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The Effect of Early Season Drought on Chlorophyll *a* Fluorescence in Sugar Beet (*Beta vulgaris* L.)

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Abstract: Two field experiments were conducted in 1998 and 1999 in the Khorasan Agricultural Research Center at Mashhad with the aim to study the effect of early season drought stress on a variety of sugar beet genotypes. The irrigation of the field was withhold for a period of about 50 days in order to impose a severe drought stress at the 8 to 10 leaf stage of sugar beet crop. The drought stress was evaluated by changes in chlorophyll fluorescence, measured in the fully expanded leaves of three beet genotypes in 1998 and nine genotypes in 1999, both in stressed and non-stressed plants.The results showed that in the 1998 experiment, both maximal (F_m) and variable (F_v) fluorescence were reduced during the growth period, but the reduction was greater under drought conditions than under non-stress conditions. The quantum yield of photochemistry in Photosystem II (F_v/F_m) remained unaffected under non-stress conditions, but reduced under drought stress. In the 1999 experiment, initial fluorescence (F_0) was not affected by drought stress, whereas chlorophyll content of stressed plant was greater than that of non-stressed. Thus, drought stress had no destructive effect on the initial fluorescence, presumably due to increase in chlorophyll content of stressed sugar beet. Under stress conditions, F_v, F_m and F_v/F_m were reduced for all genotypes, but the reduction rates varied between genotypes. The genotype 7219.P129 showed the highest reduction of F_v, F_m and F_v/F_m under drought stress conditions, that suggests the highest sensitive to drought stress. In all genotypes, F_v/F_m recovered 16 days after stress relief, although, their reactions were different. Strong correlation ($R^2 = 0.75$) between the white sugar yield and F_v/F_m has been found, suggesting that chlorophyll fluorescence can be used as a tool for screening sugar beet genotypes for early season drought stress.

Key words: Chlorophyll fluorescence, drought, genotype, stress, photosystem II, sugar beet

INTRODUCTION

It is well documented that the function of the thylakoid membrane is sensitive to several environmental stresses. Photosystem (PS) II appears to be particularly sensitive to a number of stress factors. Since different factors affect the function of PSII, directly or indirectly, fluorescence can be used as a tool, not only in revealing stress response mechanisms but also in quantifying these responses under laboratory and field conditions (Bolhár-Nordenkampf and Öquist, 1993). However, in situ measurements can provide more precise information about the effect of stress on photosynthesis (Selmani and Wassom, 1991).

The fluorescence induction curve is some time called the Kautsky curve (Bolhár-Nordenkampf and Öquist, 1993). Chlorophyll fluorescence kinetics have been used by plant breeders to quantify rapidly the response of different varieties or lines to certain stresses. According to Wilson and Greaves (1993) fluorescence based screening programs were designed to improve the chilling tolerance of maize (*Zea mays* L.) and rice (*Oriza sativa*) and heat tolerance of sunflowers(*Helianthus annuus* L.). Ranalli *et al.* (1997) have shown the potential use of chlorophyll fluorescence as a tool for screening drought tolerance of potato (*Solanum tuberosum*) germplasm. Smillie and Nott (1982) also investigated the potential of *in vivo* measurements of chlorophyll fluorescence to detect cellular responses to salinity and degrees of salt stress in leaves for sugar beet (*Beta vulgaris* L.) (Salt tolerant), sunflower (Moderately salt tolerant) and common bean (*Phaseolus vulgaris* L. cv. Canadian Wonder) (Salt intolerant). They concluded that *invivo* measurements of chlorophyll fluorescence could be used to detect salt stress in leaves and may have relevance to screening for salt tolerance.

The fluorescence rise from initial fluorescence (F_0), when the primary acceptor (Q_A) is oxidized to maximal

fluorescence (F_m), when all reaction centers become closed is called the "fast phase"(Bolhár-Nordenkampf and Öquist 1993, Ranalli *et al.,* 1997). F_0 is known to be affected by any environmental stresses that cause structural alterations at the PS II pigment level. Thermal damage of PS II is characterized by a drastic increase of F_0. By comparison, photoinhibition may lead to a slight increase in F_0. Freezing damage to thylakoids does not affect F_0 (Anonymous, 1993). There are not more documents about the effect of drought stress on F_0.

F_m would be decreased when leaves were exposed to high but not injurious temperatures. More severe heat treatment results in an increase in F_0 and a decrease in F_m accompanied by inhibition of PS II activity (Anonymous, 1993).

The difference between F_m and F_0, which termed as variable component of fluorescence (F_v) (Ranalli *et al.*, 1997) is usually lowered by environmental stresses which cause thylakid damage. Examples of these stresses are heat, freezing and photoinhibition (Anonymous, 1993).

The ratio F_v/F_m that can be shown to be proportional to the quantum yield of potochemistry (Bolhár-Nordenkampf and Öquist, 1993) depends on leaf water potential (Anonymous, 1993).

The half-rise time ($T_{1/2}$) for the rise from F_0 to F_m is an alternative simple indicator for estimating the size of the plastoquinone pool. Shade leaves with large chlorophyll antenna and a small plastoquinon pool exhibit a shorter $T_{1/2}$ compared with sun leaves, which have a small antenna size and a large plastoquinon pool (Anderson *et al.*, 1988).

This study was conducted to determine the effect of early season drought stress on chlorophyll fluorescence of sugar beet leaves in order to determine drought effects on sugar beet photosynthetic apparatus.

MATERIALS AND METHODS

The experiment was carried out in the Khorassan Agricultural Research Station field at Mashhad, NE of Iran, in 1998 and 1999. This station is located at 36° 12` N latitude with the longitude of 59° 40` E and the height of 985 meters from the sea level. Based on DeMarton classification Mashhad is regarded as a semiarid region.

A split plot design was used in which two-irrigation regimes were considered as the main plots and nine sugar beet genotypes as subplots. Irrigation treatments were: non-stress and water stress conditions. For the stress treatment, irrigation was withheld when plants reached about eight to ten-leaf stage. The duration of water stress at 1998 and 1999 was 41 (9 June- 20 July) and 53 (1 June-24 July) days, respectively. The first irrigation was in May 24 and May 10 in 1998 and 1999, respectively. Plot size was $8\times5m^2$ and row space was 0.61. Plants were thinned at 0.2 m apart at the four-leaf stage. During the stress period, no effective raining was recorded.

A chlorophyll meter model SPAD-502 was used for chlorophyll measurement. Chlorophyll was measured on middle-aged leaves considering five replications in 1998 and four replications in 1999. In 1998, five leaves and in 1999 three leaves were measured in each plot. Measurement of chlorophyll rate was repeated after sixteen days from stress removal in 1999.

The fast phase of chlorophyll *a* fluorescence variation was determined by the use of Plant Efficiency Analyzer (PEA) instrument. As dark adaptation causes reaction centers of PSII to rest, i.e. not involved in any photosynthetic reactions (Papageorgios, 1975, Lavorel and Etienne, 1977) a portion of dark-adapted leaf was measured. Dark-adaptation was inducted by a clip having a sliding opening. Measurements were made from 11.00 till 14.00 o'clock after 30 minutes of dark-adaptation. The PEA was set on the light level of 4 and the measuring time of two seconds, before starting up. Initial fluorescence (F_0), maximal fluorescence (F_m), variable fluorescence (F_v), the ratio F_v/F_m as well as the half-rise time ($T_{1/2}$) for the rise from F_0 to F_m were recorded.

In 1998, twenty-two days after stopping irrigation until re-irrigation of crops, chlorophyll *a* fluorescence variations were measured in three genotypes (7219.P69, 7233.P3 and 7233.P3) both at stress and non-stress conditions. On every plot, three plants were chosen randomly and measurements were made on one of the middle leaves of each plant. In each day, two replications were measured. The data were averaged for every irrigation level and the best regression equation was fitted for each level against time.

In 1999, the measurement of the fast phase of chlorophyll fluorescence was carried out within the final 5 days of stress period for every two replications on a daily basis. Measurements in every plot were made on the same three leaves, which their chlorophyll amount was determined. Treatment averages were obtained per day and each day was considered as one replication. The measurements were repeated 16 days after stress period, but they were made for only four days.

At harvest, root weight from five square meters of each plot was determined and their pulps were prepared. White sugar percent was also measured at each plot. White sugar yield for every plot was determined by multiplying root yield with white sugar percent.

RESULTS AND DISCUSSION

The trend of chlorophyll *a* fluorescence variation, as measured by F_0, F_m, F_V and the ratio F_v/F_m, from 22 to 40

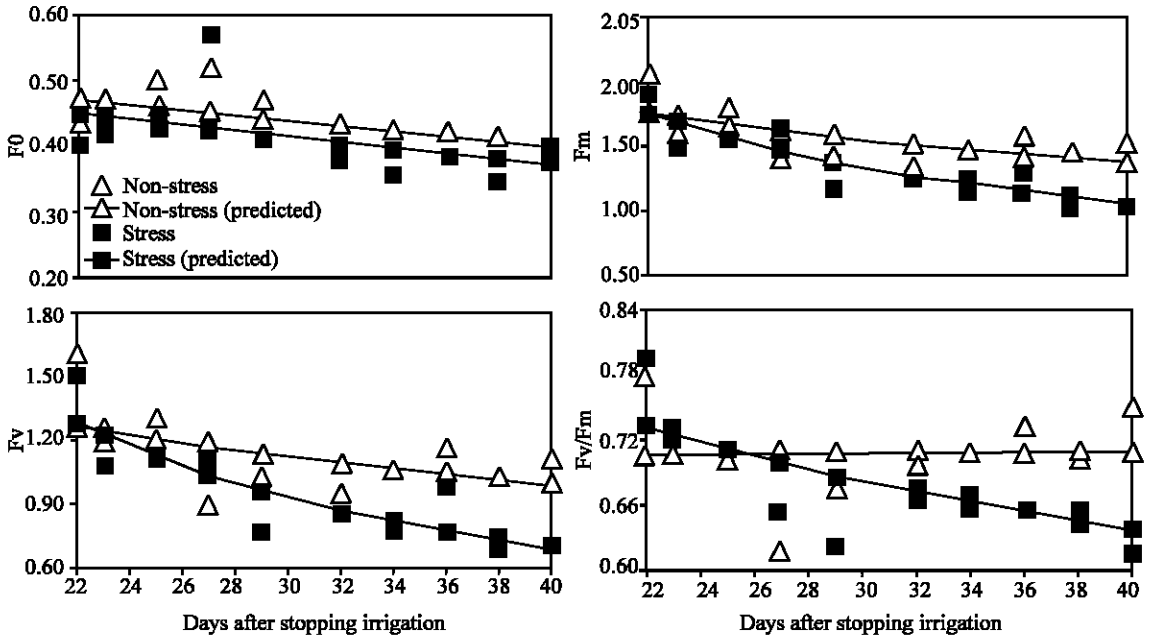

Fig. 1: Chlorophyll *a* fluorescence variations of sugar beet genotypes in water stress and non-stress conditions

days after stopping irrigation in the stress and non-stress conditions are presented in Fig. 1. In both conditions, F_0 was reduced, but the differences between them were small during this period. However, the values of F_0 in the stress condition were generally lower than that of the non-stress condition. The values of F_m and F_v also showed a reducing trend in stress and non-stress conditions, but the reduction was higher under stress condition. The quantum yield of potochemisty (F_v/F_m) decreased rapidly in the stress condition. On the other hand, a stable relation existed under the non-stress condition (Fig. 1). Our results are different from those of Flagella *et al.* (1994). This might be due to different method applied in this experiment. They cut the determinate leaves and put the control leaves in water saturation condition at the laboratory and for stress condition, they kept the leaves in filter. They controlled the water potential at -1±0.2 and -2.5±0.6 ÌPa in control and stress treatment respectively while our study was conducted in the field. According to Selmeni and Wesson's (1991), chlorophyll fluorescence measurement at the laboratory or during night could not show the permanent damage to the photosynthetic apparatus caused by drought, because under these conditions, recovery might happen if the damage of chlorophyll is not sever enough. On the other hand, Cerovic *et al.* (1996) reported a linear relationship between lifetime and yields for chlorophyll fluorescence *invivo* in studies with maize, sugar beet and kalanchoe (*Bryophyllum sp.*) under both optimal and non-optimal water conditions. Considering these, it could be concluded that water variation inside the cell during the growth period and also leaves age, could have different effects on the fast phase of chlorophyll fluorescence.

Analysis of variance related to the fast phase of chlorophyll *a* fluorescence variation before termination of stress condition is represented in Table 1. For F_0, there was not a significant difference between non-stress and stress conditions. F_0 value was only reduced by 2.4 percent under the stress condition (Table 4). Since F_0 is resulted from the fluorescence of the antenna chlorophyll's associated with PSI and PSII (Anonymous, 1993, Wilson and Greaves, 1993), thus it could be concluded that drought stress in sugar beet dose not have any damaging effects on F_0 This can be the result of increased chlorophyll concentration under the stress condition as compared to the non-stress condition. Chlorophyll assessment of all genotypes in 1998 and 1999, before the termination of stress condition, showed that chlorophyll concentration of the stress condition, was 12.45 and 13.6 percent higher than the non-stress condition, respectively (Tables 2 and 3). However, genotypes didn't differ significantly (Table 2). Sixteen days after the termination of water stress in 1999, chlorophyll concentration of genotypes under drought stress was still significantly more than the same group of genotypes under the non-stress condition ($P<0.01$, Tables of 2 and 3). Khafagi and El-Lawendy (1997) also observed that decreasing irrigation frequency increased chlorophyll and carotene concentration in sugar beet leaves. Since there is a relationship between the amount of leaf

Table 1: Mean squares from of the fast phase of chlorophyll fluorescence variations in nine sugar beet genotypes, under non-stress and drought stress conditions in early growth season

	Before irrigation termination						Afterirrigationtermination	
		MS						M.S.
Source of variations *a*	DF	F0	Fm	Fv	Fv/Fm	T1/2	DF	Fv/Fm
Replication	4	0.02**	-	-	0.019**	-	3	-
Irrigation regime (I)	1	0.001n.s.	0.946n.s.	0.912+	0.081**	332.5n.s.	1	0.001n.s.
Error a	4	-	0.325	0.237	-	-	3	-
Genotype (G)	8	0.001n.s.	0.013n.s.	0.009n.s.	0.002n.s.	986.4n.s.	8	0.001n.s.
G × I	8	0.001n.s.	0.028+	0.026+	0.004n.s.	442.3n.s.	8	0.006*
Error b	64	0.001	0.016	0.015	0.003	601.9.	48	0.002
C.V.%		11.8	14.81	20.19	7.7	18.75		6.72

Table 2: Mean squares from analyses of the chlorophyll values (SPAD) in nine sugar beet genotypes, under non-stress and drought stress conditions in early growth season in 1998 and 1999

	1998		1999		
	Before irrigation termination		Before irrigation termination		Afterirrigationtermination
Source of variations *a*	DF	MS	DF	MS	
Replication	-	-	-		-
Irrigation regime(I)	1	974.695**	1	527.945+	859.742**
Error a	8	17.48	6	101	-
Genotype(G)	8	37.87**	8	46.01 n.s.	10.16 n.s.
G ×I	8	4.12 n.s.	8	23.55 n.s.	22.59 n.s.
Error b	64	5.49	48	25.7	14.3
C.V.%		4.54		12.68	9.57

a when the main plot error and / or replication were not significant at P< 0.05, they were pooled with the error term.
DF= degrees of freedom MS= mean squares **,+ = Significant at the 0.01 and 0.1 levels, respectively n.s.= Not significant at 0.1 level

Table 3: Mean of the sugar beet chlorophyll values (SPAD) under non-stress and drought stress conditions in early growth season in 1998 and 1999

	1998		1999	
	Non-stress	Stress	Non-stress	Stress
Before irrigation termination	48.265	54.847	43.480	48.896
After irrigation termination	-	-	42.164	49.075

Table 4: Mean of the fast phase of chlorophyll fluorescence variations under non-stress and drought stress conditions in early growth season

	Before irrigation termination			After irrigation termination		
	F0	Fm	Fv	T1/2	Fv/Fm	Fv/Fm
Non-stress	0.25	0.946	0.698	132.778	0.717	0.713
Stress	0.244	0.741	0.496	128.933	0.657	0.706

chlorophyll and the level of leaf nitrogen (Piekielek and Fox, 1992, Piekielek *et al*., 1995, Giordani and Bernati, 1998), it may be stated that the amount of sugar beet nitrogen in young leaves, under the water stress condition, should generally be more than the non-stress condition. Groves and Bailey (1994) showed a negative relationship between plant nitrogen concentration and water consumption. C:N ratio of sugar beet top was also affected by crop water supply and the plants under drought stress had a lower values of C:N. The high concentration of nitrogen under the drought stress condition maybe effective in expediting the recovery from drought stress condition after irrigation.

The genotype × irrigation regime interaction (G×I) was not significant for F_0 (Table 1). In other words, all genotypes were almost equally affected by the stress treatment. It was reported that temperature damaging effect on photosystem II can be determined by a drastic increase in F_0, while photoinhibition lead to a slight increase in F_0 and freezing damage dos not affect F0 (Anonymous, 1993). Wilson and Greaves (1993) also reported that increasing light intensity caused increased F_0 value, in maize leaves. They observed different responses from six lines of maize by putting them in 40°C for 5 hours. However, no significant genetic differences in 35-40°C were observed for sunflower genotypes.

Drought stress also decreased F_m, by 22 percent (Table 4) though this decrease was not significant (Table 1). It was also been reported that F_m value decreases under high temperature conditions (Anonymous, 1993), However intensive light in maize increased F_m value (Wilson and Greaves, 1993).

The G ×I interaction was significant for F_m (Table 1). In other words, the declining values of F_m by water stress between the genotypes under study (Fig. 2A). The largest decrease of F_m was observed in the genotype 7219.P229 and the smallest decrease in 7219.P69, PC9597.P58, 7233.P3 and BPkaraj×261.

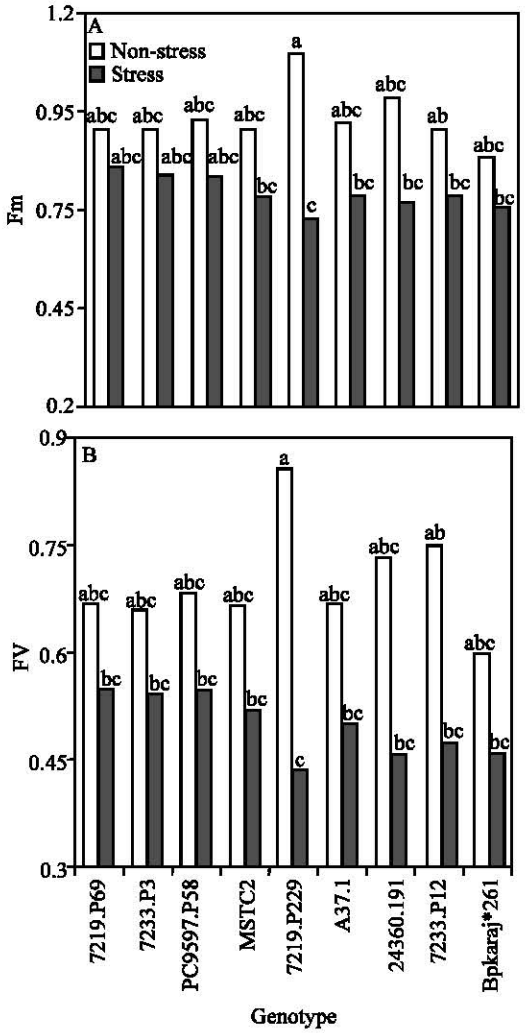

Fig. 2: Mean of fluorescence maximum (F_m) and fluorescence variable (F_v) of 9 sugar beet genotypes under water stress and non-stress conditions

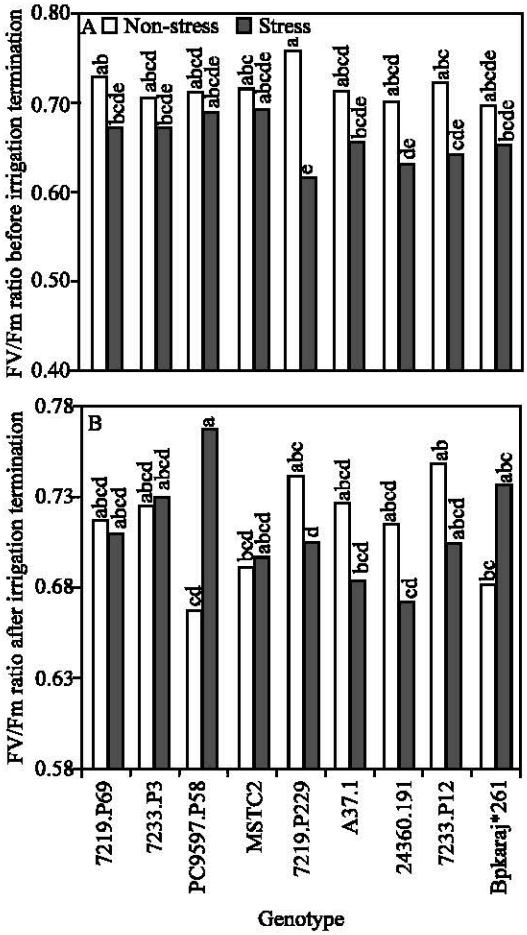

Fig. 3: Mean of the F_v/F_m ratios before and after irrigation termination for the nine sugar beet genotypes under water stress and non-stress conditions

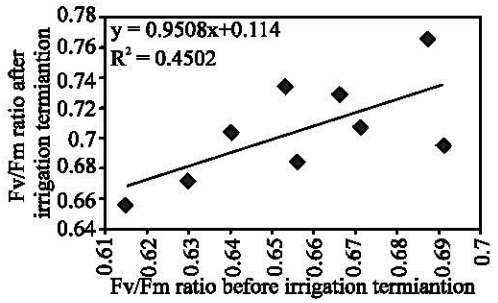

Fig. 4: The relationship between F_v/F_m ratios, before and after irrigation termination in nine genotypes of sugar beet under water stress conditions

The mean F_v values of all genotypes in water stressed plots were significantly lower (about 29%) than those in non-stressed plots (Tables of 1 and 4). The G × I interactions were also significant for F_v (Table 1). Therefore, the decrease in F_v was different among the genotypes as stress increased (Fig. 2B). Generally, F_v is sensitive to changes in the ultrastructure of the membranes and rates of electron transfer. Usually environmental stresses decrease the F_v values, as the photooxidizing side of PSII is inhibited (Wilson and Greaves, 1993). As is shown in Fig. 2B, the largest decrease in F_v observed in 7219.P229. High value of Fv indicates that electron flow is blocked beyond Q and the low value of Fv shows that electron flow is blocked on the waterside of photosystem II (Ranalli *et al*., 1997). Thus it may be stated that in 7219.P229, water stress blocked electron flow on the waterside of phtosystem II more than other genotype. Ranalli *et al.* (1997), examined chlorophyll fluorescence of potato genotypes under drought stress and non-stress conditions and stated that drought stress

Table 5: Correlation coefficient of the fast phase of chlorophyll *a* fluorescence variations of sugar beet, before and after irrigation termination under drought stress condition

	Before irrigation termination				After irrigation termination			
	F0	Fm	Fv	Fv/Fm	F0	Fm	Fv	Fv/Fm
F0	1				1			
Fm	0.3 n.s.	1			0.33 n.s.	1		
Fv	-0.036 n.s.	0.941**	1		0.246 n.s.	0.99**	1	
Fv/Fm	-0.404 n.s.	0.688*	0.858 **	1	-0.093 n.s.	0.874**	0.901**	1

**,* = Significant at the 0.01 and 0.05 levels, respectively n.s.= Non significant at 0.05 level

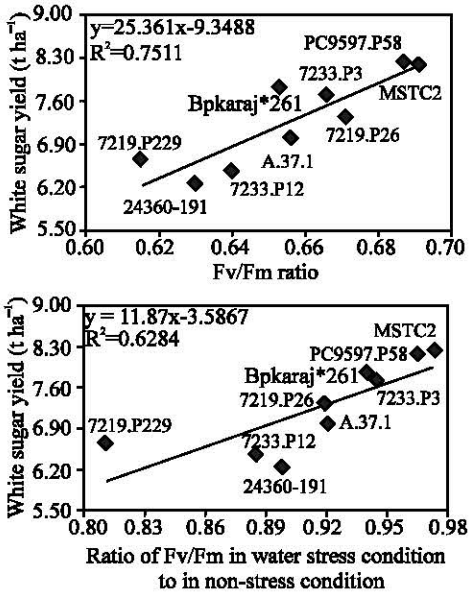

Fig. 5: The relationship between F_v/F_m ratio before irrigation termination and white sugar yield in water stress condition and between white sugar yield and the ratio of F_v/F_m in stress condition in sugar beet

may block electron flow either on the water side of photosystem II or beyond acceptor Q.

The ratio of Fv/Fm significantly decreased under drought stress (Tables of 1 and 4). This indicates the capacity of photosystem II electron transfer (Demmig and Bjorkman, 1987), which has a high relationship with quantum yield of photochemisty (Bolhár-Nordenkampf and Öquist, 1993). In spite of non significant differences in G × I regime interactions for F_v/F_m (Table 1), drought stress significantly decreased the ratio of F_v/F_m in 7219.P229 ($P<0.05$, Fig. 3A). Therefore, it seems that in 7219.P229 the photosynthetic apparatus was affected more than other genotypes by drought stress.

The mean half-rise time for the rise from F_0 to F_m ($T_{1/2}$) did not significantly affected by drought stress (Table 1), although this period was reduced by 2.4 percent under stress condition (Table 4). If electron transfer from reaction center to electron transfer chain is blocked, as the case of DCMU (a phenylura PSII inhibitor) in photosynthesis, $T_{1/2}$ value decreases intensively (Anonymous, 1993, Bolhár-Nordenkampf and Öquist, 1993). Since in our experiment the $T_{1/2}$ value did not decrease significantly, thus in the sugar beet genotypes under investigation, drought stress probably affected electron flow from the donor (water) side of photosystem II to Q more than electron flow beyond Q.

There was no significant difference between stress and non-stress conditions for F_v/F_m 16 days after terminating the stress period (Tables of 1 and 2). Basu *et al.* (1998) showed that net photosynthetic rate and F_v/F_m values decreased under stress condition in potato leaves, but after rewatering, they were restored to the values of control plants within 24 hour. The G × I interactions were significant for F_v/F_m (Table 2). It was mainly due to different reaction of the two genotypes PC9597.P58 and 7219.P229 (Fig. 3B). In PC98597.P58, F_v/F_m was significantly increased, while in 7219.P229, was significantly decreased in stressed condition (Fig. 3B). These results indicate that under water stress the electron acceptors of phtosystem II reducing velocity in PC9597P58 increased after terminating the drought stress, whereas in 7219.P229, the electron flow still was restricted on the waterside of photosystem II. Therefore it seems that photosynthesis amount of the latter genotype was not reached to the normal level yet.

Correlation coefficient between the parameters of the fast phase of chlorophyll *a* fluorescence, before and after the stress termination are shown in Table 5 in the stressed treatment. Correlation coefficients of F_v/F_m with F_m and F_v were significant before the stress termination, but correlation coefficient of F_v/F_m with F_0 was not significant. Under this condition, F_v was significantly correlated with F_m, but the correlation of F_v with F_0 and F_m with F_0 was not significant. After stress termination, F_0 had no significant correlation neither with F_m and F_v nor with F_v/F_m. F_v/F_m were significantly correlated with F_m and F_v. Under this condition F_v had a significant correlation with F_m. Thus, it could be concluded that F_0 can not indicate alone the general characteristics of fluorescence *a*.

Fig. 4 shows the relationship between F_v/F_m values before and after stress termination. The correlation was positive and significant ($r=0.671$, $P<0.05$). In other words, the genotypes having high capacity of photosystem II electron transfer under water stress condition, show also a high capacity of photosystem II electron transfer after stress termination.

The correlation coefficient between white sugar yield and F_v/F_m ratio before the stress termination and between

white sugar yield and the ratio of F_v/F_m in stress condition to F_v/F_m in non-stress condition were positive and significant (r=0.87 and r=0.79, respectively, Fig. 5). It could be inferred that the genotypes with a higher capacity of photosystem II electron transfer under water stress condition or their capacity of photosystem II electron transfer is resistant to stress condition, have a high white sugar yield. It was reported that there is a high association between tuber yield in potato and F_v/F_m under drought condition (Ranalli *et al*., 1997). As it is shown in Fig. 5, PC9597.P58, MSTC2, 7233.P3, 7219.P69, Bpkaraj×261 and A37.1 not only were high yielding in terms of white sugar but also were drought resistant.

Our results indicate that under early season drought stress, the electron flow was restricted on the waterside of photosystem II in spite of increased chlorophyll concentration in leaves of sugar beet and genotypes under study differed in this case. Therefore chlorophyll fluorescence could be used with other useful agronomic characters to screen drought resistant genotypes of sugar beet.

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