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CHLOROPHYLL ESTIMATION OF SOME POTATO CLONES WITH POTENTIAL RESISTANCE TO LATE BLIGHT DISEASE (*PHYTOPHTHORA INFESTANS*)

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ABSTRACT

*Thirteen potato genotypes with different level of resistance to potato late blight (*Phytophthora infestans) pathogen were characterized using non-invasive method to determinate the chlorophyll content and the late blight influence. *Field experiment was conducted to NIRDPS Brasov, Romania between 2021-2022. During the vegetation were applied the usual maintenance works (hilling, herbicides), including three treatments for Colorado beetle unless late blight control with fungicides. With SPAD 502 Plus meter (Chlorophyll Meter) were carried out 3 determinations on the leaves of the middle level of 3 plants from each genotype taken into the study. For the evaluation of the results, analysis of variance and Pearson's correlation analysis were used. In 2021, following the measurements, closely negative correlations were observed between SPAD values and genotypes (-0.058), respectively the date of the observations (-0.126), while in 2022, the correlation was negative between the values regarding the date of the observations (-0.065), but positive at genotypes level (0.030).*

INTRODUCTION

Potato, with high nutritional value and yield, is considered the third largest staple food in the world (Hong et al. 2017, Alva et al. 2011). Chlorophyll, which can capture and transform light energy into chemical energy for the conversion of inorganic matter to organic matter, is an important raw material in potato photosynthesis (Vesali et al. 2017). In addition, the chlorophyll content is significantly correlated with the content of nitrogen (Gaurav et al., 2019) which is one of the most important nutrients for the potato plant's healthy growth (Zhou 2018), development (Clevers et al. 2012) and production (Nigon et al. 2014, Liu et al. 2020). Therefore, the accurate detection of the chlorophyll content of potato plants is significant for precision agriculture management. In recent years, many destructive and non-destructive methods have been developed to estimate the chlorophyll content of leaves.

At present, there are many reports on potato cultivation, but due to the diversity of varieties and cultivation patterns, the complexity of soil types and natural conditions, the growth and development of potato, and the requirements of the environment are not static. For specific varieties under specific ecological conditions, the specific cultivation measures still need to be carefully studied (Li et al. 2022).

The SPAD 502 Plus (Chlorophyll Meter) is a widely used portable device for the rapid, accurate and non-invasive measurement of leaf chlorophyll concentration, which can change subtle trends in plant health long before they are visible to the human eye. This is a portable device that is based on the values measured by transmitting two wavelengths of the electromagnetic spectrum (650 nm in the red and 940 nm in the infrared) at the level of the potato leaflets (Gianquinto et al.,2004).

The advantages of nondestructive methods can be summarized in that its rapid, not expensive, and the same leaf or crop can be followed up during the agricultural season, so today chlorophyll nondestructive instruments are commonly used in agriculture to indicate the crop growth requirements especially nitrogen due to the reality that the majority of leaf nitrogen exist in chlorophyll molecule (Netto et al. 2005). With remotesensing method is that it can obtain plant canopy information on a large scale without disrupting the normal growth of plants (Tan et al., 2018; Jiao et al. 2014).

Phytophthora infestans causes late blight diseases in potato and tomato crops worldwide. It is not cause only economic losses of yield but also the quality and quantity of the crop. It is a highly researchable pathogen in plant diseases (Mehi et al. 2018). Late blight outbreaks have become more difficult to manage in recent years due to the increased genetic diversity of the pathogen in many parts of the world. The prevalent and widespread outbreaks of the devastating late blight disease have long posed a threat to global potato production and food safety (Armstrong et al., 2018). Variations in disease severity are mainly due to climatic factors (rainfall, relative humidity, temperature) and pathogen virulence.

In potato, the resistance of the varieties is horizontal and vertical. Horizontal resistance (also called field resistance race-specific, uniform, stable) is polygenic and non-specific, respectively in all races of the parasite, while vertical resistance (differentiated, race-specific, unstable) is oligogenic or monogenic, based on the concept gene -for-gene. Vertical resistance delays the onset of disease, while horizontal resistance decreases the rate of disease progression (infection rate). For practice, varieties of combined resistance, horizontal and vertical, are required.

Despite the efforts of breeders and the extensive use of fungicide control measures, there are still concerns regarding the durability and level of resistance against *Phytophthora infestans* (van der Lee et al. 2001).

MATERIAL AND METHODS

Experiments were carried out at the National Institue of Research and Development for Potato and Sugar Beet Brasov, Romania, on a cambic chernozeum soil, with 6.7 pH, humus 4.68% and clay 27%. The pre-crop was wheat and for current fertilizer was used 1000 kg/ha N:P:K:15:15:15+S.

1 Romanian variety (Rustic) and 5 foreign ones (Orchestra, Pamela, Oceania, Fribel and Florice) selected for resistance to late blight were associated with wild species (*Solanum vernei* 74, *Solanum agrimonifolium* 54, *Solanum demissum*), presenting genes of specific interest (R 3; R 6; R 7; R 8) thus resulting in clones R3SasaxRustic (clone 1), Agria x Rustic (clone1), R6Sasa x Orchestra, R3Sasa x Rustic (clone 2) R3Sasa x Rustic (clone 3), R8Sasa x Orchestra, Sasa x Pamela, R3Sasa x Orchestra, Agria x Rustic (clone 2), R3Sasa x Oceania, R7Sasa

x Orchestra, Sasa x Fribel, Sasa x Florice, which were planted in the experimental field.

The late blight development on foliage was assessed as percentage of foliage area damaged by *Phytophthora* infection. The assessment is from the international literature using a key on whole plant (Malcolmson, 1976). It assess the overall amount of necrotic tissue per plant on a scale from 1 (highly susceptible) to 9 (highly resistant). Has not been interfered with artificial *Phytophthora infestans* inoculum sprayers, using only the natural pressure of infection.

Planting distance was 75 cm between rows and 30 cm between plants per row, having 4 rows with 10 plants each one. Planting was done manually in May 3, 2021 and. April 9, 2022.

During the vegetation were applied the usual maintenance works (hilling, herbicides), including three treatments for Colorado beetle unless late blight control with fungicides.

With SPAD 502 Plus meter (Chlorophyll Meter) on the days of 2.07, 12.07 22.07.2021 and respectively, 5.07, 15.07 and 26.07. 2022, 3 determinations (readings) were carried out on the leaves of the middle layer of 3 plants from each genotype taken into the study. For interpretation of chlorophyll results was used the formula of nitrogen index (Shapiro et al., 2006): N index = readings average/control average x 100%.

Statistical evaluation of the experimental data was performed using Microsoft Excel 2013 and SPSS for Windows 13.0. For the evaluation of the results, analysis of variance and Pearson's correlation analysis were used.

RESULTS AND DISCUSSIONS

1. Meteorological data

In 2021 April month was colder than normal, with minimum negative air temperatures recorded for 10 consecutive days. Negative values were also recorded at ground level. The amount of precipitation that fell in April was 39.2 mm, which is 10.8 mm lower than the MAA. In May, the air temperature was lower by 1.3°C compared to the multiannual average, and the amount of precipitation was lower by 4.97 mm compared to the MAA value. June was a rainy month (106,1 mm), with precipitation in the first two decades even daily and with slightly higher temperatures than average (+0.8°C). in July the situation changed completely, registering much higher temperatures (+2.9°C) and a lower level of precipitation, by 28.7 mm compared to the multiannual average.

In winter period (October-March) the recorded temperatures exceeded by 1.6°C the multiannual average and the amount of precipitation (30 mm/m2) that fell approached the multiannual value (38.9 mm/m2), but without being evenly distributed. The month of April, in which the planting took place, was rich in precipitation, exceeding the multiannual amount by 14.8 mm/m^2, and the temperatures (8.3°C) were close to the multiannual average (8.5°C), ensuring a good start to potato crops. In May, temperatures were recorded with +1.2°C compared to the average and a much lower amount of rainfalls (40.0 l/m^2), but despite all this, the potato crop sprouted evenly

Table 1

Air temperature and rainfalls during the experiment

Year	Month				
	May	June	July	August	
	Air temperature (°C)				Average
2021	13.8	17.3	21.0	19.2	17.8
2022	14.8	19.0	20.6	-	
Multiannual average of air temperature	13.6	15.9	18.1	17.5	16.3
	Amount of rainfall (mm)				Total
2021	77.5	109.0	71.1	100.9	358.5
2022	48.3	62.2	20.1	-	
Multiannual average of rainfall	82.0	96.7	99.8	76.4	407.4

2.SPAD values registered in the field (2021-2022)

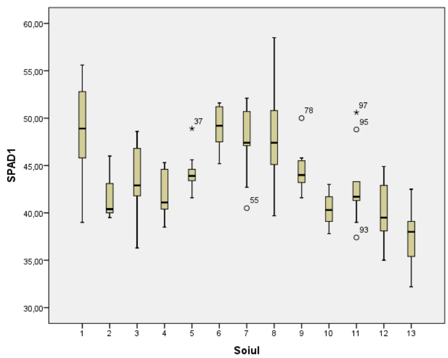

Figure 1. Box plot with reference to SPAD values (2.07.2021)

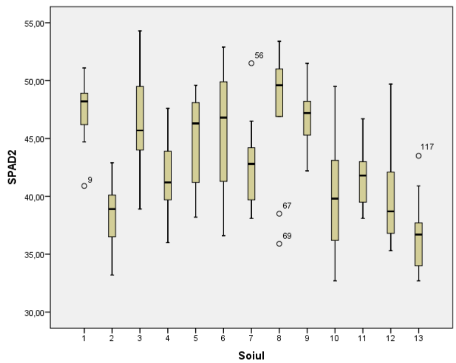

Figure 2. Box plot with reference to SPAD values (12.07.2021)

Analyzing figure 1, a very large variation of chlorophyll values can be observed to the genotypes, it gradually increases from 0.33 units (Sasa x Florice) to 0.58 units (R3Sasa x Orchestra). There are also significant decreases (R7Sasa x Orchestra and Sasa x Pamela), the obtained data not being symmetrical, and the dispersion elements present in most genotypes. Differences between medians were statistically significant.

Figure 2 shows the amplitude of the chlorophyll level on July 12, the lowest values (0.32 units) being recorded at R3Sasa x Oceania and Agria x Rustic1 (33 units), and the highest at R6Sasa x Orchestra (0.54 units). We can observe the positioning of the median in most genotypes, with the exception of R3Sasa x Oceania at different levels, higher or, as the case may be, lower, indicating a variable level of chlorophyll. The median position indicates a variable level of genotypes chlorophyll content.

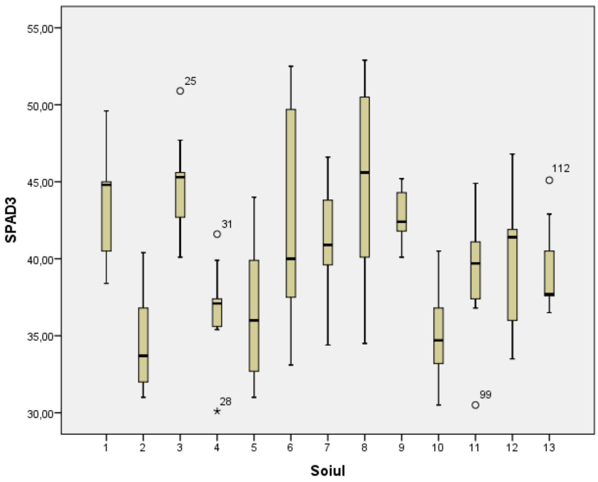

Figure 3. Box plot with reference to SPAD values (22.07.2021)

To the last assessment from July 2021 (Figure 3) can be observed a decrease in the level of chlorophyll in all genotypes as the vegetation progresses. The median is at different level and the amplitude of the variables in the case of some genotypes (R8Sasa x Orchestra) is very large.

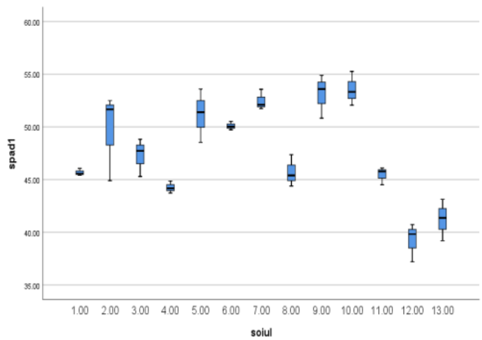

Figure 4. Box plot with reference to SPAD values (16.06.2022)

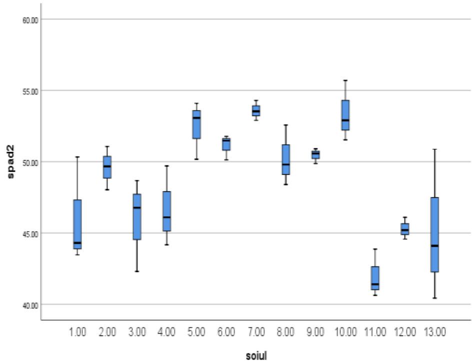

Figure 5. Box plot with reference to SPAD values (27.06.2022)

Figure 4 suggests that the genotypes have a high level of agreement with each other, with value between 0.55 and 0.43 units (R3SasaxOceania and C2R3SasaxRustic),only genotypes Sasa x Fribel and Sasa x Florice presenting a lower level of chlorophyll.

To the second assessment (Figure 5) the amplitude of some variables(SasaxFlorice) was high than the majority; being clones that presented very tight and high values (SasaxPamela – 0.54 units)

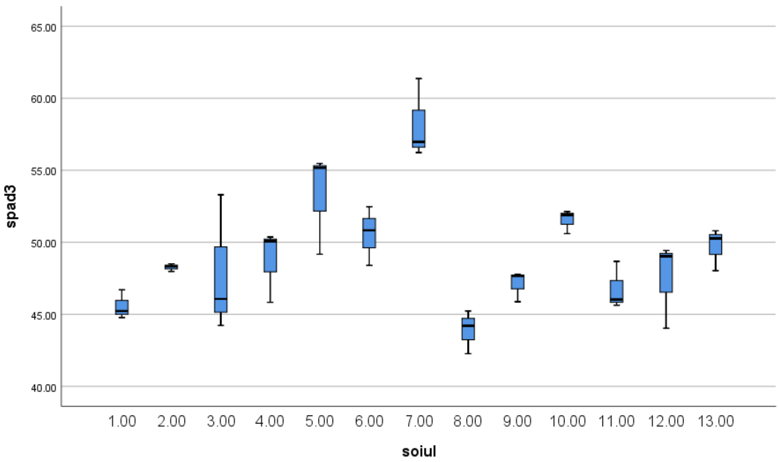

Figure 6. Box plot valori SPAD (11.07.2022)

To the last assessment, in 11 July 2022 (Figure 6) can be observed the level of the median and a high amplitude between genotypes SasaxPamela having the highest value 0.63 units and R3SasaxOrchestra the lowest level of 0.43 units, while R6SasaxOrchestra appears to have larger variability than the others genotypes. The content of chlorophyll pigments in plants is not only a species-specific feature, but also variety-specific feature (Zielewicz et al., 2021).

Table 2

Correlations of SPAD means between the genotypes and observations

SPAD correlations with:	Pearson correlation coefficients	
	2021	2022
- genotype	-0,058	0.030
- observation	-0,126	-0.065

In 2021, following the measurements, closely negative correlations were observed between SPAD values and genotypes (-0.058), respectively the date of the observations (-0.126), while in 2022, the correlation was negative between the values regarding the date of the observations (-0.065), but positive at the level of genotypes (0.030), but insignificant correlations (Table 2).

3. **The influence of late blight on the chlorophyll content**

Late blight incidence depends on environmental conditions.In 2021 date of occurrence of the disease was 24 June, relatively early due to favourable climatic conditions. The data from 2022 couldn't be used because from the middle of June the drought has been installed. Drought has sdeepened with the extremely hot days and without rainfall in July. In July very high temperatures surpassed nearly 5°C the multiannual average was combinated with almost total lack of rainfall reaching only 27.2% rainfall characteristic of the area.

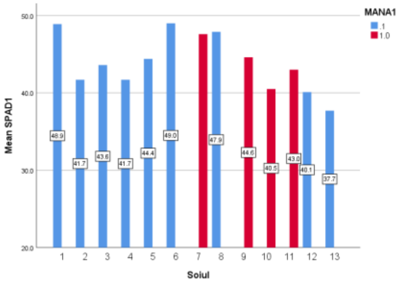

Figure 7. Association between SPAD values and late blight (15.07.21)

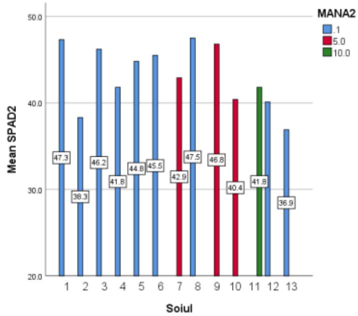

Figure 8. Association between SPAD values and late (26.07.21)

In 2021 to the first assessment (15 July 2021), there were no significant differences between the genotypes. The late blight attack started slowly, values above 1 (representing 10 spots/plant/) presented SasaxPamela, AgriaxRustic, R3SasaxOceania, R7SasaxOrchestra genotypes.

To the second assessment from 26July it was observed that the late blight attack continued to evolve in the genotypes SasaxPamela, AgriaxRustic, R3SasaxOceania (values of 10%, representing 2-5 leaflets/plant with lesions) (figure 7and 8).

Potato genotypes which developed late blight symptoms early are susceptible and genotypes that developed late blight in the crop cycle are resistant. Due to different genetic background, differential rate of disease development may have been recorded to the clones. For example, the Orchestra variety that was used as a parent for some of the genotypes has a low level of late blight resistance and requires a lot of treatments.

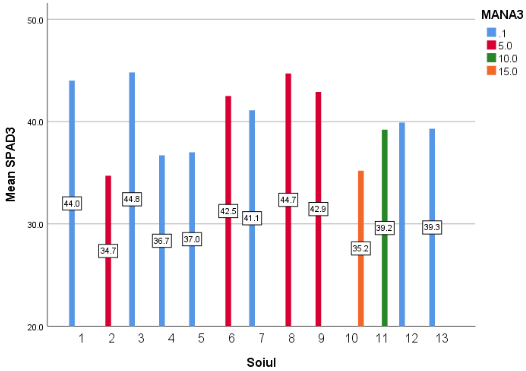

Figure 9. Association between SPAD values and late blight (5.08.21)

Chlorophyll content tends to decrease with disease stress, which leads to a drop in SPAD values.
To the third assessment (5 August), it is observed how the plant's defense system acts against the pathogen, so that genotypes with low attack values (3R6SasaxOrchestra) have a higher chlorophyll content, while a genotype (R3SasaxOceania)) with strong attack also has a low chlorophyll content.

Plants are simultaneously subjected to both the stress induced by the pathogen and the stress produced by climatic conditions (high temperatures) (Figure 9).

Table 3

Correlations between SPAD and late blight observations

		Clones	SPAD1	SPAD2	SPAD3	LB1	LB2	LB3
Clones	Pearson Correlation	1	-.485	-.419	-.092	.401	.428	.317
	Sig. (2-tailed)		.093	.155	.766	.175	.145	.291
SPAD 1	Pearson Correlation	-.485	1	.824**	.625*	.005	-.029	-.110
	Sig. (2-tailed)	.093		.001	.022	.988	.925	.721
SPAD 2	Pearson Correlation	-.419	.824**	1	.750**	-.025	-.066	-.126
	Sig. (2-tailed)	.155	.001		.003	.937	.831	.681
SPAD 3	Pearson Correlation	-.092	.625*	.750**	1	-.109	-.115	-.316
	Sig. (2-tailed)	.766	.022	.003		.723	.708	.292
LB1	Pearson Correlation	.401	.005	-.025	-.109	1	.921**	.593*
	Sig. (2-tailed)	.175	.988	.937	.723		.000	.033
LB2	Pearson Correlation	.428	-.029	-.066	-.115	.921**	1	.614*
	Sig. (2-tailed)	.145	.925	.831	.708	.000		.025
LB3	Pearson Correlation	.317	-.110	-.126	-.316	.593*	.614*	1
	Sig. (2-tailed)	.291	.721	.681	.292	.033	.025	

For the first assessment was registered a positive correlation (.005) and for the second (-0.066) and the third assesment (-0.0316) a negative one.
Out of the thirteen genotypes tested, C1R3SasaxRustic performed best for late blight resistance. Also SasaxPamela, SasaxFlorice and SasaxFribel presented a high level of resistance and ensure the possibility of use in a future breeding program.

From the results of this study, it is concluded that specificity plays a significant role in interactions between late blight and the leaves cllorophyl. This highlights the need to preserve the integrity of the foliage as long as possible so that a genotype can adapt when exposed to aggressive late blight populations.

CONCLUSIONS

Periodically estimating the chlorophyll content is essential to monitor the general health of potato. The SPAD values varied with the development of disease and the changes in reflectance reflect the disease level.

The SPAD-502 meter is a potentiall useful quick nondestructive diagnostic tool for the management of potato field. From the statistical data it can be seen how the chlorophyll content of the genotypes varied both in relation to the climatic conditions, respectively the vegetation state of the plants and to what a genotype itself presents from a phenotypic point of view.

Sustainable potato production is threatened by the aggressive spread of new clonal lines of late blight that are able to break through the disease resistance of different potato cultivars. Were observed an earlier appearance of the disease and the need to start the treatments at the end of May, with consequences both on the environment (increase in the number of treatments) and on the economic yield (higher costs per surface unit).

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