

Using an NDVI Sensor to Assess the Forage Yield Potential of Red Clover Germplasm

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ABSTRACT

Red clover (*Trifolium pratense* L.) is one of the most important perennial forage legumes in grassland agroecosystems worldwide. Due to its global utilization and high yielding potential, incorporating modern technologies into the selection process is essential to accelerate breeding programs. Therefore, the objectives of this research were to evaluate the possibilities of early estimation of forage biomass yield using a GreenSeeker handheld crop sensor and to analyze the relationship between the Normalized Difference Vegetation Index (NDVI) and yield parameters. The study was conducted during two consecutive years in Croatia, using 22 red clover cultivars and populations of different geographical origin. NDVI measurements were conducted in late spring at the early vegetative stage of the first cut and compared with harvested green forage and dry matter yields from the first cut and total annual yields. The results revealed significant variation among the investigated germplasm, with PCD exp 6, PCD exp 4, PCD exp 1, and CRO STAR achieving the highest NDVI values and biomass yields. Average values for all investigated traits were significantly higher in the second year compared to the first growing season. NDVI showed strong positive correlations with all yield-related traits ($r = 0.857-0.868$), explaining a substantial proportion of biomass variability. These findings confirm the GreenSeeker sensor as a reliable, non-destructive tool for early identification of superior genotypes and support its use as an additional selection criterion to improve forage breeding.

Keywords: red clover, vegetation index, forage yield, variation, breeding.

INTRODUCTION

Red clover (*Trifolium pratense* L.) is one of the most important perennial forage legumes in grassland agroecosystems worldwide. It is grown in temperate areas of the world, in monoculture or, more frequently as a mixture in combination with grasses such as timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* Huds.), tall fescue (*Festuca arundinacea* Schreb.), and/or perennial ryegrass (*Lolium perenne* L.) for hay, haylage, silage, grazing, or as green manure (Vleugels et al., 2019; Ergon and Bakken, 2022; Radinović et al., 2022; Nay et al., 2023). Red clover is a short-term perennial legume that grows for about two to three years and is adapted to a wide range of

climatic conditions, soil types, fertility levels, use patterns, and management strategies (Tucak et al., 2019). It has a high yielding potential, and cultivars grown in monoculture under humid climatic conditions can achieve in the second season over 80 t ha⁻¹ of green mass yield and 15 t ha⁻¹ of dry matter yield (Tucak et al., 2016).

In addition to its agronomic and nutritional value, red clover is characterized by its numerous beneficial environmental effects in terms of soil fertility preservation and reduction of groundwater nitrate pollution due to decreased inorganic fertilizer use, preservation of biodiversity, soil erosion protection, mitigation of climate change impacts, fossil fuel consumption and greenhouse gas emissions (Berti et al., 2021; Tucak et al., 2021; Osterman

et al., 2022; Dlamini et al., 2024; Purwin et al., 2024; Valujeva et al., 2025).

Biomass yield production is a primary trait of interest to agricultural producers utilizing red clover. Therefore, the major focus of most red clover breeding programs and accompanying research is increasing forage yield as well as persistence (individual plant longevity) because of its association with general adaptability and yield (Taylor, 2008; Riday, 2010; Inostroza et al., 2020). Biomass yield is a highly quantitative trait and is subject to extensive genotype-by-environment interactions, making field observations over multiple harvests and years essential for its improvement (Riday, 2009). Estimated genetic rates of gain in red clover are around 0.21 to 1.39% per year (Riday, 2010). Breeding schemes are mainly based on recurrent phenotypic selection, either to directly develop open-pollinated cultivars through population improvement or to select suitable parental plants for mutual intermating (polycrossing) and the development of synthetic cultivars (Nay et al., 2023; Caradus and Chapman, 2025). Also, in recent times, the methods and goals of breeding have been gradually changing due to the increasing use of red clover in the food, pharmaceutical, and cosmetics industries as a valuable source of bioactive compounds (Petrauskas et al., 2023; Mikulić et al., 2024).

Red clover selection to increase forage yield includes evaluation and observation, usually of a few thousand of individual spaced plants in breeding nurseries, and testing experimental populations in a larger number of cuts in one growth season over several years. Phenotypic characterization in forage legume breeding can be time-consuming, very labor- and resource-intensive (Sindic and Riday, 2020). Due to red clover's global utilization in many different types of agricultural situations, its hectareage is on a smaller scale than that of major cash commodity crops (Riday, 2023). Therefore, compared to commodity crops, clover breeding programs tend to be smaller and have limited resources, and incorporating new modern technologies and tools in the selection process could significantly contribute to the

acceleration, improvement and sustainability of these programs. Tedesco et al. (2022) emphasized that remote sensing technologies can significantly contribute to obtaining production and quality insights, providing scalability, and supporting complex decision-making in farming.

The Normalized Difference Vegetation Index (NDVI) is one of the oldest and most widely used indices for monitoring vegetation, and it has been widely applied in numerous scientific studies on different species due to its ease of use and its ability to quickly provide information on crops, such as nitrogen levels, moisture stress, grain yield, biomass yield, and other agronomic traits (Andersson et al., 2017; Naser et al., 2020; Kimaro et al., 2023; Lima et al., 2024; Hammad et al., 2025; Petrović et al., 2025; Tucak et al., 2026). Vegetation indices (especially NDVI) are closely correlated with leaf area and photosynthetic activity, and are able to identify plant growing conditions, physiological state, and yield potential (Klimek-Kopyra et al., 2018). This method provides valuable information to plant breeders and can be a very useful tool as an additional selection criterion in early yield prediction and identification of desirable genotypes and/or populations for further breeding process.

Therefore, the objectives of this research were to present the possibilities of early estimation of the forage biomass yield of 22 red clover cultivars/populations of different origin by measuring NDVI using a GreenSeeker handheld crop sensor and to analyze the relationship between the observed parameters.

MATERIAL AND METHODS

Selection of red clover germplasm

A total of twenty-two red clover cultivars and populations of different geographical origins were included in this research. Six experimental breeding populations (PCD exp 1 - 6), four local populations (LPCD 1 - 4), and two recognized cultivars, OS VIVA and CRO STAR, were created within the framework of the perennial legume breeding program on the selection experimental fields of the Agricultural Institute Osijek, Croatia

(Figure 1). Seeds of additional red clover materials were obtained from the various gene banks, breeding companies, and seed companies: *Margot Forde* Genebank, New Zealand (cultivars *Grasslands Sensation*, *Relish*, *Scarlett II*, and *Primus*), Genebank Czech Republic (cultivars *Ottawa* and *RAM*), Polish Genebank - NCPGR (cultivar *Start*), Deutsche Saatveredelung AG - DSV (cultivars *Milvus* and *Temara*), Saatzucht Steinach GmbH & Co. KG, Germany (cultivar *Lucrum*). All studied red clover materials are diploid, except for the cultivar *Temara* which is tetraploid.

Study site characteristics

The field experiment was carried out at the Agricultural Institute Osijek, situated in Osijek, eastern Croatia (Juzno predgradje 17, 31000 Osijek; 45°32'25.82" N, 18°44'12.00" E), at an altitude of approximately 90 m above sea level. Eastern Croatia belongs to the temperate continental climatic zone. According to long-term meteorological data covering the period from 1899 to 2024, the region is characterized by a mean annual air temperature of 11.18°C and an average annual precipitation of 692.7 mm. Weather conditions during the years of the experiment differed noticeably from the long-term averages. In 2024, the mean annual temperature was higher, reaching 14.29°C, while total annual precipitation amounted to 632.0 mm. Likewise, in 2025, the mean annual temperature was 13.09°C, and total precipitation decreased to 601.7 mm (Croatian Meteorological and Hydrological Service, 2026). Overall, both experimental years were warmer and drier than the established climatic norm for the region. The experimental area is dominated by eutric brown soil, classified as an eutric Cambisol. A soil analysis conducted in 2024 by the Croatian Agency for Agriculture and Food indicated a light sandy texture and a slightly acidic soil reaction (pH in H₂O = 6.1). The soil was characterized by a low humus content (1.75%), was well supplied with phosphorus (P₂O₅ = 14.70 mg kg⁻¹), and richly supplied with potassium (K₂O = 25.90 mg kg⁻¹).

Experimental design and field data collection

The field trial was conducted during the two consecutive years, 2024 and 2025, in the first and second red clover growing seasons. The experimental design was laid out in a randomized complete block with three replications. Each plot consisted of six rows, 0.20 m apart, and each row was 6 m long. The total area of the experimental plot was 7.2 m². Sowing of red clover was carried out manually at a depth of 1.5 cm, with a seeding rate of 15 kg ha⁻¹ on 10 March 2024. No chemical fertilizers, pesticides, or irrigation were applied at the trial site. Hand weeding was carried out when necessary, during the course of the experiment.

GreenSeeker Handheld Optical NDVI sensor data collection

Optical sensor readings were taken with a handheld GreenSeeker sensor (Trimble Inc., Sunnyvale, CA, USA), which measures canopy reflectance at specific bands in the red (670 nm) and near-infrared (780 nm) spectral regions and displays the Normalized Difference Vegetation Index (NDVI) data. NDVI measurements were conducted in late spring at early vegetative stage of red clover in the first cut in both years of growth (12 May 2024 and 18 May 2025) in all plots of all replicates for all studied red clover cultivars and populations. A detailed description of the data collection methodology and guidelines for using the NDVI sensor applied in this research are shown in our previous paper (Tucak et al., 2026).

Forage biomass yield data collection

Following NDVI measurements, green forage and dry matter yields were determined in both the first and second year of the study. All red clover experimental plots were harvested three times in 2024 and four times in 2025 at the flowering stage using a forage plot harvester (Hege Model 212, Wintersteiger AG, Waldenburg, Germany). Prior to each cut, subsamples of approximately 500 g of green mass were taken from the middle of each plot, weighed

fresh, dried in a dryer at 105°C for 48 h, and weighed dry to determine dry matter content in order to calculate dry matter yield. Data recorded per plot for first and other cuts were converted into tons per hectare ($t\ ha^{-1}$) to determine green forage and dry matter yields for first cuts, as well as total yields obtained by summing all cuts per year.

Statistical analyses

The analysis of the collected experimental data was processed using a two-factorial analysis of variance (ANOVA) with population and year as factors, using the

STAR v. 2.0.1 software (IRRI, 2013). Fisher's protected LSD test was used at the 0.05 and 0.01 probability levels to identify significant differences between the mean values of populations and years. Phenotypic correlations between the studied traits were calculated as Pearson's correlation coefficients, and the significance of the relationships was determined using the aforementioned statistical software. To visualize the relationships among all parameters evaluated in the present study, a correlation heat map was generated using R software version 4.3.2 (R Core Team, 2023).

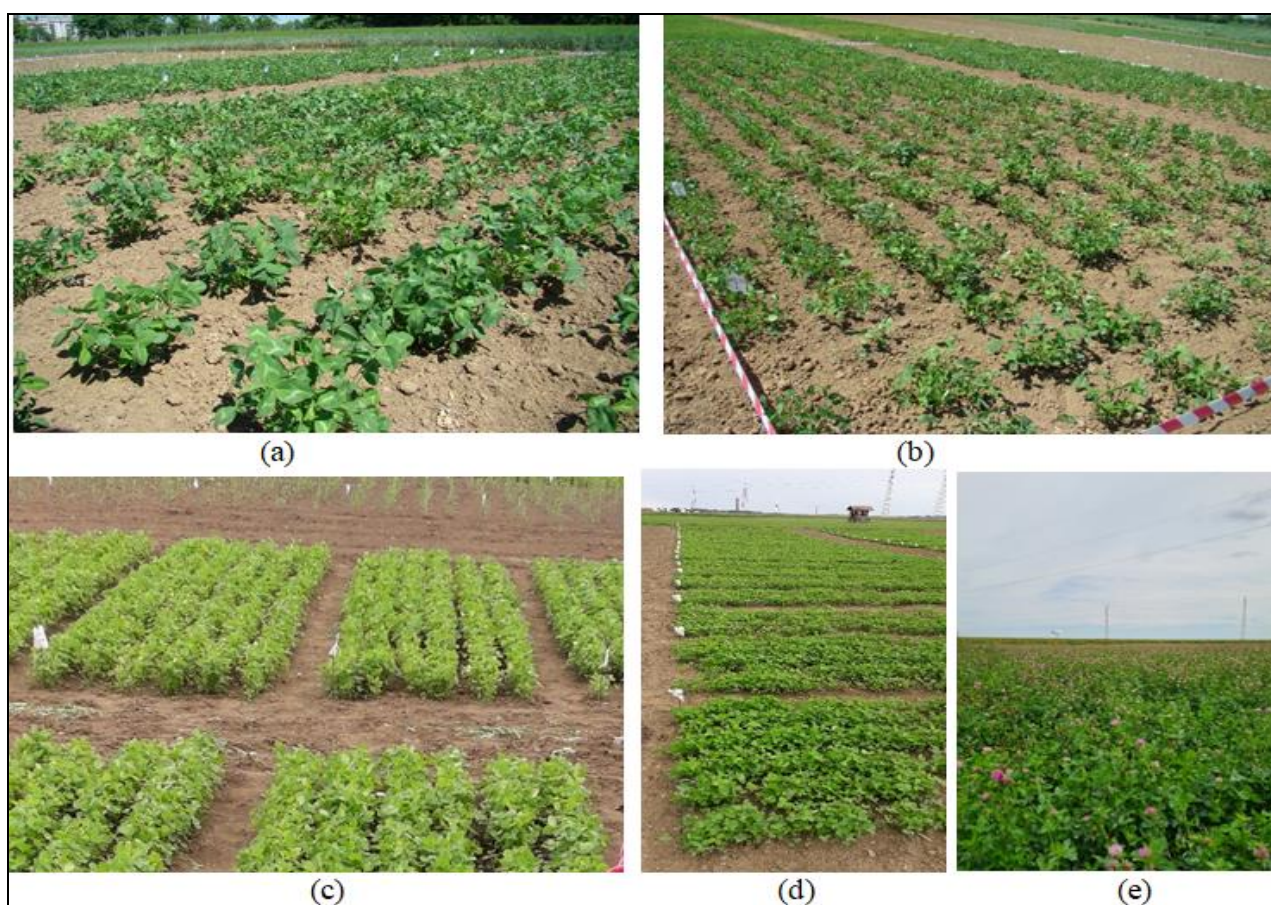


Figure 1. Types of red clover field plots used in the perennial legume breeding program at the Agricultural Institute Osijek: (a) and (b) spaced-plant red clover breeding nursery, (c) and (d) mini sward plots, (e) large sown sward plots of red clover in the phenological stage of full bloom

RESULTS AND DISCUSSION

Analysis of variance revealed statistically significant differences among the red clover cultivars/populations for all investigated

traits, except for dry matter yield in the first cut. Also, a significant influence of year and the population \times year interaction was found in the investigated red clover materials for all tested traits (Table 1).

Table 1. Results of two-way ANOVA for analyzed traits of 22 red clover cultivars/ populations

Source of Variation	DF	NDVI		GFY FC		DMY FC		TY GF		TY DM	
		MS	FV	MS	FV	MS	FV	MS	FV	MS	FV
Repetition	2	0.006	0.77 ns	9.87	1.63 ns	0.53	1.48 ns	21.54	4.89 ns	0.80	4.72 ns
Year (Y)	1	0.696	87.97*	39015	6472**	1613	4467**	58859	13367**	1540	9017**
Error year	2	0.007		6.02		0.36		4.40		0.17	
Population (P)	21	0.005	1.46*	53.64	3.28**	2.30	1.51 ns	414.41	5.60**	9.91	2.42*
Y X P	21	0.003	3.49**	16.32	3.55**	1.52	7.50**	73.93	3.24**	4.08	4.57**
Error	84	0.001		4.58		0.20		22.80		0.89	
Total	131	0.007		312.22		13.07		542.61		14.58	

DF - Degrees of freedom; MS - Mean square; FV - F values; * Significant at $p < 0.05$; ** Significant at $p < 0.01$; ns - not significant; GFY FC - Green forage yield in the first cut; DMY FC - Dry matter yield in the first cut; TY GF - Total yield of green forage; TY DM - Total yield of dry matter.

The average NDVI value of all red clover cultivars/populations at the early vegetative stage of the first cut was 0.32 (Table 2).

Table 2. Average values of normalized difference vegetation index (NDVI), yields of green forage and dry matter in the first cut (GFY FC and DMY FC), total yields of green forage and dry matter (TY GF and TY DM) of the investigated red clover germplasm during the two experimental years (2024, 2025)

Cultivar/ population	Origin	NDVI	GFY FC (t ha ⁻¹)	DMY FC (t ha ⁻¹)	TY GF (t ha ⁻¹)	TY DM (t ha ⁻¹)
PCD exp 1	CRO	0.36	30.99	6.42	68.48	12.90
PCD exp 2	CRO	0.34	29.07	5.71	65.75	12.46
PCD exp 3	CRO	0.34	27.71	6.38	60.53	12.66
Grass. Sens.	NZL	0.34	29.09	6.38	61.85	12.55
PCD exp 4	CRO	0.35	31.02	6.46	67.49	13.55
OS VIVA	CRO	0.34	28.98	5.97	66.07	12.70
Relish	NZL	0.30	29.20	6.32	64.28	12.43
PCD exp 5	CRO	0.32	28.38	6.10	65.90	12.76
Scarlett II	USA	0.29	26.54	5.52	55.44	10.89
PCD exp 6	CRO	0.36	33.56	6.75	75.07	14.12
CRO STAR	CRO	0.35	30.24	6.46	66.97	12.84
Ottawa	CAN	0.28	23.78	4.55	52.44	9.94
Start	CZE	0.32	24.76	4.99	54.61	10.59
RAM	CAN	0.28	27.30	4.56	55.70	9.81
LPCD 1	CRO	0.34	26.14	5.53	54.78	11.02
Temara	GER	0.31	28.32	5.58	65.14	12.02
Luertum	GER	0.33	30.10	5.78	65.98	12.69
Milvus	GER	0.28	29.82	5.81	65.05	12.11
Primus	BEL	0.27	27.54	5.92	57.26	12.09
LPCD 2	CRO	0.32	27.47	5.88	60.60	12.41
LPCD 3	CRO	0.26	21.51	5.00	42.51	9.60
LPCD 4	CRO	0.31	21.33	5.37	40.69	9.86
Average		0.32	27.86	5.79	60.57	11.91
LSD 0.05		0.05	2.458	ns	5.483	1.084
LSD 0.01			3.259	ns	7.627	

Over the growing season, the highest NDVI value was determined in the populations PCD exp 1 and PCD exp 6 (0.36), which was not statistically significantly higher than the values recorded in the PCD exp 4 and cultivar CRO STAR (Table 2). The lowest NDVI value was

measured in the local population LPCD 3 and the cultivar Primus. According to the results of this paper, there was significant variation among populations/cultivars in NDVI values at the studied growth stage of red clover. These results confirm the findings of previous research on different crops, in

various studies, where NDVI showed high levels of genotypic variability, whether measured in single or multiple different developmental plant stages (Klimek-Kopyra et al., 2018; Inostroza et al., 2021; Lemma et al., 2022; Yildirim et al., 2024; Nazeer and Akram, 2025; Tucak et al., 2026). Hazratkulova et al. (2012) examined NDVI variation over a two-year period at two locations, across four different plant growth stages in an elite set of thirty winter wheat germplasm, which included breeding lines, commercial cultivars, and accessions, and its relationship with grain yield, and they detected the presence of genotypic variability for NDVI at each of the observed growth stages. Mirosavljević et al. (2018) reported significant variation in NDVI values and positive relationship of NDVI with the most important agronomic properties in elite winter barley genotypes, and they indicated that NDVI measurements at the anthesis growth stage could be efficiently used for the indirect identification of productive winter barley genotypes.

Population PCD exp 6 achieved the highest average yields of green forage and dry matter in the first cut (33.56 and 6.75 t ha⁻¹), as well as total biomass yields (75.07 and 14.12 t ha⁻¹), as shown in Table 2. This population had 16% to 23% higher forage yields compared to the average values of all observed red clover cultivars/populations, which indicates its high genetic potential for yield and characterizes it as elite breeding material. High average yields of green forage and dry matter in the first cut and total biomass yields were recorded in populations PCD exp 4 and PCD exp 1 and in the cultivar CRO STAR. In most of the observed

traits, the significantly lowest values were determined in local populations LPCD 4 and LPCD 3, and in the cultivar Ottawa.

Red clover materials that were superior in forage yield had a high association with the highest NDVI values, not only in the first cut when measurements were taken but also in the total annual yield. This result indicates the possibility of using NDVI for identification and selection of desirable red clover germplasm already at a very early stage of crop development, which is in perennial legume species a very valuable and useful tool for the improvement of forage yield in breeding processes. Previous research by Kaur et al. (2025) also showed that the early-season crop reflectance sensing proved more reliable for prediction accuracy of yield and plant height due to the lower impact of senescent leaves.

The average annual values of all investigated traits of the red clover cultivars and populations were statistically significantly higher in the second year compared to the first growth season (Figure 2). NDVI values were higher by 35%, and yields of green forage and dry matter obtained in the first cut of the second growth season were approximately 75% higher compared to the values in the first cut of the establishment year of red clover. A similar trend was observed for total forage yields. The first cut of the season is the highest yielding for red clover, especially in the second year of cultivation, when the root system is well developed and penetrates deep into the soil, the root crown is wide and a larger number of shoots develop, and if there is enough moisture in the spring, the first cut has a significant share in the total forage yield.

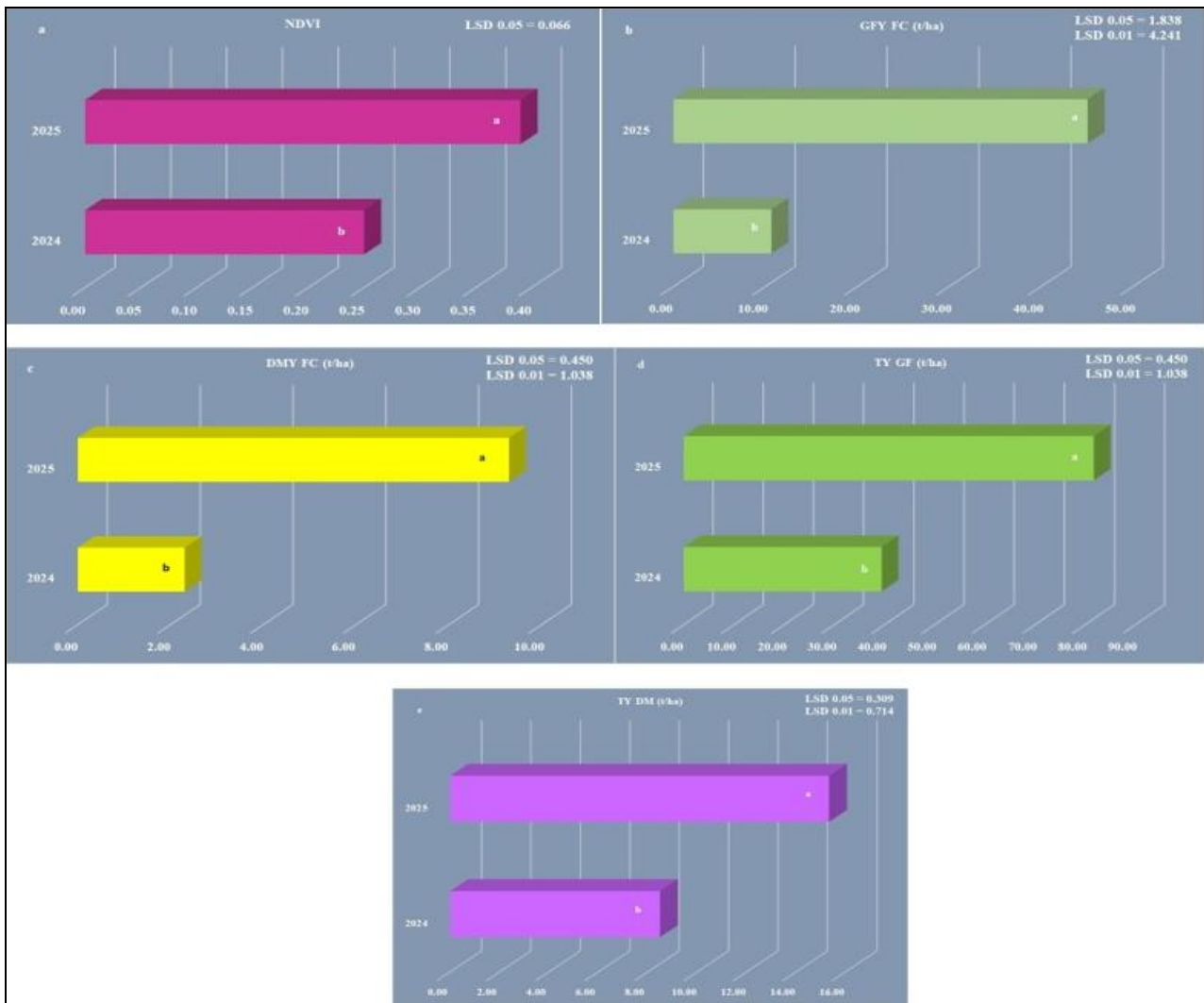


Figure 2. Average annual values for the normalized difference vegetation index (NDVI, a), yields of green forage and dry matter in the first cut (GFY FC and DMY FC, b and c), total yields of green forage and dry matter (TY GF and TY DM, d and e). Different letters indicate significant difference between years at $p < 0.05$ according to the LSD test.

To determine the relationships among all measured parameters, the Pearson correlation coefficient was calculated, and the results were visualized using a heat map in which color intensity represents the strength and direction of the correlation (Figure 3). The heat map illustrates the interrelationships among the investigated traits and reveals strong positive correlations among all evaluated parameters ($r = 0.857-0.987$), indicating a high level of trait integration. The highest correlation was observed between GFY FC and DMY FC ($r = 0.987$), indicating a linear relationship. Likewise, TY GF and TY DM were strongly correlated ($r = 0.969$), while DMY FC also showed very high correlations with TY DM ($r = 0.964$) and TY GF ($r = 0.949$), confirming the close association between fresh and dry biomass components.

NDVI exhibited strong positive correlations with all yield-related traits ($r = 0.857-0.868$), with the strongest association recorded with TY GF ($r = 0.868$). The corresponding coefficients of determination ($R^2 = 0.734-0.754$) indicate that NDVI accounted for approximately 73-75% of the variability in yield-related traits. Comparable magnitudes have been reported in maize by Hammad et al. (2025), where R^2 ranged from 0.67 to 0.97 ($r \approx 0.82-0.98$). Similarly, Kimaro et al. (2023) reported significant correlations between NDVI and rice grain yield ranging from $r = 0.82$ to $r = 0.94$ depending on growth stage and sensor height. Together, these results support the use of NDVI measured with the GreenSeeker sensor as a reliable, non-destructive indicator of biomass productivity across different crop species.



Figure 3. Heat map illustrating Pearson correlation coefficients (r) among the studied agronomic parameters (NDVI, GFY FC, DMY FC, TY GF, and TY DM). Colour intensity represents the strength of the correlation. See the description of the analysed traits in the title of the previous table or figure.

CONCLUSIONS

Significant variation was observed among the investigated red clover cultivars and populations for all traits, with the exception of dry matter yield in the first cut. Both year and population \times year interaction had a significant effect on all measured traits. The identified superior forage high-yielding populations PCD exp 6, 4 and 1 also had the highest NDVI values. NDVI showed a stronger positive association with forage biomass yields across the different sets of red clover populations/cultivars. The outcomes of this study indicate the potential for the use of NDVI sensor during the early vegetative stages for efficient screening promising germplasm in the field experiment based on their forage productivity. Given the simplicity, relatively low cost, and rapid data acquisition of NDVI sensors, this approach represents a valuable tool for advancing forage legume breeding programs.

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