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EFFECTS OF NITROGEN ON CHLOROPHYLL FLUORESCENCE AND THE RELATIONSHIP BETWEEN CHLOROPHYLL CONTENT AND SPAD VALUES IN SUGAR BEET (*Beta Vulgaris* L.) UNDER DRIP-TAPE SYSTEM

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ABSTRACT

Determining Chlorophyll Content with Spectrophotometric Devices and Chlorophyll Extraction is a Time and Cost inefficient method so in the present Study finding a relation between Chlorophyll a, b, and Total content with Spad Values is one of the objectives. Moreover, Chlorophyll Fluorescence is a Photosynthetic activity index that is used in Biotic and Abiotic Stress studies. Nutrient deficiencies (i.e., nitrogen) as an abiotic stress can decrease yield, another aim of the current study is to determine relations between Nitrogen fertilizer rate and Chlorophyll Fluorescence and also Total Nitrogen accumulation in sugar beet organs. in order to it, an experiment carried out under tape-drip irrigation in 2013 and 2014 in Karaj, Iran, in Strip factorial plot with a randomized complete block arrangement with 2 levels of plant Spacing (14 and 20 cm), 2 levels of Planting Pattern (40-60 and 40-50) and Nitrogen Fertilizer treatment in four levels was: 0, 50, 75 and 100% of what had been recommended for furrow irrigation. The results showed nitrogen causes significant Changes on Chlorophyll A, Chlorophyll Total and SPAD Value ($P< 0.5$). Means comparison indicated that using 75% and 100% of nitrogen recommended for furrow irrigation had the best impacts on these traits. Correlation between all this traits implies that Chlorophyll a, b and Total measured with Spectrophotometry and Spad Values had positive and significant relations with themselves and with Impure sugar Yield ($P< 0.01$) the results also showed nitrogen causes significant Changes on Nitrogen accumulation in different Sugar beet Organs ($P< 0.01$) in a way that the treatment with maximum and minimum fertilizer rate had the maximum and minimum nitrogen accumulated in their organs respectively. The minimum chlorophyll Fluorescence was measured in the treatments that had the minimum fertilizers rate.

Keywords: chlorophyll a and b, chlorophyll fluorescence, SPAD, tape-drip irrigation.

INTRODUCTION

Nitrogen is a structural Chlorophyll content and much of it is a part of enzymes that are connected with the metabolic activities of chlorophyll and with respect to this fact that 70% of leave nitrogen are accumulating in the Chloroplast so it can be referred that Chlorophyll has a close correlation with Nitrogen content (Schlemmer *et al*., 2005). Chlorophyll content inside the plant is related to soil available nitrogen and the ability of plant to absorb it (Jongschaap and Booij, 2004). Leaf chlorophyll content measurement can be used as a determining tools for nitrogen status inside plant so measuring the leaf chlorophyll can show possible nitrogen deficiency (Bakhshande Larimi *et al*., 2014). There are different methods that can be used for measuring chlorophyll content i.e.: 1- methods on the basis of light absorbance in the liquid extract of leaf chlorophyll and acetone that is a time consuming laboratory action. 2- methods on the basis of light reflections from leaf surface that can estimates chlorophyll content and 3- methods on the basis of absorbing and reflexing certain wavelengths with the help of chlorophyll meter (SPAD), a hand-held, self-calibrating, convenient, and nondestructive lightweight device used to calculate the amount of chlorophyll present in plant leaves (Shibghatallah, 2013). Finding a relation between Nitrogen fertilizer rate, Chlorophyll a, b, total and SPAD values in sugar beet under tape drip irrigation system is one of the objectives of this study. on the other hand, Chlorophyll florescence, is a quick experiment that was improved in 1975 by kitajima and butler (Kitajima and Butler, 1975) the light which was absorbed by the leaf has 3 paths to be entered: 1- entering to the photochemical path to produce ATP and NADPH which are used in the photosynthesis. 2- reflecting as a fluorescent beams 3- lost as a heat (Kooten and Snel, 1990). Fv/Fm is a component of chlorophyll fluorescent that can be measured by chlorophyll phlorometer. Fv/Fm test is designed in a way that let the most of light entered to the fluorescence path this test is comparing the condition of photosynthetic reaction centers of the leaf before and after acclimating the darkness (respectively F0 and Fm). in the maximum fluorescence (Fm) most of the photosynthetic reaction centers are closed by the saturated light generally, plants under stressful conditions have less open reaction centered and Fv/Fm is low (Baker, 2008) photosynthetic system functionality can be studied by determining Fv/Fm that shows the efficiency of photosynthetic reaction center(II)(Chen *et al*., 2005). Lots of studies showed that nitrogen deficiency reduced Fv/Fm and has a destructive effect on Photosystem (II) (Conming and Zang, 2000). The other objective of this study is to evaluate effects of different levels of nitrogen fertilizer on nitrogen accumulation in different sugar beet organs and on chlorophyll fluorescence.

MATERIAL AND METHOD

This study was conducted in Motahary Research Field in Karaj- Iran, in two years of 2013-14 under Split Factorial experimental design with 16 treatments including 2 factors of Nitrogen fertilizer (4 levels of 25, 50 and 100% of recommendation rate in furrow irrigation, and without fertilizer) and planting pattern (2 levels of 40-60cm and 40-50cm with the tapes between 40cm rows) in the factorial format as vertical factors and plant spacing (2 levels of 14 and 20 cm after thinning) as a horizontal factor, with 4 replications under tape-drip irrigation. after analysis of variances results showed Planting Pattern and on-row plant spacing has no significant effect on studied traits, so the results showed on the basis of randomized complete block design with just one factor (nitrogen) and each data is a representative of 4 other digits (means of 2 levels of Plant pattern and 2 levels of on- row spacing). Prior to sowing operation, soil sampling for determining its nutritional condition was held. calculating the recommended nitrogen was done based on 15 kg urea to increase 1mg nitrate in each kilogram of Soil to the depth of 30cm (Noshad, 2010) as a result in the current experiment the net amount of nitrogen added to the soil through fertigation on several occasions in every two irrigation intervals based on the nitrogen levels of the treatment were 0 kg/ha in N0, 70 Kg/ha in N1, 100Kg/ha in N2 and 130 Kg/ha in N3. Dropper spaces are 20 cm from each other with the pressure of 0.6 bar and 1.2 lit/ha. Harvest operation was conducted at the end of the growing season, and in the laboratory, roots were weighted before providing pulp from them. White sugar yield was obtained by multiplying root yield × sugar percentage. White sugar yield is the most important parameter in sugar beet production which is the amount of sugar that can be extracted from roots; it is always lower than the total sugar yield (Cooke and Scott, 1993). To obtain sugar content, the Polarimetry method by Saccharomat instrument was used which is the most common method, Sodium and potassium contents were measured by flame photometry method.

Total Nitrogen measurements: Leaf, Petiole, Crown and taproot material dried to constant weight at 70–105$^{\circ C}$ was used to calculate the dry matter content of each fraction. Dry mater was analyzed for total N content by wet (Kejeldal-method) combustion (Bremner and Mulvaney, 1982).

Chlorophyll a, Chlb b, and total measurements: The quantitative determination of chlorophyll a, Chlb b, and total in a whole pigment extract of green plant tissue by spectroscopy at the canopy closure stage after using all the nitrogen in different treatments, based on the method developed by Lichtenthaler and Wellburn, 1985 was done. Extraction of pigments was carried out in stoppered tubes. Plants organ samples were prepared with a laboratory homogenizer using about 1 g fresh material. Extraction solution was used for each sample: 100% acetone. The extraction ratio was 1:50. Homogenized mixture is separated by centrifugation at 3000 rpm, for 10 minutes. The analytical determination was performed with Helios α spectrophotometer at the following wavelengths: 645 and 663 nm, for chlorophyll a and b. Equations used for calculation are presented below.

Chlorophyll a = 11.75 A662 – 2.350 A645

Chlorophyll b = 18.61 A645 – 3.960 A663

SPAD value measurements: at the same day, and in the same rows that sampling for chlorophyll measurement content took place, with the help of chlorophyll meter (SPAD 502), chlorophyll content of 10 leaves were calculated.

Chlorophyll fluorescence: Contents of leaf fluorescence i.e. Primary fluorescence (F0), Maximum fluorescence (Fm) and Variable Fluorescence (Fv=Fm-F0), with the help of PSM mark II plant stress meter were measured. After collecting all the data and measuring all the traits, analysis of variances, means comparison and regression equations with the SAS software ver. 9.2 were analyzed and with excel software trend lines were illustrated.

RESULTS AND DISCUSSIONS

Based on the results, treatment levels of On-Row spacing and Planting Pattern didn't have any significant effect on qualitative and quantitative traits of sugar beet in tape-drip irrigation system (Table-1). Maximum Sugar and root yield were obtained in the treatment with 75 and 100 percent of nitrogen recommendation rate (Table-1.) means of two year showed that the maximum root, sugar and white sugar were more than 70, 10 and 8 T.ha^{-1} respectively and in the plots with 75% and 100% nitrogen recommendation rate (Ttable-1). So, it can be understood from this results that decreasing 25% of the amount of nitrogen from the recommendation in the sugar beet cropping system under tape-drip irrigation can be economical and environmental friendly but further decrease in the fertilizer rate may diversly affect the yield and caue yield loss, as shown in the Table-1 the minimum Root and sugar yield were in the plots without fertilizer (on avarage 59.4 and 8.5 T.ha^{-1}). So same as the previous studies, we can cincluded that nitrogen is an essential element in sugar beet growth that can positively and negatively affect the yield of this crop, the excess amount of nitrogen availebility can reduce quality of Roots and lack of this element can cause reduce in quantity of the yield (Werker *et al*., 1999).

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Table-1. Means comparisons of some qualitative and quantitative sugar beet root traits, Karaj, 2013-2014.

Treatment/ Trait	Root yield ($T.ha^{-1}$)			Sugar yield ($T.ha^{-1}$)			White sugar yield ($T.ha^{-1}$)			Sugar percentage			White sugar percentage		
Nitrogen	2013	2014	mean	2013	2014	mean	2013	2014	mean	2013	2014	mean	2013	2014	mean
0	54.1c	64.7b	59.4	8.5c	8.6b	8.5	7.0c	6.5	6.8	15.8	13.2b	14.5	13.1	10.1b	11.6
50%	61.3b	66.8a	64.1	9.6b	9.4ab	9.5	7.9b	7.3	7.6	15.7	14.1a	14.9	12.9	11.1a	12.0
75%	69.2a	72.0a	70.6	10.8a	9.7a	10.3	8.8a	7.4	8.1	15.6	13.5ab	14.6	12.7	10.4ab	11.4
100%	71.3a	68.8ab	70.1	11.3a	9.5a	10.4	9.3a	7.4	8.4	15.9	13.8ab	14.8	13.2	10.7ab	11.9
On-row Spacing															
14	64.3	68.7	66.5	10.1	9.4	9.7	8.3	7.3	7.8	15.7	13.8	14.8	12.9	10.74	11.8
20	63.7	67.5	65.6	10.0	9.1	9.6	8.2	7.0	7.6	15.8	13.5	14.7	13.0	10.43	11.7
Planting Pattern															
40-50	63.3	69.7	66.5	9.9	9.5	9.7	8.1	7.4	7.8	15.6	13.7	14.7	12.8	10.72	11.7
40-60	64.6	66.5	65.6	10.0	9.0	9.6	8.5	6.9	7.6	15.9	13.6	14.8	13.1	10.45	11.8
Mean	64.0	68.1	66.0	10.0	9.3	9.65	8.3	7.2	7.7	15.7	13.6	14.75	12.9	10.58	11.75

Analysis of variance and comparison of means were shown in Table-2. Nitrogen has a significant effect on chlorophyll a and Total which were measured by spectrophotometry and also has significant effect on Spad values and Sugar Yield ($P<0.05$). comparison of means shows that in tape drip irrigation, the most Sugar yield belongs to the plots that received 75% of the recommended nitrogen fertilizer with 9.7 $T.ha^{-1}$ Sugar Yield (Table-1) in the plots without using Nitrogen fertilizer the minimum sugar yield were obtained (8.5 $T.ha^{-1}$). Comparison of means also showed that the maximum Chlorophyll a and total measured by spectrophotometric device and the maximum SPAD values was detected in the treatments with 75 and 100% Nitrogen recommended rate and the minimum Chlorophyll a and total measured by spectrophotometric device and the minimum SPAD values was for treatments with zero and 50% Nitrogen recommended rate. changes of chlorophyll b content in different Nitrogen treatment was similar to that of chlorophyll a and total but the effect of Nitrogen was not significant statically based on Duncan test (Table-2., $P>0.05$). So it can be concluded that with increasing Nitrogen fertilizer rate, the amount of chlorophyll and Sugar content were increased. So, considering the ecological and economical impacts of Nitrogen, it can be inferred that the best level of nitrogen recommendation for having the best Sugar yield and the best condition of chlorophyll in Drip-Tape irrigation system of sugar beet is 75 percent of the amount recommends in gravity irrigation methods. it can be the result of improved Nitrogen Uptake efficiency in the fertigation by comparison with dry usages of fertilizers.

Table-2. Analysis of Variance and Means comparison of Chlorophyll and nitrogen content and Chlorophyll fluorescence(Fv/Fm) of sugar beet in tape-drip irrigation at different nitrogen levels. Karaj. 2014.

S.O.V	D.F	Means of square roots								
		Chlorophyll content				Total Nitrogen ($Kg.ha^{-1}$)				Chlorophyll fluorescence
		a	b	Total	SPAD	leaf	petiole	Root	All plant	Fv/Fm
replication	3	0.41	0.51	0.47	3.8	241.9	49.0	17.6	295.1	0.002
Nitrogen	3	*4.66	0.67	*8.8	*26.6	**2238.1	**243.8	**5044.5	**17618.9	**0.054
error	9	0.45	0.59	1.3	1.4	38.1	23.7	24.5	105.8	0.001
C.V		11.4	55.0	15.6	4.2	15.1	29.3	5.1	6.6	11.1
Nitrogen fertilizer recommendation rate based on gravity irrigation	0%	4.67b	0.99	5.67b	45.1b	21.3c	8.9b	60.8d	91.1d	0.26b
	50%	5.22b	1.14	6.36b	46.1b	23.2c	11.0b	75.5c	109.8c	0.28c
	75%	7.00a	1.89	8.91a	49.1a	46.8b	21.5a	119.3b	187.6b	0.49a
	100%	6.46a	1.57	8.04a	50.6a	71.6a	24.9a	136.0a	232.6a	0.46a

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Table-3. Correlation efficiencies among Sugar Yield, Chlorophyll contents and SPAD values in sugar beet under tape drip irrigation, Karaj, 2014.

	Sugar yield	SPAD values	Chla a	Chlb b	Chl Total
Sugar Yield	1/00				
SPAD Values	**0.77	1/00			
Chl a	**0.87	**0.89	1.00		
Chl b	**0.83	**0.83	**0.98	1/00	
Chl Total	**0.86	**0.88	**0.99	**0.99	1/00

Correlation efficiencies among studied traits illustrated in the Table-3 Showed significant positive correlations between Chlorophyll a, b, total measured with spectrophotometry and SPAD Values (P<0.01) also they have a positive and significant correlation with Sugar Yield. So it can be concluded that Spad values which can rapidly and nondestructively gave us information about pigment status (so one can judge about nitrogen level) inside leaves can be a good alternative for time consuming methods like chlorophyll extractions and spectrophotometry but to estimate the chlorophyll a, b and total contents of the leaves from SPAD values in sugar beet, Regression trends and equation were fit (Figure-1) regression coefficient for these traits are more than 90% which shows the highly possibility of using these equations to estimate Chlorophyll status of leaves with the help of SPAD the Slight differences between SPAD values and Chlorophyll contents can be related to the fact that in spectrophotometry one special wavelength for each kind of pigments are used but in SPAD the wide spectrum of light is used (Lichtenthaler and Wellburn, 1985; Jongschaap and Booij, 2004).

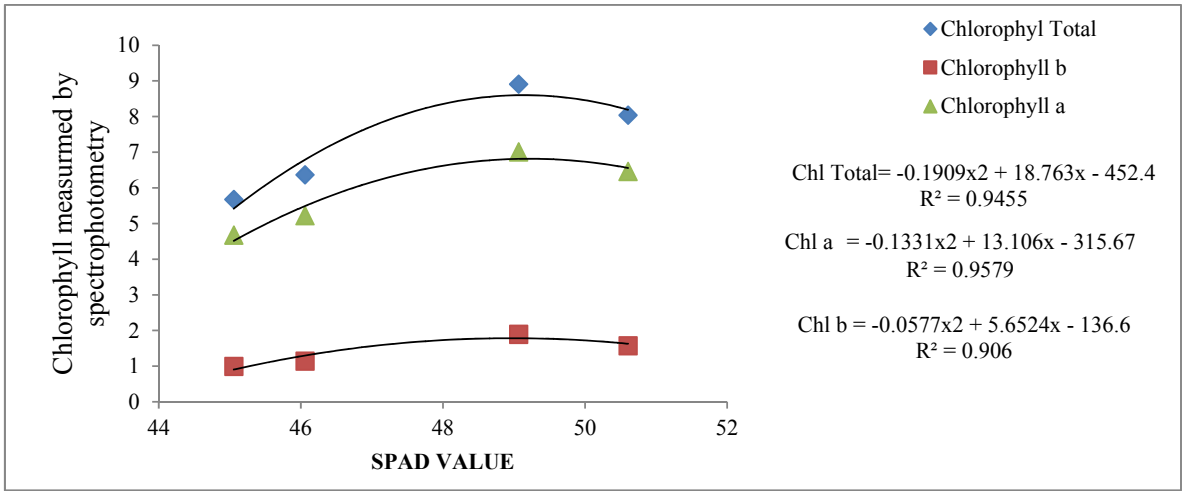

Figure-1. Regression trends and coefficients between Chlorophyll measured by Spectrophotometry and SPAD values in sugar beet, Karaj, 2014.

Nitrogen has significant effect on Chlorophyll fluorescence (Fv/Fm) and Nitrogen accumulation in different organs of Sugar beet (Table-2, P<0.01). Comparison of means showed that the most nitrogen accumulation in the organs of sugar beet under tape drip irrigation were always in the treatments with the most nitrogen fertilizer usage (71.6, 24.9, 136.0 and 232.6 in leaf, petiole, Root and all plant respectively, Table-2). it has also showed that with decreasing the amount of nitrogen fertilizer usage, the amount of accumulation in the sugar beet organs will decrease so the least Nitrogen content always were detected in the plot without adding Nitrogen (21.3, 8.9, 60.8 and 91.1 in leaf, petiole, Root and all plant respectively, Table-2) but decreasing the nitrogen from 100% recommendation to 75% recommendation rate, didn't decreased the Fv/Fm ratio. on the other word, reduction of 25% of nitrogen from the fertilizer recommendation rate not only didn't cause stress for sugar beet in the tape-drip irrigation systems but also can lead to the increase of Sugar yield (9.7 $T.ha^{-1}$ Vs. 9.5 $T.ha^{-1}$, Table-1) but further reduction in the fertilizer led to the further stress and decreased the Fv/Fm ratio (Table-2).
to estimate Nitrogen contents of leaves, Petiole and whole plat and Sugar yield from the chlorophyll fluorescence (Fv/Fm), regression trends and equations were measured and set (Figure-2) as it was illustrated in the Figure-2 the best regression coefficient is for Nitrogen available and accumulated in the petiole (R^2= 0.96; Figure-2). So it can be inferred that chlorophyll fluorescence can illustrates the nitrogen contents inside the petiole better than leaves. on

the other side, regression coefficient of the Nitrogen contents of leaves and whole plant is significant and positive, too (R^2= 0.88 and 0.94, respectively; Figure-2) so based on this results we can confidently use the Stress meter in the field as a device to ensure the nitrogen status of plant at least at the canopy closure stage (like this study) for sugar beet crops planted under tape-drip irrigation and plan the time and amount of nitrogen which is needed to prevent stress on the other side, relation between Chlorophyll fluorescence at this stage and the Sugar yield was shown in Figure-2 based on the results the regression coefficient is 0.73 and it means with the help of stress meter 73% of the changes in the sugar yield can be expressed. based on the fact that nutrition stress (importantly lack of nitrogen) can cause yield reduction (Conming and Zang, 2000) and chlorophyll fluorescence is an appropriate way to show biotic and abiotic stresses (Kitajima and Butler, 1975), So Chlorophyll fluorescence (Fv/Fm) measurement of the sugar beet for predicting the sugar yield and estimating the nitrogen contents with the proposed regression models in this study can help producers to increase their products, decrease their costs and help environment.

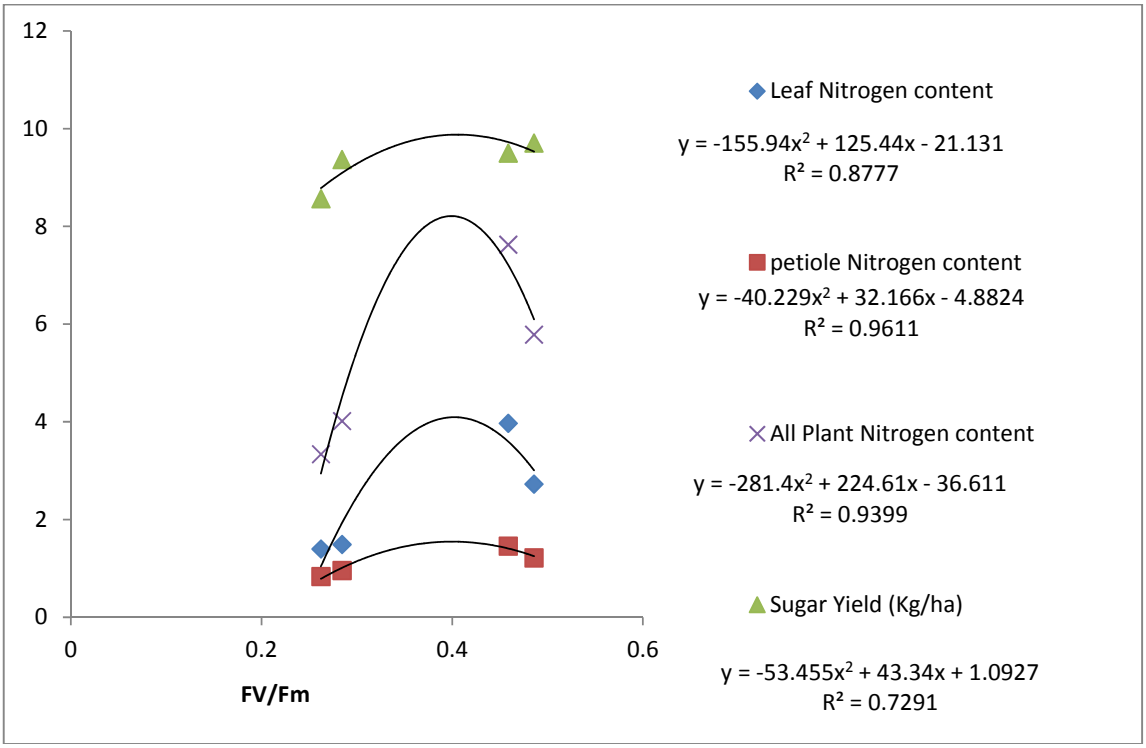

Figure-2. Regression trends and coefficient between Chlorophyll fluorescence and Nitrogen accumulation in sugar beet organs and Sugar yield. Karaj, 2014.

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