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Full Length Research Paper

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

Roza Gholamin* and Majid Khayatnezhad

Young Researchers Club, Ardabil Branch, Islamic Azad University, Ardabil, Iran.

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The drought is the most important limiting factor of the agricultural plants production in Iran and the world. Much of the Iranian lands are classified as arid and semi arid areas. To evaluate the effect of drought stress of the last season in 2007 to 2008 on the chlorophyll fluorescence, chlorophyll content of leaf and having the tolerance of five maize genotypes, this experiment was done in a randomized complete block design in four replications in two conditions. Experiment carried out from 2008 to 2010 and in two agricultural years in Ardabil and the farming lands of Arjestan area located in 16 km of northwest of Ardabil. 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.**

Key words: Arjestan, Ardabil, drought stress, maize, chlorophyll fluorescence.

INTRODUCTION

Maize crop plays an important role in the world economy and is a valuable ingredient in manufactured items that affect a large proportion of the world population (AOQP, 2006). 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 (Gowda et al., 2007). 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 (Malakouti et al., 2005). 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 (that is 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 developed 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

*Corresponding author. E-mail: Gholamein@yahoo.com.

greenhouse conditions is used (Baker and Rosenqvist, 2004; Ort, 2004; Rapacz et al., 2001; Rizza et al., 2001; Zobayed et al., 2005).

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 patterns of the drought outbreak, changing the appropriate strategies for reducing the difference of the predicted yield and yield potential of crops in these areas is necessary (Rizza et al., 2001). According to Maxwell and Johnson (2000), 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.

According to Maize hybrids importance in our life and negative effect of drought stress on Maize through increasing photosynthesis, 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 been conducted.

MATERIALS AND METHODS

In this study, 5 maize hybrids in the randomized complete blocks design in four replications and in terms of agricultural aqueous and dry years (2008 to 2010) and in two farming years in Ardabil and agricultural land area of Arjestan was located in the 16 km of northwest of Ardebil were used. The stress treatments including two levels:

1) Full irrigation (100% 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) for end season stress.

The seeds in 5 rows with 50 cm distance of each other and at the length of 2 m manually were sown. The area of each plot was equal to 4 m. After planting, the farm immediately was irrigated so 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 Opti-science company. This apparatus measures the chlorophyll content index of leaves. For measuring the amount of chlorophyll fluorescence for a month after flowering, measurements were done by the portable 'plant stress meter' (BioMonitor SCI AB) apparatus. First, after ensuring that their valves are in two complete leaves on top of the plant, two special clamps were installed until the leaves were placed in the dark and the light reaction of photosynthesis stopped. For this purpose, the leaves were kept at a dark place for about 40 min. After this time elapsed, the clamps were attached to the optic fiber of the device and the valves were opened, and after the device was started, the 695 nm modulated light was radiated through the optic fiber toward the leaf. Subsequently, the 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 (Fracheboud, 2006).

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

The stress tolerance index (Fernandez, 1992):

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

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 by the software Minitab-15, SPSS-I6 and excel.

RESULTS AND DISCUSSION

The results of average comparisons (Table 1) for F_0 showed that by applying the stress, the amount of this trait was increased. Elato and Yordanov (2005) reported that the F0 value in terms of increasing the levels of drought stress had the maximum amount compared to the non-stress conditions that shows the destruction of PSII reaction centers in the drought conditions. Havaux et al. (1998) 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. (1998) and Wilson and greaves (1993) 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 of 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% 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. Darvishi et al. (2010) reported a significant negative correlation among F_0 with Fv and F_v/F_m. The amount of F_V/F_m

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	7.5
Stress	0.364	0.797	0.65	319.1	7.16
Genotypes					
Single cross	0.375	0.9	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.

Variable	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

representing the maximum quantum efficiency of photo-system II and a criterion of 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 (Fracheboud, 2006). According to Paknejad et al. (2007a), 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% 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 (Paknejad et al., 2007a) that with the net photosynthetic, quantum yield is highly correlated (Anonymous, 1993). Therefore, reducing the amount of 18.4% of the F_v/F_m ratio showing a decline in the amount of optical protection and is a reason that the drought stress had a significant effect on the photosynthetic efficiency. Alidib et al. (1994) expressed that reducing the photon consumption efficiency by the photo-system II specifies the amount of optical deterrence under the stress conditions. 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 (Anonymous, 1993; Wilson and Greaves, 1993). 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 is not 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 (Paknejad et al., 2007b; Darvishi et al., 2010). Darvishi et al. (1389) studying the effect of the chlorophyll fluorescence changes in the maize (SC704) yield reported the similar results. 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 F_0 ratio that had a negative correlation, the leaf chlorophyll content and grain yield

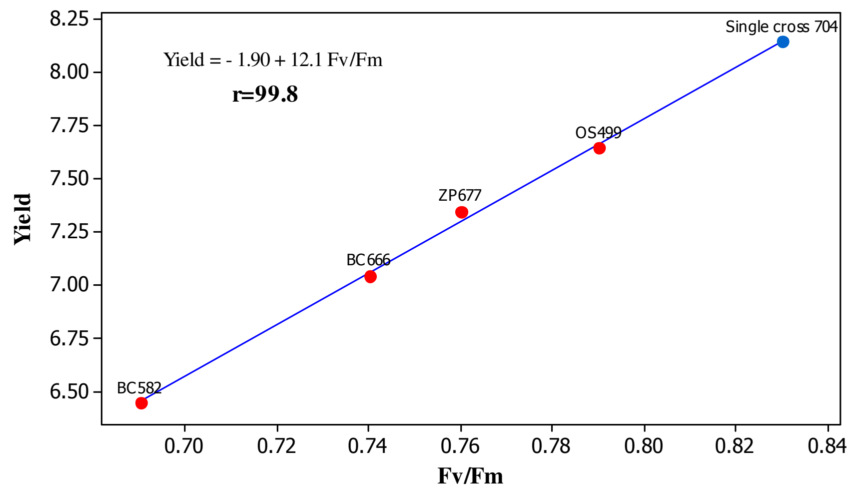

Figure 1. The relationship between the amount of F_v/F_m and yield.

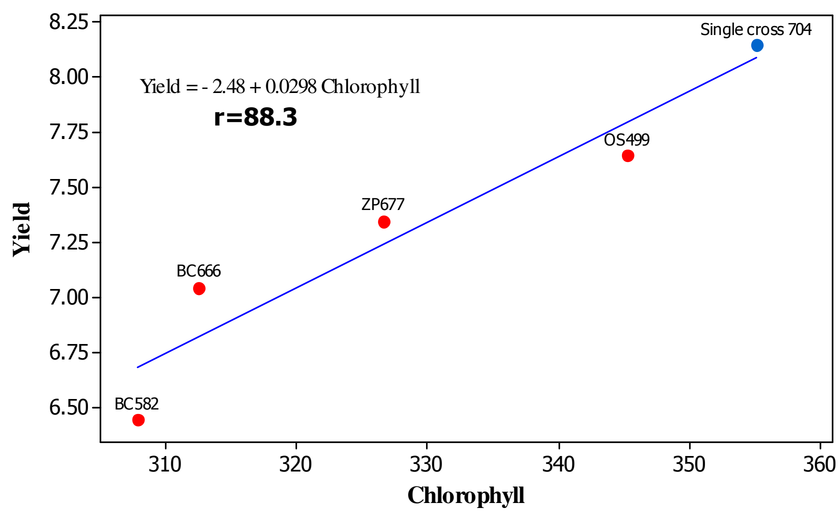

Figure 2. Relationship between leaf chlorophyll content and grain yield.

with the values of F_m, F_v showed a positive and significant correlation in the level of 0.01. Darvishi et al. (2010) also reported highly correlation with grain yield and the rate of F_v/F_m. While Araus et al. (1998) reported the lowest correlation between the ratio of F_v/F_m and the grain yield (R = 0.34). 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 Figure 1, it is 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. 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 (Zhao et al., 2007). 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. (2007) stated that severe water shortages caused the stoppage of chlorophyll making. In water deficit conditions, all enzymes activities (such as nitrate reducing enzyme) were often reduced. Figure 2 indicates that there is a linear relationship between the leaf chlorophyll content

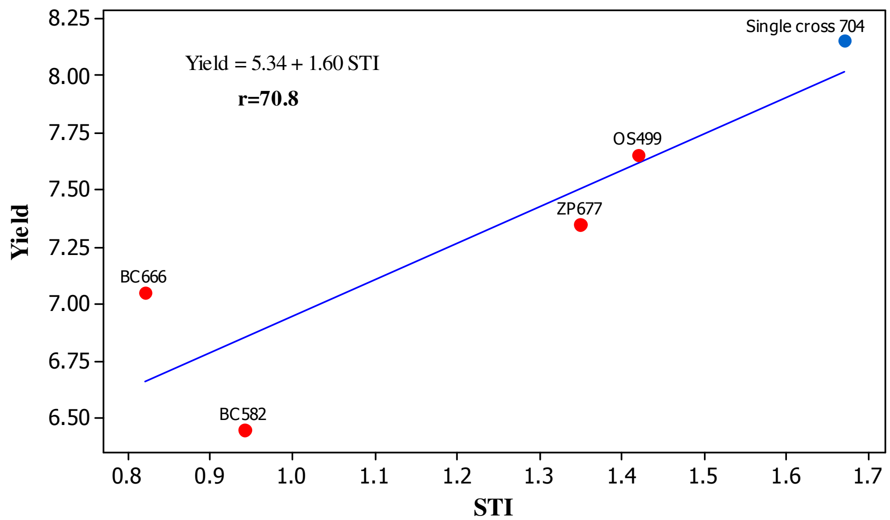

Figure 3. The relationship between STI stress tolerance index and grain yield.

and grain yield, and the existent correlation between these two traits confirms the present diagram. It was observed in this figure that the single cross genotype having the highest leaf chlorophyll content had the highest yield. There are some evidence based 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 (Ashraf et al., 2001). Ahmadi and Baker (2001) also reported that the short -term water stress that caused a typical wilt and complete halt of the net photosynthesis (Pn) in the wheat has no effect on the leaf chlorophyll but increases the chlorophyll ratio of a/b (Ashraf et al., 2001). No reduction in the chlorophyll content of wheat and sunflower plants, also increasing the chlorophyll ratio of a/b was reported in the other studies.

Antolin et al. (1995) found that the leaf chlorophyll content by increasing the drought stress was decreased but the chlorophyll ratio of a/b increases (Havaux et al., 1998). It 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 (Bohrani and Habili, 1992; Chapman and Barreto, 1997). 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, it can be concluded that single cross genotype having the highest leaf chlorophyll can be as a resistant genotype to the moisture stress. 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 (Figure 4) 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 (Figure 5) 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 high 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 (Bohrani and Habili., 1992).

Conclusion

Regarding the results of this study, it can be concluded that the drought stress through the impact on photosynthesis system (maximum quantum efficiency PS2) 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, by reducing the leaf surface of the plant 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 (Bohrani and Habili, 1992).

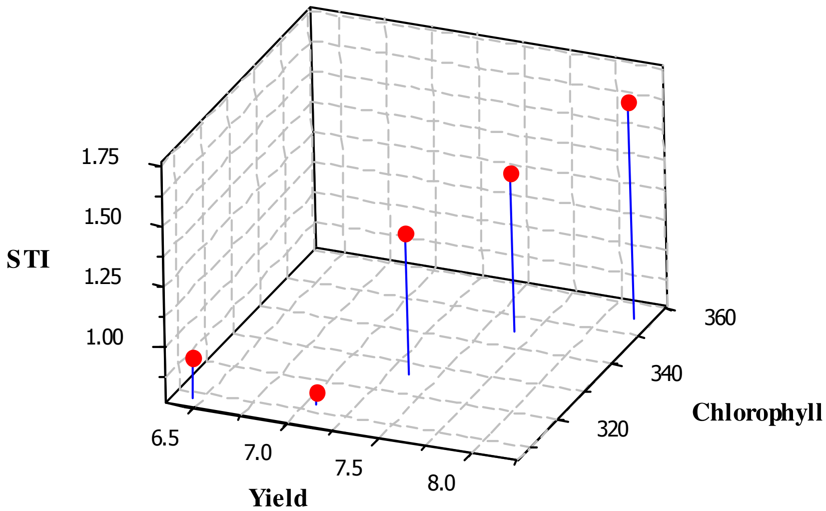

Figure 4. Three-dimensional graph of relations between the leaf chlorophyll, stress tolerance index and grain yield.

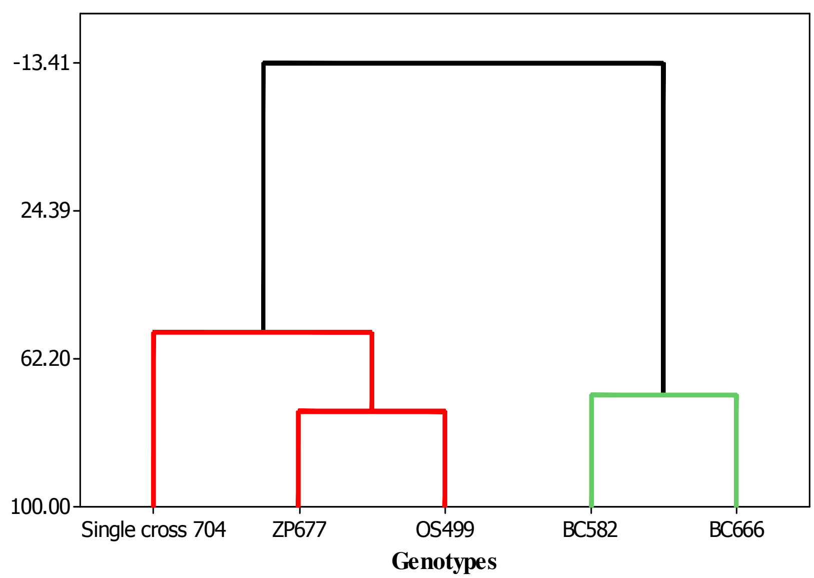

Figure 5. Grouping the investigated genotypes based on traits.

The research results have shown (Khayatneghad et al., 2010) the drought -resistant genotypes of wheat under moisture stress conditions have a higher level of carotenoids and chlorophyll, considering that the F_0 value was increased under moisture stress, suggesting this issue that presumably the rest of fluorescence factors had more impact on yield. 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. Elate and Yordanov (2005) argued that 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 (Paknejad et al., 2007b) 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 (Paknejad et al., 2007a).

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.

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