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How good are vegetation indices to assess water status and biochemical parameters in olive tree?

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Abstract: As the water is an increasingly scarce resource, the adoption of deficit irrigation (DI) strategies has become essential to optimize its use in agriculture. To implement DI, it is important to monitor the leaf water status during crop development by indicators such as Relative Water Content (RWC) and Leaf Water Potential (LWP) to optimize the crop production. However, these methods are destructive and time-consuming. Many relationships between spectral data from remote sensing observations and various biophysical and physiological crops parameters have been proposed, in which, Vegetation Indices (VIs) are widely used. This study aims to evaluate the relationship between VIs with leaf water status (RWC, LWP) and biochemical parameters, in a drip irrigated olive orchard (cv. Cobrançosa), located in the Northeast of Portugal (Alfândega da Fé). Five irrigation strategies were studied: full irrigated (FI), that received a volume of water equivalent to satisfy crop water needs, FI_{120} irrigated with 20% more than FI, two sustained deficit irrigation (SDI_{60} and SDI_{30}) and farmer-managed irrigation (FMI). A list of 20 VIs was calculated and correlated with RWC, LWP and biochemical parameters. Although no good correlations were found between VIs and total polyphenols, a good agreement were found between: Photochemical Reflectance Index (PRI) and ortho-diphenols (R^2=0.64); Green Normalized Difference Vegetation Index (GNDVI) and proline (R^2=0.74); Normalized Difference Greenness Vegetation Index (NDGI) and glucose (R^2=0.95); Transformed Chlorophyll Absorption Reflectance Index (TCARI) and LWP (R^2=0.71); TCARI divided by Optimized Soil Adjusted Index (OSAVI) and RWC (R^2=0.77). Thus, VIs appears as a useful tool to evaluate leaf water status and biochemical parameters from olive trees.

Keywords: relative water content; leaf water potential; spectral indices; proline; ortho-diphenols; glucose

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1. Introduction

Water is an essential and increasingly scarce resource for maintaining an adequate food supply and a productive environment for the human population in which, nowadays, agriculture consumes about 70% of fresh water worldwide [1]. Thus, in a global warming scenario, irrigation management is important for the optimization of water use in agriculture [2]. One way to mitigate this scarcity process consists on improvement of agriculture irrigation practices for efficient irrigation water management by adopting deficit irrigation strategies to maximize irrigation efficiency and optimize water productivity [3]. However, it is necessary to monitor the water status of the crop throughout this process to maintain its productivity and quality, since if the plant is subjected to high water stress it may die. Two methods wildly used to monitor this status consists in Relative

Water Content (RWC) [4] and Leaf Water Potential (LWP) [5]. RWC is a sensitive variable, which quickly responds to environmental conditions such as temperature, light, humidity, and water supply [4]. This indicator correlates closely with a plant's physiological activities and soil water status and is a reliable trait, e.g., for screening for drought tolerance of different genotypes [6]. In the case of olive trees, this water status indicator was used in [7] where the authors used it to control the water stress in an olive orchard composed by different irrigation strategies. The authors found that this value was quite discriminatory between full-irrigated (FI) with values of ~80% and deficit water treatments (~60%). In other hand, Leaf water potential (LWP) specifies the whole plant water status, wherein maintenance of high LWP is found to be associated with dehydration avoidance mechanisms [5]. The authors in [8] have suggested that LWP might be used as an easy and fast way to screen sorghum genotypes for drought avoidance. This water indicator was also used in [7] in olive trees under different irrigation strategies. As verified in the case of RWC, large differences among treatments were founded, where FI had values above -2 MPa and deficit water treatments reached below -6 MPa. However, these two types of measurements are destructive as it is necessary to cut off the branches and leaves of plants and cannot be intensively performed [9,10]. Moreover, biochemical parameters of the plant leaves, are also used to detect different levels of drought stress, however, this type of analysis are also destructive and time-consuming such as RWC and LWP [11].

Thus, it is necessary to create new methods capable of non-destructively estimating the water status of the plant and provide information related with RWC, LWP and biochemical parameters. This way, with the advancement of technology, authors suggested using spectral information of leaves and calculating VIs to obtain these values. VIs consists in arithmetic operations applied at different spectral reflectance's in order to obtain a single value related to the vegetation [12]. They can be used to estimate: leaf area index, biomass, stomatal conductance, water stress, chlorophyll, xanthophyll, among others parameters [14]. In [9], the authors used a NIR spectroscopy to estimate LWP in grapevines, and founded a good relationship. In other hand, the authors in [10] used the VI Photochemical Reflectance Index (PRI) to distinguish between well-watered and stressed leaves from *Sajama*, a cultivar of *Chenopodium quinoa*. The authors affirm that PRI can discriminate between two different water regimes in plants and can be considered to be a reliable water-stress index. Also, they stated that it may provide a non-destructive, low cost, non-contact optical tool for the assessment of drought intensity.

Therefore, the aim of this paper is to correlate different vegetation indices wildly used in olive growing with the water status indicators (RWC and LWP) and biochemical parameters (total polyphenols, ortho-diphenols, proline and glucose), in order to replace these destructive methods with indirect and non-destructive methods in olive trees.

2. Material and Methods

2.1. Study area description

The studied was carried out in a commercial olive orchard (*Olea europaea* L. cv "Cobrançosa") located at Vilariça Valley, near Alfândega da Fé, Portugal (Vilarelhos: 41.33° N, 7.04° W; 240 m altitude) a typical olive growing area of Northeast Portugal. The climate is typically Mediterranean with an average annual rainfall of 520 mm concentrated mainly from autumn to spring. Olive orchard area is about 1.6 ha with olive tree spacing 6 m x 6m apart and was submitted to three irrigation regimes: Full-Irrigated (FI), sustained deficit irrigation (SDI) and farmer-managed irrigation (FMI). The FI regime was divided in two water treatments, while one was irrigated with an equivalent amount of water to supply 100% estimated crop water requirements (WR), the other supplied 120% of WR. Sustained deficit irrigation regimes also include two treatments, supplying 60% and 30% of WR. To estimate the crop water requirements, the approach described in [15] was followed for this orchard.

2.2. Field data

For field data acquisition five olive trees of each irrigation strategy were randomly selected. Measurements of midday shoot water potential (Ψ), were used to evaluate tree water status. A young leafy shoot per tree was collected, from a sunny position at the crown, from 5 replicate trees per treatment. After cutting, the small leafy shoot was immediately enclosed in a plastic bag to avoid any loss of water and quickly placed into the pressure chamber (model PMS 1000, Oregon, Corvallis, USA).

Concerning RWC measurements, for each selected tree, three leaves of the year were removed and placed in a glass tube, which was sealed, placed in a cold container and transported to the laboratory. The sample was weighed on a precision balance to obtain Fresh Mass (FM). Afterwards, cold distilled water was placed into the glass and after 48 h in the dark and stored at 4 °C the leaves were again weighed to obtain the Turgid Mass (TM). Finally, the leaves were placed in a ventilated oven-drying at approximately 70 °C for 48 hours and weighed again – Dry Mass (DM). The RWC were calculated as shown in the Eq. (1).

$$RWC = 100 \times \frac{(FM-DM)}{(TM-DM)} \quad (1)$$

2.3. Spectral reflectance data and vegetation indices

From each selected tree, three leaves were randomly cut, placed in sealed bags and transported to the laboratory in a refrigerate container. Then, the leaves were analysed in the laboratory using a spectroradiometer device (HR2000, OceanOptics, UK), with a wavelength range between 200 and 1100 nm. Afterwards, with the spectral reflectance extracted from the leaves, a list of 20 different Vegetation Indices (VIs) was calculated in order to study their relationship with data collected from the field to assess leaf water status (RWC and LWC) and biochemical parameters. Appendix A shows the VIs that are the most common used in olive trees [16–18].

2.4. Quantification of polyphenols, ortho-diphenols and proline content

The methodology of Singleton and Rossi [19] was used for the quantification of total polyphenols, with minor modifications according to [20]: 20 μL of extract were added with 100 μL of Folin Ciocalteu's phenol reagent (1:10 in bidistilled H_2O) and 80 μL of 7.5% Na_2CO_3 in a 96-well microplate (Multiskan™ FC Microplate Photometer, Waltham, MA, USA). The microplate was incubated for 15 min at 45 ◦C, in the dark. Afterward, the absorbance values against a blank were recorded at 765 nm in a microplate reader (Multiskan GO Microplate Spectrophotometer, Thermo Scientific, Vantaa, Finland). A standard curve with gallic acid at different concentrations was performed and total phenolics results were expressed as mg gallic acid equivalent (GAE)/g DW as the mean ± standard deviation (SD) of three replicates.

For the quantification of ortho-diphenols the method proposed by [21] was used with some adaptations described in [20]. Firstly, 20 μL of extract were mixed with 100 μL of ultra-pure water. Then, 80 μL of phosphate buffer (pH 6.5, 0.1 M) was added, followed by 160 μL of 5% sodium molibdate ($Na_2MoO_4 \cdot 2H_2O$) solution. The microplate was left to stand in the dark for 15 min and the absorbance was measured at 370 nm against a blank reagent. Caffeic acid was used as standard to prepare a calibration curve and ortho-diphenolic content was expressed as caffeic acid equivalents per g of sample (mg CAE/g DW) as the mean ± standard deviation (SD) of three replicate.

Proline content was extracted and estimated by the method of [22] and expressed in mg g^{-1} DW.

2.5. Quantification of sugars content

Total sugars content was quantified following the method of [23] with some adaptations. Briefly, 1 mL of H_2SO_4 12M was added to a 2.5-5 mg of leaves dry mass that was incubated for 60 min at 45 ◦C. After that, 5 ml of water were added and the mixture was incubated 120 min at 100ºC for two hours. Then, 1 ml of the prepared extract was removed and 1 ml of 5% phenol reagent and 5 ml of H_2SO_4 were added. Afterward, the absorbance

values against a blank were recorded at 490 nm in a microplate reader. A standard curve with glucose at different concentrations was performed and total sugars results were expressed as mg glucose equivalent per g of dry matter sample.

3. Results and discussion

Regarding the RWC, through **Error! Reference source not found.**(a) it is possible to verify that, on average, both FI's and FMI, obtained values of approximately 88% (± 3), while SDI_{60} and SDI_{30} obtained 76% (± 3) and 62% (± 4), respectively, thus indicating that FI's and FMI had more water on the leaf. Concerning the LWP (**Error! Reference source not found.**(b)), it is also possible to verify that both FI's and FMI had higher values (-3.0 MPa, ±0.2) than SDI_{60} and SDI_{30} with -5.0 MPa (± 0.3) and -5.9 MPa (± 0.2) respectively, showing that it was necessary more pressure on the leaf of the SDI's to obtain water.

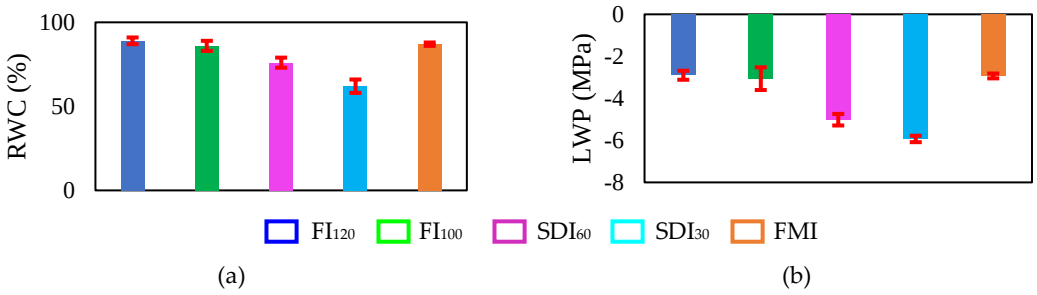


Figure 1. Mean values of the water status indicators: (a) Relative Water Content and (b) Leaf Water Potential.

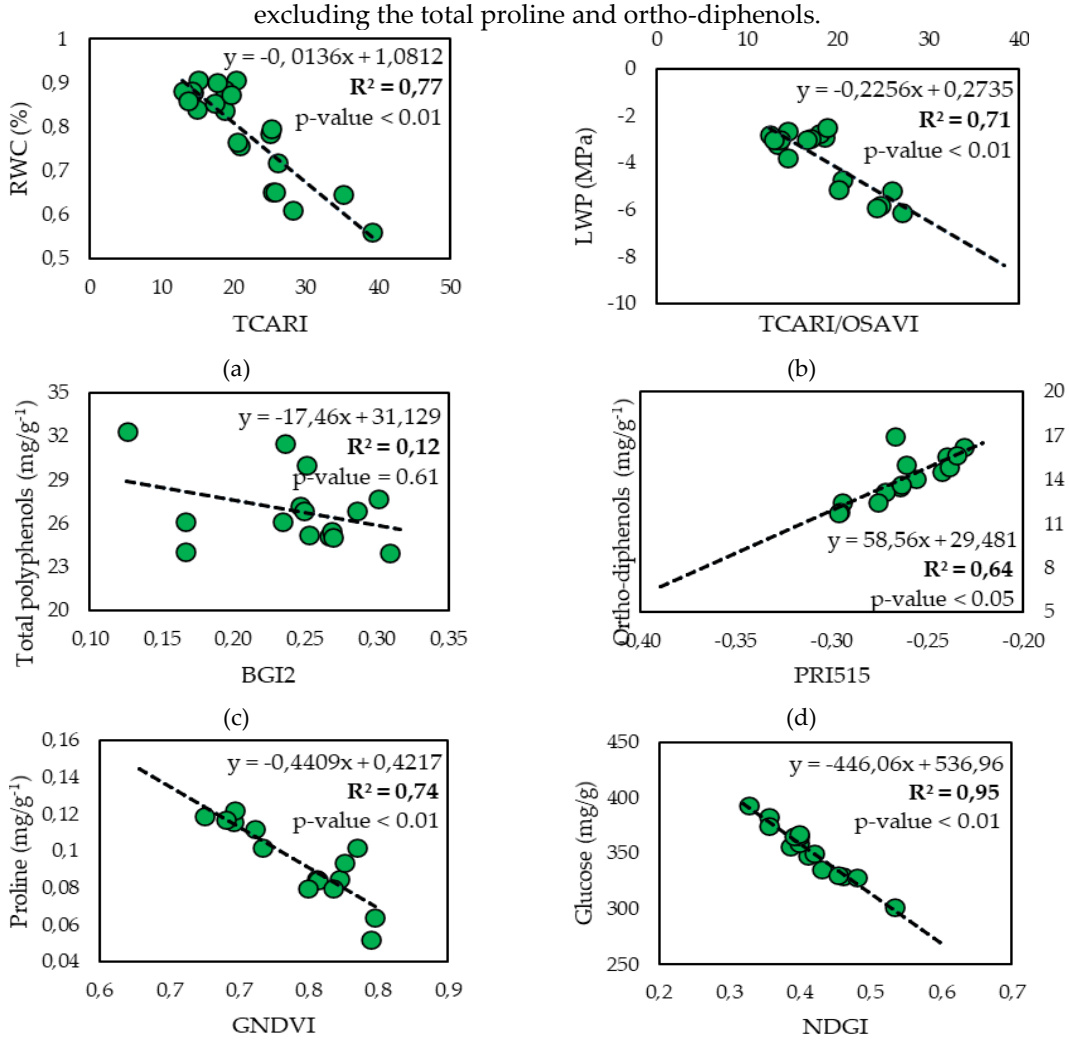
As for the biochemical parameters (**Error! Reference source not found.**), a different behaviour was verified between irrigation strategies: while FI_{120} had the highest values of total polyphenols, FMI had the lowest values; regarding the ortho-diphenols, SDI_{60} and FMI showed the highest values, whereas, FI_{120}, FI_{100} and SDI_{30} showed similar values; as for Proline, a similar behaviour with those verified in RWC and LWP was observed, in which, while both FI's and FMI showed the lowest values, SDI_{60} and SDI_{30} had the highest values; and finally, regarding the glucose values while FMI had the highest values, the SDI_{30} showed the lowest values.

Table 1. Mean values of the biochemical parameters.

Irrigation strategy	Total polyphenols (mg/g^{-1})	Ortho-diphenols (mg/g^{-1})	Proline (mg/g^{-1})	Glucose (mg/g)
FI_{120}	31.3 ± 1.1	13.4 ± 0.8	0.074 ± 0.019	346 ± 10
FI_{100}	26.1 ± 1.0	13.4 ± 1.1	0.081 ± 0.002	359 ± 8
SDI_{60}	26.7 ± 1.1	15.6 ± 0.7	0.116 ± 0.004	354 ± 14
SDI_{30}	25.7 ± 1.5	13.5 ± 1.7	0.114 ± 0.010	320 ± 16
FMI	24.7 ± 0.7	14.8 ± 1.0	0.087 ± 0.029	383 ± 9

As for the correlations between VI's, water status indicators and biochemical parameters (Appendix A), in Figure 1 are illustrated the results with the best performance by type of estimated parameter. In general, it was possible to verify that the VI's that uses the blue wavelength, such as, BGI1, BGI2 and EVI, showed the poorest correlations (R^2 = ~0.2), indicating that this spectrum is unresponsive to leaf water status and biochemical parameters variations. On another hand, the VI's that uses a combination between green, red and near infrared wavelength, such as, MTVI1, TCARI, TCARI/OSAVI and TVI showed the highest correlations (R^2 = ~0.5).

When analysing the correlations individually, the results indicates that the VI's with the best performance varies by type of measured parameter. Moreover, through Figure 1, it is possible to verify that, in general, the VI's with best performance has a negative correlation, being that, the higher the value of the VI, the lower the value of the estimated parameter (with the exception of the correlation between PRI515 and ortho-diphenols). As for the RWC estimation, the structural indices (MCARI1, MCARI2, MTVI1 and OSAVI) and chlorophyll related indices (TCARI, TCARI/OSAVI and TVI) presented the highest correlations, in which, TCARI/OSAVI showed the best performance with $R^2 = 0.77$ (Figure 1(a)). As for the LWP estimation, the aforementioned VI's presented similar results, however, MCARI1 and MCARI2 correlation values decreased. The VI with best performance estimating LWP was TCARI with $R^2 = 0.71$ (Figure 1(b)). Concerning the biochemical parameters, although no VI showed good performance estimating the total polyphenols, a good agreement was found with ortho-diphenols, proline and glucose. As for ortho-diphenols, only the chlorophyll related index PRI515 presented good agreement with $R^2 = 0.64$ (Figure 1(d)). This result indicates that the green wavelength is sensitive to ortho-diphenols variations, since PRI515 only uses this spectrum to be calculated. Regarding the proline estimation, the structural index OSAVI and chlorophyll related indices TCARI and GNDVI presented the best results, in which, GNDVI showed the best agreement with $R^2 = 0.74$ (Figure 1(e)). In the formula of these three indices, a combination of green, red and near infrared wavelength is used. Finally, the best VI's to estimate glucose was the structural indices (MCARI1 and MCARI2) and the chlorophyll related indices (TVI and NDGI), being the NDGI the VI with the best agreement with $R^2 = 0.95$ (Figure 1(f)). Surprisingly, from these vegetation indices, the NDGI is the only that don't use the near infrared wavelength, where it only uses the green and red. In overall, among all the studied VI's, the TCARI/OSAVI showed the best agreement with all the estimated parameters ($R^2 > 0.5$), excluding the total proline and ortho-diphenols.



(e) (f)

Figure 1. Results of the vegetation indices with the best performance: (a) TCARI vs RWC; (b) TCARI/OSAVI vs LWP; (c) BGI2 vs *Table 515.* vs Ortho-diphenols; (e) GNDVI vs Proline and (f) NDGI vs Glucose.

4. Conclusion

In this work, several correlations were made between vegetation indices, plant water status indicators and biochemical parameters. For this purpose, 20 vegetation indices were selected, which are the most used in the area of olive growing.

In general, it was possible to conclude that the VI's that uses the blue wavelength had the poorest results, and in the opposing way, the VI's that uses a combination of green, red and near infrared wavelength had the best performance. Moreover, a good agreement was found between different VI's and the estimated parameters, with the exception of total polyphenols, in which all VI's showed poor correlations. Furthermore, it was also possible to conclude that the VI's with best performance is dependent on the type of pretended parameter, whereas, the higher correlations by parameter type were: RWC vs TCARI (R^2 = 0.77); LWP vs TCARI/OSAVI (R^2 = 0.71); Ortho-diphenols vs PRI515 (R^2 = 0.64); GNDVI vs Proline (R^2 = 0.74); and NDGI vs Glucose (R^2 = 0.95). However, TCARI/OSAVI was the index that had the best performance with all the parameters under study ($R^2 > 0.5$), excluding total polyphenols and ortho-diphenols.

Thus, VIs poses as a good alternative to the traditional methods to estimate water status indicators and biochemical parameters in olive trees, being non-destructive, fast and effective.

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Appendix A. Vegetation indices list and coefficients of determination

VI	Formula	RWC	LWP	Total polyphenols	Ortho-diphenols	Proline	Glucose
BGI1	$\frac{R400}{R554}$	0.15	<0.01	0.03	0.46	0.06	0.27
BGI2	$\frac{R450}{R550}$	0.23	<0.01	0.12	0.31	0.13	0.24
EVI	$2.5 \times \frac{R830 - R670}{(R830 + 6 \times R660 - 7.5 \times R485) + 1}$	<0.01	0.09	0.02	0.18	0.12	0.06
GI	$\frac{R554}{R677}$	0.11	0.03	<0.01	0.40	0.01	0.47
GNDVI	$\frac{R830 - R560}{R830 + R560}$	0.21	0.08	0.06	0.05	**0.74**	0.41
MCARI1	$((R700 - R670) - 0.2 \times (R700 - R550)) \times \left(\frac{R700}{R670}\right)$	0.74	0.47	<0.01	0.04	0.25	0.79
MCARI2	$1.5 \times \frac{2.5 \times (R800 - R670) - 1.3 \times (R800 - R550)}{\sqrt{(2 \times R800 + 1)^2 - (6 \times R800 - 5 \times \sqrt{R670}) - 0.5}}$	0.68	0.34	<0.01	0.06	0.22	0.88
MSI	$\frac{R1100}{R820}$	<0.01	0.01	0.03	<0.01	<0.01	<0.01
MTVI1	$1.2 \times [1.2 \times (R800 - R550) - 2.5 \times (R670 - R550)]$	0.73	0.67	0.06	<0.01	0.71	0.57

NDGI	$\frac{R550 - R670}{R550 + R670}$	0.27	0.13	<0.01	0.10	0.29	**0.95**
NDVI	$\frac{R800 - R670}{R800 + R670}$	0.23	0.20	0.04	0.42	0.40	<0.01
OSAVI	$(1 + 0.16) \times \left(\frac{(R800 - R670)}{(R800 + R670 + 0.16)}\right)$	0.64	0.56	0.03	0.08	0.61	0.29
PRI515	$\frac{R515 - R531}{R515 + R531}$	<0.01	<0.01	0.04	**0.64**	0.11	0.16
PRI570	$\frac{R570 - R531}{R571 + R531}$	0.57	0.62	<0.01	<0.01	0.26	0.32
RDVI	$\frac{R800 - R670}{\sqrt{R800 + R670}}$	0.49	0.33	0.02	0.05	0.16	0.39
TCARI	$3 \times \left((R700 - R670) - 0.2 \times (R700 - R550) \times \frac{R700}{R670}\right)$	0.75	**0.71**	0.05	0.05	0.65	0.35
TCARI/ OSAVI	$\frac{3 \times \left((R700 - R670) - 0.2 \times (R700 - R550) \times \frac{R700}{R670}\right)}{(1 + 0.16) \times \left(\frac{(R800 - R670)}{(R800 + R670 + 0.16)}\right)}$	**0.77**	0.67	0.11	<0.01	0.59	0.56
TVI	$0.5 \times (120 \times (R750 - R550) - 200 \times (R670 - R550))$	0.73	0.62	0.03	0.02	0.39	0.83
VOG	$\frac{R740}{R720}$	0.57	0.47	0.01	<0.01	0.41	0.39
WI	$\frac{R900}{R970}$	0.20	0.17	0.05	0.20	0.26	<0.01

R: Reflectance

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