VARIATIONS IN CHLOROPHYLL, SPAD VALUES AND SOME NUTRIENT CONCENTRATIONS DEPENDING ON CHLOROSIS IN PEACH LEAVES

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Abstract

The objectives of this study was to measure greenness (SPAD), chlorophyll concentrations and Fe, Mn, Zn, Cu and Mg concentrations and examine the relations among them in the leaf having different chlorosis levels. The SPAD chlorophyll meter readings were used to measure the leaf greenness. Leaf chlorophyll contents were also determined using the acetone extraction method and other nutrients were measured with AAS. Leaf chlorophyll content, SPAD readings and Fe, Mg, and Mn concentrations increased with the leaf green colour increase. SPAD readings were significantly correlated with leaf chlorophyll content, Fe, Mn and Mg. Also, close correlation was seen between chlorophyll content and leaf Fe concentration.

Key words: Leaf greenness, SPAD, chlorophyll, nutrients

The most active pigments playing role in photosynthesis are the green pigments called chlorophylls. Also chlorophyll gives the green color to the plants. All plants have chlorophyll, which is a green pigment in leaves and stems. Chlorophyll is a light-absorbing pigment enabling plants, and some other organisms, to carry out photosynthesis by converting carbon dioxide and water to glucose and oxygen (Salisbury F.B., Ross C.W., 1992). A Chlorophyll molecule consists of an Mg as a central atom and four N atoms bounded pyrrole rings. So Mg and N are two of the main chlorophyll constituents (Kacar B., Katkat A.V., 2007). There is a close relation between leaf greenness (SPAD) and chlorophyll concentrations and for this, SPAD measurement is being used to estimate chlorophyll concentrations in the leaf of different plants in a short time and with high accuracy (Rodriguez I. R., Miller G. L., 2000; Uddling *et al*, 2007; Sharma P., 2015; Sim *et al*, 2015). In study conducted on sugarcane, it was suggested that SPAD meter readings can be used rapidly to estimate leaf chlorophyll and it can also be used to identify sensitivity of varieties to iron deficiency (Radhamini *et al*, 2016). In another studies, close correlations were found among leaf Fe concentrations, SPAD readings, active and total Fe in the leaves of apple and tomato plants (Erdal *et al*, 2008; Erdal *et al*, 2014). Although Fe is not a constituent of chlorophyll, it plays important roles in chlorophyll synthesis. So, chlorosis is closely associated with Fe deficiency and Chlorophyll contents can decrease in Fe-deficient plants (Marschner H., 2012).

The objectives of this study was to measure greenness (SPAD), chlorophyll concentrations and Fe, Mn, Zn, Cu and Mg concentrations and examine the relations among them in the leaf having different chlorosis levels.

MATERIAL AND METHODS

Leaf samples were collected from 33 peach orchards and they were grouped under 3 classifications as pale green, green and dark green depending on their greenness using Minolta-502 SPAD meter. The same aged leaves were tried to be collected to minimize nutritional differences due to leaf old.

The SPAD chlorophyll meter readings were used to measure the leaf greenness. For this 4 readings from the different side of the leaf were made and the means of them was taken as a main reading. Leaf chlorophyll contents were determined from the same leaves used for SPAD reading using the acetone extraction method. For this, 0.5 g leaf sample was homogenized in 80% acetone then filtrated and filled up 50 ml with acetone. The colour of this homogenate was read at 645 and 663 nm with spectrophotometer. Using both readings chlorophyll a and b and total chlorophyll were calculated using equations given below as in fresh weight (Witham *et al*, 1971).

Chlorophyll a (mg g^{-1}) m = [12.7 x (D663)-2.69 x (D645)] x V/ 1000 x W

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Chlorophyll b (mg g^{-1}) = [22.91 x (D645)-4.68 x (D663)] x V / 1000xW
Total chlorophyll (mg g^{-1}) = chlorophyll a + chlorophyll b
V= filtrate volume (ml); W= extracted plant weight (g); D = wave length

Leaf nutrient concentrations were determined using same leaves used for SPAD and Chlorophyll readings. For this, leaves were dried, grinded and wet digested and filtrated to 100 ml. Al nutrients in filtrate were determined using AAS (Kacar B. and Inal A., 2008). All parameters were evaluated using Co-stat statistic programs.

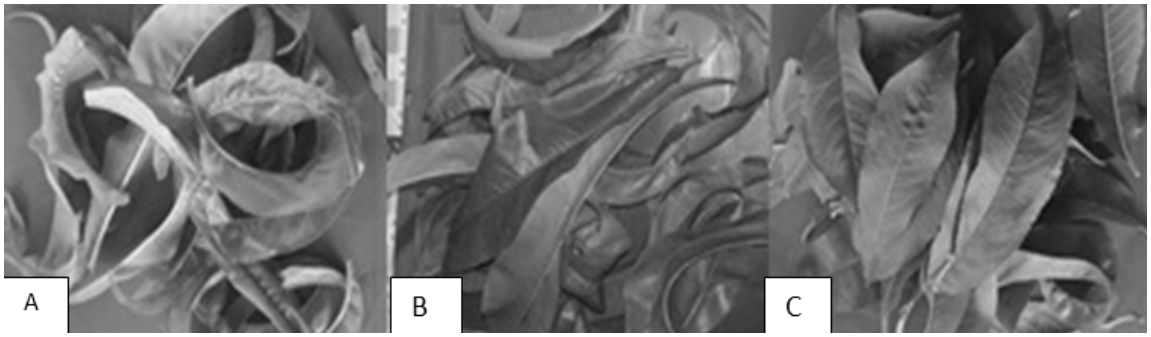

Figure 1 **Classification of leaves depending on their greenness (A: Pale green, B: Green, C: Dark green)**

Figure 2 **SPAD readings and grouping leaf samples**

RESULTS AND DISCUSSION

SPAD values and Chlorophyll contents

Leaves were collected under tree groups in terms of their SPAD readings (*figure 1 a, b, c*). SPAD readings in pale green, green and dark green leaves varied between 18 and 27, 33 and 35 and 40 and 46 with the means of 24, 34 and 43 respectively (*figure 2*). Also leaf chlorophyll contents for each group showed variation as in SPAD and the means were 2.8, 3.3 and 3.5 mg g^{-1} with the same order. Differences between the mean values for both SPAD and chlorophyll contents were significant generally. When examined the relation between SPAD and chlorophyll, it can be seen that there is a significant ($P<0.01$) positive correlation between SPAD and chlorophyll values (*figure 3*). Similar results were found in different studies (Shaahan *et al*, 1999; Sohrabi *et al*, 2015).

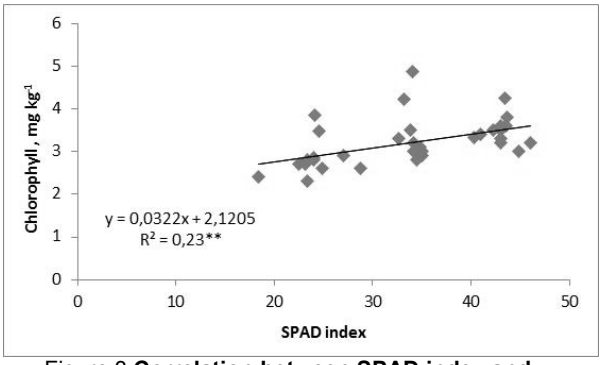

Figure 3 **Correlation between SPAD index and chlorophyll content in leaf**

Leaf mineral concentrations

Leaf Fe and Mn concentrations significantly varied depending on leaf color but Zn and Cu concentrations were not varied (*table 2*). While leaf Fe concentration in pale green leaves was 82 mg kg^{-1} which can be accepted as deficient, this value showed linear increase until sufficient levels in green and dark green leaves (Jones *et al*, 1991). When leaf Fe concentrations and the parameters related to leaf greenness were examined, it can be seen that there is significant positive correlations (*figure 4*). Similarly, leaf Mn and concentrations showed increasing tendency with the leaf greenness from 34 to 45 mg kg^{-1}. Also Mg concentrations significantly varied with leaf color and while Mg concentration was the lowest in pale green leaf, it was highest in the dark green leaves. There was a 3 fold differences between pale green and dark green leaves in terms of Mg. Being higher Fe concentration in green colored leaves is not a

surprising result because it's role in chlorophyll synthesis. There are positive correlations between leaf Mn and Mg and SPAD index (*figure 5*) As it was reported previous studies, the role of the Fe in chlorophyll formation was well described and increases in leaf greenness and chlorophyll contented were related to leaf Fe concentrations (Sanz *et al*, 1998; Shaahan *et al*, 1999; Chen *et al*, 2016). Also determining higher Mg in dark green leaves comparing to others is due to direct effect of Mg as constituent in chlorophyll (Shaahan *et al*, 1999; Kacar B., Katkat A. V. 2007).

Table 1

SPAD values and chlorophyll contents in different color group

Leaf color	SPAD			Total chlorophyll, mg g^{-1}		
	Min.	Max.	Mean	Min.	Max.	Mean
Pale green	18	27	24 C	2.4	3.9	2.8 B
Green	33	35	34 B	2.8	4.9	3.3 A
Dark green	40	46	43 A**	3.0	4.3	3.5 A**

**P<0.01

Table 2

Variations of Fe, Mn, Zn and Cu concentrations in different colored leaves

Leaf color	Fe	Mn	Zn	Cu	Mg
	mg kg^{-1}				%
Pale green	82 C**	34B*	46	13	0.4 C**
Green	95 B	39B	42	10	0.7 B
Dark green	121 A	45A	50	10	1.2 A

*P<0.05,**P<0.01

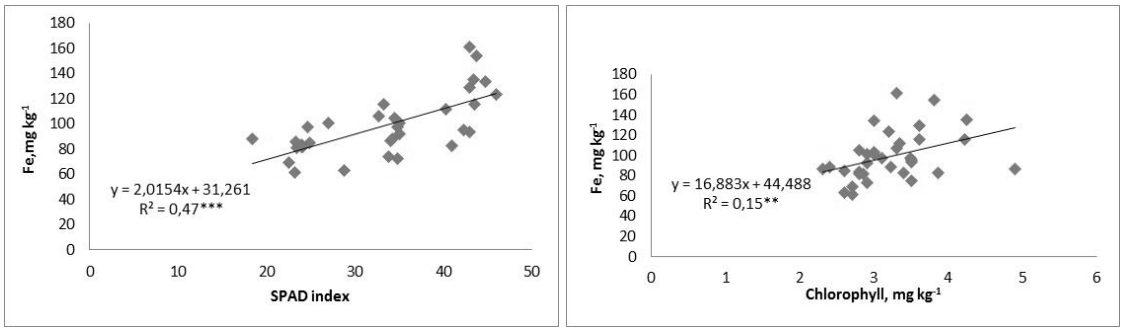

Figure 4 **Correlations leaf Fe concentrations and SPAD and Chlorophyll**

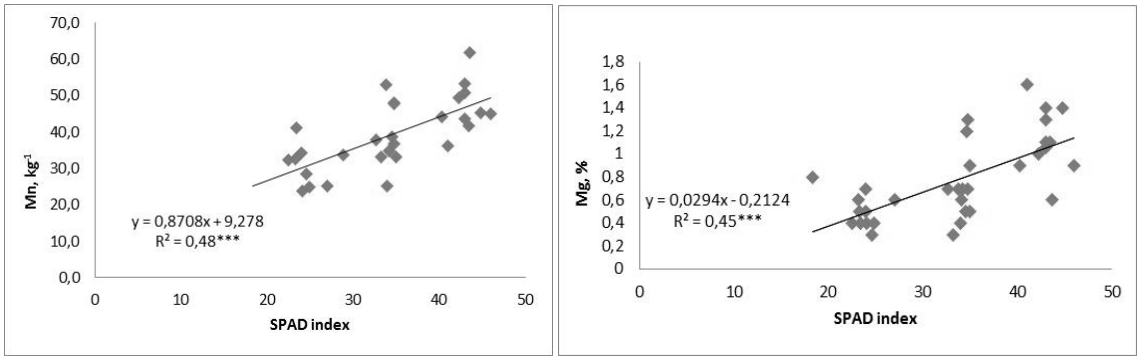

Figure 5 **Correlations leaf Mn concentrations and SPAD index**

CONCLUSIONS

Leaf greenness gave the strong correlations with SPAD readings and chlorophyll contents. Also leaf Fe, Mn and Mg concentrations showed increment with the leaf green colour. There were close correlations between SPAD reading and leaf chlorophyll, Fe, Mn, and Mg concentrations. Looking at these findings, SPAD values can be used to estimate leaf chlorophyll content and leaf Fe, Mn and Mg concentrations.

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