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; Kempenheers 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 435 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) \( \left[ \frac{(R_{400} - R_{700})}{(R_{400} + R_{700})} \right] \) (Gamon et al., 1995), PRI2 \( \left[ \frac{(R_{435} - R_{680})}{(R_{435} + R_{680})} \right] \), PRI3 \( \left[ \frac{(R_{430} - R_{680})}{(R_{430} + R_{680})} \right] \) (Zarco-Tejeda et al., 1999), Gitelson and Merzyk 1 (GM1) \( \left[ \frac{R_{670}}{R_{680}} \right] \) and GM2 \( \left[ \frac{R_{670}}{R_{680}} \right] \) (Gitelson and Merzyk, 1997), Lichtenhalter \( \left[ \frac{(R_{710} - R_{435})}{(R_{710} + R_{435})} \right] \) (Lichtenthaler et al., 1996), Simple Ratio Pigment \( \left[ \frac{R_{435}}{R_{680}} \right] \) (Peñuelas et al., 1995a), Structure Intensive Pigment \( \left[ \frac{SIP}{R_{435}} \right] \) (Peñuelas et al., 1995b), Normalized Phaeopigment (NPI) \( \left[ \frac{R_{670} - R_{560}}{R_{435} - R_{480}} \right] \) (Barnes 1992, Peñuelas et al., 1995a), Normalized Pigments Chlorophyll Ratio (NPCR) \( \left[ \frac{R_{670} - R_{560}}{R_{435} - R_{480}} \right] \) (Peñuelas et al., 1994), Modified Chlorophyll Absorption in Reflectance (MCARI) \( \frac{R_{680} - R_{650}}{R_{680} + R_{650} - 2R_{670}} \) and \( \left[ \frac{R_{680} - R_{650}}{R_{670} + R_{650}} \right] - \frac{R_{680}}{R_{670}} \) (Daughtry et al., 2000), Transformed Chlorophyll Absorption in Reflectance (TCARI) \( [3 + \frac{(R_{670} - R_{560})}{(R_{435} - R_{480})}] \) (Habudane et al., 2002) and Modified Chlorophyll Absorption in Reflectance 1 (MCARI1) \( 1.2 * \frac{2.5 - R_{650} - R_{680} - 1.3 * (R_{560} - R_{680})}{(R_{435} - R_{480})} \) (Habudane 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 light 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, Atasarı, Cinsault, Gamay, Merlot, Razakı, Semillion, Tekirdağ Cekirdekäßi 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 photosynthetic 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 hyperspectral 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 40.969184°N - 40.973562°N Latitudes and 27.461911°E - 27.477504°E longitudes (Fig. 1). Tekirdağ and its environs have old and rooted cultures of viticulture (Durgut and Arin, 2005), while Tekirdağ 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 (CO₂) consumed by the plant in unit cell area as a result of photosynthesis and expressed in μmolCO₂ m⁻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
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. 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 (r²) 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 r² value to show the relations, r value was preferred in our study) were shown in the form of correlation matrix maps (Eq. 2).

\[
\text{Eq. 1 : BR}_{i,\lambda_1,\lambda_2} = \frac{R_{\lambda_2,i}}{R_{\lambda_1,i}} \quad 400 \text{nm} \leq \lambda_1 \leq 1000 \text{nm} \\
\text{Eq. 2 : CMM}_{i,\lambda_1,\lambda_2} = r(BR_{i,\lambda_1,\lambda_2}, A) \quad 400 \text{nm} \leq \lambda_1 \leq 1000 \text{nm} \\
\text{Where: BR: Band Ratio, } A (\mu\text{molCO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}) \text{; photosynthesis rate data, } i \text{; number of varieties (9), } R_{\lambda_1,i} \text{ and } R_{\lambda_2,i} \text{; Reflectance value of the variety "i" at } \lambda_1 \text{ and } \lambda_2 \text{; Pearson correlation coefficient, CMM: correlation matrix map function, } \lambda \text{: wavelength (nm). For each variety, 601 spectral values were used within the spectral}
\]
Fig. 2: Photographs of the varieties investigated (08.08.2012 - veraison (left) and 06.09.2012 - harvest (right)).
Development of hyperspectral band ratio indices sensitive to grapevine photosynthesis

range of 400-1000 nm in order to correlate with photosynthesis rate values. The reflection values in \( \lambda_1, \lambda_2 \) 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 \( A \) and BR values data set, with 9 different varieties, were calculated using \( r(BR_{\lambda_1, \lambda_2, i}, A) \). \( N \) is the number of sample that was 9 in current study. The correlations between photosynthetic rate and BR indices values were expressed with \( r \) 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: \( \lambda_1 = 1000, \lambda_2 = 400 \).

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.

Photosynthesis measurement results: The varieties used in field (Fig. 2), their characteristics and all photosynthetic rates are given in Table 1. While the average photosynthetic rates of early stage measurements were 10.34 \( \text{A} \), but second stage measurements were around 8.35 \( \text{A} \). Standard deviation of the first period measurements was 2.88 \( \text{A} \), and that of the second period was 3.39 \( \text{A} \), 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 Nedunchezian, 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.

Table 1: Photosynthesis measurement results

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Purpose of use</th>
<th>Skin Color</th>
<th>( A (\mu \text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}) )</th>
<th>( A (\mu \text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alphonse Lavallée</td>
<td>Table Black</td>
<td>6.77</td>
<td>10.51</td>
<td></td>
</tr>
<tr>
<td>Atasarısı</td>
<td>Table White</td>
<td>10.83</td>
<td>7.04</td>
<td></td>
</tr>
<tr>
<td>Cinsaut</td>
<td>Wine-Must Black</td>
<td>8.66</td>
<td>12.53</td>
<td></td>
</tr>
<tr>
<td>Gamay</td>
<td>Wine-Must Black</td>
<td>8.15</td>
<td>4.05</td>
<td></td>
</tr>
<tr>
<td>Merlot</td>
<td>Wine-Must Black</td>
<td>9.55</td>
<td>4.53</td>
<td></td>
</tr>
<tr>
<td>Razakı</td>
<td>Table White</td>
<td>13.15</td>
<td>11.75</td>
<td></td>
</tr>
<tr>
<td>Semillion</td>
<td>Wine-Must White</td>
<td>10.15</td>
<td>14.25</td>
<td></td>
</tr>
<tr>
<td>Tekirdağ Çekirdeksizi</td>
<td>Table Black</td>
<td>11.90</td>
<td>6.91</td>
<td></td>
</tr>
<tr>
<td>Yapıncak</td>
<td>Wine-Must White</td>
<td>8.28</td>
<td>14.25</td>
<td></td>
</tr>
<tr>
<td>Avg.</td>
<td></td>
<td>10.34</td>
<td>8.85</td>
<td></td>
</tr>
<tr>
<td>Stdev.</td>
<td></td>
<td>2.88</td>
<td>3.39</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3: 08.08.2012 and 06.09.2012 dated hyperspectral measurements
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 (r²=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 (r²=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 (r²=0.849), r=0.867 (r²=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 aggregate analysis were r=0.922 (r²=0.850) and r=0.860 (r²=0.739) (Fig. 7). S-F values at 695 nm for two stages were 0.0004 (99.95% CL) and 0.0025 (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 (r²=0.973) between the photosynthetic rate values and R696/R944 [RE(NIR) BR, at veraison stage (Fig. 9). S- F value, for R696/R944 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 Rodriguez-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 \( \text{O}_2 \) by using \( \text{CO}_2 \), water (\( \text{H}_2\text{O} \)) and light energy (Seager, 2005). In the present study, as it is shown in Fig. 9, \( R_{\text{RED}}/R_{\text{NIR}} \) was found to be directly related with photosynthetic ratio, which was directly related with \( \text{H}_2\text{O} \).

At harvest stage, \( r=0.911 \ (r^2=0.827) \) correlation was determined between \( R_{\text{RED}}/R_{\text{NIR}} \) [RE/NIR] ratio and values of photosynthetic rate (Fig. 9). S-F value for \( R_{\text{RED}}/R_{\text{NIR}} \) 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.

For aggregate analysis, highest correlation with photosynthesis was found as \( R_{\text{RED}}/R_{\text{NIR}} \) [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 \( R_{\text{RED}}/R_{\text{NIR}} \) by aggregate analysis were 0.00001 (99.99% CL) and 0.0017 (99.82% CL). Another aggregate analysis result was \( \text{BR} \) \( R_{\text{RED}}/R_{\text{NIR}} \) [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 \( R_{\text{RED}}/R_{\text{NIR}} \) by aggregate analysis were 0.00001 (99.99% CL) and 0.0017 (99.82% CL) that were same as \( R_{\text{RED}}/R_{\text{NIR}} \) 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 (Penuelas et al., 1993; Kalkani 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, \( R_{\text{RED}}/R_{\text{NIR}} \) [RE/NIR], \( R_{\text{RED}}/R_{\text{NIR}} \) [red/RE], \( R_{\text{RED}}/R_{\text{NIR}} \) [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](Image)
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.

Acknowledgment

The authors would like to thank the Tekirdağ Viticulture Research Station for enabling research and using photosynthesis measuring device, furthermore to Istanbul Technical University, Satellite Communication and Remote Sensing Centre for their support with the spectroradiometer device used for hyperspectral measurements. Last but not least, the authors would like to thank the anonymous reviewers, which helped improving the paper significantly.

References


Development of hyperspectral band ratio indices sensitive to grapevine photosynthesis


McMurtrey: Estimating corn leaf chlorophyll concentration from hyperspectral vegetation indices and novel algorithms for agriculture.


Sensosy, S., M. Demircan, Y. Ulupinar and Z. Baltia: Climate of Turkey Turkish State Meteorological Services (2008).

Development of hyperspectral band ratio indices sensitive to grapevine photosynthesis


