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Full Length Article

Influence of Leaf Water Content on the Prediction of Nutrient Stress in Strawberry Leaves using Chromameter

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Abstract

Chemical soil and plant tissue analyses require considerable amount of labor, time, and cost. Non-destructive prediction of nutrient stress from chromameters may save time and labor. Previous studies did not assess multiple nutrient stresses together with the effect of leaf water content (WC). The aim of this work was to investigate the effect of leaf WC on the prediction of leaf nutrient stress from leaf color. A commercial strawberry field with a significant amount of leaf color variability was selected in Hatay province of Turkey. Forty eight leaf samples with varying colors were collected. A hand-held chromameter was used for leaf color measurements in two color systems as L*a*b* and L*C*h°. Leaf WC was determined using oven drying method. Leaf nutrient contents of N, P, K, Ca, Mg, Mn, Fe, and Cu were obtained using chemical analysis. Correlation and Partial Least Square Regression (PLSR) methods were utilized for data analysis. Leaf WC ranged from 58.3 to 65.7%. High correlation existed between color parameters and N, Ca, and WC ($|r|>0.66$). Also, correlation was high between leaf WC and leaf N content ($r=0.75$), suggesting that the leaves with high WC had also high N content. Using color data, it was possible to predict leaf N content ($R^2=0.66$), Ca content ($R^2=0.70$), and WC ($R^2=0.65$). Using WC as a variable together with the color parameters slightly improved the model performance to predict strawberry leaf nutrient concentrations. © 2018 Friends Science Publishers

Keywords: Strawberry; Leaf water content; Nutrients; Chromameter; Color determination

Introduction

Strawberry (*Fragaria* × *ananassa*) is a crop cultivated mainly for its fruit which is consumed as fresh or as preserves including juice and jam. Turkey ranks fourth in the world with yearly strawberry production of 376,070 metric tons after China, the US, and Mexico (FAO, 2014). Significant amount of the product is exported to other countries ranking Turkey seventh in the world (AKIB, 2012). Mediterranean region of Turkey is one of the main areas in strawberry production. Its cultivation, mainly in open fields, has increased in Hatay province in recent years.

In crop cultivation, fertilizer rate is ideally quantified by chemical soil analysis or leaf analysis. This process necessitates sample preparation and processing to obtain chemical solution and reading concentration of the nutrients. This process requires considerable amount of labor, time, and cost. Farmers usually apply fertilizers without an appropriate soil or leaf analysis. This frequently causes over-application of fertilizers. This case is common and may lead to toxicity in the crops as well as increased production costs, reduced profitability, and environmental pollution (Ongley, 1996; Keskin *et al.*, 2004).

Stress factors result in changes in plant leaf color (Carter, 1993; Fujita *et al.*, 2006). Thus, it could be feasible to assess water and nutrient content of leaves based on leaf color or reflectance data (Rodriguez and Miller, 2000; Keskin *et al.*, 2004). Some instruments such as chlorophyll meters, digital cameras, spectroradiometers, near infrared reflectance spectroscopy (NIRS), and color meters are used to assess the reflectance from plant leaves and estimate leaf quality or leaf nutrient contents (Rodriguez and Miller, 2000; Solari *et al.*, 2008; Akhter *et al.*, 2000). Ulissi *et al.* (2011) estimated N content of tomato leaves using VIS-NIR spectroscopy. Neto *et al.* (2017) evaluated water and chlorophyll status in sunflower leaves using Vis/NIR spectroscopy and chemometrics.

There are also several studies concerning leaf reflectance and some quality properties of strawberry leaves. Chen *et al.* (1993) estimated strawberry leaf chlorophyll content and photosynthetic activity using reflectance and fluorescence in field conditions. Saied *et al.* (2005) investigated the effect of NaCl salinity on growth, yield, color, and fruit quality of strawberries. Espana-Boquerra *et al.* (2006) predicted N content of strawberry crops from its spectral reaction. Deak *et al.* (2007) studied

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relationship of spectral features of strawberry crops and fruit quality. Fraulo *et al.* (2009) used visible/near infrared reflectance (VNIR) technique to detect two spotted spider mite damage in strawberry leaves. Li *et al.* (2010) examined strawberry fruiting efficacy and its dependence on irradiance, temperature and reflectance water index change.

Keskin *et al.* (2016) found a high association between WC and leaf color parameters for the leaf samples including both healthy green and chlorosed leaves; however, the correlation was low when the samples included only healthy green leaves with varying WC. Since the WC has an important variable with high correlation in leaf samples including healthy green and chlorosed yellow leaves, this led the researchers to ask the question if the prediction accuracy could be increased if the WC was added as a variable with color data. No literature was found to answer this question to the best of our knowledge.

Most of the previous studies did not assess multiple nutrient stresses together and the effect of leaf water content (WC) on the color or reflectance properties of the strawberry leaves and its effect on the assessment of nutrient contents of leaves from reflectance. Therefore, the purpose of this research was to investigate the effect of leaf WC on the color features of the strawberry leaves and its effect on the prediction of multiple leaf nutrient contents from leaf color.

Materials and Methods

Leaf and Soil Samples

A commercial strawberry field of about 0.3 ha area with a considerable amount of leaf color changes was selected. The field was located near Saksak village of Yayladag city in the Hatay province of Turkey (Latitude: 35.97444°, Longitude: 36.09556°, Altitude: 780 m). 48 strawberry leaf samples with varying colors were collected from the field. The samples were from the most recent mature trifoliate leaves as older and younger leaves were not selected. The variety of the strawberry was Camarosa. In addition, three soil samples, one from the area with dark green plants (Sample 1), another from the light green area (Sample 2), and the third one from the area with yellow plants (Sample 3) were obtained.

Chromameter

A hand-held color meter (Minolta CR 400, Konica Minolta Inc., Japan) was utilized for color assessment of the leaves. The chromameter works based on reflectance principle in which it sends light from its light source onto the sample and filters reflected light through RGB filters and then transforms it into voltage using photocells (Uren, 1999). It computes the color data from the reflectance. The diameter of the circular reflectance area was 8 mm for the color meter.

Leaf Color Measurements

Leaf samples were collected and transferred to a lab in plastic bags in a cooler. The color of the 48 leaf samples were measured on a lab bench using the chromameter after the device was calibrated. Each sample had five leaves stacked while the color data was obtained from the middle of the top leaf. Seven color data were acquired for each leaf sample and the average values were computed. Two different color systems as L*a*b* and L*C*h° were used for comparison purpose. Each letter in these color spaces denotes (Konica Minolta Inc., Japan):

L*: Lightness (black: 0, white: 100)
a*: Green (-60) and red (+60) color directions
b*: Blue(-60) and yellow (+60) color directions
C*: Chroma value (0-60)
h°: Hue angle (red:0°, yellow: 90°, green: 180°, blue: 270°).

Leaf Water Content Analysis

Mass of fresh leaf sample was quantified with a 0.01 g balance (Sartorius, GP 3202, Goettingen, Germany), after the color measurement was completed. An oven was used to dry the samples at 55°C for a duration of 72 h. After drying, dry leaf mass values were obtained (ASABE, 2012). The petioles were separated before weighing and drying. Water Content (WC) was calculated based on wet-basis using (ASABE, 2012):

$$WC = [(Mw-Md)/Mw]*100$$

Where:

WC: Leaf water content wet-based (WC) (%)
Mw: Mass of fresh (wet) leaf sample (g)
Md: Mass of dry leaf sample (g)

Soil and Leaf Chemical Analysis

Leaf and soil samples were evaluated at the Central Analysis Lab of Mustafa Kemal University, Antakya, Hatay, Turkey. Dry leaf samples were grounded for chemical nutrient content analysis. Nitrogen (N) content of the leaf samples was determined by Macro Kjeldahl method (Kacar and Inal, 2008). P, K, Ca, Mg, Na, Cu, Fe, and Zn contents were quantified by ICP–OES after burning with $H_2O_2 + HNO_3$ in microwave oven (Zarcinas *et al.*, 1987). Soil texture was determined by hydrometric method. Soil N was extracted by wet decomposition method with salicilic acid + salt mixture and determined according to micro Kjeldahl method (Bremner and Mulvaney, 1982). Ammonium acetate extractable K, Na, and Mg contents were measured by flame photometer as soil Mg content was determined by MP–AES (Rhoades, 1982). Available soil P level was treated with 0.5 M $NaHCO_3$ at pH 8.5 in a 1:20 soil-and-solution ratio and P level was evaluated after molibdophosphoric acid staining at 882 nm by spectrophotometer (Olsen *et al.*, 1954). Amounts of available Fe, Zn, Cu, and Mn were acquired using DTPA extraction by ICP-OES (Lindsay and Norwell, 1978).

Statistical Data Analysis

Computations were carried out to obtain correlation coefficients including the significance tests among leaf color, leaf water content (WC), and leaf nutrient contents data in spreadsheet program (MS Excel 2010, Microsoft Inc., Redmond, Washington, USA). Modelling between leaf color, WC, and nutrient contents was established using Partial Least Square Regression (PLSR) method in Unscrambler software (v9.7, Camo Software, Norway). In order to validate the prediction equations, full cross validation (FCV) method was utilized. Root Mean Square Error of Prediction (RMSEP) and R^2 values were used to evaluate and compare the models. RMSEP values were calculated using the formula given below (Esbensen, 2009):

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{N}\left(y_i - y_{i,ref}\right)^2}{n}}$$

Where; y_i: predicted value, $y_{i,\,ref}$: measured value, and *n*: number of samples.

Results

The soil texture was found to be silty clay and the soil pH level was about 7.5 (Table 1). The pH level of the soil was over the ideal value since the recommended soil pH level for strawberry plant is 6.5-7.0.

Summarized results of the leaf chemical analysis and leaf color measurements including mean, minimum, maximum, standard deviation and sufficiency levels are presented for all 48 leaf samples in Table 2. Ca, Mn, and Cu contents of the leaf samples were in normal range while N, P, and K contents were in low to normal range and Mg and Fe contents were in low range.. WC ranged from 58.3% to 65.7% (Table 2). Three plant samples with dark green leaves, light green leaves, and light yellow leaves are presented in Fig. 1. The sample with yellow color (Fig. 1, right) looked as having a general indication of Fe deficiency in which leaves change color into yellow or light green but leaf veins stay green.

Based on the leaf colors, all samples (n=48) were visually classified into five classes as dark green leaves (n=8), green leaves (n=9), light green leaves (n=5), yellow leaves (n=19), and light yellow leaves (n=7). Mean color parameters, water contents (WC), and nutrient contents of dark green, light green, and light yellow leaves are presented in Table 3. It was observed that dark green leaves have lower lightness value (35.4) as light yellow samples have higher lightness value (53.2). The mean Fe content of the dark green, light green and light yellow leaf samples were 38.7, 33.3, and 31.4 ppm, respectively (Table 3). The data revealed that the samples with lower

Table 1: Results of the soil chemical analysis

Sample No	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (ppm)	Fe (ppm)	Cu (ppm)	pH
1 (DG)	0.060	0.002	0.060	0.128	0.009	13.2	3.5	1.8	7.6
2 (LG)	0.047	0.002	0.021	0.122	0.010	7.6	1.5	1.0	7.6
3 (Y)	0.130	0.001	0.029	0.123	0.011	6.3	1.4	0.8	7.5

DG: Dark green plant area, LG: Light green plant area, Y: Yellow plant area

Fig. 1: Three strawberry plant samples with dark green leaves (left), light green leaves (medium) and yellowish leaves (right)

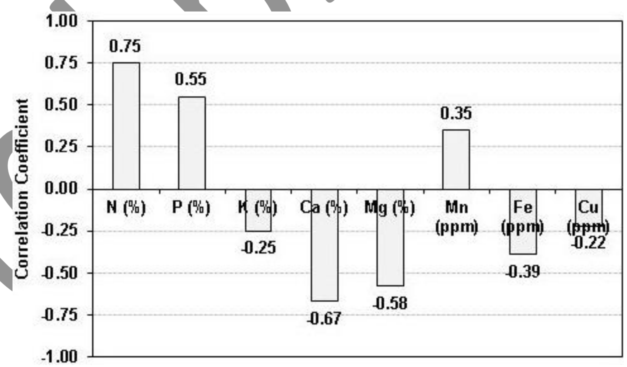

Fig. 2: Correlation coefficients between leaf water content and leaf nutrient contents (n=48)

Fe content had brighter colors (higher lightness values). Also, it was observed that the leaf samples with light yellow color had higher water contents (Table 3).

Correlation coefficients were calculated between leaf color, WC, and nutrient content. High correlation was found between color parameters and N, Ca, and WC (|r|>0.66) (Table 4). Among the five color parameters measured in the study, L*, a*, b*, and C* had similar correlations; however, h° had lower correlation for these three leaf constituents (N, Ca, and WC) (Table 4). P, K, Mg, and Mn had medium correlations with color parameters as Fe and Cu had the lowest correlations.

Correlation coefficients were also calculated between leaf water content (WC) and leaf nutrient contents. It was found that a high correlation existed between leaf N content and leaf WC content (r=0.75) (Fig. 2). It was observed that the leaves with high WC had also high N content. Leaf WC had medium level correlations with leaf P, Ca, and Mg content (r=0.55, -0.67, and -0.58, respectively) while it has low correlation with K, Mn, Fe, and Cu contents (r=-0.25,

Table 2: Results of the leaf chemical analysis and leaf color measurements (n=48)

Nutrient and Color	Min	Max	Mean	Standard Deviation	Coefficient of Variation (%)	Sufficiency Level[1]	Comment
N (%)	2.70	3.59	3.18	0.23	7.2	3.0-4.0	Low to normal
P (%)	0.16	0.27	0.22	0.03	13.6	0.2-0.4	Low to normal
K (%)	0.85	1.67	1.22	0.18	14.8	1.1-2.5	Low to normal
Ca (%)	0.53	1.05	0.74	0.14	18.9	0.5-1.5	Normal
Mg (%)	0.13	0.16	0.14	0.01	7.1	0.25-0.45	Low
Mn (ppm)	82.0	290.0	155.2	46.21	29.8	30-300	Normal
Fe (ppm)	16.2	53.8	34.05	7.07	20.8	50-300	Low
Cu (ppm)	8.0	16.0	12.46	2.10	16.9	3-15	Normal
WC (%)	58.3	65.7	62.4	1.82	2.9	-	-
L*	34.59	55.03	43.64	5.81	13.3	-	-
a*	-19.12	-9.00	-15.45	2.90	-18.8	-	-
b*	13.40	43.49	27.05	8.56	31.6	-	-
C*	16.22	47.05	31.20	8.76	28.1	-	-
h°	112.5	126.5	120.8	4.03	3. 3	-	-

[1]Sufficiency levels are based on NCDA and CS (2015)

Table 3: Mean color parameters, water contents (WC), and nutrient contents of dark green, light green, and light yellow leaves

	Dark green leaves (n=8)	Light green leaves (n=5)	Light yellow leaves (n=7)
L*	35.4	42.4	53.2
a*	-9.9	-15.6	-17.8
b*	14.1	25.3	40.2
C*	17.3	29.6	43.9
h°	125.2	121.8	113.7
N (%)	2.84	3.14	3.40
P (%)	0.19	0.20	0.24
K (%)	1.31	1.10	1.09
Ca (%)	0.91	0.66	0.59
Mg (%)	0.15	0.14	0.14
Mn (ppm)	145.5	160.6	232.4
Fe (ppm)	38.7	33.3	31.4
Cu (ppm)	13.6	11.6	12.7
WC (%)	59.4	61.9	64.2

Table 4: Correlation coefficients among water content, nutrient content, and color parameters (n=48)

	L*	a*	b*	C*	h°	Comment
N (%)	0.75***	-0.77***	0.76***	0.78***	-0.66***	High
P (%)	0.47***	-0.54***	0.49***	0.50***	-0.36*	Medium
K (%)	-0.44**	0.38**	-0.44**	-0.43**	0.45**	Medium
Ca (%)	-0.80***	0.79***	-0.82***	-0.82***	0.76***	High
Mg (%)	-0.62***	0.75***	-0.65***	-0.67***	0.52***	Medium
Mn (ppm)	0.67***	-0.39**	0.64***	0.61***	-0.73***	Medium
Fe (ppm)	-0.18^{ns}	0.33*	-0.21^{ns}	-0.23^{ns}	0.09^{ns}	Low
Cu (ppm)	-0.27^{ns}	0.35*	-0.30*	-0.31*	0.28^{ns}	Low
WC (%)	0.79***	-0.87***	0.82***	0.84***	-0.67***	High

*: Significant ($p<0.05$), **: Significant ($p<0.01$), ***: Significant ($p<0.001$), ns: not significant

0.35, -0.39, and -0.22, respectively) (Fig. 2).

Prediction models were also developed using PLSR method. The results showed that leaf N content can be predicted from L*, a*, b* color values (Table 5). Using these three color values together instead of separate variable slightly improved the model performance (RMSEP=0.15%, R^2=0.59). Also, good results were obtained for leaf Ca content; it can be predicted from L*, a*, b* color values (RMSEP=0.09%, R^2=0.66) (Table 5). In addition, the model

Table 5: RMSEP and R^2 values of the models to predict leaf water content and nutrient contents from L*, a*, b* values

	L* only		a* only		b* only		L*, a*, b*	
	RMSEP	R^2	RMSEP	R^2	RMSEP	R^2	RMSEP	R^2
N (%)	0.16	0.54	0.15	0.58	0.15	0.56	0.15	0.59
P (%)	0.03	0.18	0.02	0.25	0.03	0.20	0.03	0.20
K (%)	0.16	0.16	0.17	0.11	0.16	0.16	0.16	0.16
Ca (%)	0.09	0.62	0.09	0.61	0.09	0.66	0.09	0.66
Mg (%)	0.01	0.35	0.01	0.55	0.01	0.38	0.01	0.54
Mn (ppm)	35.8	0.41	43.6	0.13	37.2	0.37	31.5	0.55
Fe (ppm)	6.68	0.01	6.34	0.11	6.63	0.02	6.39	0.10
Cu (ppm)	2.10	0.02	2.03	0.09	2.08	0.05	2.07	0.05
WC (%)	1.15	0.60	0.93	0.74	1.08	0.66	0.95	0.73

Table 6: Performances of the models to predict leaf nutrient contents from L*, a*, b* and WC values

	L*, WC		a*, WC		b*, WC		L*, a*, b*, WC	
	RMSEP	R^2	RMSEP	R^2	RMSEP	R^2	RMSEP	R^2
N (%)	0.15	0.56	0.14	0.61	0.15	0.57	0.15	0.61
P (%)	0.02	0.20	0.02	0.27	0.03	0.21	0.02	0.22
K (%)	0.16	0.15	0.17	0.09	0.16	0.15	0.16	0.15
Ca (%)	0.09	0.63	0.09	0.60	0.09	0.66	0.09	0.66
Mg (%)	0.01	0.36	0.01	0.52	0.01	0.39	0.01	0.55
Mn (ppm)	36.2	0.40	43.8	0.12	37.3	0.36	31.5	0.57
Fe (ppm)	6.61	0.03	6.29	0.12	6.60	0.03	6.26	0.13
Cu (ppm)	2.10	0.02	2.04	0.08	2.08	0.05	2.08	0.04

to predict WC from the L*, a*, b* color parameters gave good result (RMSEP=0.95%, R^2=0.73). Adding WC to color parameters as a variable in the prediction of leaf nutrient contents did not significantly improve the model performance (Table 6). For example, RMSEP values were same (0.15 and 0.09%) in both models predicting N and Ca contents with and without WC as a variable in the model. Even if leaf WC and color parameters (L*, a*, b*) had high correlation ($|r|$>0.79-0.87) (Table 4), using WC as a variable together with the color parameters did not improve the model performance to predict nutrient contents from color parameters (Table 5 and 6).

Prediction models were also developed using L*, C*, h° color data (Table 7). Using these three color values

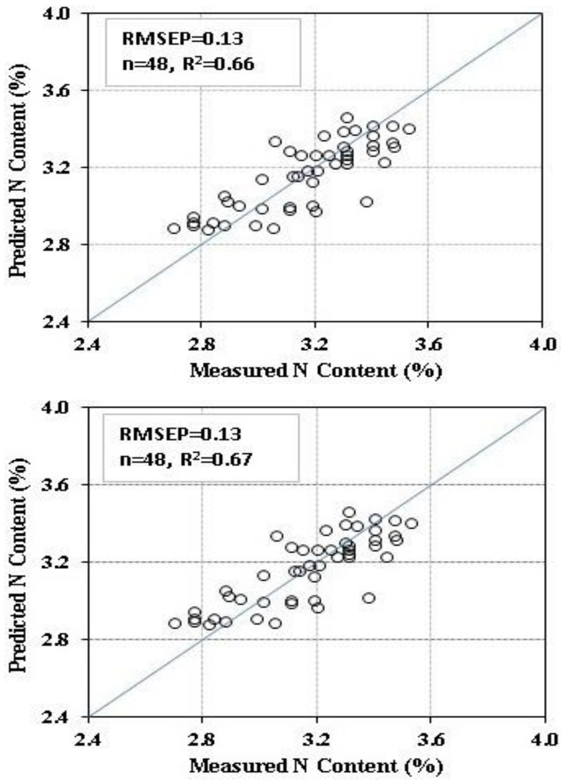

Fig. 3: Measured and Predicted N contents from the models using color parameters L*, C*, h° (top) and L*, C*, h°, WC (bottom)

Table 7: Performances of the models to predict leaf water and nutrient contents from L*, C*, h° values

	L* only		C* only		h° only		L*, C, h°	
	RMSEP	R^2	RMSEP	R^2	RMSEP	R^2	RMSEP	R^2
N (%)	0.16	0.54	0.13	0.67	0.15	0.55	0.13	0.66
P (%)	0.03	0.18	0.02	0.24	0.03	0.13	0.03	0.22
K (%)	0.16	0.16	0.16	0.15	0.16	0.19	0.15	0.19
Ca (%)	0.09	0.62	0.08	0.70	0.09	0.59	0.08	0.70
Mg (%)	0.01	0.35	0.01	0.45	0.01	0.28	0.01	0.46
Mn (ppm)	35.8	0.41	38.0	0.35	32.8	0.51	37.9	0.37
Fe (ppm)	6.68	0.01	6.43	0.04	6.62	0.03	6.45	0.02
Cu (ppm)	2.10	0.02	2.05	0.06	2.12	0.01	2.03	0.08
WC (%)	1.15	0.60	1.01	0.71	1.38	0.46	1.11	0.65

Table 8: Performances of the models to predict leaf nutrient contents from L*, C*, h° and WC values

	L*, WC		C*, WC		h°, WC		L*, C*, h°, WC	
	RMSEP	R^2	RMSEP	R^2	RMSEP	R^2	RMSEP	R^2
N (%)	0.15	0.56	0.13	0.67	0.15	0.57	0.13	0.67
P (%)	0.02	0.20	0.03	0.26	0.03	0.17	0.03	0.22
K (%)	0.16	0.15	0.16	0.15	0.16	0.19	0.16	0.19
Ca (%)	0.09	0.63	0.08	0.68	0.09	0.62	0.08	0.70
Mg (%)	0.01	0.36	0.01	0.45	0.01	0.30	0.01	0.46
Mn (ppm)	36.2	0.40	38.2	0.36	33.3	0.51	38.0	0.37
Fe (ppm)	6.61	0.03	6.37	0.05	6.42	0.03	6.43	0.03
Cu (ppm)	2.10	0.02	2.06	0.05	2.09	0.02	2.03	0.08

together instead of separate variable, slightly improved the model performance for N content (RMSEP=0.13%, R^2=0.66). Also, leaf Ca content can be predicted from L*, C*, h° color values (RMSEP=0.08%, R^2=0.70) (Table 7). In addition, the model to predict WC from only C* parameter gave good result (RMSEP=1.01%, R^2=0.71). Adding WC to color parameters as a variable in the prediction of leaf nutrient contents did not significantly improve the model performance (Table 8). For example, RMSEP values were similar (0.13 and 0.08%) in both models predicting N and Ca contents with and without WC as a variable in the model. Even if there is a high correlation between WC and color parameters (L*, C*, h°) (|r|=0.67 to 0.87) (Table 4), using WC as a variable together with the color parameters did not improve the model performance to predict nutrient contents from color parameters (Table 7 and 8). Therefore, it was found leaf WC is not needed as a prediction variable to estimate the leaf nutrient contents.

Measured and Predicted N contents from the models using color parameters L*, C*, h° and L*, C*, h°, WC are shown on Fig. 3. Results showed that leaf N content can be predicted from color parameters L*, C*, h° (RMSEP=0.13%, R^2=0.66). Using WC as a variable together with the color parameters minimally improved the model performance to predict nutrient contents from color parameters (Fig. 3).

Discussion

In the majority of the previous studies, effect of water content on the leaf nutrient estimation has not been accounted for. In our previous studies (Keskin *et al*., 2013; Keskin *et al*., 2016), we found a high correlation between water content and color of the leaf samples having both chlorotic and non-chlorotic detached crop leaves. However, low correlation existed if the leaf samples included only healthy non-chlorotic leaves (Keskin *et al*., 2016). Therefore, this present study was carried out to study the effect of leaf WC on the leaf nutrient stress estimation. In the present study, it was found that the pH value of the soil (7.5) was over the optimum value (6.5-7.0). High soil pH restricts micronutrient availability. Thus, iron deficiency and resulting iron chlorosis is a frequent problem in high pH soils decreasing the plants accessibility to iron (Cox and Koenig, 2010). Iron deficiency results in interveinal chlorosis in which leaves turn yellow or light green as leaf veins stay green (Cox and Koenig, 2010). The leaf samples with yellow color in Fig. 1 had a symptom of Fe deficiency. Fe chlorosis is a frequent problem in alkaline soils. Fe plays a crucial role in chlorophyll synthesis and makes leaves look greener (Keskin *et al.*, 2004). In such a case with iron deficiency, farmers tend to use foliar iron fertilizer application. Since farmers usually do not employ leaf analysis, they are unable to determine the nutrient deficiency on time. After the symptom is visible, it is usually late for fertilizer application. In the current study, it was found that only Ca, Mn and Cu concentrations were in normal range as N, P, and K levels were in low to normal range and Mg and Fe content are in low range (Table 2).

Results of the soil analysis showed that there was a high variability in N, K, Mn, Fe, and Cu concentrations of the soil in the study field (Table 1). Even if the area is very small (about 0.3 ha), a significant variation in some of the soil nutrients was existing. This means that applying fertilizers at a constant rate to the field results in over-application in some parts of the field which may cause increased cost, labor and crop toxicity.

In general, the greenness of a plant is usually thought to be connected to the high nitrogen content. This is not always true. Occasionally iron may have a superior influence than nitrogen on the leaf green color (Keskin *et al.*, 2004). In the current study, a similar result was found which is not mentioned in the majority of the previous studies. One of the important findings of this study is that the samples with yellowish leaf color had lower Fe content (31.4 ppm) but had higher N content (%3.40) (Table 3). In this case, even if the N content is higher, the plants looked yellow due to the lower iron content. This means that iron had a more dominant effect in the leaf color than the nitrogen content. In addition, samples with yellowish leaf color had higher P and Mn content than the dark green leaves while they had lower K, Ca, and Mg content than the dark green leaves (Table 3).

In the present study, high correlation was found between color data and N, Ca and WC (Table 4). Although Fe concentration had an important effect on leaf greenness, a very low correlation ($r \leq 0.33$) was found. One of the reasons for this could be that the variation of the iron contents of the samples was not high enough i.e., from 16.2 to 53.8 ppm (Table 2). In statistics, low variability in the samples is a crucial factors affecting the correlation and therefore, predictability (Esbensen, 2009).

In remote sensing, it is a common fact that water is absorbed in specific bands. In general, chlorophyll absorbs blue and red light and use them for photosynthesis. Water absorbs electromagnetic radiation in the bands near 940, 1100, 1350, 1900, and 2450 nm (Keskin *et al.*, 2004). Water absorption in the visible band is not reported before as far to the knowledge of the authors. However, in the current study, we found a high correlation between color properties (reflected light in visible band) and water content (WC) of the leaves ($|r| \geq 0.67$) (Table 4). In order to investigate the reason of this finding, the relationship between the color and the WC of the leaves was studied. It was found that the leaves with yellow color had high WC (64.2%) while dark green leaves had low WC (59.4%) (Table 3). This means that plants with yellow color and under stress were able to obtain water but unable to use it for photosynthesis and retain it therefore resulting in higher WC.

Adding WC to color parameters as a variable in the prediction of leaf nutrient contents did not significantly improve the model performance (Table 6). For example, RMSEP values were same (0.15% and 0.09%) in both models predicting N and Ca contents with and without WC as a variable in the model. Although a high correlation existed between WC and color data (L*, a*, b*) ($|r|$>0.79-0.87) (Table 4), using WC as a variable together with the color parameters did not improve the model performance to predict nutrient contents from color parameters (Table 5 and 6).

Conclusion

Leaf N, Ca and WC concentrations could be estimated from color data ($R2=0.66$, $R2=0.70$, $R2=0.65$ respectively). Although leaf WC and color data were highly correlated, using WC as a variable together with the color parameters slightly improved the model performance to predict nutrient contents from color parameters.

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