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Evaluation of Chlorophyll Content and Chlorophyll Fluorescence Parameters and Relationships between Chlorophyll a, b and Chlorophyll Content Index under Water Stress in *Olea europaea* cv. Dezful

E. Khaleghi, K. Arzani, N. Moallemi, M. Barzegar

***Abstract*—**This study was conducted to determine effect of water stress on chlorophyll content and chlorophyll fluorescence parameter in young `Dezful' olive trees. Three irrigation regimes (40% ETcrop, 65% ETcrop and 100% ETcrop) were used. After irrigation treatments were applied, some of biochemical parameters including chlorophyll a, b, total chlorophyll, chlorophyll fluorescence and also chlorophyll content index (C.C.I) were measured. Results of Analysis of variance showed that irrigation treatments had significant effect on chlorophylla, total chlorophyll (chl a+b), C.C.I and Fv/Fm ratio. The amount of decreased chlorophyll a and total chlorophyll in plants were received 40% ETcrop were 51.55% and 46.86%, respectively, compared with 100% ETcrop.

***Keywords*—**Evatarnspiration (ETcrop), Chlorophyll Content, Chlorophyll Fluorescence, Water stress, Olive

I. Introduction

CHLOROPHYLL is a green molecule in plant cells which plays important role in photosynthesis process. It absorbs sunlight and uses it's energy to synthesis carbohydrates from CO_2 and water. There are two types of chlorophyll in plants, chlorophyll a and b, which both of them works as photoreceptor in photosynthesis. It is well known that photosynthetic systems in higher plants are most sensitive to drought stress [1]. Indeed, Drought is one of the factors affecting photosynthesis and chlorophyll content. Some of researchers reported that chlorophyll content and chlorophyll fluorescence parameters might estimate influence of environmental stress on growth because these parameters were closely correlated with the rate of carbon exchange [2]-[4].

Also, studies showed that Fv/Fm ratio is the most commonly parameter used for water stress indeed, Fv/Fm is a dark adapted measurement that reveals the maximum quantum efficiency of PSII under existing stress conditions [5]-[7]. Therefore, the objective of this research was to determine the effect of water stress on chlorophyll parameters and determine relationships between chlorophyll a, b and chlorophyll content index (C.C.I) under water stress on young olive plants cv Dezphul.

E. Khaleghi is with the Horticultural Department, University of Tarbiat Modares, Tehran, Iran (phone: +989163107937; e-mail: e.khaleghi@ modares.ac.ir).

K. Arazni with the Horticultural Department, University of Tarbiat Modares, Tehran, Iran (e-mail: arzani_k@modares.ac.ir).

N. Moallemi is with the Horticultural Department, University of Shahid Chamran, Ahvaz, Iran, (e-mail: moalleminoor@gmail.com).

M. Barzegar is with the Food Science and Technology Department, University of Tarbiat Modares Tehran, Iran (e-mail: mbb@modares.ac.ir).

II. Material and Methods

This study was conducted under greenhouse condition at agriculture college, Tarbiat Modares University, Tehran, Iran during 26 June to 26 August 2011. Two- year old olive seedlings (*Olea europaea* cv. Dezphul) were used. Twenty seven plants were grown in 16 L pots, containing mixture of field soil (73.2% sand, 13.3% silt and 13.5% clay) and manure. Three irrigation levels were used according to amount of evatranspiration of plants (ETcrop) in this experiment. Indeed, pots were irrigated with 40% ETcrop, 65% ETcrop and 100% ETcrop (control). After 2 months from beginning of irrigation treatment, some of biochemical parameters such as chlorophyll a, b, total chlorophyll, chlorophyll fluorescence and chlorophyll content index (C.C.I) were measured.

From each pot, three mature leaves were selected. Then chlorophyll content index (C.C.I) and chlorophyll fluorescence was calculated by chlorophyll content meter (Opti-sciences CCM 200, USA) and Plant stress meter (PSM, Bio Monitor S.C.I. AB, Sweden), respectively. Immediately after measuring of CCI and chlorophyll fluorescence, for Assessment of chlorophyll concentration, three discs leaf were prepared (disk fresh weight = 0.06 mg and leaf disk area= 0.18 cm^2) and were immersed in DMSO (Dimethylsulfoxide) and kept in the dark.

A. Chlorophyll Extraction

The DMSO extraction technique of Hiscox and Israelstam (1979) was used for chlorophyll extraction. Samples were incubated at 65 °C until leaf disks were completely colorless [8]. Absorbance of the DMSO-chlorophyll extractions and blank (pure DMSO) were measured at 645 nm and 663 nm, using a spectrophotometer (Bio-RAD Smartspect^{TM} Plus Spectrophotometer). And finally, chlorophyll a, b and total chlorophyll were calculated by Arnon's equations (1), (2), (3) [9].

$$chla(mg/g) = \frac{[12.7(\mathrm{A663})\ \ 2.69(\mathrm{A645})] \times \mathrm{V}}{(1000 \times \mathrm{W})} \tag{1}$$

$$chlb(mg/g) = \frac{[22.9(\mathrm{A645})\ \ 4.68(\mathrm{A663})] \times \mathrm{V}}{(1000 \times \mathrm{W})} \tag{2}$$

$$chltotal(mg/g) = \frac{[20.2(\mathrm{A645}) + 8.02(\mathrm{A663})] \times \mathrm{V}}{(1000 \times \mathrm{W})} \tag{3}$$

V= volume of solvent W= fresh weight of tissue extracted

B. Chlorophyll Fluorescence

Chlorophyll fluorescence was measured with a chlorophyll fluorometer (Plant Stress Meter, BioMonitor SCI AB, Umeå, Sweden) at midday. Prior to the measurements, the leaves were kept in the dark for 30 min using cuvettes. A 5-s light pulse at 400 μmol m^{-2} s^{-1} was used. Maximum quantum yield of PSII was estimated by the Fv/Fm ratio [10].

C. Statistical Analysis

The data were subjected to Analysis of variance (ANOVA) and mean values were compared with Duncan's multiple range test at p<0.05 using MSTAT-C version 1.4.2 software.

III. Results

A. Effect of Water Stress on Chlorophyll Content and Chlorophyll Fluorescence

Results of Analysis of variance showed that irrigation treatments had significant effect on chlorophyll a, total chlorophyll (Chl a +b), C.C.I and Fv/Fm ratio, but no significant difference was observed on chlorophyll b (Table I).

Also, Results revealed amount of leaf chlorophyll a and total chlorophyll (chl a+b) were reduced by increasing water deficit. In fact, amount of Total chlorophyll and chlorophyll a were higher in plants were received 100% ETcrop than 65 and 40% ETcrop.

Amount of decreased chlorophyll a and total chlorophyll in plants were received 40% ETcrop were 51.55% and 46.86%, respectively, compared with 100% ETcrop (Table 2).

Anjum et al. (2003), Farooq et al. (2009) showed that water stress changes the ratio of chlorophyll 'a' and 'b'[11], [12]. Reduction in chlorophyll content was reported in drought stressed cotton [13]. Kiani et al.(2008) revealed that the chlorophyll content decreased to a significant level at higher water deficits in sunflower plants [14] . Guerfel et al. (2009a) reported water stress had significant effect on total cholorophyll in `Chemlali' and `Che toui'; olives and the amount of reduction of total chlorophyll were 29% and 42% for in `Chemlali' and `Che toui'; olives respectively under water stress [15] Arji and Arzani (2008) indicated that the water stress had a significant effect on the amount of chlorophyll a and b in some of olive cultivars [16]. Arzani and Yazdani (2008) and Arji et al. (2003) showed that the amount of chlorophyll a, b significantly decreased under drought stress [17], [18].

TABLE I
ANALYSIS OF VARIANCE OF SOME CHLOROPHYLL PARAMETERS AS AFFECTED BY IRRIGATION REGIMES

Source	Degree of freedom	Mean square				
		Chlorophyll a	Chlorophyll b	Chl a+b	C.C.I	Fv/Fm
Irrigation	2	0.481*	$0.155^{n.s}$	1.188*	545.854*	0.015*
Error	6	0.056	0.079	0.083	457.534	0.002
Total	8					

*and ^{ns} show significant difference at 5% levels and non-significant difference, respectively

Results showed that Fv/Fm ratio was reduced in plants which was received less water (Table II).this research showed that Fv/Fm ratio between 100 and 65 % ETcrop were not significantly difference whiles Fv/Fm ratio between plants were received 40% ETcrop compared with plants were received 100 and 65 % ETcrop were significantly difference.

Guerfel et al. (2009b) stated leaves of olive seedlings that received water stress had lowest maximal efficiency of PSII photochemistry (Fv/Fm) compared with control [19]. Demmig Adams and Adams (1996) reported that decrease in fv/fm ratio could be associated with an increase in energy dissipation in the PSII antennae [20].

According to Arji et al. (2003), initial chlorophyll fluorescence (Fo), maximum chlorophyll fluorescence (Fm), variable chlorophyll fluorescence (Fv) and Fv/Fm ratio were increase, decrease and decrease, respectively under drought stress [18]. Bacelar (2006) reported in `Arbequina', `Blanqueta' ,`Cobrançosa' ,`Manzanilla' and `Negrinha' cultivars of olive, Chlorophyll fluorescence measurements showed a down-regulation of photochemical efficiency of photosystem II around midday, revealing the occurrence of a dynamic photoinhibition [21]. Faraloni et al. (2011) showed that the Fv/Fm ratio decreased by 90% in the "susceptible" cultivars of olive, whereas the "tolerant" ones did not show any decrease in Fv/Fm [22].

TABLE II
COMPARISONS OF MEANS OF TOTAL CHLOROPHYLL AND CHLOROPHYLL A, B AND C.C.I AT DIFFERENT IRRIGATION REGIMES

Treatment	Means				
	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total chlorophyll (mg g^{-1})	C.C.I	Fv/Fm
100% ET_{crop}	1.773±0.16 a	0.644±0.46 a	2.422±0.32 a	80±3.08 a	0.6660±0.01 a
65% ET_{crop}	1.521±0.13 b	0.263±0.17 a	1.788±0.17 b	69.07±1.60a	0.6740±0.06 a
40% ET_{crop}	0.914±0.04 c	0.231±0.11 a	1.135±0.09 c	35.32±27.87b	0.5473±0.05 b

Means followed by different letter within column indicate significant differences at p<0.05

Chlorophyll content index (C.C.I) decreased under water stress. Not only this study showed that amount of CCI in 100% ETcrop and 65% ETcrop were not significant difference but also Lowest chlorophyll content index obtained in plants were irrigated with 40% ETcrop (Table II). Amount of decrease of C.C.I, in plants were received 40% ETcrop, was very more compared with two other treatments.

B. Relationships between Chlorophyll a, b, total Chlorophyll with C.C.I under Water Stress

The relationships between chlorophyll a, b, and total chlorophyll with C.C.I reading under water stress were presented in Figs 1, 2, 3. This study showed that relationship between chlorophyll a and C.C.I was significantly linear, with R^2 indicating 69.7 % of variation was explained by a liner model (Fig. 1) It's mentionable that, each reading (each point) by chlorophyll meter is mean of five readings taken on one leaf (n=45). Also, there was better liner relationship between total chlorophyll and C.C.I reading (R^2=0.753, P<0.001) (Fig. 3) but this relationship was not strong between chlorophyll b and C.C.I (R^2=0.323) (Fig. 2). Cate and Perkins (2003) reported that extractable chlorophyll was strongly correlated with C.C.I ($P < 0.001$, $R^2 = 0.72$, $n = 64$) in sugar maple plant (*Acer saccharum*) [23]. Previous studies showed that there is strongly correlation between C.C.I readings (using C.C.M) and amount of chlorophyll in annual crops [24]-[27]. Ghasemi et al. (2011) reported that there is linear correlation between CCI readings and chlorophyll a

(R^2=0.7183), chlorophyll b (R^2=0.8523), total chlorophyll (R^2=0.90), and total nitrogen content (R^2=0.76) in Asian pear leaves [28].

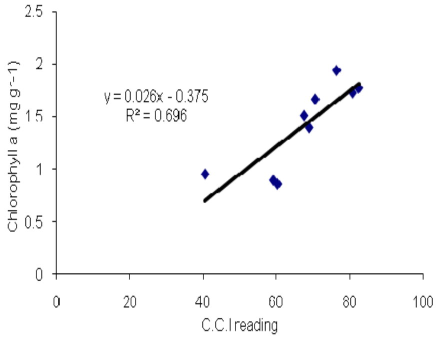

Fig. 1 Relationships between chlorophyll a and C.C.I reading

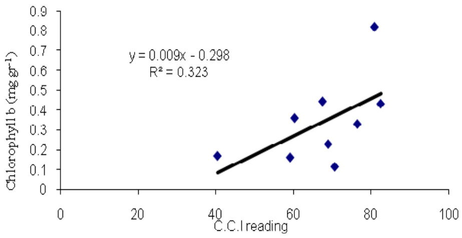

Fig. 2 Relationships between chlorophyll b and C.C.I reading

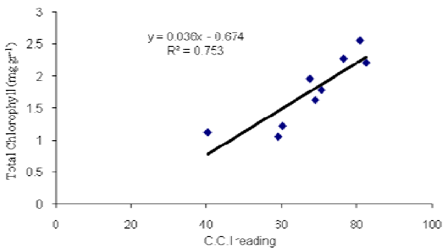

Fig. 3 Relationships between total chlorophyll and C.C.I reading

IV. Conclusions

Some of researchers believed that one of typical symptoms of oxidative stress is decreasing of chlorophyll content which may be the result of chlorophyll degradation or be due to chlorophyll synthesis deficiency or changes of thylakoid membrane structure [29], [30]. Indeed, Water stress can cause an oxidative stress due to the inhibition of the photosynthetic activity and imbalance between the light capture and its utilization [31]. Also, the decrease in chlorophyll at decreasing leaf water potentials can be attributed to the sensitivity of this pigment to increasing environmental stresses, especially to salinity and drought, which has been reported by several researchers [32], [33]. Therefore, we can suggest that an important approach for evaluating the photosynthetic process under water stress is determination chlorophyll content and chlorophyll fluorescence parameters and this method can be a rapid technique for detecting plants tolerance to drought stress and according to the regression data, we can suggest CCM-200 Device can be a good, simple, rapid non-distractive method to estimate of chlorophyll content in olive trees.

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