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The effect of drought stress on the chlorophyll content, chlorophyll fluorescence parameters and yield in the maize cultivars

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Abstract: The drought is the most important limiting factor of the agricultural plants production in Iran and the world and much of the Iranian lands among the arid and semi arid areas are classified. To evaluate the effect of drought stress of the last season on the chlorophyll fluorescence, chlorophyll content of leaf and having the tolerance against the stress of maize genotypes using the five genotypes of experimental maize in form of the randomized complete block design in four replications and in terms of aqueous and dry conditions in the agricultural years from 2016 to 2018 and in two agricultural years in Semnan was carried out. The results of analysis of variance showed the significant differences among genotypes as well as all the traits which were evaluated in two years of testing that showed the genetic richness of cultivars. The result of the mean comparisons showed with applying the stress , on the amount of F_0 was added, but the amount of chlorophyll and the ratio of F_v/F_m and ultimately the amount of grain yield was reduced that shows a negative effect of the drought stress on the chlorophyll parameters. The studies showed that the cultivars having the more chlorophyll are also stress tolerant, according to the results, single cross genotype having the highest rate of chlorophyll and chlorophyll fluorescence parameters as the stress tolerant genotype were selected. Finally it was found that due to high correlation between the F_v/F_m ratio and also the leaf chlorophyll with the yield (respectively: $R=0.88^{**}$ and $R=0.745^{**}$), these parameters can be used to assess the stress intensity and selecting the most tolerant genotype.
Keywords: drought stress, maize and chlorophyll fluorescence.

Introduction

Maize crop plays an important role in the world economy and is valuable ingredient in manufactured items that affect a large proportion of the world population [1-4].

Drought tolerance has come to the forefront of agronomic research in recent years due to dwindling irrigation reserves and increased costs associated with irrigation application [5-12].

Plant physiologists have suggested chlorophyll fluorescence as a means for understanding photosynthetic metabolism and thus identify plants, or at least genotypes, that vary in tolerance to moisture deficit. Using the chlorophyll fluorescence technique, the imbalance between metabolism and the production process can be observed [13-17]. The study of the chlorophyll fluorescence parameters is a simple and non- destructive technique and can be quickly measured. In F_0, the photochemical application potency of raised energy is Maximum and therefore photochemically reducing the fluorescence is Maximum. When the light intensity is sufficient, the fluorescence of the F0 value to its maximum amount (i.e. F_m) will increase. This increase represents the gradual increase in the fluorescence yield and concurrent with reducing the acceleration of photochemical reactions.

Measuring the chlorophyll fluorescence relatively is a new technology that in recent years for studying the effects of different stresses including drought, salinity and temperature on the photosynthetic efficiency of leaves in the farm and greenhouse conditions is used [18-24]. Climate change in recent decades has led to a decrease in rainfall amount and distribution in the arid and - regions of the world and including the Middle East. So it seems according to the changing the patterns of the drought outbreak, changing the appropriate strategies for reducing the difference of the ac yield and yield potential of crops in these areas is necessary [23].

According to Maxwell and Johnson [25], fluorescence analysis has become a powerful and widely used technique among plant physiologists and ecophysiologists. The value of fluorescence measurement lies in its relationship to photosynthesis since light absorbed by plants that does not drive the production of carbohydrates is dissipated as heat or re-emitted as light in the form of fluorescence. Physiologists and plant breeders now seek to relate fluorescence measurements and genotype specific responses to stress.

This study for evaluating the effect of drought stress on the chlorophyll fluorescence parameters and the relationship between chlorophyll content and drought tolerance in maize genotypes in Ardabil region has conducted.

Material and Methods

In this study, 5 genotypes of maize in the form of randomized complete blocks design in four replications and in terms of agricultural aqueous and dry years (2016 to 2018) and in two farming years in Semnan.

The stress treatments including two levels:

1 - Full irrigation (100 percent of the water used by plant, based on the maize varieties' requirement in the different growth stages).

2 – The limited irrigation (Supplying the aqueous needs of plant to the pollination phase and then applying the drought stress by the method of stopping the irrigation of the pollination phase to the end of maize developmental stage).

The seeds in five rows with 50 cm distance of each other and at the length of 2m manually were sown. The area of each plot was equal to 4 meters.

After planting, the farm immediately was irrigated for that the soil moisture profile in the development area of the root to be saturated and based on all treatments to be the same and in addition the germination easily to be done. The chlorophyll contents of the leaves of flag were measured by the chlorophyll meter device (CCI-200) which was manufactured by the Optic-science company. This apparatus measures the chlorophyll content index of leaves. For measuring the amount of chlorophyll fluorescence for a month after flowering e measurements by the portable Plant Stress Meter (BioMonitor SCI AB) apparatus s done, the first, after ensuring of their valves in two complete leaves from top of the plant ,two special clamps were installed until the leaves to be placed in the dark and the light reaction of photosynthesis to be stopped and for this purpose the leaves for about 40 minutes were at the dark place, And after that this time was elapsed, the clamps were attached to the optic fiber of the device and the valves were opened and after the device was started , was radiated the the 695 nm modulated light through the optic fiber toward the leaf and fluorescence parameters such as the initial fluorescence (F_0), the maximum fluorescence (F_m), the variable fluorescence (F_V) and the yield potential (F_V/F_m), which appeared on the devices were noted and written.

In order to determine the sensitivity and the resistance of the lines which evaluated under drought, the following indicators were used:

The stress tolerance index [26]:

$$STI= (Y_{Pi})(Y_{Si})/(Y_P)^2$$

In this formula,

Y_{Pi}: the genotype yield in the surface without stress (adequate irrigation).

Y_{Si}: the genotype yield in the surface of stress (lack of surface irrigation).

YP: the average of yields in the level without stress.

The traits, measured according to the average of two-year data and based on a randomized complete block design instruction were analyzed their variance and the average of treatments also by the LCD method were compared. Furthermore, for testing data analysis of data was used of the software Minitab-15, SPSS-I6 and Excel.

Results and dissociations

The results of average comparisons (Table 1) for F_0 showed that by applying the stress, the amount of this trait was increased.

Havaux et al, [27] reported that the drought stress solely does not create the significant changes in F0 and usually heat stress alone or in combination with drought stress can cause the destruction or damage to PSII reaction centers and thus F0 can be increased.

The results by Araus *et al* [28], and Wilson and greaves [29], have also been reported. Considering that the sampling in the late of spring and in a high temperature was done thus it seems that likely the heat stress increases the drought stress and eventually destroys more the PSII reaction centers and therefore the amount of F_0 was increased.

Among the genotypes, also in the maximum value of F0 was related to the single cross genotype. However in the stress conditions, the maximum amount was related to the BC582 genotype that showed the sensitivity of this variety to the moisture stress conditions.

The results of Correlation Analysis (Table 2), showing that F_0 with the amount of F_m has a significant positive correlation in the 0.05 percent level (R = 0.45 *). Furthermore, a significant negative correlation (R = - 0.61 *) among F0 and F_v/F_m was observed, which means that by increasing the amount of F0, the amount of F_v/F_m is reduced. khayatnezhad et al., [14], were reported a significant negative correlation among F_0 with Fv and F_v/F_m.

The amount of F_V/F_m representing the maximum quantum efficiency of photo-system II and a criterion that how the plant photosynthesis operates, so that the value of this parameter for the most common plant species in the environmental conditions is about 0.83. According to Paknejad *et al* [30], the drought stress will reduce the quantum yield. The value of F_v/F_m decreased with stress. So that, applying the stress caused the value of F_v/F_m to show a decline about 18.4 percent compared to the full irrigation conditions. Considering that the amount of F_m in terms of irrigation and stress was almost constant, thus the decreasing trend of F_v/F_m can be attributed to the changes in F_0.

The value of F_v/F_m represents PSII electron transport capacity [30], that with the net photosynthetic quantum yield is highly correlated [28]. Therefore, reducing the amount of 18.4 percent of the F_V/F_m ratio showing a decline in the amount of optical protection and is a reason for that the drought stress had a significant effect on the photosynthetic efficiency. They also expressed that the decreased photochemical efficiency of the photo-system II is mainly due to the sharp rise of energy excitation in the chlorophyll receptors. Due to the significance of F_0 in the stress conditions on, we can conclude that among the conditions of moisture stress, there is a significant difference compared to the chlorophyll yield [29]. Furthermore, due to the difference significance of F_v/F_m under moisture stress, the difference in the amount of yield is attributed to the difference in quantum yield.
Due to the impairment in transfer course of Electron and tissue destruction associated with photosynthesis under the plant stress conditions isn't able to use suitably the substrate and energy and according to this, the substrate consumption efficiency and energy under such conditions can severely be reduced that can be the reason of reducing the yield in recent test [31].

Between the genotypes which have been studied, the maximum amount of F_v/F_m ratio belongs to the single cross genotype and then OS499 that showed the resistance of genotypes to the stress condition (Table 1).

The results of correlation analysis (Table 2) showed that the F_v/F_m ratio except the F0 ratio that had a negative correlation, the leaf chlorophyll content and grain yield with the values of F_m, F_v showed a positive and significant correlation in the level of 0.01.

By drawing the relationship chart between the amount of F_v/F_m and grain yield (Figure 1) showed that a linear relationship existed between these two traits and with increasing the amount of $F_v/F_{m,}$ also added on the value of yield. According to the Figure 1, considered that a single cross genotype separate itself from other groups and has the highest ratio of F_v/F_m and yield.

Considering the result of average comparisons for the amount of leaf chlorophyll (Table 1), showed that the water stress conditions affected the chlorophyll content of leaves and by applying the stress declined the levels of chlorophyll. Zhao et al [32], by studying the effect of salinity on chlorophyll fluorescence of oat leaf reported that increasing the levels of salinity increases the amount of chlorophyll fluorescence and the leaf chlorophyll content consequently is reduced. So it seems that drought stress and salinity caused inhibition of chlorophyll synthesis and or increasing its breakdown. It shows the decline of chlorophyll efficiency in performing photosynthesis at times of stress. Zhao et al [32], stated that severe water shortages caused to stop the making of chlorophyll. In a water deficit conditions, often all of enzymes activities (such as nitrate reducing enzyme) are reduced.

The Figure 2 indicates that a linear relationship was between the leaf chlorophyll content and grain yield, and the existent correlation between these two traits is confirming the present diagram. And observed in this figure that the single cross genotype having the highest leaf chlorophyll content had the highest yield. There is some evidence based on that the water stress reduces the chlorophyll content (Ashraf *et al*, 2001). While in other studies such a reduction in the chlorophyll has not been observed in the stress conditions [33]. No reduction in the chlorophyll content of wheat and sunflower plants also increasing the chlorophyll ratio of a/b was reported in the other studiesIt is noteworthy that some researchers know the increase of the chlorophyll ratio of a/b causes to darken the leaves and increase the number of Chlorophyll meter [28, 34, 35]Shahriari (1999), stated that in the plants under the drought stresses, the chlorophyll green tissue in leaves of resistant cultivars shows an increase. Due to this problem, can be concluded that single cross genotype having the highest leaf chlorophyll can be as a resistant genotype to the moisture stress [21]. The calculation results of Fernandez stress tolerance index (Figure 3) showed that a linear relationship exists among the yield and STI stress tolerance index and the amount of the stress tolerance of single cross genotype is more than others.

Drawing three-dimensional charts to confirm a linear relationship between stress tolerance rate, chlorophyll and grain yield showed that by increasing the amount of chlorophyll the stress tolerance will be increased and eventually causes to increase the yield.

The Grouping of genotype according to the characteristics under study has been done and eventually they were divided in two groups, according to expectation the genotypes with more leaf chlorophyll content and chlorophyll fluorescence were in one group and showing that a group of high stress tolerance was between these cultivars. So it seems that to be higher the content of chlorophyll meter and its maintenance under the drought stress means increasing the effect intensity of stress on the plant and reducing the leaf area. In fact, the plants by reducing the leaf surface under the stress conditions reduces the transpiration area to prevent the wasted water and therefore, despite reducing the total amount of chlorophyll in leaves, the chlorophyll content increases per unit of leaf area [34, 36, 37].

Conclusion:

Regarding the results of this study can be concluded that the drought stress through the impact on photosynthesis system (maximum quantum efficiency Mtv systm II) reduces the yield components and ultimately yield in a tangible form. Therefore, also it seems that the increased content of chlorophyll meter and keeping it in drought conditions causes to increase the effect severity of stress on the plant and reduce more the leaf area. In fact, the plant by reducing the leaf surface in stress conditions reduced the transpiration level to prevent the wasted water and therefore, despite reducing the total amount of chlorophyll in leaves, the chlorophyll content increases per unit of leaf area [34]. Therefore, to evaluate drought tolerance, traits such as Fv and Fv/Fm are more reliable that even with the yield also has a high correlation. the F_v/F_m parameter is a well characteristic to determine the difference between the control and water stress conditions. Since the ratio of Fv/Fm represents the PSII electron transport capacity [31] which has been highly correlated with net photosynthetic quantum yield and can be concluded that whatever the amount is higher in the chlorophyll content, chlorophyll conditions for electron transfer would be better from PSII photo-system, and ultimately leads to a higher net photosynthetic quantum yield [31]. Finally it was found that due to high correlation between the ratio of F_v/F_m and the leaf chlorophyll, these parameters can be used for evaluating the stress intensity and selecting the most tolerant genotype.

Table 1 – the result of average comparisons for the evaluated traits

year	F_0 (ms)	F_v (ms)	F_v/F_m	Chlorophyll (mg m^{-2})	Yield
1	0.345	0.957	0.81	378.6	7.49
2	0.367	0.934	0.79	368.9	6.97
condition					
Normal	0.321	0.915	0.77	339.3	7.50
Stress	0.364	0.797	0.65	319.1	7.16
genotypes					
Single cross	0.375	0.90	0.83	355.1	8.15
ZP677	0.324	0.81	0.76	326.6	7.35
BC582	0.305	0.77	0.69	307.8	6.45
BC666	0.345	0.86	0.74	312.5	7.05
OS499	0.355	0.89	0.79	345.3	7.65

Table 2 – the result of correlation analysis between the evaluated traits

	F_0(ms)	F_m(ms)	F_v(ms)	F_v/F_m	Chlorophyll	Yield
F_0(ms)	1					
F_m(ms)	0.453*	1				
F_v(ms)	0.324	0.869**	1			
F_v/F_m	-0.615*	0.856**	0.908**	1		
Chlorophyll	0.401	0.862**	0.888**	0.995**	1	
Yield	-0.372	0.612**	0.745**	0.745**	0.88**	1

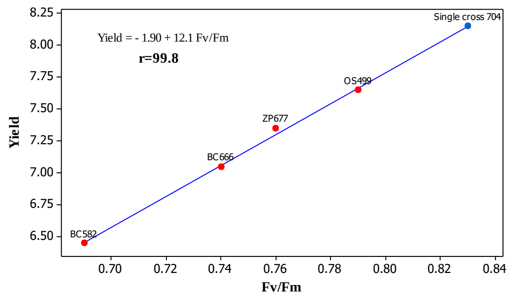

Figure 1. The relationship between the amount of F_v/F_m and yield.

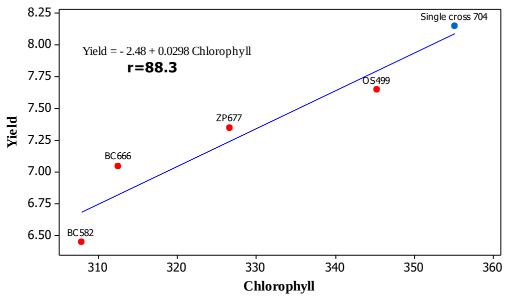

Figure 2. Relationship between leaf chlorophyll content and grain yield

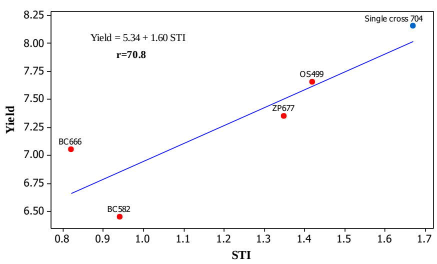

Figure 3. The relationship between STI stress tolerance index and grain yield

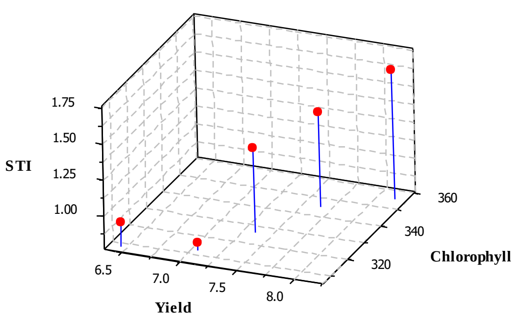

Figure 4. Three-dimensional graph of relations between the leaf chlorophyll , stress tolerance index, and grain yield

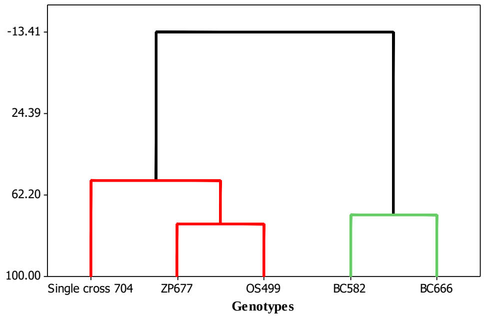

Figure 5. Grouping the investigated genotypes based on traits

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