurrence in foliage is less well documented. Browning et al.,

(2002) [20] reported a similar level of detection of PMTV by ELISA in leaflets with no symptoms and those with symptoms. In a survey of 633 poato plants in Costa Rica, Montero-Astua et al (2008) [21] detected PMTV in 28% of plants with symptoms and 11.5% of those with no symptoms. By contrast, our preliminary study show that all the plants without PMTV symptoms were infected and these one had higher level of anthocyanin content and level of chlorophyll content very close to those of negative controls.

Historycally, the effect of PMTV on yield has been assessed by comparing the yield of plants derived from tubers with spraing symptoms and those with no symptoms without taking account of whether individual plants were affected by foliar symptoms [7]. The maximum reduction in total yield recorded by Kurppa (1989) [7] was7% whch was greater than that reported by Calvert (1968) [4] . In our study, the total weight and number of tubers produced by a plant was generally less for plants with foliar symptoms than for plants with no symptoms (Table 2) and this reduction was in proportion to the severity of the foliar symptoms.

In the two years, in case of cvs. Carrera, Temerar and Hermes most of symptomatic plants were stunted and mopped and this symptom was accompanied by an average reduction in total tuber yield of 64%, 65% respectively 48.7% and in tuber numbers of 57%, 36.8%, respectively 32.5%.

Table 2. The total weight and number of tubers produced by plants with foliar symptoms pf Potato Mop Top virus infection and with no symptoms (in 2020 and 2021)

| Cultivar | Weight of tubers (g/plant) | | Number of tubers/plant | |
|---|---|---|---|---|
| | No symptoms | Foliar symptoms | No symptoms | Foliar symptoms |
| Carrera | 294±42 e* | 105±10 d | 10.4±2.5 cd | 4.4±1.0 e |
| Gared | 402±18 c | 276±26 b | 12.8±1.7 c | 10.0± 1.0 c |
| Hermes | 472±31 b | 242±31 c | 16.5±1.0 b | 10.8±1.2 c |
| Marvis | 593±12 a | 416±26 a | 17.9±1.2 a | 14.3±1.4 a |
| Temerar | 372±54 cd | 129±14 d | 11.7±2.4 de | 7.4± 1.3 d |

*Values not followed by the same letter are significantly different (P=0.05) according to Duncan's test.

By contrast, the total yield of plants of cv. Marvis with mild foliar symptoms did not differ from that of plants with no symptoms. With cvs Gared and Marvis on which foliar symptoms were intermediate in severity, the mean reduction in total yield was 31.1%, respectively 29.5% and that in tuber numbers was 21.9%, respectively 21.4%.

Hirst et al. (1973) [22] and Hide and Read (1990) [23] recorded that plants adjacent to gaps or diseased plants will compensate in terms of tuber yield for missing or diseased neighbours and that this compensation was greatest when gapping or differences in growth occurred early in crop growth. It is therefore, possible that differences in tuber yield between plants with foliar symptoms and those with no symptoms may have been influenced by this compensatory effect because it was not recorded whether the plant with no symptoms in a replicate pair was immediately adjacent to diseased plant [16]. Nevertheless, it is clear that a serious reduction in crop yield may be expected when foliar symptoms are severe and prevalent, as in case of cv. Carrera. Additional losses may occur if spraing develops in infected tubers as with the tubers of cv. Carrera or Hermes.

These preliminary results suggest that the PMTV infected was present in the tubers planted from material with primary and secondary infection and the plants from this material presented no symptoms despite the level of infection.

## IV. CONCLUSION

In this preliminary study, the relative contents of the photosynthetic pigments was estimated to be able to interpret in the future the correlations with different parameters of plants growth and with yield specific indicators. Between the experimental variants were observed differences concerning the development symptopms of plants, depending on the cultivars and on the level of chloropyll , anthocianin content. Regarding the content of anthocyanin, it was found small increase of anthocyanin content in case of PMTV infected material without symptoms (but the values were not statistically supported). As opposed the anthocyanin, monitoring the chlorophyll content indicated significant correlations with the type of the tested material.

In case of cvs. Carrera, Temerar and Hermes most of symptomatic plants were stunted and mopped and this symptom was accompanied by an average reduction in total tuber yield. As opposite, the total yield of plants of cv. Marvis with mild foliar symptoms did not differ from that of plants with no symptoms.

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