Non-destructive estimation of chlorophyll content of peanuts grown at different soil texture and salinity levels

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ABSTRACT

Chlorophyll is a significant biochemical component and can be determined in the laboratory (destructive) and using various chlorophyll content measuring devices (non-destructive). In this study, destructive and non-destructive methods were used to determine chlorophyll content and compared in peanut (*Arachis hypogaea* cv. NC-7) grown under different soil texture and saline water applications. The experiment was carried out in a complete randomized block design in pots using two soil textures (clay-loam and sandy) and three irrigation water salinity (0.7, 2.1 and 3.3 dS m^{-1}). While the chlorophyll contents (Chl-a , Chl-b, Chl-a+b, Chl-a/b) were determined with the acetone extraction procedure, which is classified as destructive methods under laboratory conditions, the Chlorophyll Content Index (CCI) values were measured with the hand-held chlorophyll meter device (Apogee CCM-200), which is a non-destructive method. While irrigation water salinity decreased all types of chlorophyll contents (Chl-a, Chl-b, Chl-a+b) (mg cm^{-2}), it did not cause a statistical difference in Chl-a/b. Linear and polynomial models were fitted between the different chlorophyll contents and the CCI values under different soil textures and saline water levels. Model performances were slightly better with the polynomial model compared to the linear model in all experimental treatments. Since the difference between model performances is small, it is recommended to use the linear model due to its ease of use. In addition, the total chlorophyll content can be safely estimated under saline conditions by using portable chlorophyll meters.

1. Introduction

Chlorophyll (Chl) is a significant biochemical component in the molecular system responsible for the photosynthesis of peanuts (Patane and Vibhute 2014). Chlorophyll a is the most abundant form of chlorophyll within photosynthetic organisms and, for the most part, gives plants their green color. There are also other forms of chlorophyll, coded b, c, and d, enlarging the overall fluorescent signal (Patane and Vibhute 2014). The amount of chlorophyll a, b and a/b ratio in plants varies depending on species, organ (Perera and Smith 2013), agronomic practices, growth stage (Manolopoulou et al. 2016), drought stress (Khaleghi et al. 2012; Tezcan et al. 2018), salinity stress (Taibi et al. 2016), temperature stress (Martinazzo et al. 2012) and light intensity (Hazrati et al. 2016).

Various methods were developed to determine leaf chlorophyll content (Patane and Vibhute 2014). Of these methods, the spectrophotometric method is accepted as the most accurate and reliable method for determining leaf chlorophyll content (Parry et al. 2014). But the most important disadvantages of this method are time-consuming measurement process, destructive and requires well trained personnel. In recent years, nondestructive, in situ, optical techniques have become widely used to give a relative indication of leaf chlorophyll concentration (Parry et al. 2014) and provide a quick, reliable alternative to in vitro techniques (Wood et al. 1993). For this purpose, chlorophyll meters were developed

which indirectly determines the amount of chlorophyll. These devices measure the transmittance of the red and infrared radiation emitted by leaves. The transmitted light is detected by the sensor as the analog signal and the inbuilt mini-processor converts this signal into SPAD or index (Chlorophyll Content Index (CCI)) by a defined arithmetic operation (Lunagaria et al. 2015). The CCM-200 uses absorbance to estimate the chlorophyll content in leaf tissue. Two wavelengths are used for absorbance determinations. One wavelength falls within the chlorophyll absorbance range while the other serves to compensate for mechanical differences such as tissue thickness. The CCM-200 measures the absorbance of both wavelengths and calculates a CCI value that is proportional to the amount of chlorophyll in the sample (Apogee 2017).

In more than 30 published papers to determine chlorophyll content for a large number of plants, the two types of chlorophyll meter developed by Minolta, (model SPAD-502) and Opti-Sciences, (model CCM-200) were commonly used (Parry et al. 2014). Chlorophyll contents were determined under water stress, salinity stress and fertilizer stress using various chlorophyll content measuring devices. Chlorophyll contents of various plants under salinity stress for beans (Beinsan et al. 2009), rice (Chandramohanan 2014), and soybean (Sabagh et al. 2015), and under water stress for olive (Khaleghi et al., 2012), cotton (Kazgöz Candemir and Ödemiş 2018), elaeagnus (Ahani

et al. 2015), canola (Meskini-Vishkaee et al. 2015), sesamum (Baştuğ et al. 2016) strawberry (Ödemiş et al. 2020), and peanut (Ngulube et al. 2018) were determined using chlorophyll meters.

On the other hand, the performance of chlorophyll content measuring devices for olives (Khaleghi et al. 2012), Asian Pear (Ghasemi et al. 2011), grape (Filimon et al. 2016), paper birch (Richardson et al. 2002), and quercus (Silla et al. 2010) under different stress conditions was evaluated and compared with spectrophotometric measurements. In this study, it was aimed to determine the chlorophyll content of peanut (*Arachis hypogaea* cv. NC-7) grown under different soil texture (clay-loam and sandy) and salinity levels (0.7, 2.1 and 3.3 dS m^{-1}) using the destructive method (spectrophotometer), and to compare them with the nondestructive method (CCI) for evaluating the performance of the nondestructive method.

2. Materials and Methods

The study was carried out at the Research and Application Farm of Agricultural Faculty at Akdeniz University between 27.05.2016 and 19.10.2016. The experimental area where the Mediterranean climate is prevailing was located at a latitude of 36°, 53', 45" N, a longitude of 30°, 38', 17" E, and an altitude of 30 m. The temperature and relative humidity ranged between 18.3-33.1°C and 21.9-82.9% at the growing season in 2016, respectively (MGM 2017).

The experiment was carried out in a complete randomized block design in pots using two soil textures, clay-loam and sandy, and began on 27.05.2016 with the planting of NC-7 (*Arachis hypogaea* cv. NC-7) peanut seeds. The crops were grown in 80 L plastic pots, 0.53 (upper diameter) × 0.41 (bottom diameter) × 0.46 (height) m deep, filled with clay-loam and sandy soils, and each pot had 4 plants. The physical characteristics of the soil were given in Table 1.

The amount of irrigation water was based on soil water deficit in four days. To prevent salt accumulation in the pots, 20% extra irrigation as leaching water was applied. Ayers and Westcot (1985) reported threshold values corresponding to 0% (EC$_i$=2.1 dS m^{-1}) and 50% (EC$_i$= 3.3 dS m^{-1}) yield reduction for peanut. Depending on the study of Ayers and Westcot (1985), three different salinity levels (EC$_i$= 0.7, 2.1, 3.3 dS m^{-1}) were applied including the control treatment (EC$_i$= 0.7 dS m^{-1}). Saline irrigation water was prepared by mixing specific ratios of three different salts (NaCl, MgSO$_4$ and CaCl$_2$) in tap water.

To determine the chlorophyll content in different salinity treatments and soil textures, a total of 54 leaves, 3 from each replication were selected and marked. The chlorophyll content index (CCI) value in the marked leaves was measured by Apogee CCM-200 portable chlorophyll meter. Similar to Pereyra et al. (2014) all measurements were carried out early in the morning to avoid variations caused by the movement of chloroplasts during the course of the day. Following CCI measurement, the marked leaves were cut and leaf chlorophyll contents (Chlorophyll-a (Chl-a) (mg cm^{-2}) and Chlorophyll-b (Chl-b) (mg cm^{-2}) were determined by UV-V (PG T60 UV VIS) spectrophotometer in the laboratory conditions, then

Chlorophyll-a+b (Chl-a+b) (mg cm^{-2}) and Chlorophyll-a/b (Chl-a/b) were calculated. The acetone extraction method (Williams 1984) was used to directly determine leaf chlorophyll content. Since acetone extraction procedure is classified as destructive methods under laboratory conditions, for chlorophyll measurements, leaf samples were cut from the plant and analyzed only at the end of the experiment. As soon as the leaves were cut, they were transported to the laboratory in a thermal bag and chlorophyll content analysis was performed.

The experimental data were statistically analyzed by the general linear model (GLM). Means were compared using Duncan's Multiple Range Test, if necessary, to separate the means of the data at 0.05 level of significance. Regression analysis was performed between chlorophyll content (Chl-a, Chl-b Chl-a+b) obtained in vitro and CCI values measured in situ in order to determine the performance of chlorophyll meter at different salinity levels.

3. Results and Discussion

In order to evaluate the effect of soil textures (ST) and salinity level (SL) on Chl-a, Chl-b, Chl-a+b, Chl-a/b variance analysis results were given in Table 2.

The mean of Chl-a, Chl-b, Chl-a+b, and CCI decreased due to salinity stress. Compared to the control treatments (0.7 ds m^{-1}), the Chl-a content decreased by 37.9% and 62.1% in the 2.1 ds m^{-1} and 3.3 ds m^{-1} treatments, respectively, while the Chl-b content decreased by 32.8% and 58.0%, respectively. (Table 2). Because previous studies (Jamil et al. 2007; Sabagh et al. 2015) showed that salt stress causes an increase in the chlorophyll-degrading enzyme (chlorophyllase), thereby inducing the destruction of the chloroplast structure and instability of the pigment-protein complexes. Since salts tend to attach to clay particles in the soil, salt accumulation occurs more in clay soils than in sandy soils under the same conditions (IAARD 2008). Considering these two conditions, it would be expected that the amount of chlorophyll would be low since salt accumulation was high in clay soils. However, this research showed that the mean of Chl-a, Chl-b, Chl-a+b and CCI contents were higher in clay-loam soil than sandy soil. This phenomenon can be explained by the relationship of soil texture with plant nutrients and water. Because clay soils have finer particles that can hold soil water and nutrients better than sandy soil (Dou et al. 2016). For this reason, in this study, crop development was positively affected and increased chlorophyll content due to the fact that crops benefited more from water and nutrients in clay soil.

Different levels of irrigation water salinity and soil textures did not affect the ratio of Chl-a and Chl-b (Chl-a/b) statistically (Table 2). The Chl-a/b ranged from 1.37 to 3.48, depending on the different treatments (Data not shown). Banks and Eskins (1981) reported that the ratio of Chl-a and Chl-b was between 2.5 and 3.5 in 5 different peanut varieties. However, Monge et al. (1987) declared that this value for peanuts varied between approximately 1.2 and 2.0. The difference between Chl-a/b values in different experiments shows that this ratio is affected by many environmental factors and variety differences.

Table 1. Physical characteristics of soils used in the experimental pots

No	Sand (%)	Silt (%)	Clay (%)	Texture Class	Field Capacity (%)	Permanent wilting point (%)	Bulk density (g cm^{-3})	pH	EC (dS m^{-1})
1	23.7	27.6	48.7	Clay-Loam	27	13.8	1.20	6.9	0.989
2	94	1.5	4.5	Sandy	8.5	2	1.45	7.9	0.293

Table 2. Effect of different soil textures and salinity levels on Chl-a, Chl-b, Chl-a+b, Chl-a/b and CCI content

	Soil texture	Salinity level			P > F	Mean of ST
		0.7 (ds m^{-1})	2.1 (ds m^{-1})	3.3 (ds m^{-1})		
Chl-a (mg cm^{-2})	**Clay-Loam**	$^{£}$0.38 Aa	0.22 b	0.14 c	**	†0.25 A
	Sandy	0.20 B	0.15	0.08	ns	0.14 B
	P > F	*	ns	ns		
	Mean of SL	†0.29 a	0.18 b	0.11 c		
	Significance	Soil Texture (ST): **	Salinity Level (SL): **	ST x SL: ns		
		0.7 (ds m^{-1})	2.1 (ds m^{-1})	3.3 (ds m^{-1})	P > F	Mean of ST
Chl-b (mg cm^{-2})	**Clay-Loam**	$^{£}$0.140 a	0.093 b	0.063 b	**	†0.099 A
	Sandy	0.099 a	0.062 ab	0.039 b	*	0.067 B
	P > F	ns	ns	ns		
	Mean of SL	†0.119 a	0.080 b	0.050 c		
	Significance	Soil Texture (ST): **	Salinity Level (SL): **	ST x SL: ns		
		0.7 (ds m^{-1})	2.1 (ds m^{-1})	3.3 (ds m^{-1})	P > F	Mean of ST
Chl-a+b (mg cm^{-2})	**Clay-Loam**	$^{£}$0.52 a	0.31 b	0.21 b	**	†0.35 A
	Sandy	0.30	0.22	0.12	ns	0.21 B
	P > F	ns	ns	ns		
	Mean of SL	†0.41 a	0.26 b	0.16 c		
	Significance	Soil Texture (ST): **	Salinity Level (SL): **	ST x SL: ns		
		0.7 (ds m^{-1})	2.1 (ds m^{-1})	3.3 (ds m^{-1})	P > F	Mean of ST
Chl-a/b	**Clay-Loam**	2.72	2.31	2.31	ns	†2.45
	Sandy	1.99	2.49	2.02	ns	2.16
	P > F	ns	ns	ns		
	Mean of SL	2.36	2.40	2.16		
	Significance	Soil Texture (ST): ns	Salinity Level (SL): ns	ST x SL: ns		
		0.7 (ds m^{-1})	2.1 (ds m^{-1})	3.3 (ds m^{-1})	P > F	Mean of ST
CCI	**Clay-Loam**	$^{£}$17.1 Aa	11.6 Ab	10.5 Ab	**	†12.8 A
	Sandy	10.3 B	8.3 B	7.0 B	ns	8.6 B
	P > F	*	*	*		
	Mean of SL	†13.4 a	10.0 b	8.8 c		
	Significance	Soil Texture (ST): **	Salinity Level (SL): **	ST x SL: ns		

$^{£}$: In the italicized section, means followed by the different small letters in each row or capital letters in each column are significantly different at 5% level by Duncan test.
†: In the bold section, means followed by the different small letters in each row or capital letters in each column are significantly different at 5% level by Duncan test. *: $P<0.05$ (significant); **: $P<0.01$ (highly significant). ns: non-significant.

Linear and polynomial functions were fitted to estimate Chl-a, Chl-b, Chl-a+b content from portable chlorophyll meter (CCI) measurements in sandy and clay-loam soils. The mathematical equations of linear and polynomial functions and the determination coefficients (R^2) of these equations were given in Figure 1.

The relationship between CCI and chlorophyll content was described linearly in Asian pear tree by Ghasemi et al. (2011), in wheat by Lunagaria et al. (2015), in sugar maple by Van Den Berg and Perkins (2004), and was described non-lenearly by Richardson et al. (2002) in paper birch. Parry et al. (2014), on the other hand, studied 30 different studies and examined the relationship between CCI and chlorophyll content for 22 different plants and this study highlights the enormous differences in chlorophyll distribution among species and even within species.

There was a high relationship between CCI and all chlorophyll contents in both soil textures ($R^2>0.80$) (Figure 1). Depending on the increase in chloroplast uniformity, the efficiency of red light absorption increases (Parry et al. 2014). Parry et al. (2014) reported that the transmission of light through a leaf is affected by pigment concentration and pigment spatial distribution in leaves. The authors also claimed that non-uniform chlorophyll distribution caused decreases in

transmission of light at lower chlorophyll concentrations. Therefore in each chlorophyll content, R^2 values of linear and polynomial curves of clay soils were higher compared to sandy soils.

When the different regression fitted models (linear and polynomial) were examined, the R^2 of the polynomial equations showed higher performances for all soil and chlorophyll content types. On the other hand, the R^2 of all fitted model equations was very close to each other in the same treatments. For this reason, it is recommended to use the linear equation to determine the chlorophyll contents because it is easier to use.

4. Conclusion

This study was conducted to determine the effects of soil texture and salinity level on the chlorophyll content in peanuts. In addition, the determination coefficients of linear and polynomial fitted curves were determined between the chlorophyll contents obtained by destructive and non-destructive methods. In this study, the chlorophyll contents (Chl-a, Chl-b, Chl-a+b and CCI) of peanuts varied statistically depending on the salinity level of the irrigation water and soil texture. The results showed that increasing salinity levels decreased the chlorophyll contents. In addition, the chlorophyll content on clay-loam soil treatments was higher compared to
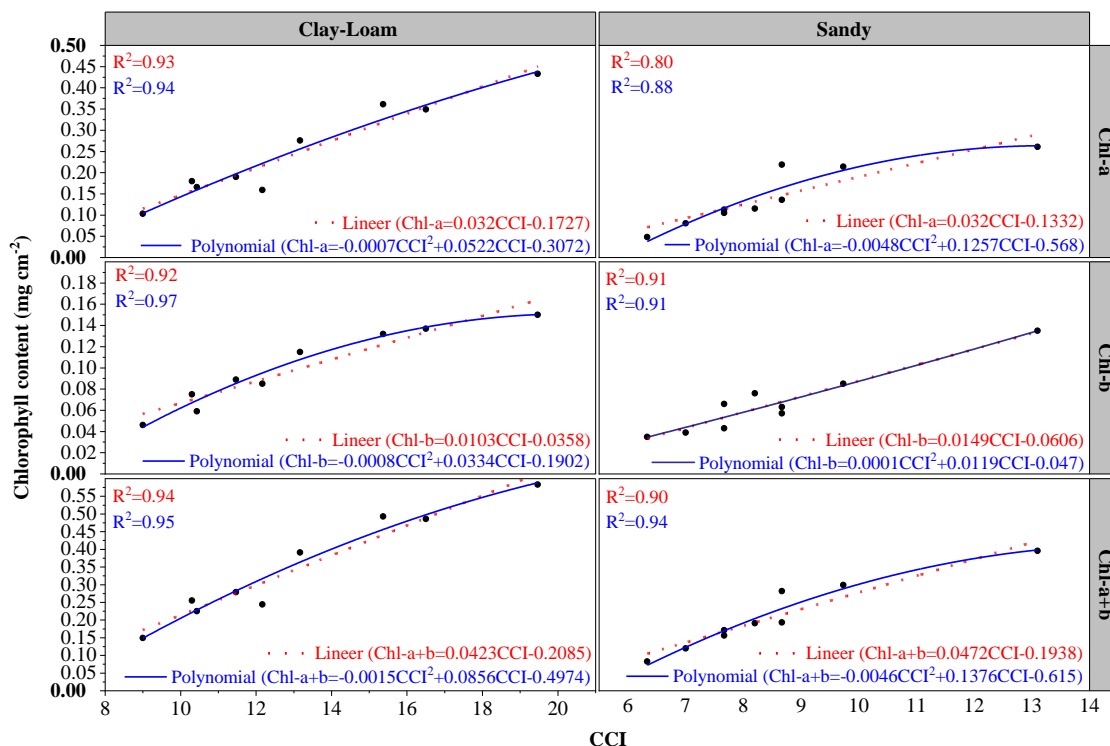
Figure 1. Equations based on linear and polynomial fitted curves between Chl-a, Chl-b and Chl-a+b and CCI values and the coefficients of determination of these equations.

sandy soil. The linear and polynomial models had high performance in all soil textures and chlorophyll contents. Although the polynomial model showed higher performance compared to the linear model, it is recommended to use the linear model to estimate CCI because there is no big difference in performance and it is easier to use. According to these results, the total chlorophyll content can be easily and safely estimated under the saline conditions by using the portable chlorophyll meter.

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