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USE OF THE SPAD METER TO ESTIMATE CHLOROPHYLL CONTENT IN DIFFERENT SESSILE OAK (*Quercus petraea* (Matt.) Liebl.) PROVENANCES IN SERBIA

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Abstract: *Chlorophyll is the main participant in the process of photosynthesis, which content can tell about plant physiological health condition. In this research, the SPAD values of the one-year-old Sessile oak seedlings of 5 Serbian provenances were measured during June, August, and October of 2021. The measuring was performed by the SPAD device, and the data were presented through the SPAD index values. The research results confirmed that the environmental conditions where the plants grow have a dominant effect on the chlorophyll content, concerning the origin of the individuals. In further research, it is necessary to accurately determine on a greater plant sample the relationship between chlorophyll content and SPAD index values.*

Key words: sessile oak, provenances, SPAD, chlorophyll.

PRIMENA SPAD U PROCENI SADRŽAJA HLOROFILA RAZLIČITIH SRPSKIH PROVENIJENCIJA HRASTA KITNJAKA

Izvod: *Hlorofil je glavni učesnik procesa fotosinteze, čiji sadržaj govori o fiziološkom stanju biljke. U ovom radu merene su SPAD vrednosti jednogodišnjih sadnica hrasta kitnjaka 5 srpskih provenijencija tokom juna, avgusta i oktobra 2021. godine. Merenje je obavljeno pomoću SPAD uređaja, a podaci predstavljeni kroz vrednosti SPAD indeksa. U ovom radu potvrđeno je da dominatan efekat na sadržaj hlorofila imaju uslovi*

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sredine u kojoj se biljke nalaze, u odnosu na poreklo jedinki. U daljem istraživanju neophodno je precizno utvrditi odnos koncentracije hlorofila i SPAD jedinica, na većem uzorku.

Ključne reči: hrast kitnjak, provenijencije, SPAD, hlorofil.

1. INTRODUCTION

Photosynthesis is a fundamental phytochemical process on Earth that enables the development of complex organisms. The central participant in the process is chlorophyll, the *green pigment* existing in all organisms capable of absorbing light photons and converting this energy into the chemical form of carbohydrates that make it available to all other heterotrophs. Higher plants have chlorophyll a and b, essential for primary photosynthetic reaction. Therefore, the chlorophyll content in plant organs especially leaves as primary photosynthetic organs, is of major importance for their metabolism. However, the variability of chlorophyll content among plant species, functional plant groups, and natural forest communities particularly on high scales, is not been completely clarified until today (Li et al., 2018). Chlorophyll synthesis is a complex, multi-stage process, which is largely affected by temperature, water, and nutrient availability, i.e. soil characteristics, etc. Considering the involvement of numerous enzymes, the whole process is polygenically determined.

The quality and intensity of sun irradiation can vary significantly during a certain time in a location. Plants inhabited the land about half a billion years ago and being the sessile organisms, they have developed sophisticated adaptation mechanisms on the environment, through communication with other organisms (microorganisms primarily), but also by controlling their own processes. Hence, plants are capable to adapt to different light intensities and qualities, and depending on available wavelengths at the time, can properly adjust the chlorophyll's relation to provide the existence.

In spite of multiple factors affecting chlorophyll content, its investigation is an important parameter in overall plant health state perception. The collected data can indicate the stressor influence which is very important in plant production, or the individual or group differences and similarities in terms of the photosynthesis process, its efficacy, and sunlight energy processing in general. Ling et al. (2011) report that chlorophyll leaf concentration is an indicator of chloroplast development, photosynthetic capacity, nitrogen leaf content, and plant health in general.

As one of the modes of fast, efficient, and completely safe chlorophyll content measurement in a plant leaf, the SPAD-502 Plus (Soil Plant Analysis Development; Konica-Minolta, Inc. Japan) device is being used. It measures the difference between red (650 nm) and infrared (940 nm) emitted light transmission, with the help of two diodes, presenting the final SPAD value, or index. The red light of 650 nm wavelength presents the value of chlorophyll absorption, while the 940 nm wavelength presents the factor of correction for leaf thickness of different plant species. The idea in SPAD device appliance is that increased leaf chlorophyll

concentration increases also absorption of red light, and since all leaves transmit a high fraction of close infrared light, the relationship between SPAD and chlorophyll can be seen theoretically as cause and effect (Wang, Li, 2018).

The chlorophyll content dynamics in leaves of Sessile oak (*Quercus petraea* (Matt.) Liebl) in Serbia have not been investigated so far using the SPAD device, according to the knowledge of the authors. After the Common oak, the Sessile oak is the most common oak species in the growing stock of the Republic of Serbia. It is an autochthonous species, inhabiting elevations between 300 and 1300 m a.s.l. The Sessile oak forms more than 20 forest types, and it is one of the most valuable forest species in terms of the area of occurrence, volume, application in the economy, and wood quality. The Sessile oak is also one of the most common oak species cultivated in seedling nurseries in Serbia (Popović et al., 2019). Concerning the wide tolerance range and related to climate change, it is assumed that Sessile oak will expand its area in the future. The Sessile oak individual trees of different provenances are locally adapted to present environmental conditions. Physical modifications lead to different physiological patterns for the adaptation purpose, which all impact final plant fitness.

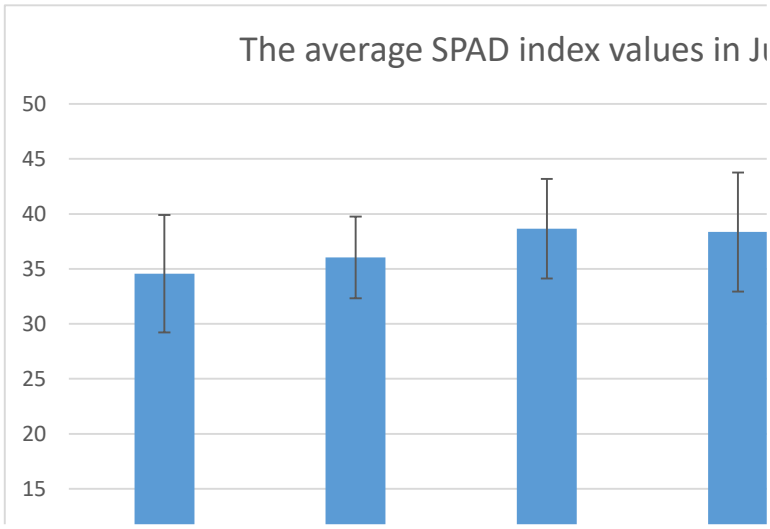
This research aimed to investigate chlorophyll content variability by measuring the leaf SPAD index of containerized one-year-old Sessile oak seedlings of 5 populations, originating from Serbia, during one growing season.

2. MATERIAL AND METHODS

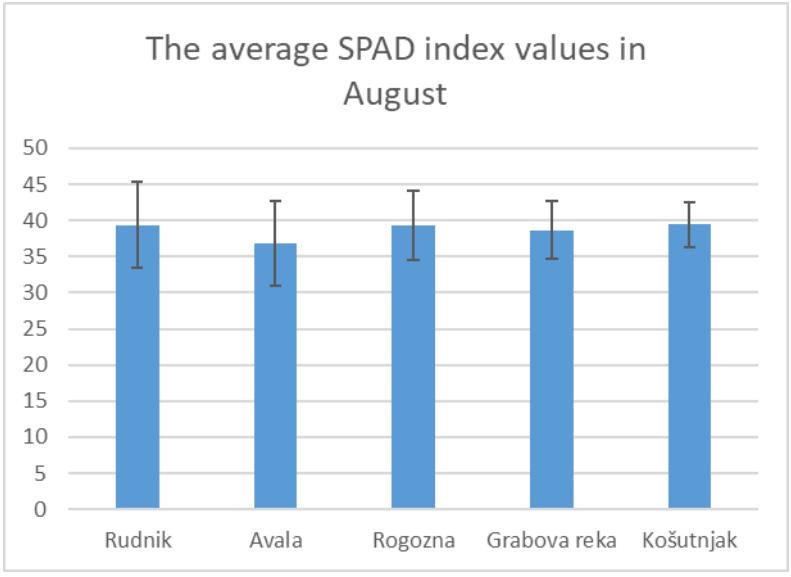
During the autumn of 2020, the Sessile oak seeds were collected in 5 natural stands of different provenances, as follows: Rudnik, Avala, Rogozna, Grabova reka, and Košutnjak. The acorns were stratified in the sand and left to overwinter on 4 °C. In spring 2021, the containerized seedlings were produced from seeds (container type HIKO-V-350), and peat was used as a substrate (Freepeat). Plants were nurtured in semi-controlled conditions in the seedling nursery of the Institute of Forestry in Belgrade (44°46’55”N 20°25’21”E), in half-shadow. They were watered regularly, without any fertilization treatment. The SPAD leaf measurements were taken by the SPAD chlorophyll meter (SPAD-502 Plus, Minolta, Inc.) on a sample of 10 plants per provenance, during one growing period. The dates were chosen to follow vegetation’s crucial phases and seasonal dynamics, but also 3 phases of leaf age. The measurements were performed on the following dates: on June 23 – at the beginning of the season when the young developed leaves were measured; on August 3, – in the middle of the season, when large, fully developed leaves were measured; and on October 21, at the end-season when the leaves had occasional signs of chlorophyll degradation. The SPAD sensor was placed randomly on leaf mesophyll, avoiding leaf nerves. The measured area was 2 mm x 3 mm, and the measurement of small leaves was also enabled.

3. RESULTS AND DISCUSSION

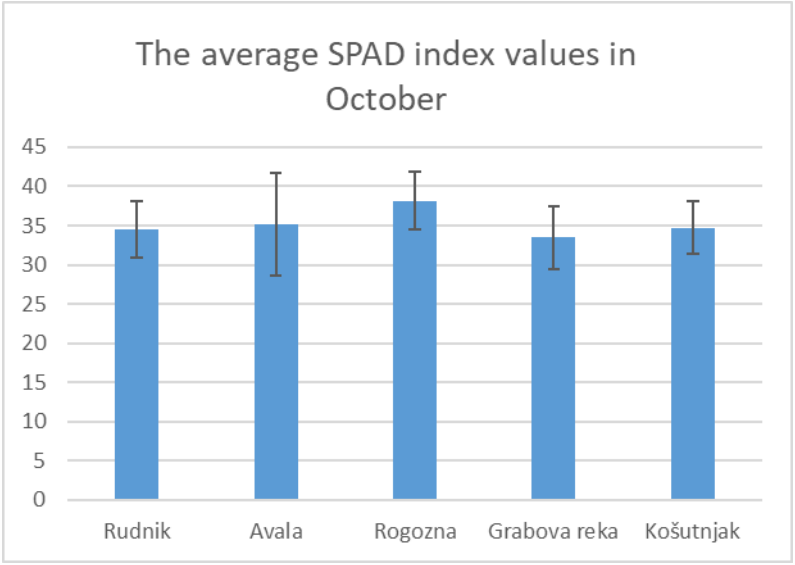
Average SPAD index values of one-year-old Sessile oak seedlings different in origin, measured over the months are presented on graphs 1, 2, and 3.



Graph 1. *Average SPAD index values of all provenances with standard deviation in June*



Graph 2. *Average SPAD index values of all provenances with standard deviation in August*



Graph 3. *Average SPAD index values of all provenances with standard deviation in October*

The average values of meteorological data for June, August, and October on the territory of Belgrade are presented in Table 1.

Table 1. *Average values of some meteorological parameters for June, August, and October on the territory of Belgrade*

Month	June	August	October
Average value of maximal monthly temperatures	29.6°C	29.7°C	16.9°C
Precipitation (mm)	34.2	38.2	73.4

In a habitat, plants are affected by numerous factors, and the light is spatially and temporarily the most variable (Pearcy, 1999). Therefore, plants have evolved mechanisms of adaptation. Plants tested in this experiment, although different in their origin, demonstrated a relatively uniform response to sun irradiation exposition during all investigated months of one growing season. The highest average values of the SPAD index are reported for August in the "Košutnjak" provenance. The highest individual value was also noted in August, in the population of "Rogozna", numbering 49.4.

The lowest average SPAD index values are detected in October, in the "Grabova reka" provenance, valuing 33.48. When analyzing individually, the lowest value was reported in the "Grabova reka" population in June, numbering 24.9 SPAD units. Interestingly, the highest average SPAD unit value in June, at the beginning of the season, had seedlings of "Rogozna" population, as well as in October, at the season's end. The lowest average values in June had seedlings of the "Rudnik" provenance, while in October the lowest average values had the "Grabova reka" provenance seedlings.

Atar et al., (2020) investigated in their research the SPAD values of *Quercus hartwissiana* Steven species, in May and October, and obtained the average maximal SPAD value of 32.7 for May, and 28.8 for October. Jagiełło-Leńczuk et al. (2015) reported maximal values for Common oak in mid-July, numbering 49.9 SPAD units, while the average value of measurements taken in the period from May to November was 31.8.

When analyzing the SPAD unit values per provenance over the year, the highest average values had the "Rogozna" population and the lowest the "Avala" population.

The highest standard deviation of SPAD values was detected in June, in the "Košutnjak" provenance.

During the season, plant dieback was also detected. The provenance "Košutnjak" lost the highest number of individuals, two, while one individual lost "Avala" and "Grabova reka" provenances. The causes of plant dieback are high temperatures, the occurrence of oak powdery mildew, and attacks of insects from the genus *Corythucha*.

The total average SPAD values of all provenances during the vegetation season showed a small variation range. The highest values were in August when meteorological conditions were optimal and also the leaf was in a stage of full development and capacity. On the other hand, the total leaf chlorophyll amount and specific leaf area increased with increasing shadow (Niinemets, 2010) to compensate for photosynthetic losses (Popović et al., 2016). Bearing in mind the fact that containerized seedlings were grown in the half-shadow conditions, and their canopies overlapped, these conditions were one of the influence factors which was also reported in the research of Bielinis et al. (2015).

The lowest average values were in October when plants were slowly entering the phase of leaf rejection.

Similar results were obtained by Bielinis et al. (2015) who investigated chlorophyll content in Sessile oak: the total amount of chlorophyll increased from May to July, with a peak in July to August, and during September the concentration values decreased. Louise et al. (2009) in their research of two-year-old Sessile oak seedlings reported an increase in chlorophyll content, which achieved the plateau phase at the end of May when leaves were wholly formed. Afterward, the chlorophyll concentration gradually decreased from July to September, with the leaf aging.

Chlorophyll is very important for plants. Hence, the general opinion is that leaf chlorophyll content is mainly influenced by environmental factors, concerning phylogenetic ones, and that plant adapts to them to optimize the photosynthetic process (Li et al., 2018). This was also confirmed by Arab et al. (2020) who investigated the effect of the Sessile oak seedling origin on a foliar response to arid conditions, where great phenotypic plasticity has been detected, and only a small number of leaf characteristics were genetically fixed.

Also, the leaf structure parameters change with aging, undoubtedly leading to a change in leaf optical properties (Silla et al., 2010). In this research there can be seen a regular pattern in all provenances, where the SPAD unit values have been increasing from the beginning of the season towards its middle, correlating with the leaf development and chlorophyll content, and the SPAD index values decrease at

the end of the growing season, in parallel with leaf drying processes. The results also correlate with the dynamics of changing average temperature values. The measured values of SPAD units can also be influenced by changes in growth conditions, which could lead to the redistribution of chloroplasts in leaf mesophyll cells (Naus et al., 2010). The negative impact could be caused by a common oak pathogen – fungi *Erysiphe alphitoides* (Griffon et Maubl.), that provokes oak powdery mildew disease. Ashy scrum on the adaxial side of the leaf is the fungus mycelium which changes the leaf transmission, consequently affecting the measured SPAD values. In the presented research, during the growing season, there have been detected 2 strong attacks of this pathogen – at the beginning of June and during September.

4. CONCLUSIONS

The SPAD chlorophyll meter is a practical technical device that enables fast and simple measurement of a great number of plant samples in a short time interval, without making any kind of damage to a plant organism. In this research, the SPAD values of the one-year-old Sessile oak seedlings of 5 different Serbian provenances were measured. Similar average values were noted among all individuals with minor differences. It has been confirmed that environmental conditions have a dominant impact on the chlorophyll content, in relation to the origin of plants. In further research, it is necessary to accurately determine the relationship between chlorophyll content and SPAD index values in a greater plant sample.

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Summary

Chlorophyll is the main participant in the photosynthetic process, which content indicates plant physiological condition. In this research, the SPAD values of the one-year-old Sessile oak seedlings of 5 Serbian provenances were measured during June, August, and October of 2021. The measures were taken by the SPAD device and the data were presented in the SPAD index values.

The greatest average values were recorded during August in the "Košutnjak" provenance. The lowest average SPAD index values have been detected in October, in the "Grabova reka" provenance. When analysing the values of the SPAD units during the year, the population of "Rogozna" had the highest mean values, and the "Avala" population had the lowest. Total average SPAD values of all provenances during the growing season manifested a small variation range.

In this research, it has been confirmed that chlorophyll content is mainly affected by environmental conditions where plants are nurtured, in relation to their origin. In spite of their different ,,genetic content'', the plants showed a small variation range in chlorophyll quantity and adapted to the environs, where they were living. In further research, it is necessary to accurately determine the relationship between chlorophyll content and SPAD units, in a greater plant sample.

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Rezime

Hlorofil je glavni učesnik procesa fotosinteze, čiji sadržaj govori o fiziološkom stanju biljke. U ovom radu merene su SPAD vrednosti jednogodišnjih sadnica hrasta kitnjaka 5 srpskih provenijencija tokom juna, avgusta i oktobra 2021. godine. Merenje je obavljeno pomoću SPAD uređaja, a podaci predstavljeni kroz vrednosti SPAD indeksa.

Najveće prosečne vrednosti zabeležene su tokom avgusta meseca, u provenijenciji Košutnjak. Najmanje prosečne vrednosti SPAD indeksa ustanovljene su u oktobru, u provenijenciji Grabova reka. Kada posmatramo vrednosti SPAD jedinica provenijencija tokom godine, najveće prosečne vrednosti imala je populacija Rogozna, a najmanje Avala. Prosečne ukupne SPAD vrednosti svih provenijencija tokom vegetacione sezone ispoljile su mali opseg varijacija.

U ovom radu potvrđeno je da dominatan efekat na sadržaj hlorofila imaju uslovi sredine u kojoj se biljke nalaze, a ne poreklo jedinki. Biljke, uprkos svom različitom ,,genetskom sadržaju", ispoljile su mali opseg varijacija količine hlorofila i prilagodile okolini u kojoj su sve obitavale. U daljem istraživanju neophodno je precizno utvrditi odnos koncentracije hlorofila i SPAD jedinica, na većem uzorku.