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Usporedba različitih metoda praćenja vodnoga stresa kod soje (*Glycine max* (L.) Merr.).

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COMPARISON OF VARIOUS WATER-STRESS MONITORING METHODS IN SOYBEAN (*Glycine max* (L.) MERR.)

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SUMMARY

The research was conducted in a greenhouse at the Agricultural Institute Osijek in 2022. The study aimed to evaluate and compare different methods for detecting soil water deficit and plant water-stress responses in soybean (Glycine max (L.) Merr.). The evaluated methods included soil water content sensors, physiological parameters, and machine learning models. Two irrigation treatments were implemented: optimal soil water content (control treatment, n1, 100 % of field capacity, FC) and water stress (n2, 50% FC) applied during the flowering (f1) and grain-filling (f2) stages. TDR300 and AT sensors exhibited the most rapid response to changes in soil water content (% vol.), indicating high sensitivity to early water deficit. Among physiological indicators, LRCC and RC/CS₀ were the most sensitive during flowering, while PI_{ABS} and PI_{TOTAL} showed the highest responsiveness during grain filling. The k-Nearest Neighbors (kNN) model demonstrated the highest performance, characterized by high classification accuracy (CA = 0.921) and AUC (0.976). The results emphasize the importance of selecting stage-specific indicators for water stress detection and provide a basis for the development of future integrative monitoring frameworks in soybean production.

Keywords: soybean, water stress, soil moisture sensors, physiological indicators, machine learning models

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is one of the most important oilseed crops globally. In 2023, global soybean production was estimated at approximately 398 million tons, with Brazil and the United States being the largest producers and exporters, while China remained the largest importer, confirming the significance of soybeans in international trade and food supply chains (Volkova and Smolyaninova, 2024).

Along with 20% to 25% oil, soybean seeds contain approximately 40 % to 50 % high-quality protein utilized in human nutrition as a substitute for animal products (Rotundo et al., 2024; Singer et al., 2023) and as the primary source of plant protein in concentrated livestock feed (Ali et al., 2020).

Soybeans' indispensable role in global agricultural production and trade is dependent on their sensitivity to water stress, particularly during flowering and grain filling. Water deficits significantly increase oxidative stress, while simultaneously decreasing pigment content and Photosystem II (PSII) efficiency, resulting in impaired biomass development (Wang et al., 2022; Falcioni et al., 2025). Drought also disrupts reproductive processes and limits assimilate translocation to grain, leading to lower seed set, reduced seed size, and yield losses. Grain quality can also be altered by water deficit, which increases protein and decreases oil content (Pereira et al., 2021; Tavares et al., 2022).

Given these impacts, accurate monitoring of water stress is essential. Research on soybean responses to water stress is extensive, but most studies focus on individual monitoring approaches, such as soil-based

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measurements or plant physiological indicators, without systematically comparing their sensitivity under the same experimental conditions. This lack of comparative evaluation constrains the selection of optimal indicators for use in future integrative water stress monitoring frameworks. Water stress is usually monitored and assessed through the analysis of meteorological conditions and the measurement of soil water content (SWC) using soil moisture sensors (SMSs) or profile probes. Effective monitoring of SWC is a cornerstone of precision agriculture and sustainable resource management (Loconsole et al., 2025). Although SMS are traditionally utilized (Robinson et al., 2008), they often fail to capture early physiological responses of the plant. Thus, modern research increasingly emphasizes the importance of physiological indicators, such as chlorophyll fluorescence and gas exchange, which enable the early detection of stress at the physiological level (da Silva et al., 2024). Existing machine learning models, such as k-Nearest Neighbors (kNN) or more advanced algorithms like Support Vector Machine (SVM), facilitate high precision in the classification of stress but frequently rely on single-source datasets and primarily focus on yield prediction. According to studies by Li et al. (2021) and Tan et al. (2024), the integration of SMS and physiological data with machine learning models facilitates high precision in the classification of stress states and the prediction of grain nutritional composition.

Given the limited comparative evaluation of water stress monitoring approaches in the same experimental conditions, the objective of this study was to assess the sensitivity and suitability of different soil-based measurements (Watermark and Tensiometer, TDR300 and AT SM150 sensors), plant physiological indicators (leaf relative chlorophyll content, TR_0/ABS, RC/CS_0, PI_{ABS}, PI_{TOTAL}) and machine learning models (Support Vector Machine, k-Nearest Neighbors, Logistic Regression, and Simple Neural Network) for detecting soil water deficit and soybean water stress responses during critical developmental stages (flowering and grain filling), to identify the most appropriate indicators for future integrative applications.

MATERIALS AND METHODS

The study was conducted in a greenhouse at the Agricultural Institute Osijek with soybean cultivar OS Nevena (maturity group 0). It included the control (n1), where soybean plants were grown under optimal soil water content (SWC, 80 % to 100 % of field capacity, FC), and water deficit treatment (n2, 50 % FC) implemented during two developmental stages: flowering (f1) and grain filling (f2). Air temperature (°C) and relative humidity (%) were recorded daily using a LOG32 data logger (Dostmann electronic GmbH, Germany). During May, June, and July, mean daily air temperatures ranged from 20.6–29.7 °C, 24.8–31.9 °C, and 24.8–31.9 °C, respectively. Corresponding relative humidity levels were 41.7–73.4 %, 53.2–64.4%, and 45–63.7 %. The experimental pots were filled with soil sampled from the surface arable layer (≤30 cm), with following properties: unstable macroaggregates, very stable microaggregates, moderate water capacity (36.61% vol.), a pore volume of 41.8%, air capacity of 5.3%, specific bulk density of 1.5 g cm^{-3}, pH values of 7.5 (H2O) and 6.8 (KCl), and a humus content of 1.6% (Marković, 2013). The soybean was sown on April 25, 2022. Six seeds were sown in each vegetation pot, with two seeds placed at each corner of an equilateral triangle with 10 cm sides. At the V2 developmental stage (Fehr and Caviness, 1977), the plants were thinned to maintain three plants per pot. There were five repetitions/ pots per treatment (n=15 plants per treatment). The irrigation rate was adjusted according to the water stress treatment, developmental stage, and SWC. The soil drying period for the n2f1 occurred from June 7 to June 10, while the drying period for the n2f2 took place from July 5 to July 8. The drought treatment period length was chosen based on the results of a previous drought treatment study by Markulj Kulundžić et al. (2022) on soybean in similar conditions, in which plants initiated a defense mechanism after the second day of water withholding, followed by drastic changes in electron flow in photosystem II (PSII).

Various types of SMS were utilized in the study, including the Watermark and Tensiometer (Irrometer Company, Inc., Riverside, USA), TDR300 (Specmeters, Spectrum Technologies, Aurora, USA), and AT SM150 (Delta-T Devices Ltd, Cambridge, United Kingdom). The chlorophyll *a* fluorescence was measured on all three plants per repetition by the saturation pulse method (Kalaji et al., 2014) on a middle leaflet of the last fully developed trifoliate with the Handy Plant Efficiency Analyzer (PEA, Hansatech Instruments, King's Lynn, Norfolk, UK). Measurements were made from June 7 to June 10 during the flowering stage (R2) on n1 and n2f1 treatment plants and from July 4 to July 7 during the grain-filling stage (R6) for n1 and n2f2 treatment plants, in the morning, between 07:00 and 09:00. Dark-adaptation clips were placed on the leaves 30 minutes before measurement to ensure the opening of reaction centers, which is a prerequisite for measuring the minimum fluorescence intensity (F_0). The following chlorophyll *a* fluorescence parameters were measured: F_0 (fluorescence intensity after 50 μs, step O), F_{300} (fluorescence intensity after 300 μs), F_J (fluorescence intensity after 2 ms, step J), F_I (fluorescence intensity after 30 ms, step I), and Fm (maximum fluorescence intensity, step P). The analyzed OJIP test parameters, calculated according to Strasser et al. (2004) and Yusuf et al. (2010) using Microsoft Office Excel 2010, are presented in Table 1.

Table 1. Analyzed chlorophyll *a* fluorescence parameters *(expressed in relative units)*

Tablica 1. Analizirani parametri fluorescence klorofila a (izraženo u relativnim jedinicama)

Label / *Oznaka*	**Parameter description** / *Opis parametra*	**Parameter equation** / *Jednadžba parametra*
TR_0/ABS	The maximum quantum yield of PSII photochemistry *Maksimalan kvantni prinos fotosustava II*	$[1 - (F_0/F_m)]$
RC/CS_0	Density of active PSII RCs per cross-section *Gustoća aktivnih reakcijskih središta*	$F_v/F_m \times (V_J/M_0) \times ABS/CS_0$
PI_{ABS}	Performance index on absorption basis *Indeks fotosintetske učinkovitosti na bazi apsorpcije*	$(RC/ABS) \times (TR_0/DI_0) \times [ET_0/(TR_0\text{-}ET_0)]$
PI_{TOTAL}	Performance index for energy conservation from exciton to the reduction of PSI end acceptors *Indeks fotosintetske učinkovitosti pretvorbe energije od ekscitona do redukcije krajnjega akceptora elektrona na PSI*	$PI_{ABS} \times RE_0/ET_0/(1 - RE_0/ET_0)$

On the same leaf where the chlorophyll *a* fluorescence was recorded, the leaf relative chlorophyll content (LRCC) was also measured using a Chlorophyll Content Meter CL-01 (Hansatech, United Kingdom). Furthermore, measurements were conducted using an AMS (ams-OSRAM AG, Austria) AS7263 sensor module, featuring six spectral bands (3 x 2 photodiode array) sensitive to wavelengths in the red and near-infrared spectrum (610, 680, 730, 760, 810, and 860 nm) with a full width at half maximum (FWHM) of 20 nm. An analysis and development of machine learning models was conducted to classify the data into two classes: favorable SWC (control treatment, n1) and water stress conditions (n2). Four machine learning models were utilized, including Support Vector Machine (SVM), k-Nearest Neighbors (kNN), Logistic Regression, and a Simple Neural Network. Model performances were evaluated using measures such as Area Under the Curve (AUC), Classification Accuracy (CA), F1-score, Precision, and Recall. To evaluate differences between control and water deficit conditions for physiological parameters, analysis of variance (ANOVA) and Fisher's Least Significant Difference (LSD) test were performed separately for each developmental stage using Statistica 12.0 software (StatSoft Inc., Tulsa, Oklahoma).

RESULTS AND DISCUSSION

The SWC dynamics, monitored by SMS, are presented in Figure 1. During May, the net irrigation rate (NIR) was 76 L for n1 and 68 L for n2f1 and n2f2. During June and July, water stress was induced. In June, the NIR was 154 L (n1) and 126 L (n2f1 and n2f2), and in July it was 128 L (n1), 120 L (n2f1) and 98 L (n2f2). SMS responded to the alternating dry and wet phases of the soil across all research treatments, with the most rapid response recorded by the TDR300 and AT sensors.

a) b) c) d)

Figure 1. Soil moisture dynamics measured by a) AT, b) Watermark, c) Tensiometer, d) TDR300, irrigation rate (dcl) under the n1 (control treatment), n2f1 (water stress at flowering stage), and n2f2 (water stress at grain-filling stage) treatments.

Grafikon 1. Dinamika vlažnosti tla mjerena s pomoću a) AT-a, b) Watermarka, c) Tensiometera, d) TDR300, obroci navodnjavanja (dcl) na n1 (kontrolni tretman), n2f1 (zasušivanje u fazi cvatnje) i n2f2 (zasušivanje u fazi nalijevanja zrna).

More consistent results under optimal moisture conditions (n1) were recorded with the Tensiometer and Watermark sensors, which aligns with the findings of Yadav et al. (2016). In this study, a slower reaction of the tensiometer was observed under the water stress treatment (n2), which contradicts the findings of Hanson et al. (2000), who reported a slower response time for Watermark sensors compared to Tensiometers. According to the authors, possible factors contributing to these differences include a lag in the response of the Watermark block to changes in soil moisture content, small-scale spatial variability in soil texture and infiltrated water between instrument locations, and differences in the response characteristics among a given set of instruments.

Two-way ANOVA and LSD test were used to compare the differences between leaf relative chlorophyll content (LRCC) and chosen chlorophyll a fluorescence parameters for plants in control (n1) and water deficit conditions (n2) in flowering (f1) and in grain filling (f2) separately (Table 2).

Table 2. ANOVA for leaf relative chlorophyll content (LRCC), maximum quantum yield of photosystem II (TR_0/ABS), density of active reaction centers (RC/CS_0), photosynthetic performance index (PI_{ABS}) and performance index for energy conservation from exciton to the reduction of PSI end electron acceptors (PI_{TOTAL}) determined for soybean variety OS Nevena in optimal soil water content (n1) and water stress (n2) conditions. Measurements were taken during four days (D1–D4) in flowering (f1) and grain filling (f2) stages.

Tablica 2. ANOVA za relativni sadržaj klorofila u listu (LRCC), maksimalni kvantni prinos fotosustava II (TR_0/ABS), gustoću aktivnih reakcijskih centara (RC/CS_0), indeks fotosintetske učinkovitosti na bazi apsorpcije (PI_{ABS}) i indeks fotosintetske učinkovitosti pretvorbe energije od ekscitona do redukcije krajnjega akceptora elektrona na PSI (PI_{TOTAL}) za sortu soje OS Nevena u uvjetima optimalnoga sadržaja vode u tlu (n1) i vodnoga stresa (n2). Mjerenja su provedena tijekom četiri dana (D1–D4) u fazama cvatnje (f1) i nalijevanja zrna (f2).

Sources of variation / *Izvori varijabilnosti*	df	LRCC		TR_0/ABS		RC/CSO		PI_{ABS}		PI_{TOTAL}		RC/CS_0	
		f1	f2	f1	f2	f1	f2	f1	f2	f1	f2	f1	f2
Treatment (n) / *Tretman (n)*	1	**	**	**	**	**	**	**	**	ns	**	**	**
Days of measurement (d) / *Dani mjerenja (d)*	3	**	**	**	**	**	**	**	**	**	**	**	**
Interaction (n x d) / *Interakcija (n x d)*	3	**	**	**	**	ns	**	ns	**	ns	**	ns	**

** - Highly significant; ns - Non-significant ($p < 0.01$) –** - visoko značajno, ns – nije značajno ($p < 0,01$)

As expected, all parameters had higher average values in control (n1) than water stress treatment (n2), except TR_0/ABS in f1 (Figure 2b). The differences between treatments for LRCC increased with the measurement days (Figure 2a), but in the flowering stage, the greatest difference was observed on the third day. The results indicated that LRCC was sensitive enough to show differences between treatments in both investigated developmental stages from day two of the onset of water withholding. The negative impact of drought on LRCC observed in this study has been established in numerous previous studies (Chowdhury et al., 2017; Dong et al., 2019; Iqbal et al., 2019). Although there were some differences in f1 between individual n1 and n2 TR_0/ABS values per day, they were not statistically significant (Figure 2b). In f2, the significant differences between individual n1 and n2 TR_0/ABS values occurred on day three (D3; Figure 2b). Although TR_0/ABS is one of the most commonly used parameters for determining the impact of environmental stressors on photosynthetic activity (Kalaji et al., 2016), numerous studies show that it is not a good choice for monitoring early symptoms of drought stress (Woolery et al., 2010; Markulj Kulundžić et al., 2022). This explains the fact that statistically significant differences between n1 and n2 TR_0/ABS occurred on the third day of measurements in f2. In f1, n2 RC/CS_0 values were statistically lower compared to n1 from day two (D2; Figure 2c). In f2, individual n2 RC/CS_0 values were statistically lower compared to n1 values on the third (D3) and fourth day (D4). PI_{ABS} had significantly lower values in n2 compared to n1 only on D3 in f1 (Figure 2d), while there were no differences between treatment values in f1 for PI_{TOTAL} (Figure 2e). However, there were significant differences between n1 and n2 PI_{ABS} and PI_{TOTAL} for all days of measurement in f2 (Figure 2d and 2e, respectively). The difference increased with days of measurement (Figures 2d and 2e, respectively). A similar impact of water stress was confirmed by Umar and Siddiqui (2018) in sunflower and by Markulj Kulundžić et al. (2022) in soybean. In this study, the lack of differences in PI_{ABS} and PI_{TOTAL} values during the flowering stage indicates that the tested soybean plants can withstand moisture deficiency at this developmental phase, whereas they exhibit higher sensitivity during grain filling. In the research conducted by Matoša Kočar et al. (2021), PI_{TOTAL} was identified as the most sensitive chlorophyll *a* fluorescence parameter for detecting the average effect of drought across five developmental stages of soybean (V2, R1, R4, R5, and R6). The authors further state that this parameter is sufficiently informative to exclude materials with the poorest photosynthetic apparatus functionality in breeding for abiotic stress tolerance, thereby increasing the efficiency of the selection process when evaluating a large number of genotypes.

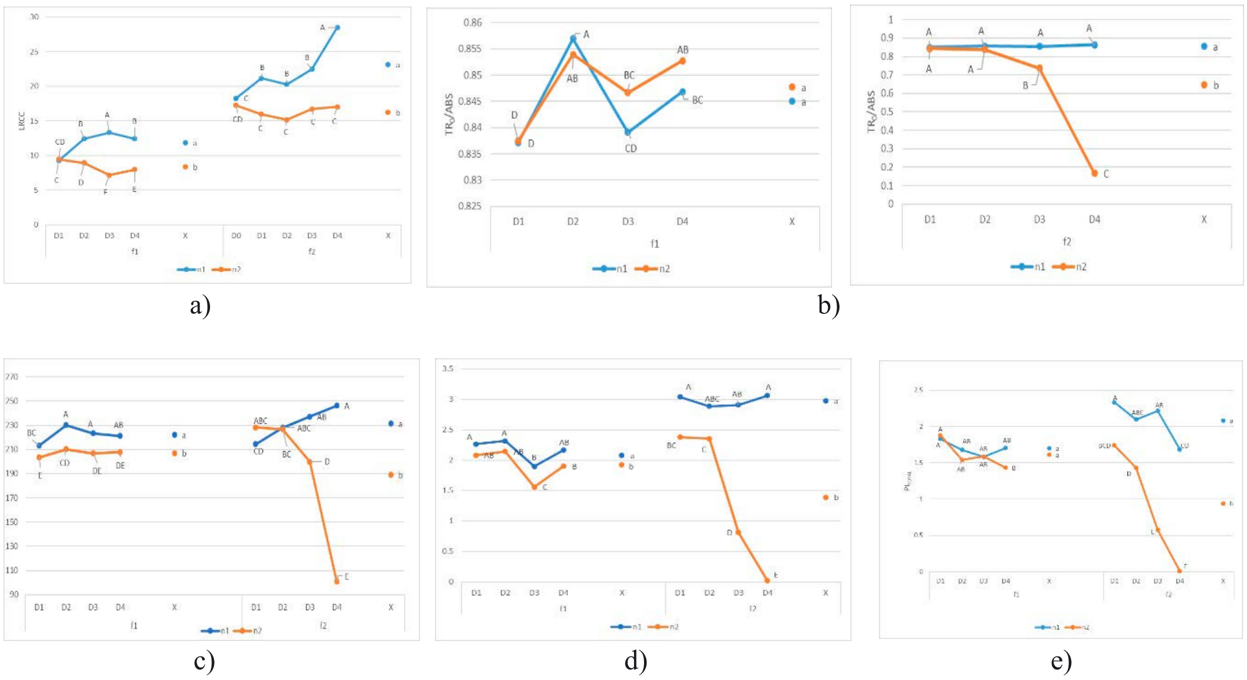

Figure 2. a) Leaf relative chlorophyll content (LRCC); b) Maximum quantum yield of photosystem II (TR_0/ABS); c) Density of active reaction centers (RC/CS_0); d) Photosynthetic performance index (PI_{ABS}); and e) Performance index for energy conservation from exciton to the reduction of PSI end electron acceptors (PI_{TOTAL}) determined for soybean variety OS Nevena in optimal soil water content (n1) and water stress (n2) conditions. Measurements were taken during four days (D1–D4) in flowering (f1) and grain filling (f2) stages. Markings with the same capital letter are not significantly different from each other. Markings with the same lower-case letter are not significantly different from each other.

Grafikon 2. a) Relativan sadržaj klorofila u listu (LRCC); b) Maksimalan kvantni prinos fotosustava II (TR_0/ABS); c) Gustoća aktivnih reakcijskih centara (RC/CS_0); d) Indeks fotosintetske učinkovitosti na bazi apsorpcije (PI_{ABS}); i e) Indeks fotosintetske učinkovitosti pretvorbe energije od ekscitona do redukcije krajnjega akceptora elektrona na PSI (PI_{TOTAL}) za sortu soje OS Nevena u uvjetima optimalnoga sadržaja vode u tlu (n1) i vodnoga stresa (n2). Mjerenja su provedena tijekom četiri dana (D1–D4) u fazama cvatnje (f1) i nalijevanja zrna (f2). Oznake s istim velikim slovom nisu statistički značajno različite međusobno. Oznake s istim malim slovom nisu statistički značajno različite međusobno.

The dataset contained 1,099 measurements, evenly distributed between the two classes, with 549 samples in class "0" (no water stress) and 550 samples in class -1 (water stress). The data were divided using the standard 70/30 hold-out method; 770 instances (70%) were used to train the models, while the remaining 329 instances (30%) were used for evaluating how well the models perform on new, unseen data. Four algorithms (SVM, kNN, Logistic Regression, and the Simple Neural Network) were evaluated on the same training and test partitions; the observed performance differences reflect the algorithms themselves and no variation in the data split. All four models yielded very high AUC values, indicating robust class separation. The kNN and Neural Network models achieved the highest AUC values. Regarding the CA metric, the kNN model achieved the best performance. In terms of the F1-score, which accounts for both precision and recall, the kNN model again demonstrated the superior value. Furthermore, the kNN and Neural Network models achieved the highest values for precision (0.926 and 0.899, respectively) and recall (0.921 for kNN and 0.897 for the Neural Network). The performance of the models on the test dataset varied significantly compared to their performance on the training set. The kNN model achieved high performance with an AUC value of 0.994 and a precision of 0.959 on the test set. Conversely, the SVM model exhibited lower performance relative to the other models, with an AUC value of 0.821 and a precision of 0.794 (Table 3).

Table 3. Machine learning models and results on the test dataset

Tablica 3. Modeli strojnoga učenja i rezultati na testnome skupu

Model / Model	AUC	CA	F1	Precision / Preciznost	Recall / Osjetljivost
kNN	0.9998	0.9922	0.9922	0.9923	0.9922
SVM	0.8435	0.6844	0.6618	0.7670	0.6844
Neutral Network	0.9882	0.9376	0.9376	0.9381	0.9376
Logistic Regression	0.8730	0.8090	0.8089	0.8092	0.8090
Results on the test dataset/ Rezultati na testnom skupu					
kNN	0.9940	0.9574	0.9574	0.9593	0.9574
SVM	0.8210	0.7021	0.6674	0.7940	0.7021
Neutral Network	0.9787	0.8966	0.8959	0.9007	0.8966
Logistic Regression	0.8377	0.8085	0.8081	0.8084	0.8085

CONCLUSION

The results of this research highlight the critical importance of selecting the most sensitive measurement methods to be included in the integrative detection of water stress levels in soybeans in critical stages of development, i.e. flowering and grain filling. The most rapid response in water deficit conditions was observed in sensors measuring volumetric soil water content (TDR300 and AT), indicating their suitability for early detection of soil water depletion. The best option among physiological parameters for detecting plants' response to water deficit was LRCC and RC/CS_0 in flowering, and PI_{ABS} and PI_{TOTAL} in grain filling, as they were the most sensitive from the early onset of water withholding. The k-Nearest Neighbors (kNN) model proved to be the most effective method for water stress classification, demonstrating superior efficiency in identifying nearest neighbors within the training dataset. The successful integration of highly sensitive physiological indicators with accurate machine learning classification offers great value for agricultural practice. Relying solely on soil moisture sensors and a traditional approach can sometimes result in a delayed response to drought conditions. By continuously monitoring the plant's physiological changes and utilizing a highly reliable algorithm like kNN to classify the data, producers can detect the early onset of water stress before irreversible damage occurs. The next step in the study would be testing the methods in field conditions and using the most informative ones to be incorporated into future integrative frameworks for precise and timely water stress monitoring in soybean.

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USPOREDBA RAZLIČITIH METODA PRAĆENJA VODNOGA STRESA KOD SOJE (*Glycine max* (L.) MERR.)

SAŽETAK

Istraživanje je provedeno u zaštićenome prostoru na Poljoprivrednome institutu Osijek 2022. godine. Cilj istraživanja bio je procijeniti i usporediti različite metode za otkrivanje deficita vode u tlu i odgovora biljaka na vodni stres kod soje (Glycine max (L.) Merr.). Proučavane su različite metode za praćenje vodnoga stresa kod soje, uključujući senzore za mjerenje sadržaja vode u tlu, fiziološke pokazatelje, statističke modele i strojno učenje. Dva tretmana navodnjavanja uključivala su optimalan sadržaj vode u tlu (kontrolni tretman, n1, 100 % retencijskoga kapaciteta tla za vodu, Rkv) i vodni stres (n2, 50 % Rkv) primijenjen u fazama cvatnje (f1) i nalijevanja zrna (f2). TDR300 i AT senzori imali su najbržu reakciju na promjene sadržaja vode u tlu (% vol.), što ukazuje na visoku osjetljivost na rani deficit vode. Među fiziološkim pokazateljima, LRCC i RC/CS_o bili su najosjetljiviji tijekom cvatnje, dok su PI_{ABS} i PI_{TOTAL} pokazali najveću osjetljivost tijekom nalijevanja zrna. Model kNN pokazao je najbolje performanse, s visokom točnošću klasifikacije (CA = 0,921) i AUC-a (0,976). Rezultati naglašavaju važnost odabira specifičnih pokazatelja za detekciju vodnoga stresa i pružaju osnovu za razvoj budućih integrativnih okvira praćenja u proizvodnji soje.

Ključne riječi: soja, vodni stres, senzori za mjerenje vlažnosti tla, fiziološki pokazatelji, modeli strojnog učenja

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