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GROWTH AND CHLOROPHYLL FLUORESCENCE UNDER SALINITY STRESS IN SUGAR BEET (*BETA VULGARIS* L.)

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Abstract

This study was carried out in the General Commission for Scientific Agricultural Research (GCSAR), Syria, at Der EzZour Agricultural Research Center, from 2008-2010, to examine the effect of salt conditions on some growth attributes and chlorophyll fluorescence in 10 Sugar Beet (*Beta vulgaris* L.) genotypes under salinity stress. Sugar beet plants were irrigated with saline water, having electrical conductivity ranged from 8.6-10 $dS.m^{-1}$during first year and 8.4-10.4 $dS.m^{-1}$during second year. A randomized completely block design with three replicates was used. The results showed that all studied growth attributes, leaf area, leaf number, relative growth rate, and net assimilation rate were decreased in salinity stress conditions compared to the controlled state. The findings indicated that salinity caused a decrement of light utilizing through increased values of fluorescence origin (fo), decreased values of fluorescence maximum (fm), and maximum yield of quantum in photosystem-II (fv/fm). Genotypes differed significantly in all studied attributes except in leaf number. Under salt conditions, Brigitta (monogerm) achieved an increase in net assimilation rate, while Kawimera (multigerm) achieved the lowest decrement in quantum yield in photosystem-II. Further studies are necessary to correlate the yield with yield components under similar conditions to determine the most tolerant genotype.
Key words: Growth, Chlorophyll fluorescence, Salinity stress, Sugar Beet (*Beta vulgaris* L.).

Introduction

Salinity is considered as a global environmental challenge, affecting crop production on over 800 million hectares, or a quarter to third of all agricultural land on earth (Rengasamy, 2010). The 21stcentury is marked by global scarcity of water resources,

environmental pollution, and increased salinity of soils and waters (Djilianov *et al.*, 2005). The problem is particularly severe in irrigated areas (Zhu, 2001), where as much as one-third of global food production occur (Zhang *et al*., 2010), and also where infiltration of highly saline sea water observed (Flowers, 2004). However, salinity is also increased in dry land agriculture in many parts of the world (Rengasamy, 2006). Development of crops with improved salt tolerance is proposed as part of solution to this problem (Zhu, 2001).

Plants follow different behaviors to combat salinity. Detailed reviews about salinity tolerance mechanisms in different species are presented by Ashraf (2004) and Sairam and Tyagi (2004). Sugar beet (*Beta vulgaris* L., family; *Chenopodiaceae*), has halophytes ancestors. Its tolerance threshold to salinity is high (7 dS m^{-1}) (Katerji *et al*., 1997). It is a salt sensitive species during seed germination period and seedling emergence, and a salt tolerant with variations in its genotypes (Sadeghian *et al*., 2000; Ghoulam *et al*., 2002, Abbas *et al*., 2009). Sugar beet plant has a good ability in modifying its osmotic potential as a response to salt stress (Abbas *et al*., 2012).

Salinity decrease growth and net photosynthesis of higher plants (Long and Baker, 1986), which may open the possibility of using photosynthetic parameters in salt-tolerance screening. The rationale for the view that changes in leaf photosynthetic parameters may be used to carry out screening of stress-resistant cultivars is that such parameters would reflect any constraint acting on the photosynthetic processes. Therefore, more stress-tolerant cultivars are expected to exhibit photosynthetic parameters during stress periods (Belkhodja *et al*., 1994).

Chlorophyll fluorescence could be an excellent tool for screening, since it is easy to measure and may allow the screening of large numbers of genotypes in a short time span. This approach was used in screening several sugar beet genotypes for drought and salinity tolerance (Abbas, 2011), to characterize the changes in the efficiency of photosynthetic energy conversion occurring in Fe-deficient sugar beet plants (Morales *et al*., 1991). The technique is also used to study the changes in quantum yield under sulfur spray on sugar beet foliage (Abbas and Seedo, 2010), and zinc sulfate application (Abbas, 2012). Results of Abbas and Seedo (2010) and Abbas (2012) showed that foliar application of sulfur and zinc sulfate accelerated the yield of quantum in photosystem-II. The purpose of the present study is to study the effect of salinity stress on some growth parameters and chlorophyll fluorescence in 10 sugar beet genotypes.

Materials and Methods

Two field trials were tested on $7^{th}$and 9^{th}August during (2008-2009, 2009-2010) growing seasons. The experiments were carried out in the General Commission for Scientific Agricultural Research (GCSAR) at Der EzZour Agricultural Research Center, Syria. The area is dry with an irrigation facilities for the sugar beet production. The aim of these trials was to evaluate the response of ten sugar beet genotypes (five monogerms and five multigerm) (Table 1) under salinity stress and control conditions. The investigated genotypes were obtained from different breeding companies. Nitrogen fertilization was added at the rate of 446 kg ha^{-1}. Phosphorous at a rate of 180 kg P_2O_5 and Potassium at a rate of 185 kg K_2O were added during sowing and after thinning. Mechanical and chemical analysis of the soil at the experimental site was carried out (Table 2).

Plants were irrigated with saline water under saline stress conditions, having electrical conductivity ranged from 8.6 to 10 $dS.m^{-1}$(first year) and 8.4 to 10.4 $dS.m^{-1}$(second year). It is also important to mention that the first three emergent were irrigated with pure water, and the same plants were fed with saline water during growing season. For this, randomized completely block design with three replicates was used. The size of each plot was 24 m^2, consisted of 6 ridges (8m long, 50cm wide) and hills were 20 cm apart from each block.

Table 1. Source, germity and salt tolerance of sugar beet genotypes

No	Genotype	Source	Germity	Poloidy	Type	Salt tolerance *
1	Dita	Belgium	monogerm	Diploid	N	tolerant
2	Brigitta	Germany	monogerm	Diploid	NZ	tolerant
3	Progress	USA	monogerm	Diploid	N	Mid-tolerant
4	Rifle	Belgium	monogerm	Diploid	N	sensitive
5	Concept	USA	monogerm	Diploid	NE	sensitive
6	Tigris	Denmark	multigerm	Polyploid	N	sensitive
7	Montebaldo	Germany	multigerm	Triploid	N	tolerant
8	Prestibel	Belgium	multigerm	Polyploid	NE	Mid-sensitive
9	Waed	Germany	multigerm	Diploid	N	tolerant
10	Kawimera	Germany	multigerm	Triploid	N	tolerant

* Abbas *et al*. (2011)

Table 2. Soil properties of study area

Soil Sample	Particle size distribution			Chemical analysis of soil paste extraction		
Season	Sand %	Silt %	Clay %	CaCo3 %	EC (mmhos/cm) (25^0C)	pH
2008-2009	33.3	36.4	30.3	19.4	1.8	8.1
2009-2010	29.3	40.7	29.6	20.7	1.9	8.2

Two samples were selected during the growth period i.e. 120 and 150 days during sowing period. Five guarded plants were chosen at random from each sub-plot to determine:

Leaf area index (LA) ($cm^2.plant^{-1}$): The disk method was followed using 10 disks of 0.91 cm. diameter according (Watson, 1958). Leaf number (LN) Only number of green leaves with a lamina length greater than 6 cm was considered (Rinaldi, 2003).

Relative growth rate (RGR) in ($g.g^{-1}.day^{-1}$) (Watson, 1958)

$$RGR = \frac{\log_e W_2 - \log W_1}{T_2 - T_1}$$

Net Assimilation Rate (NAR) ($gm^{-2}day^{-1}$)(Radfords, 1967)

$$NAR = \frac{(W_2 - W_1)(\log_e A_2 - \log_e A_1)}{(T_2 - T_1)(A_2 - A_1)}$$

Where W1,W2 and A2 refer to dry weight to plant, and leaf area at time T1 and T2, respectively.

-Chlorophyll fluorescence was measured in middle-aged leaves after 150 days from sowing time. The fast phase of chlorophyll *a* fluorescence variation was determined by Plant Efficiency Analyzer (PEA, Handsatech Instruments Ltd., King's Lynn, Norfolk PE32 IJL England). Leaves were exposed to dark state for 30 minutes before measurements,

as dark phase stimulates reaction centers of photosystem II to rest (not involved in any photosynthetic reactions (Lavorel and Etienne, 1977). Dark adaptation was inducted by a clip having a sliding opening. Measurements were taken from 11 am till 2 pm after 30 minutes of dark state. Measurements included:

- Fo (Fluorescence Origin): Dark adapted initial minimum fluorescence.
- Fm (Fluorescence maximum): Maximal fluorescence measured during first saturation pulse after dark adaptation.
- Fv/Fm = (Fm – Fo) / Fm.The dark adapted test used to determine maximum quantum yield. This ratio is an estimate of maximum portion of absorbed quanta used in PS-II reaction centers.

Data for each treatment were statistically analyzed and presented as ANOVA. The combined analysis for four evaluated planting dates was done for each season (Gomez and Gomez, 1984). Treatment means were compared using the Least Significant Difference (LSD) method.

Results

Leaf Area (LA) and Leaf Number (LN)

Under salinity stress, Leaf area (LA) in all genotypes decreased by 8.94% as compared to control after 120 days from sowing period. Indeed, the genotypes differed significantly in this trait ($p<0.01$). The decrement in LA ranged from 4.87% in Montebaldo and 17.67% in Tigris. Leaf numbers per plant decreased (0.94-6.79%) but the decrements were not significant under saline conditions. However, the mean decrement in all genotypes was 2.37% compared to control. The results depicted that leaf number was less affected than leaf area by salinity (Table 3).

Table 3. Leaf Area ($cm^2.plant^{-1}$) and Leaf Number (leaf/plant) for 10 sugar beet genotypes under control and salinity stress conditions

Genotype	Leaf Area (120 days)			Leaf Number (150 days)		
	Control	Salt conditions	Comparison with control (±%)	Control	Salt conditions	Comparison with control (±%)
Dita	4352	4239	-6.47	34.67	34.33	-0.94
Brigitta	4987	4692	-5.93	33.50	32.67	-2.47
Progress	4878	4459	-8.54	32.83	31.83	-3.05
Rifle	4610	3957	-14.12	33.17	32.00	-3.45
Concept	4708	3991	-15.21	33.17	31.67	-4.46
Tigris	4620	3805	-17.67	34.17	31.83	-6.79
Montebaldo	5101	4849	-4.87	34.83	34.50	-0.98
Prestibel	4413	4040	-8.44	32.33	30.83	-4.64
Waed	4493	4344	-3.31	33.00	32.00	-3.02
Kawimera	5257	5000	-4.88	35.00	34.17	-2.37
Mean	4742	4338	-8.94	33.67	32.58	-3.22
Leaf area (LSD0.01=442.3 **), leaf number (ns)						

Relative Growth Rate (RGR)

RGR decreased in all genotypes by an average of 34.85% as compared to control. The decrement ranged from 8.14% in Brigitta and 78.35% in Tigris (Table 4). *Net Assimilation Rate (NAR)*

NAR decreased also in all genotypes by an average of 26.47% as compared to control, which was increased in Brigitta by 2%. The decrement ranged between 1.81% in Dita and 73.49% in Tigris (Table 4).

Table 4. RGR ($g.g^{-1}.day^{-1}$), and NAR($g.m^{-2}.day^{-1}$) for 10 sugar beet genotypes under salinity stress conditions during (120-150) days period after sowing

Genotype	RGR			NAR		
	Control	Salt condition	Comparison with control (±%)	Control	Salt condition	Comparison with control (±%)
Dita	0.015	0.013	-13.23	5.57	5.45	-1.81
Brigitta	0.014	0.012	-8.14	4.77	4.86	+2
Progress	0.014	0.01	-26.69	4.63	3.49	-24.1
Rifle	0.013	0.005	-61.66	4.58	1.93	-57.67
Concept	0.014	0.006	-58.82	4.92	2.41	-51.3
Tigris	0.014	0.003	-78.35	4.79	1.26	-73.49
Montebaldo	0.015	0.012	-21.65	5.35	5.09	-4.51
Prestibel	0.015	0.009	-38.07	5.69	4.19	-25.63
Waed	0.014	0.011	-20.85	5.59	4.75	-14.42
Kawimera	0.014	0.011	-21.09	4.98	4.28	-13.72
Mean	0.014	0.009	-34.85	5.09	3.77	-26.47
RGR (LSD0.01=0.003**), NAR (LSD0.05=0.679 **)						

Chlorophyll fluorescence

Chlorophyll a fluorescence as measured by fo, Fm, and Fv/Fm ratio at 150 days after sowing is the stress and non-stress conditions are presented in Table 5.Fo was increased under salt stress condition in all genotypes by an average of 30.25% compared to control, but Fm decreased in all genotypes by 23.54%, so the ratio Fv/Fm also decreased by 15.62%. The differences among genotypes were significant.

Table 5.Fo, Fm,Fv/Fm for 10 sugar beet genotypes under salinity stress conditions

Genotype	**Fo**			**Fm**			**Fv/Fm**		
	Control	Salt condition	Comparison with control (±%)	Control	Salt condition	Comparison with control (±%)	Control	Salt condition	Comparison with control (±%)
Dita	659	794	20.82	3585	2934	-18.08	0.816	0.729	-10.66
Brigitta	639	731	14.63	3773	2957	-21.51	0.831	0.752	-9.45
Progress	670	844	26.28	3575	2939	-17.78	0.812	0.712	-12.34
Rifle	591	951	61.77	3426	2518	-26.33	0.827	0.622	-24.76
Concept	640	891	39.54	3844	2494	-35.1	0.833	0.642	-22.94
Tigris	589	920	57.09	3586	2202	-37.72	0.835	0.58	-30.46
Montebaldo	640	755	18.12	3671	2982	-18.4	0.825	0.746	-9.61

Prestibel	644	886	38.21	3806	2590	-31.87	0.83	0.655	-21.08
Waed	688	755	9.76	3513	2841	-19.2	0.804	0.743	-8.72
Kawimera	661	764	16.23	3671	3308	-9.49	0.82	0.769	-6.23
Mean	642	829	30.25	3645	2776	-23.54	0.823	0.694	-15.62
Fo (LSD0.01=74.96 **), Fm (LSD0.01=339.5 **), Fv/Fm (LSD0.01=0.026 **)									

Disscussion

The leaf number was less affected than leaf area by salinity. It is suggested that most of the reduction in plant leaf area was caused by the inhibition of leaf expansion. This is consistent with the results of previous researches, which showed that high levels of salinity decreased the leaf area due to combination of decrease in cell number and cell size (De-Herralde *et al*., 1998; Dadkhah and Grrifiths, 2006). Munns and Termaat (1986) demonstrated that for a given amount of NaCl transport to the shoot, reduction in leaf expansion results in the same proportional increase in the leaf NaCl concentration. Salt stressed barley plants produced smaller leaf areas, which caused a higher Na^+ accumulation in specific leaf area (Munns, 1985). Witkwski and Lamont (1991) reported that plants might reduce water loss by reducing their evaporation surface. Therefore, leaves tend to be smaller and thicker under saline conditions.

Halophytes tolerate the saline conditions and show a resistance to higher salt concentrations with a reduction in growth rate. Different cultivars of the same plant had different behavior toward salt tolerance (Flowers and Hajibagheri, 2001; Qadir *et al*., 2001). Our results indicated that RGR and NAR of all genotypes decreased significantly under salt condition. The decreased biomass weights of plants under saline conditions are correlated with the reduced leaf area, which results in decreases of photosynthetic area (Yang *et al.*, 2008). It is thought that a decreased photosynthesis under stress could have reduced the shoot growth and development, leading to lower biomass production compared to control (Campbell and Nishio, 2000). Greenway and Munns (1980) reported that the effect of salinity on leaf area was greater than dry weight, as salt accumulation in the shoot occurs via transpiration stream, which is highest in old leaves killing them. This proves that Brigitta genotype showed an increase in net assimilation rate under salinity stress.

Many studies have concluded that reduction in photosynthesis in response to salinity reduce stomatal conductance and consequently restrict the availability of CO_2 for carboxylation (Everard *et al*., 1994).

In control plant, there is no significant difference in chlorophyll fluorescence measurement, but in the presence of salinity the significant differences represented the differences in the efficiency of photosystem II in sugar beet cultivars. The fluorescence suggested that the rate of energy translocation or light capture might be limited by salinity (Long and Hallgern, 1993). We suggested that 10 genotypes experienced some degree of photo inhibition. Moreover, lower Fv/Fm was observed in salt-stressed conditions compared to control plants, which indicated that RuBP(Ribulose-1,5-bisphosphate) regeneration, which needs adequate electron translocation from photosystem II to electron acceptors, might be disturbed by salinity.

In terms of genotype tolerance, the genotypes differed significantly in all studied attributes except for LN. Under salt conditions, Brigitta (monogerm) achieved an increasing in (NAR), while Kawimera (multigerm) achieved the lowest decrement in (fv/fm). Tigris (monogerm) shows the highest reduction in all parameters, so, we consider Tigris the most non-tolerant genotype. And further studies must be done in future to study the correlations with yield and yield components of these genotypes under the same conditions to determine the most tolerant genotype.

Conclusion

The foregoing discussion showed that all studied growth attributes, leaf area, leaf number, relative growth rate, and net assimilation rate was decreased in salinity stress conditions compared to the controlled state, we think these could be returned to the decrement of light utilizing through increased values of fluorescence origin (fo), decreased values of fluorescence maximum (fm), and maximum yield of quantum in photosystem-II (fv/fm). Genotypes differed significantly in all studied attributes except in leaf numbers. Under salt conditions, Brigitta (monogerm) achieved an increase in net assimilation rate, while Kawimera (multigerm) achieved the lowest decrement in quantum in photosystem-II. Tigris (monogerm) shows the highest reduction in all parameters, so, it considered the most non-tolerant genotype.

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