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Investigation of grapevine photosynthesis using hyperspectral techniques and development of hyperspectral band ratio indices sensitive to photosynthesis

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Abstract

The photosynthetic rate of 9 different grapevines were analyzed with simultaneous photosynthesis and spectroradiometric measurements on 08.08.2012 (veraison) and 06.09.2012 (harvest). The wavelengths and spectral regions, which most properly express photosynthetic rate, were determined using correlation and regression analysis. In addition, hyperspectral band ratio (BR) indices sensitive to photosynthesis were developed using optimum band ratio (OBRA) method. The relation of BR results with photosynthesis values are presented with the correlation matrix maps created in this study. The examinations were performed for both specific dates (i.e. veraison and harvest) and also in aggregate (i.e. correlation between total spectra and photosynthesis data). For specific dates wavelength based analysis, the photosynthesis were best determined with -0.929 correlation coefficient (r) 609 nm of yellow region at veraison stage, and -0.870 r at 641 nm of red region at harvest stage. For wavelength based aggregate analysis, 640 nm of red region was found to be correlated with 0.921 and -0.867 r values respectively and red edge (RE) (695 nm) was found to be correlated with -0.922 and -0.860 r values, respectively. When BR indices results were analyzed with photosynthetic values for specific dates, -0.987 r with R_{696}/R_{944} at veraison stage and -0.911 r with R_{648}/R_{715} at harvest stage were found most correlated. For aggregate analysis of BR, common BR presenting great correlation with photosynthesis for both measurements was found to be R_{632}/R_{971} with -0.974, -0.881 r values, respectively and other R_{610}/R_{760} with -0.976, -0.879 r values. The final results of this study indicate that the proportion of RE region to a region with direct or indirect correlation with photosynthetic provides information about rate of photosynthesis. With the indices created in this study, the photosynthesis rate of vineyards can be determined using *in-situ* hyperspectral remote sensing. The findings of this study would enable cost-efficient, rapid and effective control of viticulture activities.

Key words

Grapevine, Photosynthesis, Hyperspectral reflectance, Optimum band ratio, Correlation matrix map

Introduction

Photosynthesis is a vital energy process, standing at the top of the phenomena, required for healthy growth of plants at all phenological stages (Chen and Li, 2010). The photosynthetic ability of a plant directly affects the productivity and quality of agricultural products (Vu, 2005; Gust, 2006; Zobiole *et al.*, 2010). It is very important to predetermine the slowdown of photosynthetic rate, by phenomena such as drought and improper agricultural practices and their destructive effects on plants (Gust, 2006). The effect of many parameters such as wavelength of light used, amount of carbon dioxide, light intensity, amount of photosynthetic pigments, temperature, amount of mineral substances, width of leaf surface on the rate of photosynthesis could be determined and analyzed using spectroradiometric (hyperspectral remote sensing) methods

(Verma *et al.*, 2002; Oppelt and Mauser, 2004; Kempeneers *et al.*, 2010; Cho *et al.*, 2010).

In hyperspectral studies, the spectral range in which photosynthesis is most effective and photosynthetic pigments can be detected is called Photosynthetically Active Radiation (PAR) (Hatchell, 1999; Sims and Gamon, 2002; Mishra *et al.*, 2010). While, in some studies, PAR is described between 400-700 nm, where it is also defined as Photosynthetic Flux Density (PFD) (Hatchell, 1999), in some others it is described between 400 nm and end of the red edge, which is between 670-780 nm (Jayaraman and Srivastava, 2002; Fitzgerald *et al.*, 2006). The red edge (RE) region is related with plant's chlorophyll content, biomass, plant stress and water content, which directly affects photosynthesis (Zarco-Tejeda and Miller, 1999; Thenkabail *et al.*, 2004; Liu *et al.*, 2004). In addition, RE is an important spectral region for determining plant and water stress in grapevine (Rodríguez- Pérez *et al.*, 2007). It is a strong and marked reflection region related to chlorophyll content as compared to green region that gives green color to plant near 500 nm (Seager 2005). Chlorophyll a pigment absorbs light at 430 nm and 662 nm, while chlorophyll b pigment absorbs light at 453 nm and 642 nm. Intensity of these pigments directly affect the rate of photosynthesis (Hatchell, 1999; Seager 2005).

Although there are several generated indices sensitive to photosynthesis (Zarco-Tejeda *et al.*, 2004; Rodríguez-Pérez *et al.*, 2007) such as Photochemical Reflectance Index 1 (PRI1) $[(R_{528}-R_{567})/(R_{528}+R_{567})]$ (Gamon *et al.*, 1995), PRI2 $[(R_{531}-R_{570})/(R_{531}+R_{570})]$, PRI3 $[(R_{570}-R_{539})/(R_{570}+R_{539})]$ (Zarco-Tejeda *et al.*, 1999), Gitelson and Merzlyak 1 (GM1) $[R_{750}/R_{550}]$ and GM2 $[R_{750}/R_{700}]$ (Gitelson and Merzlyak, 1997), Lichtenthaler (Lic) $[(R_{800}-R_{680})/(R_{800}+R_{680})]$ Lic1$[R_{440}/R_{690}]$, Lic2 $[R_{440}/R_{740}]$ (Lichtenthaleret et al., 1996), Simple Ratio Pigment (SRPI) $[R_{430}/R_{680}]$ (Peñuelas *et al.*, 1995a), Structure Intensive Pigment (SIPI) $[(R_{800}-R_{445})/(R_{800}+R_{680})]$ (Peñuelas et al., 1995b), SIPI1$[(R_{800}-R_{450})/(R_{800}+R_{650})]$, Normalized Phaeophytinization (NPQI) $[(R_{415}-R_{435})/(R_{415}+R_{435})]$ (Barnes 1992, Peñuelas *et al.*, 1995a), Normalized Pigments Chlorophyll Ratio (NPCI) $[(R_{680}-R_{430})/(R_{680}+R_{430})]$ (Peñuelas *et al.,* 1994), Modified Chlorophyll Absorption in Reflectance (MCARI) $R_{700}-R_{670}-0.2*(R_{700}-R_{550})]*(R_{700}/R_{670})]$ (Daughtry *et al.*, 2000), Transformed Chlorophyll Absorption in Reflectance (TCARI) $[3*(R_{700}-R_{670}-0.2*(R_{700}-R_{550})]*(R_{700}/R_{670}))]$ (Haboudane *et al.*, 2002) and Modified Chlorophyll Absorption in Reflectance 1 (MCARI1) $1.2*[2.5*R_{800}-R_{670}-1.3*(R_{800}-R_{550})]$ (Haboudane *et al.,* 2004), we preferred to produce new photosynthesis hyperspectral band ratio (BR) indices using a new popular method which is called optimum band ratio (OBRA). OBRA, a simple and useful technique, helps in identifying the most correlated band ratio (BR) indices related to corresponding related data (Legleiter *et al.*, 2009), which was river bathymetry in the study of Legleiter *et al.*, (2009) and photosynthesis rate in the present study.

In the present study, photosynthetic rate of 9 different grape varieties (Alphonse Lavalle, Atasarısı, Cinsaut, Gamay, Merlot, Razakı, Semillion, Tekirdağ Çekirdeksizi and Yapıncak) were analyzed using simultaneous photosynthesis and *in-situ* hyperspectral measurements on grape vine leaves, in two different phenologic periods on 08.08.2012 (veraison) and 06.09.2012 (harvest). Most correlated wavelengths, BR indices with photosythetic rates were determined using correlation and regression analysis and correlation maps. The methodology followed in the present study differed from the previous studies in the area of viticulture hypespectral remote sensing.

Materials and Methods

The present study was carried out in 4 steps which are as follows: (1) *In-situ* measurement of hyperspectral and photosynthesis data of 9 grape varieties; (2) hyperspectral and photosynthesis data were analyzed with correlation and regression analysis to find the highest related wavelengths; (3) all possible BR indices were created and correlated with photosynthetic values and (4) the relationship of BR indices with photosynthetic rate values were determined using correlation and regression and the relations were shown with correlation maps (Gong *et al.*, 2003; Legleiter *et al.*, 2009; Legleiter and Roberts, 2009; Yu *et al.*, 2012; Joyce *et al.*, 2013).

Study area : The study was conducted in vineyard of Tekirdağ Viticulture Research Station, located in Northwest of Turkey (in Thrace), within the coordinates 40.969184ºN - 40.973562ºN Latitudes and 27.461911ºE - 27.477504ºE longitudes (Fig. 1). Tekirdağ and its environs have old and rooted cultures of viticulture (Durgut and Arin, 2005), whileTekirdağ Viticulture Research Station had more than 1200 grape varieties. The Marmara climate, which is a transition climate between the Continental, the Black Sea and the Mediterranean climates, prevailed in the study area. The general characteristics of Marmara climate was : summers with less rain than the Black Sea climate and more rain than Continental climate, and winters were cooler than the Mediterranean climate and not warmer than the Continental Climate (Sensoy *et al.*, 2008). The altitude of the study area approximately ranged between 20 to 40 m and was approximately 2 km away from the sea.

Photosynthesis measurements : Photosynthesis measurements were performed using Li-Cor LI-6400XT portable photosynthesis and fluorescence system. LI-6400XT produces a ratio which was related to the amount of carbon dioxide (CO_2) consumed by the plant in unit cell area as a result of photosynthesis and expressed in $\mu molCO_2m^{-2}$ leaf area (s^{-1}) and letter A. Photosynthetic ratio was measured in leaves, which were fully grown, healthy, exposed to sunlight and reflected the best phenological stage (Li - Cor, 2013).

Hyperspectral measurements: For hyperspectral measurements, ASD hand held field spectroradiometer, able to measure within a

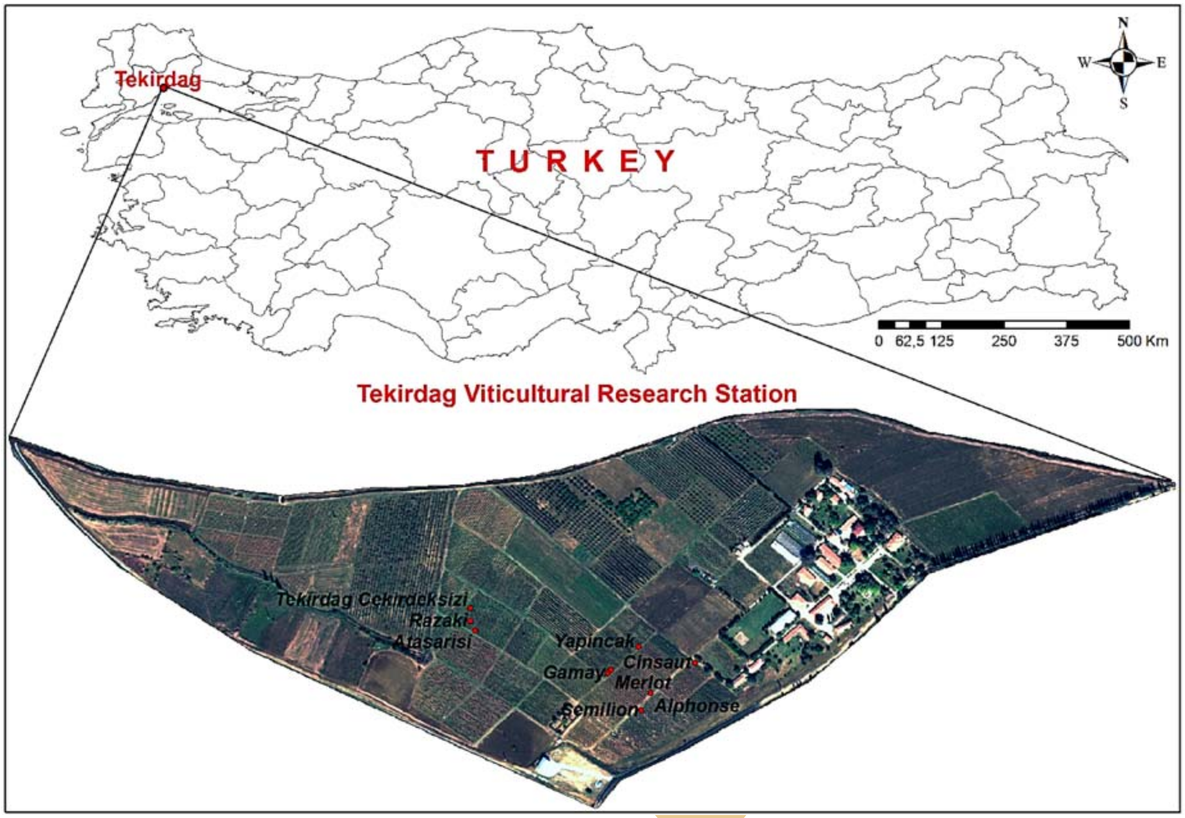

Fig. 1 : Study area and the locations of the measurement points

wavelength range of 325-1075 nm was used. The hyperspectral (*i.e.* spectroradiometric) measurements were performed simultaneously with photosynthesis measurement on same leaves, which were fully grown, healthy, exposed to sunlight and reflected the best phenological stage. At the beginning of each measurement, optimization and irradiance measurement (using white reference panel given by ASD) applications were performed to eliminate the day illumination changes. Each calibration and measurement was performed on the target (white object and leaf) from same distance (10 cm) and nadir. The measurement parameters with 1 degree lens from a distance of 10 cm were: "field of view" = 1°, diameter of scanned circle = 0.1745 cm and the scanned area of 0.0239 cm^2. 10 spectra were measured for each variety in each hyperspectral measurement and the hyperspectral curve of each variety was created by taking the average of these values .

Statistical analysis: The relation between the rate of photosynthesis and hyperspectral reflectance values (wavelength based analysis), within the range of 400-1000 nm, was analyzed with correlation and regression analysis. Pearson correlation coefficient (r), coefficient of determination (r^2) and significance F(S-F), which gives information about the confidence level (CL) (that means 100x(1 - (S-F value)) as percentage) of the correlations by using Analysis of Variance (ANOVA) , values were shown individually for each analysis.

Relation between the photosynthetic rates and hyperspectral BR values was analyzed in three steps: (1) Equation 1 was used in order to create all the possible BR indices within the spectral range of 400-1000 nm, using OBRA method; (2) the BR indices correlated to photosynthesis were determined by correlation and regression analysis (again the S-F values were mentioned), and finally (3) distribution of the correlations (differently from OBRA, which shows the r^2 value to show the relations, r value was preferred in our study) were shown in the form of correlation matrix maps (Eq. 2).

$$\text{Eq. 1 : } BR_{(\lambda 1,\lambda 2,i)} = \left\{\frac{R_{(\lambda 1,i)}}{R_{(\lambda 2,i)}}\right.$$

$$\text{Eq. 2 : } CMM_{(\lambda 1,\lambda 2,i)} = r(BR_{(\lambda 1,\lambda 2,i)}, A_i)$$

$$\left\{\begin{array}{c} 400nm \leq \lambda_1\ 1000nm \\ 400nm \leq \lambda_2\ 1000nm \\ \lambda_1 \neq \lambda_2 \\ 1 \leq i \leq N \end{array}\right.$$

Where; BR: Band Ratio, A (µmolCO_2.m^{-2}.s^{-1}): photosynthesis rate data, i: number of varieties (9), $R_{(\lambda 1,i)}$ and $R_{(\lambda 2,i)}$: Reflectance value of the variety "i" at λ_1 and λ_2, r: Pearson correlation coefficient, CMM: correlation matrix map function, λ: wavelength (nm). For each variety, 601 spectral values were used within the spectral

Fig. 2 : Photographs of the varieties investigated (08.08.2012-veraison (left) and 06.09.2012 - harvest (right))

range of 400-1000 nm in order to correlate with photosynthesis rate values. The reflection values in λ_1 , λ_2 defined as the index pair within the range of 400-1000 nm were used in calculating BR indices by using $BR_{(\lambda 1,\lambda 2,i)}$ (Eq.1). The r values between photosynthesis A_i and BR values data set, with 9 different varieties, were calculated using r $(BR_{(\lambda 1,\lambda 2,i)}, A_i)$. N is the number of sample that was 9 in current study. The correlations between photosynthetic rate and BR indices values were expressed with $r_{(\lambda 1,\lambda 2)}$ in correlation matrices maps with a size of $\lambda_1 \times \lambda_2$ (601x601). The starting coordinate of the maps created was designed to be: $\lambda_1 = 400$, $\lambda_2 = 400$, their maximum coordinate in x axis: λ_1=400, λ_2 = 1000 and their maximum coordinate in y axis:(λ_1=1000, λ_2= 400).

Results and Discussion

In this part of the study, the photosynthetic and hyperspectral data were analyzed individually after the correlations between the photosynthetic and hyperspectral (wavelength and BR based) data set were examined and finally the results were associated and discussed in line with existing literature.

Table 1 : Photosynthesis measurement results

			08.08.2012	06.09.2012
Varieties	**Purpose of use**	**Skin Color**	**A (µmol $CO_2 m^{-2}s^{-1}$)**	**A (µmol $CO_2 m^{-2}s^{-1}$)**
Alphonse Lavallée	Table	Black	6.77	10.51
Atasarısı	Table	White	10.83	7.04
Cinsaut	Wine-Must	Black	12.40	8.07
Gamay	Wine- Must	Black	6.15	4.05
Merlot	Wine- Must	Black	15.05	4.53
Razakı	Table	White	8.56	12.53
Semillion	Wine- Must	White	13.15	11.75
Tekirdağ Çekirdeksizi	Table	Black	8.28	14.25
Yapıncak	Wine- Must	White	11.90	6.91
		Avg.	10.34	8.85
		Stdev.	2.88	3.39

Photosynthesis measurement results : The varieties used in field (Fig. 2), their characteristics and allphotosynthetic rates are given in Table 1. While the average photosynthetic rates of early stage measurements were 10.34 A, but second stage measurements were around 8.35 A. Standard deviation of the first period measurements was 2.88 A, and that of the second period was 3.39 A, respectively. The reason for such differences between two periods could be explained due to the following reasons: the phenological development of plants during harvest period had slowed down, their maturation reached its end, their photosynthetic activities started decreasing, their stress values increased, and physiological differences between varieties emerged (Carbonneau, 1998; Bertamini and Nedunchezhian, 2003; Deloire *et al.* , 2004).

Hyperspectral measurement results : Due to noise at wavelengths lower than 400 nm and higher than 1000 nm, spectral values within the range of 400- 1000 nm were used in this study. Fig. 3 shows the hyperspectral measurements dated 08.08.2012 and 06.09.2012. In the first stage (veraison) , the spectral differences between the species themselves mostly started to be apparent after 700 nm (RE) and near infrared (NIR)) wavelength. After that biggest differences were observed near 550 nm (green region) of the visible region. In the second stage (harvest), the biggest differences were observed after 700 nm. But during this period, spectral differences had emerged between the varieties through out the visible region. The reason for this could be explained due to the differences observed between varieties during the harvest time .

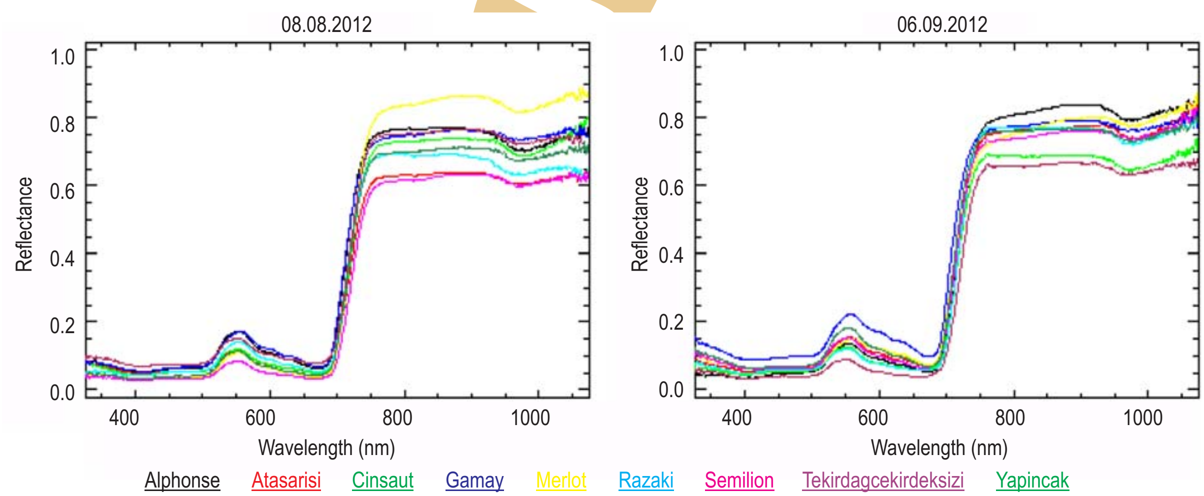

Fig. 3 : 08.08.2012 and 06.09.2012 dated hyperspectral measurements

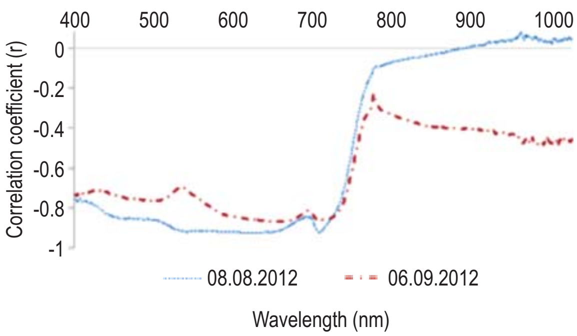

Fig. 4 : Correlation distribution between hyperspectral measurements and photosynthetic rates

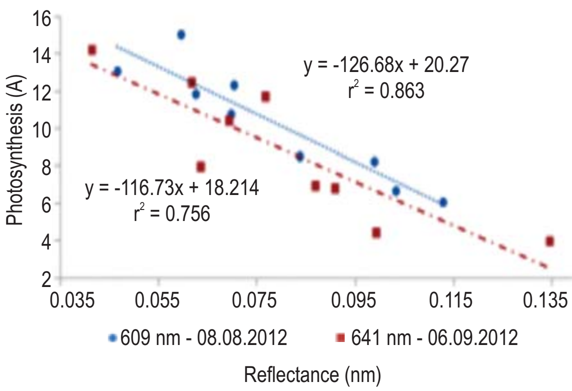

Fig. 5 : Relation between photosynthetic rate and the reflectance of 609 nm and 641 nm, respectively

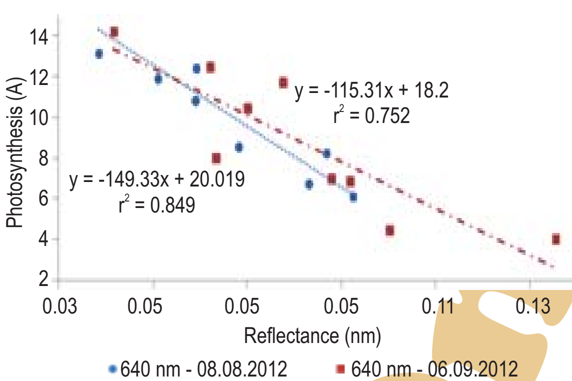

Fig. 6 : Relation between photosynthetic rate and reflectance of 640 nm, respectively

Wavelength based analysis : A strong relation , on the basis of wavelengths, was determined between photosynthetic rate and hyperspectral values particularly in the visible region of spectrum. Fig. 4 shows distribution between 400-1000 nm of the r value between the hyperspectral and photosynthesis measurement values of the first and second stage. The most correlated wavelengths were determined using linear regression for both specific dates (*i.e.* veraison and harvest) and also in aggregate.

At veraison stage, a linear regression of r=-0.929 (r^2=0.863) was found between photosynthetic rate and wavelength of 609 nm (yellow region) (Fig. 5). S-F value of ANOVA at 609 nm was 0.0003 (*i.e.* 99.97% CL), which showed that linear models supplied a consistent and statistically significant fit for veraison. At harvest stage, a linear regression of r=-0.870 (r^2=0.756) was found between photosynthetic rate values and 641 nm wavelength (red region) (Fig. 5), where S-F value was 0.0023 (*i.e.* 99.77% CL).

Regions giving highest correlation in aggregate were analyzed, and their values were r=-0.921 (r^2=0.849), r=-0.867 (r^2=0.752) at 640 nm wavelength (red region) (Fig. 6). S-F values at 640 nm was 0.0004 (i.e. 99.96% CL) and 0.0025 (i.e. 99.75% CL), which showed that the linear models supplied a consistent and statistically significant fit for the aggregate analysis. At 695 nm wavelength (RE region), the values for agg regate analysis were r=-0.922 (r^2=0.850) and r=-0.860 (r^2=0.739) (Fig. 7). S-F values at695 nm for two stages were 0.0004 (99.95% CL) and 0.0029 (*i.e.* 99.70% CL), respectively.

Band ratio (BR) based analysis : Relation between BR indices and photosynthetic rate was presented using correlation matrix maps (Fig. 8). Fig. 8 a and b shows, r distribution between BR indices and photosynthetic rate values on 08.08.2012 and 06.09.2012. Negative correlations were expressed by negative colors with -1 on dark blue, and positive correlations were expressed by warm colors with +1 on dark red.

Initially, the following comments can be made respectively for the BR indices of the specific dates' analysis in the most apparent way in general. At veraison stage, band ratio of "the region near about 700 nm" to the region within "the range of 720-1000 nm" [(RE) / (RE and NIR)] or vice versa, and band ratio of "the region near about 550-650 nm" to the region within "the range of 720-1000 nm" [(green, yellow and red) / (RE and NIR)] were found to be highly correlated with BR indices' computation spectral regions". At harvest stage, the ratio of " the region near about 700 nm " to the region within " the range of 720-1000 nm " [(RE)/(RE and NIR)] or vice versa and the ratio of "the region near about 610-670 nm" to the region within "the range of 720- 1000 nm" [(green, yellow and red)/(RE and NIR)] or vice versa were found to be highly correlated BR indices ' spectral computation regions.

For specific date analysis, again, a linear regression was determined with values of r=-0.986 (r^2=0.973) between the photosynthetic rate values and R_{696} /R_{944} [RE/NIR] BR, at veraison stage (Fig. 9). S- F value, for R_{696}/R_{944}, at veraison was 0.000001 (99.99% CL). The sensitivity of RE region including 696 nm wavelength to photosynthetic have been explained earlier. Sims and Gamon (2003) and Rodríguez- Pérez *et al.* (2007) proved that the region near 950-970 nm was a major water absorption region. 944 nm wavelength determined in the present study, lies in the region defined by Penäuelas *et al.* (1997) and Sims and Gamon (2003). Photosynthesis is a mechanism that produces glucose and O_2 by using CO_2, water (H_2O) and light energy (Seager, 2005). In the present study, as it is shown in Fig. 9, R_{696}/R_{944} was found to be directly related with photosynthetic ratio, which was directly related with H_20.

At harvest stage, r=-0.911 (r^2=0.827) correlation was determined between R_{648}/R_{715} [RE/NIR] ratio and values of photosynthetic rate (Fig . 9). S- F value for R_{648}/R_{715} at the harvest was 0.0006 (99.93% CL). Red and RE areas were found directly related to photosynthesis. A sudden increase from 648 nm, which is a strong chlorophyll absorption area, to 715 nm was determined as an indicator of photosynthesis in this study.

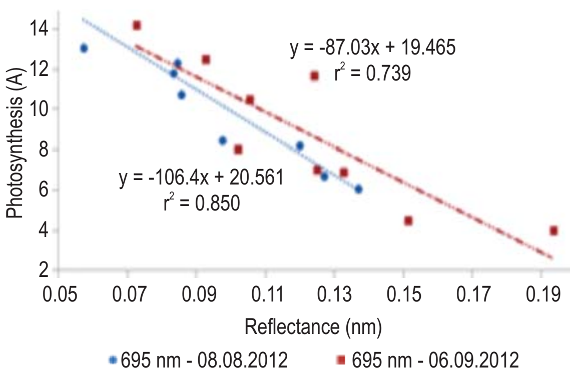

Fig. 7 : Relation between photosynthetic rate and reflectance of 695 nm

For aggregate analysis, highest correlation with photosynthesis was found as R_{632}/R_{971} [RE/NIR] with r=-0.974 (r^2=0.950), r=-0.881 (r^2=0.776) values, respectively for the veraison and harvest stages (Fig. 10). S-F values by R_{632}/R_{971} by aggregate analysis were 0.00001 (99.99% CL) and 0.0017 (99.82% CL). Another aggregate analysis result was BR R_{610}/R_{760} [yellow/RE] that was found with r=-0.976 (r^2=0.952), r=-0.879 (r^2 =0.773) values respectively at veraison and harvest stages (Fig. 11). S-F values for R_{610}/R_{760} by aggregate analysis were 0.00001 (99.99% CL) and 0.0017 (99.82% CL) that were same as R_{632}/R_{971} aggregate analysis. The BR of yellow/ RE can be explained as follow: yellow area lies in the visible area and the center of photosynthetic events; and RE is related with photosynthesis and plant stress, as a result the ratio of yellow/RE generates BR indices sensitive to photosynthesis. In addition, 760 nm, as indicated in the previous studies, was a sensitive wavelength to leaf water content, related with photosynthetic activity (Peñuelas *et al.*, 1993; Kakani *et al.,* 2007; Rodríguez- Pérez *et al.,* 2007).

In the present study, a high correlation was noted between photosynthetic values and 609 nm (yellow region), 641 nm (red region), 640 nm (red region) and 695 nm wavelength (RE region). In addition, $R_{696/944}$ [RE/ NIR] , $R_{648/715}$ [red/RE] , $R_{632/971}$ [RE/ NIR] and $R_{610/760}$ [yellow/RE] BR indices were found to be the most related indices with photosynthesis. These designated spectral regions, wavelengths and BR indices were determinative in monitoring photosynthesis and other physiological parameters

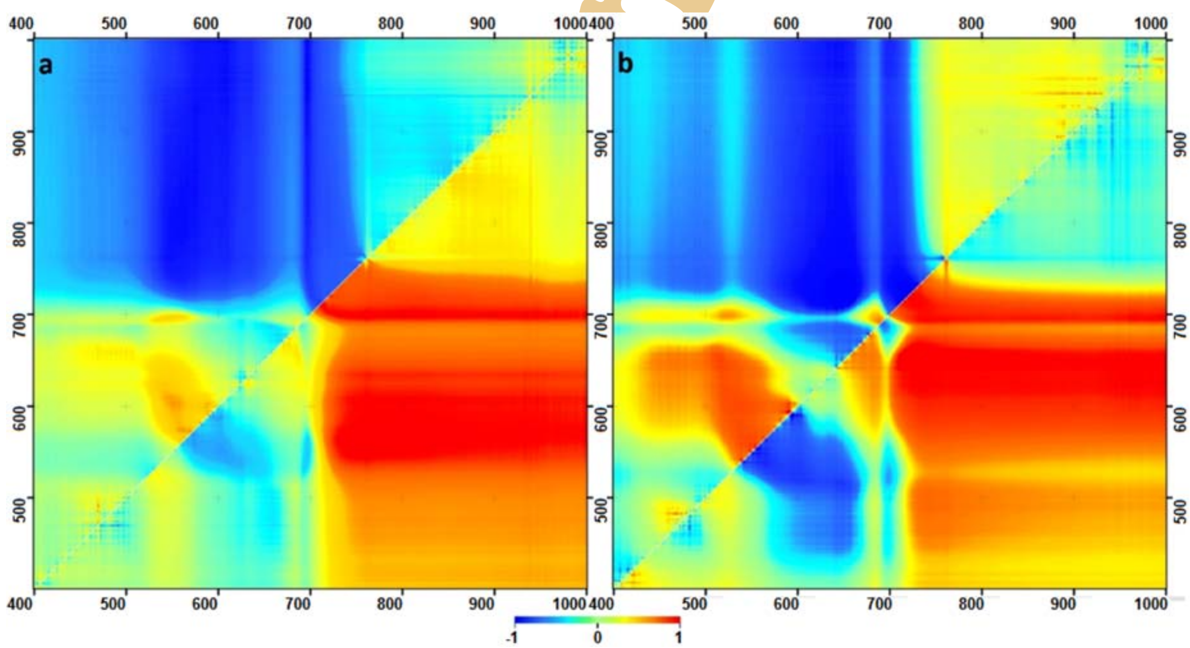

Fig. 8 : The correlation matrixes maps for 08.08.2012 (a) and 06.09.2012 (b) which show the r between BR indices and photosynthesis values

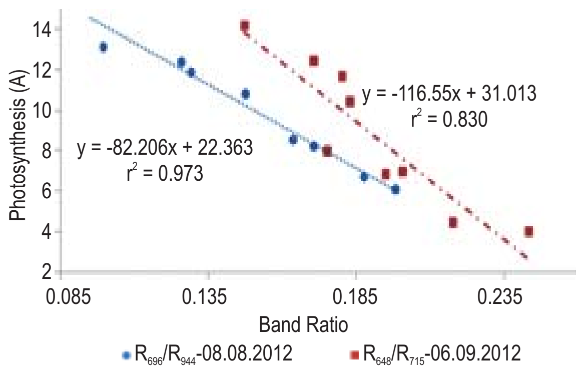

Fig. 9 : Relation between photosynthesis rate and BR indices of $R_{696/944}$ and $R_{648/715}$ values

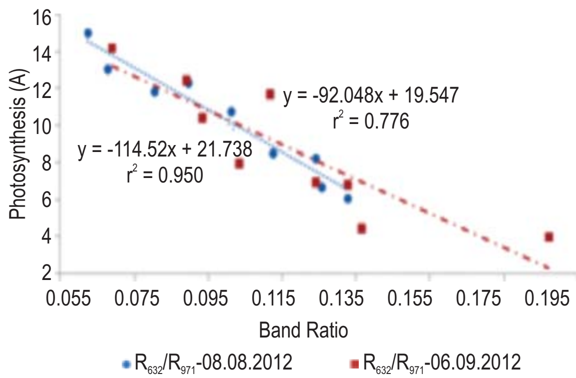

Fig. 10 : Relation between photosynthesis rate and $R_{632/971}$ BR values

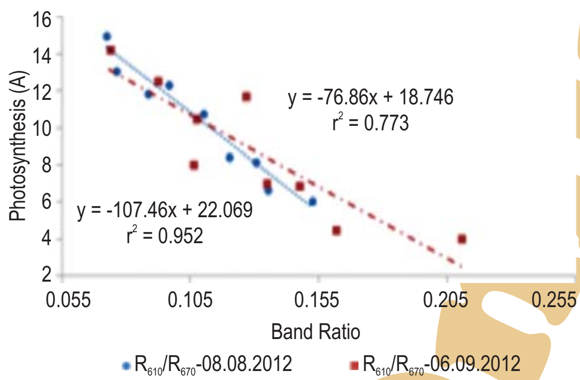

Fig. 11 : Relation between photosynthesis rate and $R_{610/670}$ BR values

such as water stress, water content and photosynthetic pigment content.

In details, most correlated spectral region with photosynthetic rate was found to be between 600-700 nm (yellow-RE). The center of 600-700 nm is the chlorophyll pigments' maximum absorption region and sensitive to photosynthesis (Seager, 2005). In BR indices, the RE region, which is the transition range formed due to chlorophyll content from the absorption area (red) to the high reflection region within the NIR, definitely exists as a ratio. The results show that, the steepness and width of the RE formed due to this transition or the wavelength it starts express the photosynthetic state of a plant.

Using the indices created in this study, the level of photosynthetic rate of grapevines can be determined through *in-situ* spectroradiometric/hyperspectral measurements. The correlation matrix maps for analyzing photosynthesis rate values and BR indices provide great advantages for viewing the dimension, distribution and location of relations. The results, which were found to be statistically significant, indicate the consistency of the proposed method. The methods and indices developed and used in this study can enable cost efficient, rapid and effective control of sensitive agricultural activities in viticulture.

The approach of our study allows producing the most usable hyperspectral results due to the corresponding study area such as agriculture, forestry, mineralogy and others. In other words, this method may be used as an optimization of hyperspectral studies and decrease the dependency to the known indices which are proven and should be definitely used to verify the results of the performed study.

For future research, the findings obtained can be worked out along with the results of other parameters such as water stress, stomatal conductivity, leaf temperature and soil water content, which affect plant physiological processes. Correlation of the position and changes in RE region and photosynthesis can be examined comprehensively. The hyperspectral BR indices created can be compared with the other indices that are presented in the literature review of the present study and also adapted according to outputs of multispectral and hyperspectral sensors through *in-situ*, airborne and satellite remote sensing.

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