Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development
Vol. 22, Issue 1, 2022
PRINT ISSN 2284-7995, E-ISSN 2285-3952

RESULTS ON THE INFLUENCE OF PLANTING DISTANCE AND MEASUREMENT DATE, ON SPAD VALUES IN *PRIMULA OFFICINALIS* HILL. SPECIES

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Abstract

The aim of this research was to measure the amount of chlorophyll leaf content of the Primula officinalis Hill. Species during the vegetation period. Evaluations were made using the chlorophyll meter SPAD 502 (Chlorophyll Meter SPAD 502), with three determinations in dynamics. At each variant repetition / row, three uniform plants in size were analyzed, with each plant performing three readings on three different leaves, resulting a total number of 729 readings and 243 analyzed plants on the occasion of each determination. The SPAD 502 measuring device allows quick and easy measurement of leaf chlorophyll concentrations, without damaging the analyzed plant leaf. The amount of chlorophyll in a plant shows how healthy that plant is. Estimating the concentration of chlorophyll leaf content in real time can be used to determine the optimal period of administration of nutritional supplements as well as their quantity, thus ensuring higher quality yields. Chlorophyll Meter SPAD 502 measuring device is used to determine the plants' vegetation period and at the same time, based on the obtained results, the optimal harvesting time can be determined.

Key words**:** *Chlorophyll Meter SPAD 502, Primula officinalis Hill*

INTRODUCTION

This paper aims to study some physiological plant aspects, to determine the concentration of chlorophyll in the leaves of *Primula officinalis* Hill., during vegetation period, using the Chlorophyll Meter SPAD 502 device.

According to Richards [11], the genus *Primula* has its genetic centers, mainly in the temperate or subalpine areas of the northern hemisphere, with the main center of species diversity in the Sino-Himalayan region and secondarily in the large mountain ranges of the Circumboreal region.

Primula is the largest genus of Fam. *Primulaceae,* and the heterogeneity of morphological and cytological characters has led different authors to conclude that many, if not most of the remaining genera, are derived members of it [10].

From a taxonomic point of view, species *Primula officinalis* Hill. is classified as follows: Kingdom: *Plantae*; Subregnum: *Viridiplantae*; Order: *Primulales*; Family: *Primulaceae;* Genus: *Primula*; Species: *Primula officinalis* Hill. or *Primula veris* L. [15].

Genetically, *Primula officinalis* Hill. $2_n = 22$. Phylogenetically, framed in *Querco-Fagetea, Arrhenatheretea* [7], [2].

Primula officinalis Hill. ("cowslip" or "cowslip primrose" in popular language) is a well-known endemic plant, it grows spontaneously in Romania, on poor, calcareous soils, with southern exposure, at a certain altitude. Being an endangered species, due to irrational harvesting, it is necessary to cultivate it in areas of natural plant growth [9].

main morphological characteristics and active substances in the vegetative and generative organs of the species *Primula officinalis* Hill.: the root is a cylindrical rhizome 10-15 cm

long, 0.5 cm thick, thin, white-yellow, contains 5-10% triperpenic saponins (primrose, primic acid A and other saponins), heterosides (primveroside, primulavezoride), volatile oil (0.1-0.25%), starchy substances, enzymes, etc.; the stem is cylindrical, 15-30 cm height, erect, hairy, without leaves, finished with inflorescence; the leaves are arranged in a basal rosette, are ovate, with a crenate or wavy edge, 12-15 cm long and 5 cm wide, with prominent ribs on the underside, green on the upper side and gray-green on the underside, contain ascorbic acid (vitamin C), beta-carotene, etc.; the inflorescences are arranged in umbels, 6-18 at a time, on type 5, persistent calyx, gamopetal corolla, golden-yellow, contain saponins and flavones; the fruits are denticulated capsules, with a length of 6-10 mm, have a persistent calyx [14], [7].

In Romania, *Primula officinalis* is found in hilly forested areas, on pastures and alpine meadows, up to almost 2,300-2,400 meters height, according to chronological map attached below (Fig.1), regarding the presence of *Primula officinalis* Hill. Species in our country [13].

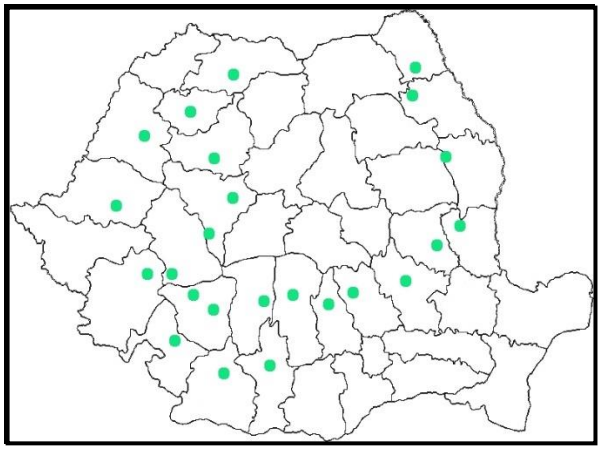

Fig. 1. Chronological map of *Primula officinalis* Hill. Species on the territory of our country
Source: [13].

The SPAD-502 chlorophyll meter is a portable, nondestructive, lightweight device designed to estimate foliar chlorophyll [12].

This meter records optical density measurements at two wavelengths, converts them into digital signals, and then into a SPAD value [4].

Chlorophyll meters have been used to estimate leaf chlorophyll content, and therefore nitrogen (n) status and fertilization requirements for several crops. A field study was conducted in 1995 and 1996 to evaluate the potential of chlorophyll (SPAD) meter readings to determine switchgrass n concentration and herbage yield. Meter readings were taken on the top most fully expanded leaves of switchgrass grown on a free draining sandy clay [6].

Recent studies have demonstrated the use of a hand-held chlorophyll meter (SPAD 502 meter, Minolta Corp, NJ) for evaluation of N sufficiency or management strategies. This technique is based on the fact that leaf chloroplasts contain 70% of leaf N and, as a result, chlorophyll content is well correlated with N content [16].

The SPAD-502 chlorophyll meter is a portable, nondestructive, light-weight device designed to estimate foliar chlorophyll [12].

The chlorophyll meter SPAD-502 is for simple, rapid and non destructive estimation of chlorophyll content in tomato leaves [3]. Studies of Madakadze et al. [6], showed that the relationship between N concentration and SPAD readings was linear (r^2=0.62–0.93; $p<0.01$) for the cut systems. Except for the 1st cut under the 4 week harvest regime, there were positive correlations between dry matter yield and SPAD meter readings (r^2=0.58–0.96; $p<0.01$). These results indicate that SPAD meter readings can be used to measure N concentration and/or monitor N availability for seed production, and when N is the limiting factor, to estimate yield of switchgrass (*Panicum virgatum* L.)

MATERIALS AND METHODS

In order to highlight the biological aspects of the plant, the aim was to determine the concentration of chlorophyll in the leaves of *Primula officinalis* Hill. synonymous with *Primula veris* L., where non-invasive measurements were made using the portable device Chlorophyll Meter SPAD 502.

The researches were carried out at The National Institute of Research and Development for Potato and Sugar Beet

Brașov, Technology and Good Practices in Agriculture Department, Laboratory of Medicinal and Aromatic Plants. The biological material, on which the researches were made, was brought in the spring of 2016 from the spontaneous flora of Brașov County; this study focused on aspects of biology and technology regarding the introduction into the culture of the species *Primula officinalis* Hill. being part of the author's doctoral thesis.

The experiment was bifactorial, set according to the model of randomized blocks, in three repetitions, being established by seedling in the fall of 2016, with the aim of determining the optimal nutrition space for the species *Primula officinalis* Hill.

Factor A – distance between rows with the following graduations: 25 cm, 50 cm, 75 cm;

Factor B – distance between plants per row, with the following graduations: 10 cm, 25 cm, 50 cm;

Interaction with 10/25 density is considered the Control of this research.

Experimental device: the surface of the plots from the factor a_1 = 4.5 m^2; the surface of the plots in factor a_2 = 9 m^2; the surface of the plots in factot a_3 = 13.5 m^2; total experimental surface including paths (27 m^2 * 3 + 13.5 m^2 * 2) = 108 m^2; number of plants on plots - b_1; (2 m/10 cm) = 20 * 9 = 180 * 3 = 540; number of plants on plots - b_2; (2 m/25 cm) = 8 * 9 = 72 * 3 = 216; number of plants on plots - b_3; (2m / 50 cm) = 4 * 9 = 36 * 3 = 108; total number of plants per experiment: 540 + 216 + 108 = 864 [8].

SPAD-502 PLUS Chlorophyll Meter can measure relative content of chlorophyll, non-destructive to leaves. Measured datum will be displayed in trend graph. It can be used to enhance the utilization rate of nitrogen fertilizer [1].

Chlorophyll content is an indicator of plant health and can be useful to optimize the time and amount of application of additional fertilizers, ensuring higher crop yields and superior quality. It is an easy-to-use device, being at the same time very useful to food industry because it can be used both by farmers during harvest, for determing the optimal time for harvesting, but it can also be used in cold warehouses, to determine the state of vegetables and fruits' full growth. SPAD 502 Plus measuring device quantifies subtle changes or new prospects in plant health long before they are visible to the human eye.

The device has a high level of precision: for values between 0-49, 0 error; for values between 50-99, errors less than 0.1 [5].

Advantages of using the Chlorophyll Meter SPAD-502: non - invasive method; the measurement is done in real time (less than 2 seconds); the measuring area is very small, 2 mm x 3 mm, which allows the analysis of small leaves, and the thickness of the leaves can have dimensions up to 1.2 mm; it is water resistant and can be used in adverse weather conditions. SPAD value: the relative chlorophyll content index indicated by the device has values between -9.9 and 199.9 [5].

Working method: turn on the device and calibrate by holding the two flaps between which the leaf is to be inserted in contact, until the value 0 is indicated; the leaf is inserted in locations intended for these, between the two flaps, so that this also completely covers the measuring window; press with your finger to close the measuring end, holding it closed until you hear a sound, when the result is displayed on the screen and stored automatically [5].

Measurement principle: The measured values with the SPAD 502 Plus Chlorophyll Meter correspond to the amount of chlorophyll present in the leaf. They are calculated based on the amount of light transmitted by the leaf in the two wavelengths, in which the absorption is different. The leds in the lighting system emit red and infrared waves. The light passes through the sample and passes through the receiver, which transmits the light into analog electrical signals. These signals are then amplified and converted to digital signals by the A / D converter [5].

RESULTS AND DISCUSSIONS

Three determinations in dinamics were made, using the SPAD 502 Chlorophyll Meter. At each variant/ repetition/ row three uniform plants were analyzed, three readings on three

different leaves were made, resulting in a total of 729 readings and 243 analized plants on each measurement.

The SPAD 502 Device was calibrated before use by pressing the measuring head, after which the actual reading was performed by inserting the leaf into the measuring area. The values were read approximately two seconds after closing the measuring cap.

The choice of leaves for reading was made following a visual control on all leaves/ plants, choosing three plants from each variant/repetition/row.

Results on SPAD values were statistically processed by analyzing the variance and interpreted by the "t" test for the user significance thresholds p 5%, p 1% and p 0.1%.

Assessing the measurement date on SPAD values (Table 1) from *Primula officinalis* Hill. leaves in 2018, there is a slight decrease in the values measured on 03.05.2018 by 0.17 units compared to the first measurement considered as a control; the measurements made on 11.05.2018 indicate an average value of 45.73, distinctly significantly positive, with a difference of 2.48 from the control.

Table 1. The influence of measurement date on SPAD values in *Primula officinalis* Hill. leaves experimental year 2018

No.	Date	SPAD average values	Value (%)	Difference	Signif.
1	25.04.2018	43.26	100.0	0.00	MT
2	03.05.2018	43.04	99.6	-0.17	-
3	11.05.2018	45.73	105.7	2.48	**
DL 5%				1.47	
DL 1%				2.43	
DL 0.1%				4.54	

Source: own calculation.

Table 2. The influence of measurement date on SPAD values in *Primula officinalis* Hill. leaves experimental year 2019

No.	Date	SPAD average values	Value (%)	Difference	Signif.
1	25.04.2019	35.92	100.0	0.00	Mt.
2	13.05.2019	40.69	113.3	4.77	*
3	20.05.2019	42.98	119.6	7.06	**
DL 5%				3.16	
DL 1%				5.23	
DL 0.1%				9.80	

Source: own calculation.

In 2019, SPAD values gradually increased, at the same time with the senescence of the plants, the highest SPAD value (42.98) being registered on 20.05.2019, distinctly significant compared to the control variant (Table 2).

SPAD values showed a great state of plant health, falling within the parameters at which the measuring device has 0 error.

Studying the influence of planting distance on SPAD values in *Primula officinalis* Hill. leaves, can be observed the fact that measured values increase both in the variants planted at a distance between rows of 50 cm and in those planted at 75 cm, with very significant values compared to the control variants, planted at a distance of 25 cm between rows (Table 3).

Table 3. The influence of planting distance on SPAD values in *Primula officinalis* Hill. leaves experimental year 2018

No.	Distance between rows	SPAD average values	Relative value(%)	Difference	Signif.
1	25	41.51	100.0	0.00	MT
2	50	46.43	111.9	4.92	***
3	75	44.13	106.3	2.62	***
DL (p 5%)				1.15	
DL (p 1%)				1.61	
DL (p 0.1%)				2.28	

Source: own calculation.

The influence of planting distance (Table 4) on SPAD values, in 2019, registered a vegetation state of the plants with very significant differences at planting distances of 75 cm between rows, reaching an average of 3.37 units higher values than the 25 cm between rows variant

Table 4. The influence of planting distance on SPAD values in *Primula officinalis* Hill. leaves experimental year 2019

No.	Distance between rows	SPAD average values	Relative value(%)	Difference	Signif.
1	25	38.53	100.0	0.00	Mt.
2	50	39.16	101.6	0.62	-
3	75	41.90	108.7	3.37	***
DL (p 5%)				1.64	
DL (p 1%)				2.30	
DL (p 0.1%)				3.25	

Source: own calculation.

From the data in Table 5, where results of interaction between planting distance and SPAD measurement fom 2018 were presented, resulted that planting distance of 50 cm between rows had the highest values, regardless of the measurement date, with very

significant values, compared to the control planted at a distance of 25 cm between rows.

Table 5. Interaction between planting distance and SPAD measurement date in *Primula officinalis* experimental year 2018

No.	Planting distance (cm)	Date of measurement	SPAD average values	Relative value (%)	Differ. ±	Signif.
1	25	25.04.2018	40.93	100.0	0.00	MT
2	50		45.43	111.0	4.50	***
3	75		43.40	106.0	2.47	*
4	25	03.05.2018	41.37	100.0	0.00	MT
5	50		45.23	111.3	3.87	***
6	75		42.67	103.1	1.30	-
7	25	11.05.2018	42.23	100.0	0.00	MT
8	50		48.63	115.2	6.40	***
9	75		46.33	109.7	4.10	***
DL (p 5%)					1.99	
DL (p 1%)					2.80	
DL (p 0.1%)					3.95	

Source: own calculation.

In 2019, the results of the interaction between planting distance and the date of SPAD measurements were significant at both planting distances: 50 and 75 cm (Table 6).

Table 6. Interaction between planting distance and SPAD measurement date in *Primula officinalis* experimental year 2019

No	Planting distance (cm)	Date of measurement	SPAD average values	Relative value (%)	Difference±	Signif.
1	25	25.04.2019	35.50	100.0	0.00	MT
2	50		33.07	93.1	-2.43	-
3	75		39.20	110.4	3.70	*
4	25	13.05.2019	39.13	100.0	0.00	MT
5	50		40.20	102.7	1.07	-
6	75		42.73	109.2	3.60	*
7	25	20.05.2019	40.97	100.0	0.00	MT
8	50		44.20	107.9	3.23	*
9	75		43.77	106.8	2.80	-
DL (p 5%)					2.84	
DL (p 1%)					3.98	
DL (p 0.1%)					5.63	

Source: own calculation.

The following conclusions can be drawn from the table of comparisons of the interaction between the SPAD measurement date and the planting distance: in the case of variants planted at 25 cm between rows, the differences are insignificant for both analyzed data; the variants with graduations of 50 cm between rows present insignificant values on 03.05.2018 and significant values on 11.05.2018; the same meanings are registered in the case of the variants with 75 cm between rows, compared to the control variant analyzed on 25.04.2018 (Table 7).

Table 7. Interaction between SPAD measurement date and planting distance to *Primula officinalis* experimental year 2018

No.	Date	Distance between rows	Average SPAD	Values (%)	Differ. ±	Signif
1	25.04.2018	25cm	40.93	100.0	0.00	MT
2	03.05.2018		41.37	101.1	0.43	-
3	11.05.2018		42.23	103.2	1.30	-
4	25.04.2018	50cm	45.43	100.0	0.00	MT
5	03.05.2018		45.23	99.6	-0.20	-
6	11.05.2018		48.63	103.1	2.93	*
7	25.04.2018	75cm	43.40	100.0	0.00	MT
8	03.05.2018		42.67	98.3	-0.73	-
9	11.05.2018		46.33	106.8	2.93	*
DL (p 5%)					2.17	
DL (p 1%)					3.26	
DL (p 0.1%)					5.25	

Source: own calculation.

Comparing the interaction between measurement date of SPAD values and the planting distance in 2019, it is found that on 20.05.2019 all planting variants registered significant differences in planting distances of 25 and 75 cm between rows and very significant in the variant planted at 50 cm between rows (Table 8).

In conclusion, the distance of 50 cm and 75 cm between plants, gives them an optimal space for photosynthesis and the ability to show their true productive potential.

Table 8. Interaction between SPAD measurement date and planting distance in *Primula officinalis* experimental year 2019

No.	Date	Distance between rows	Average SPAD	Values (%)	Differ. ±	Signif.
1	25.04.2019	25cm	35.50	100.0	0.00	MT
	13.05.2019		39.13	110.2	3.63	-
3	20.05.2019		40.97	115.4	5.47	*
4	25.04.2019	50cm	33.07	100.0	0.00	MT
5	13.05.2019		40.20	121.6	7.13	**
6	20.05.2019		44.20	133.7	11.13	***
7	25.04.2019	75cm	39.20	100.0	0.00	MT
8	13.05.2019		42.73	109.0	3.53	-
9	20.05.2019		43.77	111.6	4.57	*
DL (p 5%)					3.89	
DL (p 1%)					6.05	
DL (p 0.1%)					10.29	

Source: own calculation.

CONCLUSIONS

The influence of the measurement date on the SPAD values on the leaves of *Primula officinalis* Hill., in 2018, registers a slight decrease of the values measured on 03.05.2018 by 0.17 units compared to the first measurement, considered as a control (25.04.2018); the measurements made on

11.05.2018 indicate an average value of 45.73, this being distinctly significantly positive, with a difference of 2.48 compared to the control. In 2019, the SPAD values increased gradually, with the plants' strength, the highest SPAD value (42.98) being registered on 20.05.2019.

The results of the interaction between planting distance and SPAD measurement date in 2018 show that the variants planted at a distance of 50 cm between rows at the highest values, regardless of measurement date, with very significant values compared to the control planted at 25 cm between rows. In 2019, the results of the interactions between the planting distance and the data of the SPAD measurements were significant at both planting distances (50 and 75 cm).

SPAD measurements on the leaves of Primula officinalis Hill, with high values in the variants whose distance between rows was 50 cm, show a good health of the plants and recommend this planting distance.

ACKNOWLEDGEMENTS

The study was conducted as part of research PhD programme of the first author. The first author is thankful to NIRDPSB Brasov for support during PhD programme.

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