BIOLOGIA (PAKISTAN)
June, 2016, 62 (1), 95-102

PKISSN 0006 – 3096 (Print)
ISSN 2313 – 206X (On-Line)

Establishment of Relationships among changes in Fluorescence, Gaseous Exchange and Growth Attributes under Drought Stress in Maize Cultivars (*Zea mays* L.)

IJAZ AHMAD[1], MUMTAZ HUSSAIN[1], TANVEER HUSSAIN[2], MISHAL IFTIKHAR [3], ABDUL GHANI[3], SADAF HONEY GHOURI[3], MUJAHID HUSSAIN[3], MUHAMMAD IKRAM[3] & IFTIKHAR AHMAD[3].

[1]Department of Botany, University of Agriculture, Faisalabad, Pakistan
[2]Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan
[3]Department of Botany, University of Sargodha, Sargodha, Pakistan

ABSTRACT

To study physio-biochemical responses of maize cultivars (*Zea mays* L.) during drought stress conditions, a pot experiment was performed at Botanical Garden, Department of Botany, Agriculture University Faisalabad. Experiment was conducted with 7 maize cultivars i.e., Sadaf, Pak Afgoi, EV-1098, EV-5098, sahiwal-2002, Agaiti-85, Agaiti-2002, and two drought stress (60% field capacity and control) treatments with 4 replications. The results showed that water stress significantly affected the photosynthetic rate (*A*) of all maize cultivars. Different maize cultivars showed differential response to water stress. In cvs. Sahiwal-2002, EV-5098 and Agaiti-2002 photosynthetic rate increased while in all other cultivars it decreased significantly. Drought stress also reduced Transpiration Rate (*E*), Sub – stomatal carbon dioxide (CO_2) concentration (*Ci*), *Ci/Ca* ratio, F_m (maximal chlorophyll fluorescence), (fluorescence) F_v, F_v/F_o ratio and F_v/F_m ratio of all maize cultivars. However, *A/E* (water use efficiency) and F_o (chlorophyll fluorescence) values and F_o/F_m ratio were significantly increased with the imposition of water stress.

Key Words: Drought resistance, *Zea mays,* biochemical responses, photosynthetic rate, transpiration rate, chlorophyll fluorescence

INTRODUCTION

The main abiotic factor is water, which acts as a limiting factor in different crop production regions of the World (Araus *et al*., 1998). Shortage of water due to irregular and low rainfall in different regions (less than 100 mm) cause heavy crop losses in Pakistan. Any change in low availability of fresh water or rainfall causes increase in aridity and causes the greater crop loss **(**Athar & Ashraf, 2005; Parry *et al*., 2006; Tambussi *et al*., 2007). Drought is the major abiotic factor that has adverse affect on the production of agricultural crops (Lea *et al*., 2004; Ramachandra *et al*., 2004).

Drought stress has two main types i.e., physiological and physical. Few plats use escaping mechanism in which plants complete their life cycle before physiological water deficiency occurs. Some plants show avoidance mechanism in which plants avoiding dehydration of plant tissues by maximizing water uptake or minimizing water loss, while the others have tolerance mechanism which involves osmotic balance by maintaining high concentrations of solutes or osmo-protectants in living cells (Chaves *et al*., 2003).

At cellular levels and for the whole plants tolerance to abiotic factor is very difficult (Ashraf & Harris, 2004). This is because the interactions between drought stresses, various molecular, physiological and biochemical processes are affecting growth and plant development which ultimately reduce the crop production (Zhu, 2002). The production of stress tolerant crop plants is considered as a best tool to fulfill the demands of food in many parts of the world. However, for the production of these plants requires the knowledge about the genetic traits and physiological mechanisms at different developmental stages of plants. The abiotic stress tolerance mechanism in biotechnology field has provided much information in plants at molecular level in past two decades, (Zhu, 2001). The stress tolerance mechanisms during different stages of plants vary in plant species (Foolad, 1999b; Ashraf, 1994). Most of cereal plants have a range of morpho-physiological adaptations, or processes in order to respond to water stress. However, the physiological attributes are useful and are reliable sources during drought tolerance cultivars/genotypes selection (Tambussi *et al*., 2007). On the basis of physiological,

Author's Contribution: I.A., & M.H., Designed and planned research work; **T.H., & M.I**., Collected and identified plants; **A.G., & S.H.G.**, Analyzed data statistically: **M.H., M.I. & I.A.**, Wrote and edited manuscript.
**Correspondence author: Mishaliftikhar19@gmail.com*

biochemical and growth attributes, present experiment was conducted to determine drought tolerance in maize cultivars.

MATERIALS AND METHODS

A study was performed in botanical Garden of Botany Department, Agriculture University, Faisalabad to study physiological response of maize cultivars. There were 7 maize cultivars i.e., Sadaf, Pak-Afgoi, EV-1098, EV-5098, Sahiwal-2002, Agaiti-85, Agaiti-2002, and two drought stress (60% field capacity and control) treatments with 4 replicas.

Seed Sowing and Experimental Design

Completely Randomized Design (CRD) was used to carry out experiment with 4 (four) replications. Four pots of each cultivar were kept as control and in the other four pots, water stress was applied at grain filling stage. Plant thinning was done after 8 days of germination.

Determination of field capacity

The Formula used to calculate field capacity:

Field Capacity = Saturation Percentage/2

Water Stress Treatments

Drought stress treatments (field capacity and control) and irrigation (at 60% field capacity) were started at grain filling stage. During drought, the moisture contents in crop were maintained and regularly monitored by visiting experimental place. The weight of every pot was maintained to 60% (sixty percent) Field Capacity by adding irrigation water.

Soil Analysis

Soil analysis was done using standard soil analysis protocols in the Institute of Soil & Environmental Sciences located in Agriculture University, Faisalabad. The data regarding different characteristics of soil were recorded and presented in Table I.

Table I: Physiochemical properties of soil

Property	Value	Property	Value	Property	Value
Sand	69%	Soluble Ca^{2+} + Mg^{2+}	14.301 meq L^{-1}	Soluble SO_4^{2-}	1.981 meq L^{-1}
Clay	21%	SAR	0.086 meq L^{-1}	Soluble Na^{+}	2.451 meq L^{-1}
Silt	10%	Soluble HCO_3^{-}	4.93 meq L^{-1}	SP	34%
Textural classes	Sandy clay loam	Soluble Cl^{-}	8.521 meq L^{-1}	Soluble CO_3^{2-}	Traces
$CaCO_3$	2.71%	***pH***	7.80	Available P	8.6 ppm
Organic matter	0.95%	***Ece***	2.53 $dS.m^{-1}$	Total N	0.73%
CEC	17.4 meq 100 g^{-1}				

Physiological Attributes

a- Leaf Water Potential

After 15 days of water stress treatment second fully expanded leaf was excised and the Water Potential of Leaf was estimated with (Scholander type) pressure chamber (from 6 am to 8.30 am).

b- Leaf osmotic potential

Second (2^{nd}**)** excised leaves were frozen for seven (7) days in freezer at -20^{o}C, and then frozen leaves were thawed. Cell saps were extracted with the use of syringe and were directly used to estimate Osmotic Potential by an Osmometer (Wescor 5500).

c- Leaf Turgor potential (Ψp)

Leaf Turgor potential was estimated by difference between **Ψs** (osmotic potential) and Ψ_w (water potential) values.

Leaf Turgor Potential (Ψp)= Ψ_w(water potential) **-** **Ψs**(osmotic potential)

d- Gas exchange characteristics

Gas exchange analysis was performed by using (an open system, LCA-4, ADC portable) Infrared Gas Analyzer (Analytical Development Company of Hoddesdon, England).

e- Leaf Chlorophyll Fluorescence

The Polyphasic Rise of Fluorescence transients were measured by a plant efficiency

analyzer (Handsatech Instruments Ltd., PEA, King's Lynn, UK) (Strasser *et al.,* 1995).
F_o= Minimum Fluorescence, F_m= Maximum Fluorescence, F_v= Variable Fluorescence, Fv/F_m= Maximal Quantum Yield of PSII

f- Cell Membrane Stability or Measurement of Cellular Injury

The cell membrane stability was estimated using this equation:

(CMS) Cell Membrane Stability =
$[1-(1-T_1/T_2)/(1-C_1/C_2)]\times100$

Where T is treatment; C is control while 1 and 2 is conductivity.

g- Leaf Relative (H_2O) Water Contents

During the period of drought stress, water (H_2O) status was calculated by RWC (relative water content), calculated according to the method Bars & Weatherly (1962).

(RWC) Relative Water Content (%) =
(FW – DW)/ (TW – DW) x 100.

Statistical Analysis

Statistical analysis was done using the MSTAT-C computer program (MSTAT development team, 1989). Difference among mean values was done by Duncan's new multiple range test at 5% level of Probability (Steel *et al*., 1997).

RESULTS

Analysis of variance (ANOVA) of this data showed that all parameters studied varied significantly with the water limitation. The photosynthetic rate (*A*) of all maize cultivars was highly affected under water stress. In cvs. Sahiwal-2002, EV-5098 and Agaiti-2002 photosynthetic rate increased while it decreased significantly in all other cultivars. Photosynthetic rate was reduced due to water stress recorded in cvs Sahiwal-2002, Agaiti-85 and EV-1098. Similarly, significant reduction in transpiration rate (*E*) was observed in all maize cultivars except cvs Sahiwal-2002 due to water stress. All the cultivars had significant difference in this attribute. Transpiration rate was maximum in cvs. Sahiwal-2002 and Agaiti-2002 both under non-stressed and stressed conditions, the minimum value of transpiration rate was seen in EV-5098.

Water stress had significant reduction in the sub-stomatal carbon dioxide (CO_2) concentration. All cultivars showed similar behavior to water stress in relation to this attribute. Higher value of (sub-stomatal CO_2 concentration) *Ci* was observed in cvs.Sahiwal-2002 and Agaiti-2002. Stomatal Conductance (g_s) of all maize cultivars showed significant reduction because drought stress. Maximum values of stomatal conductance were observed in cvs. Sahiwal-2002 and Agaiti-2002.

Data for water use efficiency (*A/E*) showed that water stress imposition significantly increased the WUE in all cultivars except cv. Sadaf in which it decreased. All cultivars differed significantly in this attribute because of water stress. A maximum increase in WUE was observed in Sahiwal-2002, Agaiti-2002 and EV-1098. *Ci/Ca* ratio of all the cultivars of maize significantly decreased except in cv. Agaiti-85 in which it remained unchanged. The maximum values of *Ci/Ca* ratio were recorded in cvs. Sahiwal-2002 and Agaiti-2002 followed by Agaiti-85. However, the cv. Pak Afgoi was minimum in this attribute as compared to the other cultivars. Minimal chlorophyll fluorescence (F_o) values of all maize plant cultivars increased significantly because of water stress imposition. A maximum increase in F_o was observed in cvs. Ev-5098 and Pak-Afgoi. However, the minimum increase in F_o value due to water stress was recorded in cvs. Agaiti-2002 and Sahiwal 2002. Water stress imposition significantly decreased the maximal chlorophyll fluorescence (F_m) of all maize cultivars under observation. The maximum values for F_m under non-stress and stress conditions were observed in cvs. Sahiwal-2002 and Agaiti-2002, however, the cvs. Pak-Afgoi and EV-5098 were minimum in this attribute as compared to the other cultivars under drought stress conditions.

ANOVA showed that (F_v) of all maize cultivars decreased significantly because of drought stress imposition. Low reduction in Variable Chlorophyll Fluorescence was estimated in Agaiti (2002) as compared to other cultivars. Cultivar Agaiti-85 showed maximum reduction in F_v because of drought stress. F_v/F_m ratio of all maize cultivars was significantly reduced because of drought stress. Reduction in F_v/F_m ratio was maximum in cv. Agaiti-85. However, cultivar Agaiti-2002 and Sahiwal-2002 showed minimum reduction in F_v/F_m ratio. F_o/F_m ratio of all maize cultivars showed increase due to drought stress. F_o/F_m ratio was maximum in cvs. Pak-Afgoi and EV-5098 followed by EV-1098 and Agaiti 85. However, cultivars Agaiti-2002 and Sahiwal 2002 showed a minimum increase in F_o/F_m ratio under drought stress conditions. F_v/F_o ratio of all maize cultivars was reduced significantly because of drought stress. However, the maximum values for F_v/F_o ratio during water stress were observed in Sahiwal-2002 and Agaiti-2002.

DISCUSSION

Photosynthesis play very important role in physiological processes that have great contribution in plant growth and development. Water deficiency

and increased temperature have adverse effect on rate of Photosynthesis. The reduction in photosynthesis of plant reduced the utilization efficiency of light energy, altered chlorophyll Fluorescence, caused the Photo inhibition of Photosystem II (PSII) and reduction in adenosine triphosphate (ATP) synthesis (Athar & Ashraf, 2005). In plants, the small decrease in water potential resulted in closing of stomata, leading to decrease in CO_2 photosynthetic assimilation intensity. Due to decrease in water level of plants, photosynthetic apparatus suffer with functional changes of plants that cause loss of its structures. In this study, water stress decreased the photosynthetic rate in all maize cultivars and correlated positively with the production of plant biomass. Similar trend was found between photosynthetic capacity and growth in different crops as in turnip rape (Mäkelä *et al*., 1999), *Brassica* species (Nazir *et al*., 2001), and wheat species (Ashraf & Bashir, 2003). The decline in Assimilation Rate of the Cultivars in the present study linked with declining leaves water potential. Sahiwal-2002 and Agaiti-2002 maintained higher water level status under drought and also highest Photosynthetic Rates than all other cultivars.

The importance of intensity of CO_2 assimilation is an integral character of photosynthesis. Water deficiency suppresses the photosynthetic assimilation of CO_2 as a rule. The extent of inhibition differed in various cultivars and species that were dependent on water content availability**.** Photosynthesis was more stable in the leaves of these resistant cultivars (Agaiti-2002 and Sahiwal-2002). It was due to closing of plant stomata and maximum rolled of the leaves, which prevented loss of water.

Drought stress affected photosynthetic rate, although there was no direct correlation observed in certain cases (Zholkevich *et al*., 2001). According to this study, there was increase in stomata resistance due to dehydration of maize plants which inhibited photosynthesis completely. Under drought stress, maize cultivars had gas exchange limiting factor which dropped transpiration rate. In addition, the stability of the photosynthetic apparatus and the intensity of photo-assimilation under drought stress conditions might be associated with additional factors that had no relation with stomata. In this experiment, the response of *E* (transpiration rate) and CO_2 stomatal conductance to drought were in general analogous to plant pattern (shown by *A*). The strong relationship between *Ci* and *A* suggests closing of stomata might be the reason for decline in carbon dioxide (CO_2) assimilation rate.

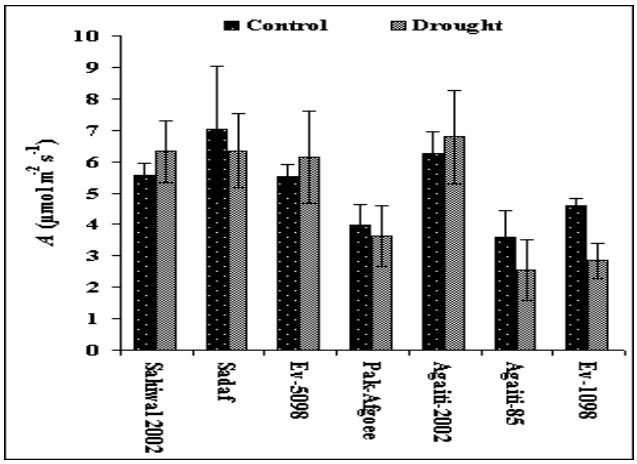
Analysis of Chlorophyll Fluorescence reflects the state of PS II. In this experiment, Quantum Yield of PSII that measured as F_v/F_m was reduced under water drought conditions. These results were similar to the findings of Colom & Vazana (2003) in which they found that water stress reduced the quantum yield of PSII (F_v/F_m) in *Eragrostis curvula*. While working with *Phaseolus vulgaris*, Cornic & Briantais (1991) observed that water stress reduced the F_v/F_m values even when values of *A* remained unaffected at elevated level of CO_2.

Agaiti-2002 and Sahiwal-2002 under stress conditions showed significant Leaf Water (H_2O) Potentials than EV-5098 and EV-1098 while other maize cultivars showed intermediate response. Moreover, Relative Water Contents (RWC) of the maize cultivars correlated with the Leaf Water (H_2O) Potential. So it was concluded that the reduction in the Leaf Water (H_2O) Potential in maize cultivars might be the result of Osmoregulation.

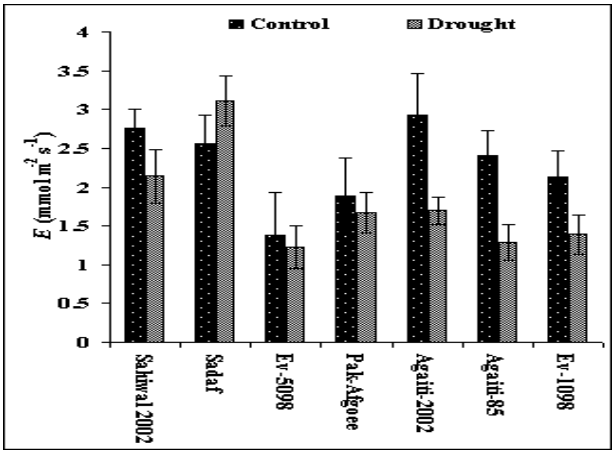
Table II: ANOVA showing various physiological activities of different maize (*Zea mays* L.) cultivars subjected to water deficit conditions at grain filling stage for 15 days.

Source of variations	d.f.	Photosynthetic rate	Transpiration rate	Stomatal Conductance	Sub-stomatal CO_2	Water Use Efficiency (*A*/*E* ratio)	C*i*/C*a*
Drought (D)	1	2355.871 ***	5.058 ***	51202.973 ***	325358.79 ***	1022.776 ***	2.783 ***
Cultivars (Cv)	6	25.824 ***	0.096 ns	4261.164 ***	11117.175 ***	41.772 ***	0.071 ***
D x Cv	6	33.336 ***	0.172 **	1247.143 ***	8290.508 ***	58.167 ***	0.064 ***
Error	42	4.948	0.046	227.381	1533.019	2.627	0.013

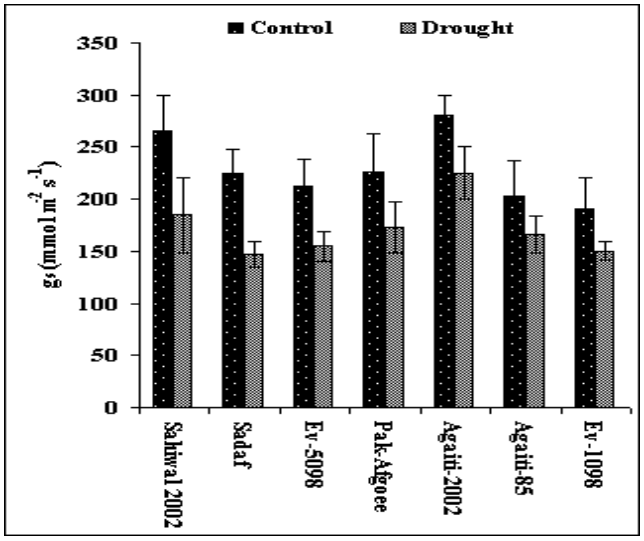
, *, ns = significant at 0.01, 0.001 levels respectively and non-significant



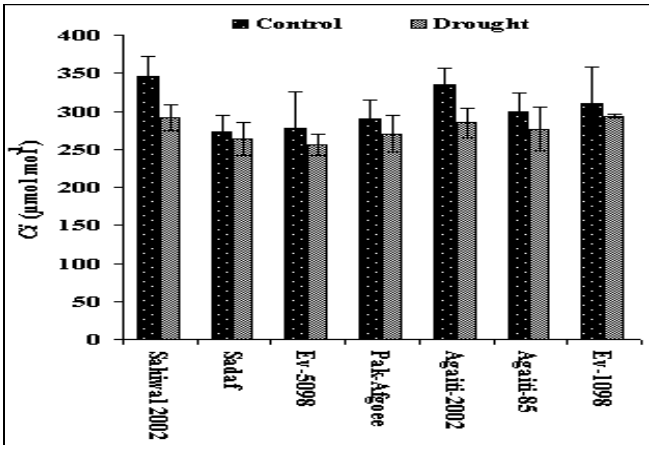
Photosynthetic rate



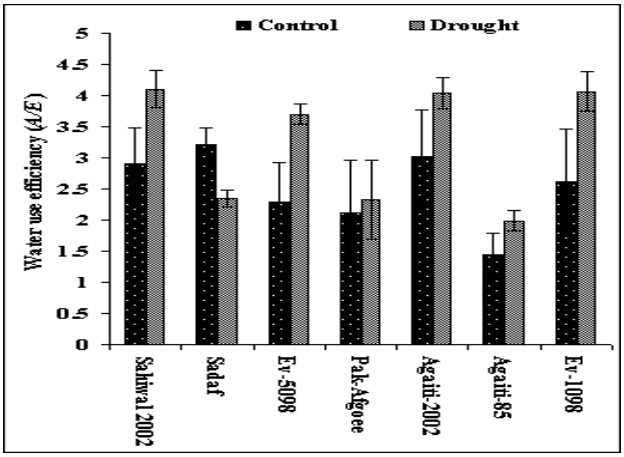
Transpiration rate



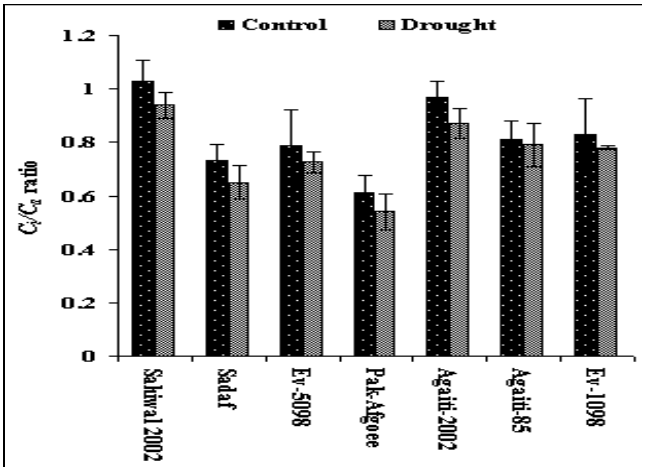
Stomatal Conductance



Sub-stomatal CO_2



Water Use Efficiency



Ci/*Ca*

Fig.,1*:* Phsiological attributes of different maize (*Zea mays* L.) cultivars when plants were subjected for 15 days to water deficit conditions at grain filling stage

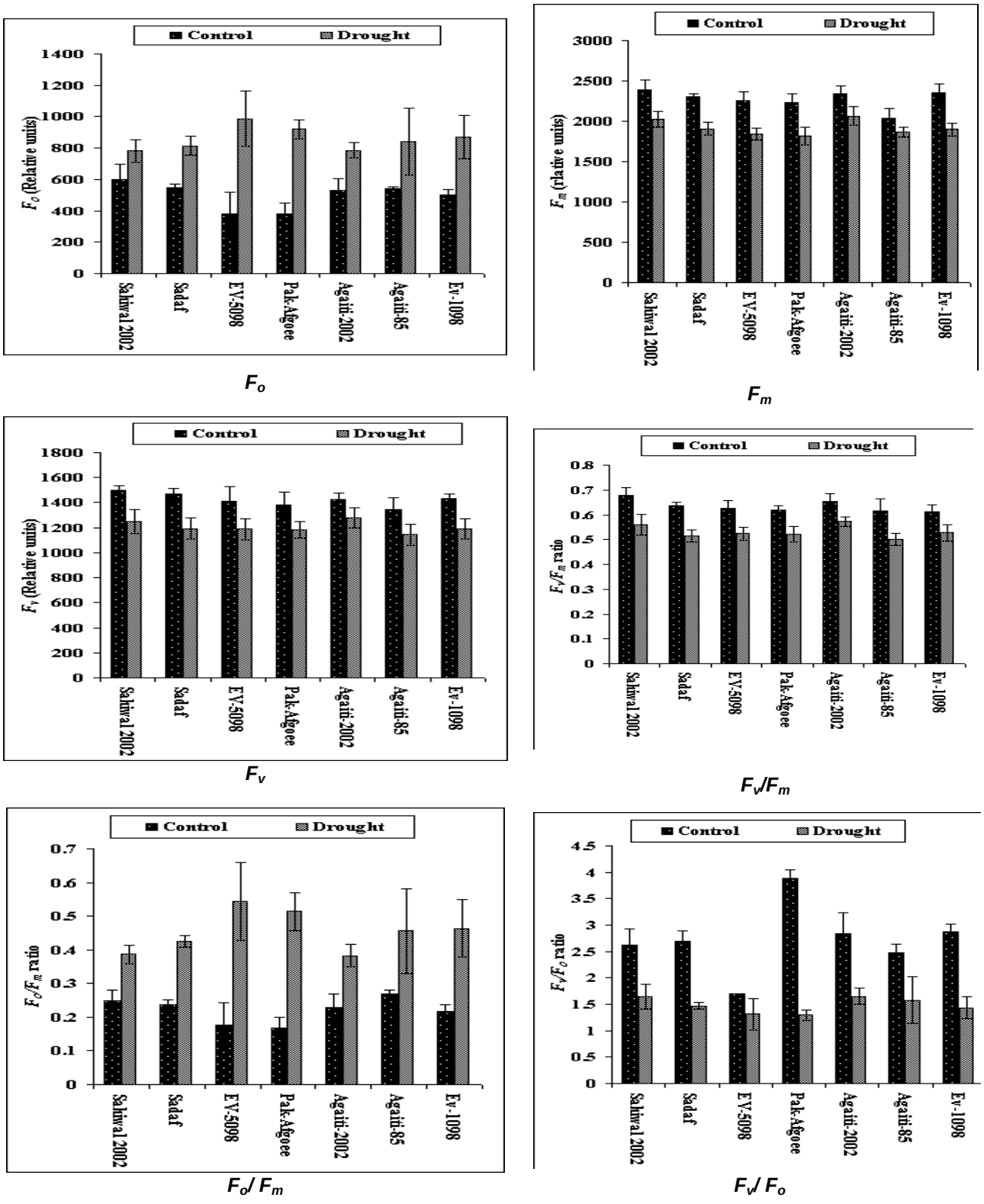


Fig., 2: Different chlorophyll fluorescence parameters of different maize (*Zea mays* L.) cultivars when plants were subjected for 15 days to water deficit conditions at grain filling stage

Table III: ANOVA for various chlorophyll fluorescence parameters of different maize (*Zea mays* L.) cultivars subjected to water deficit conditions at grain filling stage for 15 days

Source of variations	d.f	F_o	F_m	F_v	F_v/F_m	F_o/F_m	F_v/F_o
Water stress (WS)	1	1817641.4 ***	1771101.4 ***	69484.45 ***	0.1474994 ***	0.7579984 ***	163.4392 ns
Variety (V)	6	2162.1607 ns	70235.143 *	15406.869 ns	0.0044637 ns	0.003378 ns	54.846839 ns
WS X V	6	49179.53 ns	19657.821 ns	3396.4048 ns	0.00059 ns	0.0167638 ns	58.430595 ns
Error	42	32768.911	29518.351	19110.589	0.0027327	0.0108597	56.395691

, *, ns = significant at 0.01, 0.001 levels respectively and non-significant

REFERENCES

Araus, J.L., Amaro, T. Casadesús, J. Asbati, A. & Nachit, M.M., 1998. Relationships between ash content, carbon isotope discrimination and yield in durum wheat. *Aus. J. Plant Physiol*., **25**: 835–842.

Ashraf, M. & Bashir, A., 2003. Salt stress induced changes in some organic metabolities and ionic relations in nodules and other plant parts of two crop legumes differing in salt tolerance. *Flora*, **198**: 486-498.

Ashraf, M. & Harris, P.J.C., 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci*., **166**: 3-16.

Ashraf, M., 1994. Breeding for salinity tolerance in plants. *CritRev*. *Plant Sci*., **13**: 17-42.

Athar, H., & Ashraf, M., 2005. Photosynthesis under drought stress. In: *Hand Book Photosynthesis*, 2nd (ed.) by M. Pessarakli. C. R. C. Press, New York, USA, pp: 795-810.

Barrs, H.D. & Weatherley, P.E. 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Aust J of Biol Sci*., **15**: 413-428.

Chaves, M. M., Maroco, J.P. & Pereira, J.S., 2003. Understanding plant response to drought: from genes to the whole plant. *Funct. Plant Biol*., **30**: 239-264.

Colom, M.R. & Vazaana, C., 2003. Photosynthesis and PS-II functionality of drought resistance and drought sensitive weeping lovegrass plants. *Environ. Exp. Bot*., **49**:135-144.

Cornic, G. & Briantais J.M., 1991. Partitioning of photosynthetic electron flow between CO2 and O2 reduction in a C3 leaf (*Phaseolus vulgaris* L.) at different CO2 concentrations and during drought stress. *Planta*, **183**: 178–184.

Foolad, M.R., 1999b. Genetics of salt tolerance and cold tolerance in tomato: quantitative analysis and QTL mapping. *Plant Biotechnol*., **16**: 55–64.

Lea, P. J., Parry, M.A.J. & Medrano, H., 2004. Improving resistance to drought and salinity in plants. *Annal. App. Biol*., **144**: 249-50.

Makela, P., Kontturi, M., Pehu, E. & Somersalo, S., 1999. Photosynthetic response of drought and salt-stressed tomato and turnip rape plants to foliar applied glycine betaine. *Physiol. Plant*., **105**: 45-50.

Nazir, N., Ashraf, M. & Ejaz, R., 2001.Genomic relationships in oilseed Brassicas with respect to salt tolerance-photosynthetic capacity and ion relations. *Pak. J. Bot*., **33**: 483-501.

Parry, H., Evans, A.J. & Morgan, D., 2006. Aphid population response to agricultural landscape change: a spatially explicit, individual-based model. *Ecol. Model*., **199**(**4**): 451–463.

Ramachandra Reddy, A., Chaitanya, K.V. & Vivekanandan, M., 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *J. Plant Physiol*., **161**: 1189-1202.

Steel, R.G.D., Torrie, J.H. & Dickey, D.A. 1997. *Principles and Procedures of Statistics. A biometrical approach.* (3rd Ed) McGraw-Hill, New York, pp: 1-633.

Strasser, B.J. & Strasser, R.J., 1995. Measuring fast fluorescence transients to address environmental questions. *Biosphere*, **5**: 977–980.

Tambussi E. A., Bort, J. & Araus, J.L., 2007. Water use efficiency in C3 cereals under Mediterranean conditions: a review of

physiological aspects. *Ann. Appl. Biol*., **150**: 307-321.

Zholkevich, V. N., Gusev, N.A. & Kaplya, A.V., 2001. Plant Water Exchange, Nauka, Moscow (1989) (in Russian) parameter. *Ann. Bot*., **89**: 895-905.

Zhu, J. K., 2001. Plant salt tolerance. *Trends in Plant Sci*., **6**: 66-71.

Zhu, J. K., 2002. Salt and drought stress signal transduction in plants. *Annu. Rev. Plant Physiol*. *Plant Mol. Biol*., **53**: 247–273.

Received: 08-02-2016 **Revised: 15-04-2016** **Accepted: 03-05-2016**