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Original Paper

PRELIMINARY ASSESSMENT OF THE RELATIONSHIP BETWEEN PIGMENTS IN OLIVE LEAVES AND VEGETATION INDICES

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Content of leaf pigments such as chlorophyll a and b, chlorophyll a + b *and carotenoids can provide valuable insight into the physiological performance of plants. These compounds have selective proprieties for light absorption and reflectance in the visible spectra that can be used to evaluate alternative methods to biochemical to estimate their content. Numerous studies in the literature have established correlations between these compounds, spectral reflectance and vegetation indices. Nevertheless, the appropriate use of these indices depends on plant species and cultivars. Thus, the objective of this study was to assess the most common vegetation indices for the estimation of chlorophyll a and b, chlorophyll* a + b *and carotenoids of three olive tree cultivars (*Olea europaea *L, cv. Cobrançosa, cv. Verdeal Transmontana and cv. Madural) under six irrigation treatments, using spectroscopy. The results showed that the correlation between leaf pigments and vegetation indices depends not only on the type of pigment but also on the cultivar. Among the studied cultivars, cv. Cobrançosa showed the best correlation between the "M Locherer chlorophyll" index (MLO) and chlorophyll a content (r^2 = 0.66) and for the carotenoid reflectance index (CRI) 2 and carotenoids content (r^2 = 0.87). Although the results are preliminary, it seems that vegetation indices could be a useful tool for leaf pigment evaluation, and to give information about plant interactions with biotic and abiotic environmental stress conditions.*

Keywords: *chlorophyll, carotenoids, spectral indices, precision agriculture.*

INTRODUCTION

In plants, chlorophyll *a* (Chl_a), chlorophyll *b* (Chl_b) and carotenoids are the main photosynthetic pigments. The chlorophyll molecules absorb blue and orange-red wavelengths as indicated by their peaks around 450–475 nm and around 650–675 nm, but not green; these pigments are essential in photosynthesis. Although, both Chl_a and Chl_b absorb light, Chl_a plays a unique and crucial role in converting light energy to chemical energy (Rabinowitch and Govindjee, 1965). Carotenoids absorb in the short-wavelength blue region, as is indicated by its peaks around 450–475 nm, and reflect the longer yellow, red and orange wavelength. In photosynthesis, carotenoids help capture light, but they also have an important role in removal of excess light energy.

These pigments provide mechanisms for solar light utilisation in photosynthetic reactions, thus, its presence in leaves and canopies can be expected to be a key indicator of physiological status — photosynthetic capacity, developmental stage, productivity and stress (Lichtenthaler, 1998). On the other hand, carotenoids (which protect the photosynthetic reaction centres from excess light) contribute to harvesting light energy for photosynthesis and play a key role in the photoprotection of the photosynthetic apparatus by their ability to dissipate excessive light energy. Thus, they

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are an indicator of plant physiological status (Edge *et al*., 1997). This type of pigment is also known to be closely correlated with plant stress and photosynthetic capacity (Blackburn, 1998). Thus, the estimation of the of these pigments can provide valuable information about biotic and abiotic plant stress, such as, nutrient disorders, water stress and plant pest/disease, which negatively affect plants, as in healthy plants capable of maximum growth it is generally expected to have higher content of chlorophyll pigments than in unhealthy plants (Wu *et al.*, 2008). However, traditional methods to estimate leaf pigments, such as spectrophotometry and high-performance liquid chromatography, are time consuming, expensive and destructive (Gitelson *et al*., 2006).

As a result of the selective light absorption of leaf pigments in the visible spectra, the colour changes for a single leaf are related to proportions of pigments present, which can allow to estimate the content of leaf pigments by employing leaf reflectance/transmittance (Zhang *et al.*, 2020). For instance, nitrogen, iron and magnesium deficiencies in leaves can affect its reflectance and transmittance capacity for sunlight, as these elements are needed for chlorophyll biosynthesis, and consequently, increase its transmittance at visible wavelengths (Blackmer *et al.*, 1996). Thus, relationships between pigment content and spectral reflectance have been studied and a large number of spectral vegetation indices (VIs) have been developed (Datt, 1998; Gamon and Surfus, 1999; Sims and Gamon, 2002) to estimate content of these leaf pigments as an alternative to biochemistry methods. However, in most cases, these indices have been tested for only one or at most a few related species, such as maize (Peng and Gitelson, 2011), wheat (Wu *et al.*, 2009), potato crops (Kooistra and Clevers, 2016), and sugar beet crops (Jay *et al.*, 2017), among others, but it is not clear whether they can be applied across species with varying leaf structural characteristics.

The aim of this study is to assess the relationship of different chlorophyll and carotenoid related vegetation indices (VIs) in three different olive cultivars, in order to analyse and determine the best performance of the VIs between the cultivars.

MATERIAL AND METHODS

Study area description. The study was carried out in three different commercial olive orchards (*Olea europaea* L.). The cv. Verdeal Transmontana (41.25° N, 7.17° W; 460 m altitude) and cv. Madural (41.24° N, 7.13° W; 350 m altitude) are located at Mirandela, Portugal, and cv. Cobrançosa (41.33° N, 7.04° W; 240 m altitude) are located at Vilariça Valley, near Alfândega da Fé, in the Northeast of Portugal. The olive orchard area is about 2 ha with olive tree spacing 6 m × 6 m apart. The area had six irrigation regimes during late Spring to early Autumn (June–October): i) full irrigated (FI_{100}), the control treatment, that received an amount of water equivalent to 100% of estimated evapotranspiration (ET); ii) over full irrigated (FI_{120}) that received 120% of estimated ET; iii) two sustained deficit irrigation (SDI) with 60% (SDI_{60}) and 30% (SDI_{30}) of FI_{100}; iv) a regulated deficit irrigation, irrigated equally to SDI_{60} except in the pit hardening period in which irrigation was cut off (end of July to the third week of August). After that, this irrigation regime was irrigated equally to SDI_{60}; and v) farmer irrigation (FMI), where, in cv. Madural, the olive trees were irrigated during 5 hours twice a week with emitters of 4 $l \cdot h^{-1}$; and in cv. Cobrançosa, the farmer irrigated the olive orchard for 8 hours twice a week with drippers of 16 $l \cdot h^{-1}$. In cv. Verdeal Transmontana the regime FMI was not implemented. To estimate crop evapotranspiration (ET) the approach described in previous studies was followed (Fernandes-Silva *et al.*, 2010).

Data on the measured parameters presented in the next chapters, such as, soil moisture, water stress indicators, spectral data and pigment content, were collected in the period from 28 August to the day of the year (DOY) 240 (~1 week after the reestablishment of irrigation in RDI_{60}) in cv. Verdeal Transmontana and cv. Madural, and, from July 30th – DOY 211 – (~1 week after the cut off in the RDI_{60}) in cv. Cobrançosa.

Soil water content. To determine soil moisture, two access tubes were installed at a deep of ~70 cm, which is the maximum depth of the soil (Eutric Leptosols developed on metamorphic schists rocks of sandy loam), under three olive trees per irrigation treatment (with the exception of FMI), at distance 50 cm from the olive tree trunk and close to an emitter. The measurements were made using a portable TRIME-FM3 (IMKO GmbH, Germany) with probe T3 at three depths (20, 40, and 60 cm).

Plant water status indicators. Measurements of midday shoot water potential (Ψ_{MD}), were used to evaluate tree water status. A young leafy shoot was collected from a sunlit position of the crown from each of five representative olives trees in each irrigation treatment. After cutting, the small leafy shoot was immediately enclosed in a plastic bag to avoid any loss of water and quickly placed in a pressure chamber PMS 1000 (PMS Instrument, USA). Also at midday, in the same trees, the stomatal conductance (g_s) was measured using a SC-1 porometer (Decagon Devices Inc., USA) for two leaves from each of the five olive trees. Each measurement was made for a one-year-old leaf from a sunny position at the crown and in auto mode for 30 seconds. Lastly, for relative water content (RWC) measurement, for each selected tree, three one-year-old leaves were detached 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). Then, cold distilled water was placed into the glass and after 48 h in the dark at 4 °C the leaves were again weighed to obtain the turgid mass (TM). Finally, the leaves were placed in a ventilated drying oven at approximately 70 °C for 48 hours and weighed again – dry mass (DM). RWC was calculated as shown in the Eq. (1).

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

Spectral reflectance data and vegetation indices. From the same trees selected for the measurement of water status, three healthy one-year-old leaves were randomly cut, placed in sealed bags and transported to the laboratory in a refrigerated container. Then, the leaves were analysed in the laboratory using a HR2000 (OceanOptics, UK) spectrometer at wavelength range between 200 and 1100 nm. The reflectance was measured in the centre of the leaf, near the main vein. Then, for each tree, a mean value of the three collected leaves was calculated. The relationships between spectral reflectance and 27 VIs of pigments were determined. Table 1 provides a list of the VIs used in this study.

Table 1. Vegetation indices list

VI	Name	Formula	Sensitivity	Reference
ARI	Anthocyanin reflectance index	$\frac{1}{R550} - \frac{1}{R700}$	Carotenoid	(Gitelson, Merzlyak and Chivkunova, 2001)
CRI	Carotenoid reflectance index	$\frac{1}{R510} - \frac{1}{R550}$	Carotenoid	(Gitelson *et al.*, 2002)
CRI 2	Carotenoid reflectance index 2	$\frac{1}{R510} - \frac{1}{R700}$	Carotenoid	(Gitelson *et al.*, 2002)
CSI 1	Carter stress index 1	$\frac{R695}{R420}$	Chlorophyll	(Carter, 1993)
CSI 2	Carter stress index 2	$\frac{R695}{R760}$	Chlorophyll	(Carter, 1994)
GI	Greenness index	$\frac{R554}{R677}$	Chlorophyll	(Zarco-Tejada, Ustin and Whiting, 2005)
GM 1	Gitelson & Merzlyak index 1	$\frac{R750}{R550}$	Chlorophyll	(Gitelson and Merzlyak, 1997)
GM 2	Gitelson & Merzlyak index 2	$\frac{R750}{R700}$	Chlorophyll	(Gitelson and Merzlyak, 1997)
GNDVI	Green normalized difference vegetation index	$\frac{R830 - R560}{R830 + R560}$	Chlorophyll	(Gitelson, Kaufman and Merzlyak, 1996)
MCARI	Modified chlorophyll absorption in reflectance index	$((R700 - R670) - 0.2 \times (R700 - R550)) \times \left(\frac{R700}{R670}\right)$	Chlorophyll	(Haboudane *et al.*, 2004)
MLO	M Locherer chlorophyll	$\frac{R531}{R645}$	Chlorophyll	(Locherer *et al.*, 2015)
NDVI	Normalized difference vegetation index	$\frac{R800 - R670}{R800 + R670}$	Chlorophyll	(Rouse *et al.*, 1974)
NPQI	Normalized phaeophytinization index	$\frac{R415 - R435}{R415 + R435}$	Chlorophyll	(Barnes *et al.*, 1992)
PRI	Photochemical reflectance index	$\frac{R528 - R567}{R528 + R567}$	Chlorophyll	(Gamon, Peñuelas and Field, 1992)
PSSRa	Pigment specific simple ratio for Chl_a	$\frac{R800}{R675}$	Chlorophyll *a*	(Blackburn, 1998)
PSSRb	Pigment specific simple ratio for Chl_b	$\frac{R800}{R650}$	Chlorophyll *b*	(Blackburn, 1998)
PSSRc	Pigment specific simple ratio for carotenoids	$\frac{R800}{R500}$	Carotenoid	(Blackburn, 1998)
SIPI	Structural independent pigment index	$\frac{R445 - R800}{R670 - R800}$	Carotenoid	(Peñuelas *et al.*, 1995)
SR_{Chla}	Simple ratio for Chl_a	$\frac{R675}{R700}$	Chlorophyll *a*	(Datt, 1998)
SR_{Chlb}	Simple ratio for Chl_b	$\frac{R675}{R650 \times R700}$	Chlorophyll *b*	(Datt, 1998)
SR_{Chlb2}	Simple ratio for Chl_b 2	$\frac{R672}{R708}$	Chlorophyll *b*	(Datt, 1998)
SR_{Chltot}	Simple ratio for Chl total	$\frac{R760}{R500}$	Chlorophyll *a*+*b*	(Datt, 1998)
SR705	Simple ratio at 705 Index	$\frac{R650}{R705}$	Chlorophyll	(Sims and Gamon, 2002)
TCARI	Transformed chlorophyll absorption in reflectance index	$3 \times \left((R700 - R670) - 0.2 \times (R700 - R550) \times \frac{R700}{R670}\right)$	Chlorophyll	(Haboudane *et al.*, 2002)
VOG 1	Vogelmann index 1	$\frac{R740}{R720}$	Chlorophyll	(Vogelmann, Rock and Moss, 1993)
VOG 2	Vogelmann index 2	$\frac{R734 - R747}{R715 - R720}$	Chlorophyll	(Vogelmann, Rock and Moss, 1993)

Quantification of chlorophyll a, b and total carotenoid pigment content. All pigments analyses were made in samples of ten leaves with the same age, which were collected from the previous selected trees for each irrigation treatment. All measurements were made in triplicate. The content of chlorophyll and carotenoid pigments were quantified according to the procedure described in (Lichtenthaler and Wellburn, 1983; Warren, 2008). For the extraction of pigments, 100 mg of leaf dry mass was placed into centrifuge tubes to which 5 ml of 80% acetone was added and shaken for five minutes at 30 hz. The samples were centrifuged for 5 minutes (4000 rpm) and 200 µl of the supernatant of each sample was transferred to a 96-well microplate. Measurement of absorbance was made in a microplate reader at different at 470, 645, and 662 nm against blank.

For the calculation of pigment content, the absorbance of 200 µl of each sample in a microplate (470, 645, and 662) was converted into a 0.5 cm pathlength-corrected absorbance as follows:

Abs_{470}, 1cm = (A_{470},microplate – blank) / pathlength

Abs_{645}, 1cm = (A_{645},microplate – blank) / pathlength

Abs_{662}, 1cm = (A_{662},microplate – blank) / pathlength

The pigment content was calculated from the 0.5 cm corrected pathlength as follows:

Chl_a (µg·ml^{-1}): $Chl_a = (11.75 \times Abs_{662}) - (2.350 \times Abs_{645})$

Chl_b (µg·ml^{-1}): $Chl_b = (18.61 \times Abs_{645}) - (3.960 \times Abs_{662})$

Total carotenoids (µg·ml^{-1}): $((1000 \times Abs_{470}) - (2.270 \times Chl_a) - (81.4 \times Chl_b)) / 227$

Statistical analysis. The statistical analysis of the data was performed using software SPSS Statistics 26 (IBM, USA). The significance of differences in Ψ_{MD}, g_s, RWC and pigment content (Chl_a, Chl_b, Chl_{a+b} and carotenoids) between irrigation treatments was evaluated using the conventional analysis of variance (ANOVA) with the Tukey HSD test at $p < 0.05$.

RESULTS

Soil moisture and plant water status indicators. As expected, in all olive orchards, the well-watered treatments showed higher soil moisture than deficit irrigation treatments (Fig. 1). Among the studied olive orchards, the highest soil moisture in the soil profile was observed in FI_{120} with ~0.27 m^3 in cvs. Verdeal Transmontana and Cobrançosa, and the lowest in SDI_{30} with ~0.05 m^3 in cv. Verdeal Transmontana. The data collection in cv. Cobrançosa was carried out 1 week after the interruption of the irrigation in RDI_{60}, and therefore this irrigation treatment showed the lowest values of soil moisture, which were even lower than in SDI_{30}.

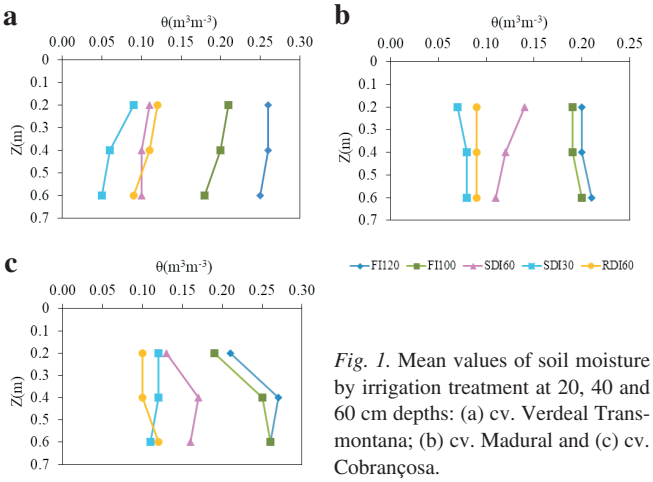


Fig. 1. Mean values of soil moisture by irrigation treatment at 20, 40 and 60 cm depths: (a) cv. Verdeal Transmontana; (b) cv. Madural and (c) cv. Cobrançosa.

The values of plant water status indicators (RWC, Ψ_{MD} and g_s) are presented in Figure 2. In general, these indicators showed coherence with soil water content in the irrigation treatments for the three olive cultivar.

Pigments. The estimated pigment contents are presented in Figure 3. In cv. Verdeal Transmontana (Fig. 3a), the lowest pigment Chl_{a+b} content was observed in the treatment RDI_{60}, which was significantly different from the other irrigation treatments. The highest carotenoid content (~115 µg·g^{-1}) was observed in SDI_{60} and SDI_{30} irrigation treatments, and RDI_{60} had the lowest (49 µg·g^{-1}), representing a reduction of 58%. In contrast, in cv. Madural (Fig. 3b), no

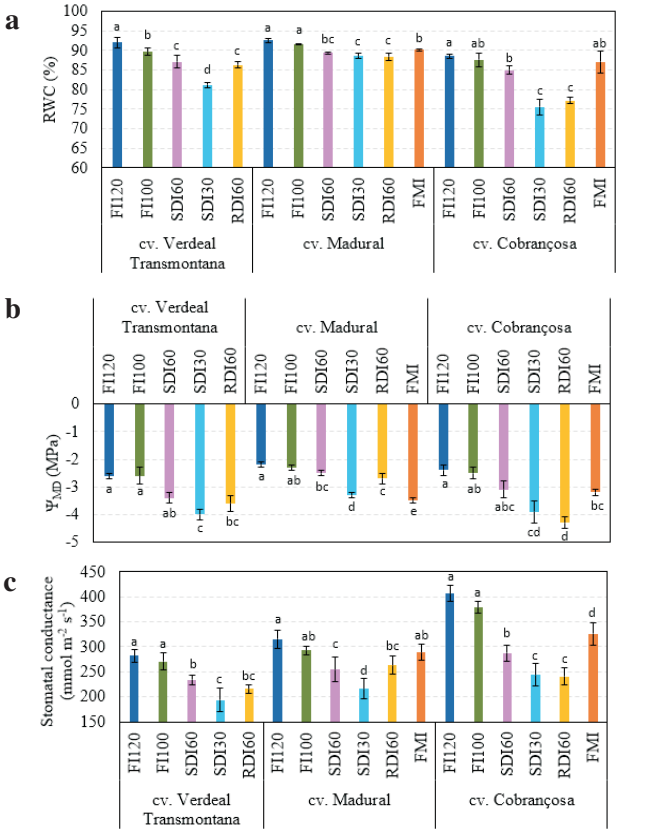


Fig. 2. Mean values of water stress indicators in the three olive orchards: (a) Relative water content (RWC); (b) midday shoot water potential (Ψ_{MD}); (c) stomatal conductance. Standard deviation is shown at the top of the bars.

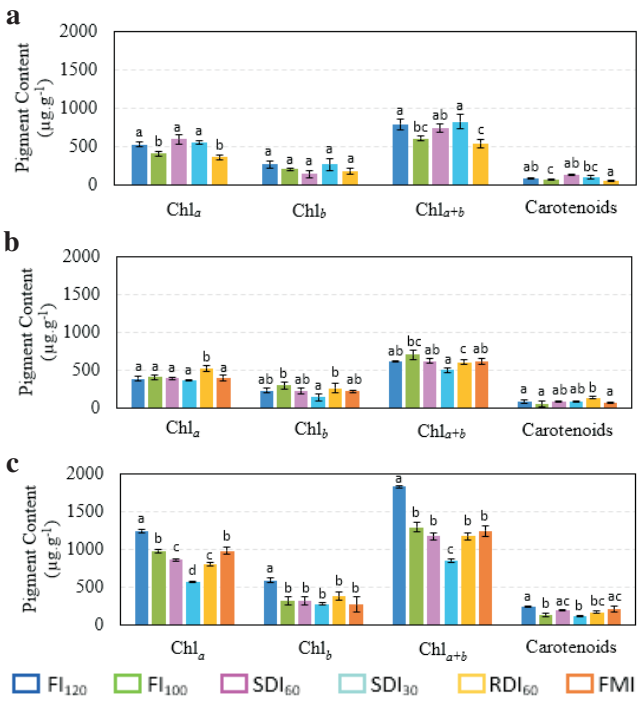


Fig. 3. Mean values of pigment content: (a) cv. Verdeal Transmontana; (b) cv. Madural and (c) cv. Cobrançosa.

significant differences in Chl_a were observed between irrigation treatments, except for RDI_{60}, which had the highest values, while SDI_{30} was lower by 31%. In contrast to cv. Verdeal Transmontana (where RDI_{60} had the lowest values), the carotenoid content in cv. Madural in RDI_{60} had the highest values (~134 $\mu g \cdot g^{-1}$) and FI_{100} had the lowest (~50 $\mu g \cdot g^{-1}$), representing a reduction by 63%. Lastly, in cv. Cobrançosa (Fig. 3c), the highest values of Chl_{a+b} were observed in the treatments that received more water, with no statistically significant differences between the FI_{100} and the deficit irrigation treatments, except for SDI_{30}, which had the lowest values, with a reduction by 55% compared to FI_{120}.

Vegetation indices. In general, the chlorophylls have high reflectance in the red and blue wavelength, and the blue wavelength is not used to estimate the chlorophyll since it overlaps with the absorbance of the carotenoids (Wu *et al.*, 2008). Thus, the blue wavelength is commonly used in VIs for the estimation of carotenoid content. These VIs include the carotenoid reflectance index (CRI), CRI 2, pigment specific simple ratio for carotenoids (PSSRc) and structural independent pigment index (SIPI). From the VIs used in this study to estimate carotenoid content, the anthocyanin reflectance index (ARI) was the only one that did not use the blue region in its formula. Nevertheless, this VI showed poor results for the estimation of the carotenoids in the three cultivars. The application of the red wavelength by itself to estimate chlorophyll may not be adequate, as the absorption in the 660–680 nm tends to saturate at low chlorophyll content (Sims and Gamon, 2002). Thus, the reflectance around the 550 nm and 700 nm regions are largely used in VIs, where absorption is saturated at higher chlorophyll.

DISCUSSION

Regarding the water status indicators, as expected, the SDI_{30} and RDI_{60} of cv. Cobrançosa had the lowest values for RWC (75.5% and 77.1%, respectively) and Ψ_{MD} (–3.1 MPa and –3.9 MPa, respectively), indicating moderate water stress (Fernandes-Silva *et al.*, 2016). The lower values in RDI_{60} were only observed in cv. Cobrançosa in response to a severe water deficit imposed by the cut of irrigation, as the data collection in this olive orchard was done one week after stopping irrigation in this treatment, while in the other olive orchards, the data was collected one week after the re-establishment of irrigation in the RDI_{60} treatment. Thus, in cvs. Verdeal Transmontana and Madural, the RDI_{60} was already recovering from the water stress imposed by the interruption of the irrigation. The water deficit caused the reduction of the minimum g_s (Fig. 2c). In cv. Cobrançosa, the midday reduction ranged from 28% (SDI_{60}) to 39% (SDI_{30} and RDI_{60}) of that in the well irrigated (FI_{120} and FI_{100}) treatment. For cv. Madural, despite the less differences in the values of RWC and Ψ_{MD} in deficit irrigation treatments, the values of g_s were statistically significantly different only for the (SDI_{30}), which experienced a reduction of 26% when comparing to the well irrigated treatment (FI_{100}). For cv. Verdeal Transmontana, the water deficit caused a significant reduction of the midday values of g_s in the treatments RDI_{60} and SDI_{30} by 21 and 29%, respectively. Considering the close relationship between g_s and leaf net CO_2 assimilation (net photosynthesis, A), stomatal closure might be one of the factors responsible for the reduction, as observed in other studies of A (Fernandes-Silva *et al.*, 2016).

As for the pigments, the results showed that water stress had negative effect on the Chl_a, Chl_b and Chl_{a+b} content. A greater water deficit resulted in lower content of photosynthetic pigments, as water deficit limits photosynthesis (Fernandes-Silva *et al.*, 2016). Water stress causes the stomatal closure, which leads to a lower internal CO_2 concentration and consequent shortage of reducible coenzymes results in photoinhibitory conditions (Horton *et al.*, 1996). This negative impact of water stress on chlorophyll was also observed in other cultivars of *Olea europaea* L. such as cv. Chemlali and cv. Chétoui (Guerfel *et al.*, 2009), cv. Dezful (Khaleghi *et al.*, 2012) and in other plants — oriental lily (Zhang *et al.*, 2011), sugarcane (Jangpromma *et al.*, 2010), barley (Anjum *et al.*, 2003), among others.

Regarding the effect of water stress on carotenoid content, different behaviours between cultivars was also observed. The deficit treatments had higher values than well-watered treatments in cv. Verdeal Transmontana and cv. Madural, while the opposite effect was observed in cv. Cobrançosa. This different response to water stress has also been described for other cultures and cultivars. For example, carotenoid content increased in beans (Ramalho *et al.*, 2014), alpine plants (Buchner *et al.*, 2017) and olive trees of cv. Koroneiki (Doupis *et al.*, 2013), while it decreased in tomatoe (Hassan *et al.*, 2015), wheat (Hammad and Ali, 2014) and sorghum (Arivalagan and Somasundaram, 2015). Moreover, both an increase and reduction of carotenoids

was observed in 24 sugarcane genotypes (Jain *et al.*, 2017) and in four cultivars of mung bean (Kumar *et al.*, 2013). An increase in carotenoid content in response to water stress can be associated with the activation of the xanthophyll cycle (Ma *et al.*, 2013).

As for the correlation between vegetation indices and pigments content, between the studied cultivars, the VIs had better and more consistent results in cv. Cobrançosa (6 4), where the correlation coefficients were higher for all pigment types (r^2 > 0.6). Moreover, the Vogelmann index (VOG) 2 appears to be a useful VI for the estimation of Chl_a, Chl_b, and Chl_{a+b} in this cultivar, as its correlation with content of the different pigments (with exception of carotenoids) were satisfactory and homogeneous with r^2 > 0.5. Zarco-Tejada *et al.* (2005), reported that VOG 2 also showed good correlation with the estimation of Chl_a, Chl_b, and Chl_{a+b} in vineyards, where, the linear and 3rd order polynomial achieved the best performance. Regarding the estimation of Chl_a in cv. Verdeal Transmontana, none of the VIs assessed in this study were effective. For the estimation of Chl_a in cv. Madural and cv. Cobrançosa, the VIs with the best performance were photochemical reflectance index (PRI) (Figure 5a) and M Locherer chlorophyll (MLO) (Fig. 4a). PRI uses only the green spectrum in its formula, while MLO uses a combination of green and red wavelengths. Moreover, PRI is strongly affected by short-term changes in the xanthophyll epoxidation state and over longer time-scales by shifts in the size of carotenoid pools (Sims and Gamon, 2002). Thus, this result indicates that the water stress affected more the xanthophyll epoxidation state in cvs. Verdeal Transmontana and Cobrançosa than cv. Madural.

The specific VIs for the estimation of Chl_b, such as simple ratio for Chl_b (SR_{Chlb}) and pigment specific simple ratio for Chl_b (PSSRb), achieved a good agreement in the cultivars Verdeal Transmontana and Cobrançosa with r^2 = 0.72 (Figure 6b) and r^2 = 0.68 (Fig. 4b), respectively. In the literature, there are similar VIs to SR_{Chlb}, such as SR_{Chlb2}; the SR_{Chlb} VI uses the reflectance at 675 nm, while the alternatives use the reflectance bands at 672 nm, 550 nm and 415 nm. However, different authors reported that for the Chl_b estimation, the reflectance at 675 nm can achieve best performance, in case of soybeans and eucalyptus (Chappelle, Kim and McMurtrey, 1992; Datt, 1998), as also found in this study on olive trees. Values of PSSRb were in good agreement for the estimation of Chl_b in wheat and different deciduous tree species (Blackburn, 1998; Jin *et al.*, 2012). Regarding the estimation of Chl_b in cv. Madural, none of the VIs used in this study had good correlation.

As expected, the relationships between VIs and the Chl_{a+b} proved to be less effective than the estimation of a specific pigment (*a* or *b*). This occurs, since if the index has a good agreement with either Chl_a or Chl_b (or vice-versa), and thus its estimation for the pigment group will be low. For example, the Gitelson & Merzlyak index (GM) 1 and SR_{Chlb} showed a good performance for estimating Chl_b content in

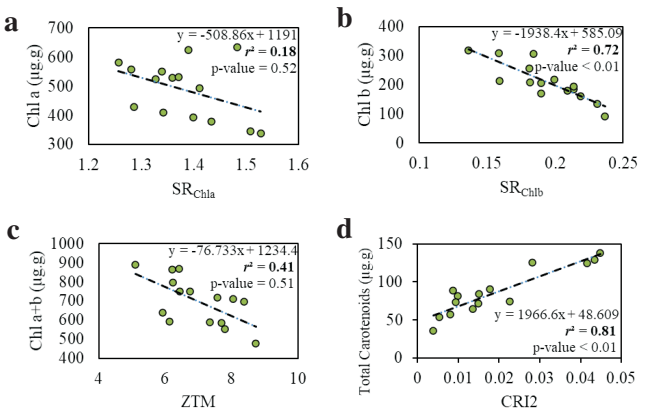


Fig. 4. Vegetation indices with the best performance by pigment in cv. Cobrançosa: (a) MLO vs Chl_a; (b) PSSRb vs Chl_b; (c) VOG2 vs Chl_{a+b} and (d) CRI2 vs Carotenoids.

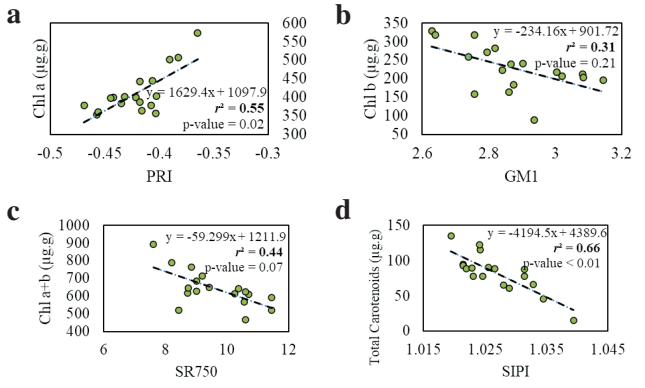


Fig. 5. Vegetation indices with the best performance by pigment in cv. Madural: (a) PRI vs Chl_a; (b) GM1 vs Chl_b; (c) SR705 vs Chl_{a+b} and (d) SIPI vs Carotenoids.

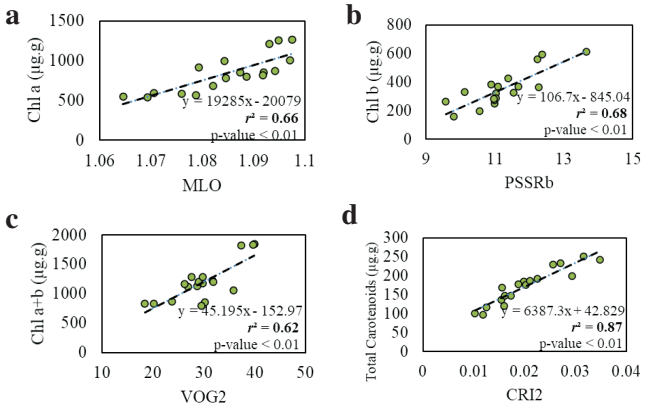


Fig. 6. Vegetation indices with the best performance by pigment in cv. Verdeal Transmontana: (a) SR_{Chla} vs Chl_a ; (b) SR_{Chlb} vs Chl_b ; (c) ZTM vs Chl_{a+b} and (d) CRI 2 vs Carotenoids.

cv. Verdeal Transmontana (r^2 = 0.72), but not for Chl_a content (r^2 = 0.15). Thus, the effectiveness of this VI in the estimation of Chl_{a+b} in cv. Verdeal Transmontana was low (r^2 < 0.34). Consequently, as the results for the estimation of Chl_a and Chl_b was poor in cvs. Verdeal Transmontana and Madural, respectively, the estimation of Chl_{a+b} in these cultivars was weak for all of the VIs used in this study. In cv. Cobranēosa, as VOG 2 showed better results for the es-

Table 2. Correlations between vegetation indices and pigment content by olive cultivar

VI	cv. Verdeal Transmontana				**cv. Madural**				**cv. Cobrançosa**			
	Chl_a	Chl_b	Chl_{a+b}	Carot	Chl_a	Chl_b	Chl_{a+b}	Carot	Chl_a	Chl_b	Chl_{a+b}	Carot
ARI	-	-	-	0.14	-	-	-	0.06	-	-	-	0.03
CRI	-	-	-	0.16	-	-	-	0.05	-	-	-	0.06
CRI 2	-	-	-	**0.81**	-	-	-	0.11	-	-	-	**0.87**
CSI 1	0.1	0.59	0.26	-	0.14	0.28	0.16	-	0.01	0.01	0.01	-
CSI 2	0.07	0.68	0.3	-	0.25	0.12	0.19	-	0.01	0.01	0.02	-
GI	0.08	0.22	0.12	-	0.45	0.04	0.19	-	0.01	0.01	0.01	-
GM 1	0.15	**0.72**	0.34	-	0.16	**0.31**	0.2	-	0.26	0.14	0.25	-
GM 2	0.06	0.32	0.14	-	0.41	0.05	0.18	-	0.02	0.01	0.01	-
GNDVI	0.06	0.32	0.14	-	0.01	0.01	0.01	-	0.6	0.26	0.6	-
MCARI	0.09	0.07	0.08	-	0.26	0.12	0.22	-	0.29	0.14	0.27	-
MLO	0.08	0.02	0.03	-	0.16	0.22	0.15	-	**0.66**	0.19	0.57	-
NDVI	0.09	0.06	0.07	-	0.06	0.07	0.04	-	0.42	0.13	0.35	-
NPQI	0.01	0.56	0.12	-	0.27	0.07	0.13	-	0.05	0.01	0.02	-
PRI	0.05	0.69	0.23	-	**0.55**	0.04	0.23	-	0.1	0.01	0.05	-
PSSRa	0.14	0.05	0.1	-	0.06	0.09	0.05	-	0.21	0.06	0.18	-
PSSRb	0.01	0.36	0.07	-	0.09	0.08	0.05	-	0.39	**0.68**	0.57	-
PSSRc	-	-	-	0.17	-	-	-	0.1	-	-	-	0.08
SIPI	-	-	-	0.02	-	-	-	**0.66**	-	-	-	0.17
SR_{Chla}	**0.18**	0.21	0.36	-	0.05	0.04	0.03	-	0.33	0.27	0.31	-
SR_{Chlb}	0.15	**0.72**	0.29	-	0.11	0.13	0.2	-	0.09	0.12	0.01	-
SR_{Chlb2}	0.11	0.14	0.17	-	0.14	0.19	0.11	-	0.39	0.43	0.43	-
SR_{Chltot}	0.06	0.67	0.26	-	0.22	0.11	0.15	-	0.01	0.01	0.02	-
SR705	0.14	0.6	0.3	-	0.49	0.08	**0.44**	-	0.02	0.01	0.01	-
TCARI	0.09	0.07	0.08	-	0.22	0.2	0.18	-	0.42	0.15	0.36	-
VOG 1	0.08	0.69	0.3	-	0.42	0.05	0.13	-	0.09	0.01	0.05	-
VOG 2	0.07	0.16	0.12	-	0.36	0.26	0.34	-	0.51	0.53	**0.62**	-
ZTM	0.17	0.56	**0.41**	-	0.41	0.06	0.17	-	0.07	0.01	0.03	-

Bold values represent the vegetation index with the best performance by pigment type between cultivars

timation of Chl_a and Chl_b, it also achieved a good agreement with Chl_{a+b} ($r^2 = 0.62$) (Fig. 4c).

As previously mentioned, the carotenoid related vegetation indices commonly use the blue wavelength, since its absorbance occurs in this spectrum. However, among the VIs used in this study for carotenoids content estimation, the ARI was the only one that did not use the blue wavelength, but used the green and red spectrum. Nevertheless, this VI showed poor results in the estimation of carotenoids content in the three cultivars ($r^2 < 0.14$). It is more recommended for the estimation of flavonoids (Gitelson *et al.,* 2001). Regarding the estimation of carotenoids in cvs. Verdeal Transmontana and Cobrançosa, the vegetation index CRI 2 achieved the best performance with $r^2 > 0.8$ (Figs. 6d and 4d). This VI also showed good relationship with the carotenoid content in other crops such as maple, chestnut and beech (Kira *et al.,* 2015). On the other hand, CRI 2 was not effective for estimation of carotenoid content in cv. Madural, however, but the vegetation index SIPI showed good correlation with carotenoid content ($r^2 = 0.66$) (Fig. 5d). Thus, the results indicate that the combination of the red and blue spectrum are not sufficient for carotenoid content estimation in this cv., and it is necessary to add the near infrared wavelength at 800 nm. Sun *et al.* (2014), also reported a good relationship between SIPI and carotenoid content in olive trees of cv. Leccino. Moreover, the authors verified that PRI was more effective than SIPI, but this was not confirmed in this study.

CONCLUSION

The study found several significant correlations between vegetation indices and olive leaf pigment content of three different cultivars. Twenty-seven vegetation indices were selected, which are the most used chlorophyll and carotenoids related vegetation indices. In general, the best performance between vegetation indices and pigment content was achieved for cv. Cobrançosa, in which the correlation coefficients of the best VIs was $r^2 > 0.6$ for all analysed pigments. Moreover, cv. Cobrançosa also achieved the best correlation of the estimated parameters between CRI 2 and carotenoid content with $r^2 = 0.87$. The poorest correlations were observed in Chl_a of cv. Verdeal Transmontana and Chl_b of cv. Madural, in which all of the used VIs showed a

$r^2 < 0.3$. These preliminary results are interesting and should be further investigated in order to make solid conclusions about the robustness of the VIs to estimate leaf pigment content in olive trees.

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OLĪVU LAPU PIGMENTU UN VEĢETĀCIJAS RĀDĪTĀJU SAISTĪBAS PROVIZORISKS NOVĒRTĒJUMS

Lapu pigmentu, tādu, kā hlorofils a un b, hlorofils a + b un karotinoīdi, koncentrācija var sniegt vērtīgu informāciju par augu fizioloģisko stāvokli. Šiem savienojumiem ir selektīvas īpašības attiecībā uz gaismas absorbciju un atstarošanu redzamajos spektros, ko var izmantot, lai to koncentrāciju noteikšanai izveidotu bioķīmiskajai analīzei alternatīvas metodes. Vairākos pētījumos ir atrastas korelācijas starp minēto savienojumu atstaroto spektru un veģetācijas indeksiem. Tomēr saistība ir atkarīga no konkrētām augu sugām un to šķirnēm. Šī pētījuma mērķis bija, izmantojot spektroskopiju, novērtēt izplatītākos veģetācijas indeksus hlorofila a un b, hlorofila a + b un karotinoīdu noteikšanai trim olīvkoku šķirnēm pie sešiem apūdeņošanas veidiem. Iegūtie rezultāti apstiprināja, ka korelācija starp lapu pigmentiem un veģetācijas rādītājiem ir atkarīga ne tikai no pigmenta veida, bet arī no šķirnes. Lai gan rezultāti ir provizoriski, tie norāda, ka veģetācijas indeksi varētu būt noderīgi lapu pigmentu novērtēšanai, kas savukārt sniegtu informāciju par augu mijiedarbību ar biotiskiem un abiotiskiem vides stresiem.