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Full Length Research Paper

Effect of salinity and silicon application on photosynthetic characteristics of sorghum (*Sorghum bicolor* L.)

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Supplementing Si to the soil is one of the alternative strategies for overcoming the negative effects of salinity on crop yield. Therefore, a field experiment was conducted to investigate the effects of silicon application on photosynthesis characteristics of two sorghum (*Sorghum bicolor* L.) cultivars (CV) (Omidbakhsh and Sepideh) under three levels of salt stress (5.2, 10.5 and 23.1 dS m^{-1}), two levels of Si (1.44 and 1.92 $g.kg^{-1}$soil) application and control (0 $g.kg^{-1}$ soil) arranged as a split, split plot design. Salinity significantly decreased photosynthesis rate, chlorophyll a (Cha), chlorophyll b (Chb), and dry matter accumulation (DM) of the sorghum plants, indicating that plants suffered from stress. In addition, salinity imposed a remarkable decrease on variable fluorescence (Fv) and quantum yield. At highest level of salinity supply of 1.92 $g.kg^{-1}$soil Si, alleviated negative effects of salinity and increased photosynthetic rate (24%), transpiration rate (19%), quantum yield (38%), total pigments (22%) and dry matter accumulation (65%) compare with 1.44 $g.kg^{-1}$soil Si application. Correlation between shoot dry weight, Cha and Chb were no significant. In the relative salt sensitive CV (Sepideh) leaf Cha, Chb content and Cha/Chb, was higher than salt tolerance CV (Omidbakhsh), however, Omidbakhsh showed higher photosynthetic rate and dry matter accumulation.

Key words: Salinity stress, photosynthesis, chlorophyll fluorescence, transpiration rate.

INTRODUCTION

Salinity is one of the most important environmental factors limiting crop production mainly in arid and semi-arid areas. Salinity problem can be alleviated by adding reclamation substances, drainage and breeding salt-tolerant crops but the cost of engineering management of salinity is high and attempts to improve the salt tolerance crops had very limited success (Flowers and Flowers, 2005, Tuna, et al., 2008). Supplement Si is one of the alternative strategies for overcoming the negative effects of salinity on crop yield.

Silicon (Si) is the second most abundant element on earth and is a beneficial element for plant growth. All plants contain Si in their tissues, yet its role in plant biology and physiology has not been understood clearly. It is not counted among the essential elements for higher plants, apart from some species belonging to Poaceae and Cyperaceae (Liang et al., 2006). However, the Si content of the plant varies greatly in different plant species, ranging from 0.1 to 10.0 % of dry weight (Ma et al., 2006). Nevertheless, it was commonly accepted that silicon can positively affect growth and health status of some plants under biotic (Ma, 2004) and abiotic stresses (Ranganathan et al., 2006). Silicon has been shown to ameliorate the adverse effects of salinity on plants (Mohsenzadeh, et al., 2011). In the soil solution, Si occurs mainly as monosilicic acid (O_4SiH_4) at concentrations ranging from 0.1 to 0.6 mM and is taken up by plant in this form (Ma and Takahashi, 2002). After the uptake, Si accumulates on the epidermis of various tissues mainly as a polymer of hydrated amorphous silica (Ma, 2004).

Sorghum is moderately salt tolerant plant and is widely grown in semi arid areas on soils prone to salinity. In response to salinity, different genotypic variation exists among sorghum cultivars (Bavei et al., 2011).

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Table 1. Main chemical properties of the waters and soil (0-30cm) at the study site.

	Na	Ca	Mg	K	SO_4	CO_3	HCO_3	Cl	EC
					($meq.l^{-1}$)				$dS.m^{-1}$
Water 1	32.50	8.60	9.20	0.23	15.00	0.40	2.40	34.40	5.20
Water 2	67.10	16.40	22.20	0.38	25.00	0.00	3.00	75.60	10.50
Water 3	179.80	27.00	46.80	0.31	56.10	0.00	3.20	172.40	23.10
Soil	31.10	10.60	10.20	0.75	31.30	0.00	1.80	26.80	5.80

Measurement of photosynthesis characteristics including net CO_2 uptake, chlorophyll fluorescence, chlorophyll content, chlorophyll pigments and transpiration rate for monitoring plant responses to salt stress instead of yield response is recommend by many authors (Parveen and Ashraf, 2010; Ashraf et al., 2008; Ashraf, 2009). The decline in growth under salinity stress, associates with a decrease in the photosynthetic rate through effect on chemical and nonchemical mechanisms (Parveen and Ashraf, 2010). Chlorophyll fluorescence is one of the main important factors in order to assess stress levels on photosynthesis apparatus (Najafpour 2012).

Higher plants have an important involvement with silicon (Raven, 2003) and satisfactory results of Si supply to alleviate NaCl stress showed in different experiments (Mohsenzadeh, et al., 2011, Parveen and Ashraf, 2010; Ali et al., 2009). Studies show that in salt stress condition supply of Si could improve photochemical efficiency of PSII by increased chlorophyll content, limiting the transpiration rate and detoxifying ROS by accumulation of silicon in leaves (Mohsenzadeh, et al., 2011; Al-aghabary et al., 2004). Classical methods of screening for salt tolerance are based on yield response and are time consuming and expensive. Photosynthesis characteristics are the possible tool for salinity tolerance screening in crops genotypes (Ashraf et al., 2008).

The objectives of this study were to investigate effects of exogenous Si on photosynthesis characteristics of a relatively salt sensitive and a relatively salt tolerant sorghum under different levels of salt stress in the field condition.

MATERIAL AND METHODS

Plant materials and growth conditions

Sorghum (*Sorghum bicolor L.*) seeds were kindly supplied by the Agriculture and Natural Resources Research Center of Khorasan Razavi. Two Sorghum cultivars including: Omidbakhsh, a relatively salt-tolerant, and Sepideh, a salt-sensitive cultivar were selected. Seeds were directly sown in the soil at the Research Farm of the Center of Excellence for Special Crops of the Ferdowsi University, located 20 km east of Mashhad, Iran, in June 2008. This station, located at latitude 36°18´11´´N and longitude 59° and 46´´19´´E, has a geographical altitude of 985 m above sea level. The climate of the experiment site is dry, with annual precipitation of 259 mm and annual reference-crop evapotranspiration of more than 1300 mm (Dinpashoh, 2006). The average relative humidity of the location during the growth period of Sorghum (June to September) was 34%. During the course of the experiment, rainfall did not exceed 2 mm, and the soil–water reserve was ignorable. Therefore, the total water requirement of the plants was matched by the irrigation water. The soil had a loamy–silty–clay texture, with clay, silt, and sand contents of the soil were 39%, 46%, and 15%, respectively, and the acidity and electrical conductivity (EC) of the soil extract were 7.7 and 5.80 dS m^{-1}, respectively. The source of irrigation water for low-level of salinity was the water pumped from a deep well near the site (Table 1). For the remaining two higher levels of salinity, water was transferred by tankers from ground sources in the same basin, within a distance of 5 km. Chemical analysis of the water resources in terms of the three levels of salinity was carried out (Table 1). Low salinity level (EC= 5.20 dS m^{-1}) played the role of control because previous experiments have shown that sorghum showed no significant yield reduction under moderate salinity (6.8 dS m^{-1}) compared to that in fresh water (1.5 dS m^{-1}) (Igartua *et al*., 1995). Volume of irrigation water in each plot was monitored by volumetric counter.

SiO_2 used as the Si source is composed of 97.59% SiO_2 and other minor elements such as $AlSO_3$ (0.37%), $FeSO_3$ (0.73%), CaO (0.26%), Na_2O (0.1%), K_2O (0.06%), MgO (0.13%) and P_2O_5 (0.11%).

1.1. Treatments

The experiment was arranged as a split-split plot based on randomized complete block design with three replications. Saline waters (5.2, 10.5, and 23.1 dS m^{-1}), silicon concentration (0, 1.44, and 1.92 $g.kg^{-1}$soil) and two sorghum cultivars (Omidbakhsh and Sepideh) were allocated as main, sub and sub-sub plots, respectively. Seeds were sown at 0.75 × 0.2 m distances between and within rows on 13 June in 2008. Plants were grown under non-saline and non-silicon conditions up to four fully expanded leaves were appeared. Soil fertilized by application of di-ammonium phosphate at a rate of 50 kg h^{-1} before treatments were applied.

Photosynthetic rate, chlorophyll fluorescence and chlorophyll content (SPAD value as well as chemical measurement) and photosynthetic rate, were measured

Table 2. simple effect of different levels of salinity, Si and sorghum cultivars on photosynthetic rate (A) (µmol $m^{-2}s^{-1}$), transpiration rate (E) (mmol $m^{-2}s^{-1}$), intercellular CO_2 concentration (Ci) (ppm), chlorophyll fluorescence yield (Yq) and chlorophyll content (SPAD value).

	Salinity(dS m^{-1})			LSD† 0.05	Silicon(g.kg^{-1}soil)			LSD 0.05	Cultivar		LSD 0.05
Parameters	5.2	10.5	23.1		0	1.44	1.92		O	S	
A(µmol $m^{-2}s^{-1}$)	18.0	16.2	15.9	1.22	17.5	16.0	16.6	1.56	19.0	14.4	0.77
E(mmol $m^{-2}s^{-1}$)	2.4	2.3	2.5	0.37	2.4	2.5	2.5	0.16	2.6	2.3	0.08
Ci (ppm)	314.0	307.1	311.0	9.57	311.6	312.3	308.2	5.09	309.7	311.6	3.15
F0	281.9	309.7	297.9	37.05	283.9	310.8	294.8	30.21	286.7	306.3	20.10
Fm	513.8	513.6	498.4	18.23	483.7	519.1	522.9	62.32	473.3	543.9	36.73
Fv	231.9	204.0	200.4	48.77	199.8	208.4	228.1	38.40	186.6	237.6	31.22
Yq	0.395	0.371	0.359	0.06	0.374	0.356	0.395	1.55	0.358	0.392	0.03
SPAD	44.4	44.9	44.2	1.81	45.1	44.2	44.1	0.04	44.4	44.6	1.20

†Least significant different

on the youngest fully expanded leaves of each treatment twice a week at soon after treatment up to the end of the treatments period. Net photosynthetic rate (A) was measured by a portable photosynthetic system (LCA4). Leaf chlorophyll content was measured using a hand-held chlorophyll meter (SPAD-502 Japan). At each measurement, the content was measured in three parts from leaf tip to base and the average was used for analysis. Chlorophyll fluorescence emission from the upper leaf surface was measured by a pulse amplitude modulation fluorimeter (PAM 101–103 H Walz, Effeltrich, Germany).

Sampling and harvest procedure

Fresh samples were randomly taken from fully matured leaves. All the measurements that needed fresh samples were carried out during the flowering stage. Youngest fully expanded leaves were sampled for biochemical analysis and were kept in -80°C freezer, until determinations. For dry weight determination at maturity, samples were dried in an oven at 70°C until constant mass was reached.

Chlorophyll content

Chlorophyll a, b and total carotenoid was determined according to Dere et al. (1998). One-hundred mg of fresh leaf material taken from the youngest fully expanded leaf and extracted with 99% methanol and read absorption recorded using spectrophotometer (Jenway Model 6305) at 653 and 666 nm wavelengths, for chlorophyll a and b, respectively. Chlorophyll concentrations were calculated by using the below equations (Dere et al., 1998):

Ch a = $15.65\ A_{666} - 7.340\ A_{653}$ and Ch b= $27.05\ A_{653} - 11.21\ A_{666}$

1.2. Statistical analysis

The data compiled were submitted to an analysis of variance (ANOVA) using statistical program SAS 9.1 and the mean comparison was performed by Duncan's multiple-range test (P ≤ 0 05).

RESULTS

Photosynthetic rate (A)

Photosynthesis rate significantly decreased 10.3 and 12.1 %, respectively, when plants were subjected to salt stress of 10.5 and 23.1 dS m^{-1} compare to control (Table 2). Low level of Si application imposed a significant reduction (8.6%) in net CO_2 uptake rates but at high level of Si application this rate was increased compare to non-Si application. The photosynthesis rate in salt- tolerant CV (Omidbakhsh) was significantly (P≤0.05) higher (23.8%) than the relative salt- sensitive CV (Sepideh) (Table 2). Photosynthetic rate was also significantly (P≤0.01) decreased in later growth stages. No significant decrease in photosynthesis was obtained in the presence of salinity a week after salt application but after this period, more salt accumulation in the leaves might caused a reduction in photosynthetic activity. Interaction effects of salinity and Si on photosynthetic rates showed that in low level of salinity Si application did not change the photosynthetic rate significantly, but at higher levels of salinity photosynthetic activity decreased significantly. Application of 1.44 g.kg^{-1}soil Si at 10.5 dS m^{-1} salinity level, increased photosynthetic rate by 4.7 % and 7.7% compare to other two levels of Si application. At highest level of salinity, Si application alleviated negative effects of salinity, so that photosynthetic activity increased 19.4% compare to non Si application (Table 4). Result showed that photosynthetic activity at 21 days after salt application in low level of salinity was higher than other salinity levels but at 63 days after salt application, there were no significant differences among salinity treatments (Table 6). Interaction effects of CV and time of measuring on photosynthesis rates were significant (P≤0.01).

Table 3. Trend of photosynthetic rate (A) (μmol $m^{-2}s^{-1}$), transpiration rate (E) (mmol $m^{-2}s^{-1}$), intercellular CO_2 concentration (Ci) (ppm), chlorophyll fluorescence yield (Yq) and chlorophyll content (SPAD value) of sorghum varieties under salinity and Si treatments at different sampling from 7 to 63 days after salinization.

	Days after treatment					LSD† 0.05
Parameters	7	21	35	49	63	
A(μmol $m^{-2}s^{-1}$)	24.1	20.1	16.3	12.1	10.9	1.22
E(mmol $m^{-2}s^{-1}$)	4.2	2.9	2.4	1.5	1.1	0.12
Ci (ppm)	261.7	297.4	299.7	352.6	342.1	4.98
F0	242.8	172.4	274.5	420.1	372.7	33.01
Fm	347.2	250.4	486.2	773.2	685.9	59.19
Fv	104.4	78.0	211.7	353.0	313.2	48.96
Yq	0.285	0.292	0.419	0.444	0.435	0.61
SPAD	46.0	45.8	46.5	43.6	40.5	1.90

†Least significant different

Table 4. Interaction between different levels of salinity and silicon on the photosynthetic rate (A) (μmol $m^{-2}s^{-1}$), transpiration rate (E) (mmol $m^{-2}s^{-1}$), intercellular CO_2 concentration (Ci) (ppm), chlorophyll fluorescence yield (Yq) and chlorophyll content (SPAD value) of the two sorghum varieties.

	Salinity(dS m^{-1})									
	5.2			10.5			23.1			
	Silicon(g.kg^{-1}soil)									LSD† 0.05
Parameters	0	1.44	1.92	0	1.44	1.92	0	1.44	1.92	
A(μmol $m^{-2}s^{-1}$)	19.4	17.5	17.2	16.1	16.9	15.6	16.9	13.7	17.0	1.63
E(mmol $m^{-2}s^{-1}$)	2.4	2.3	2.4	2.4	2.6	2.7	2.4	2.6	2.5	0.16
Ci (ppm)	319.0	316.7	306.2	310.8	306.3	304.3	305.0	313.8	314.2	6.68
F0	275.1	292.0	278.7	305.2	311.9	311.9	271.5	328.5	293.8	44.29
Fm	502.8	536.8	501.8	480.6	522.0	538.2	467.7	498.6	528.8	79.41
Fv	227.8	244.8	223.0	175.5	210.1	226.3	196.2	170.1	235.0	65.69
Yq	0.405	0.401	0.378	0.349	0.370	0.394	0.368	0.295	0.414	0.08
SPAD	46.2	44.4	42.6	45.2	44.1	45.3	44.0	44.0	44.5	2.55

†Least significant different

Transpiration rate (E)

Transpiration rate was not affected by salinity and silicon treatments (P≤0.05) but result showed a remarkable transpiration difference in different levels of salinity (Table 2). Increased transpiration rate from 5.2 to 10.2 dS m^{-1} salinity was observed, and after that with increase salinity up to 23.1 dS m^{-1} this rate decreased (Table 2). Transpiration rate of leaves received 1.44 g.kg^{-1}soil Si was lowest and transpiration rate of Si application of 1.92 g.kg^{-1} soil was highest (Table 2). Interaction of salinity and Si application showed no significant decrease in transpiration rate. However, at high levels of salinity and 1.92 g.kg^{-1}soil Si application, the highest rate of transpiration was obtained (Table 4). Transpiration rate in relative salt tolerant (Omidbakhsh) was (10.72%) higher than the relative salt sensitive (Sepideh) (Table 2). Transpiration rate of salinity and cultivar showed that increase in salinity up to 10.5 dS m^{-1}, caused higher transpiration rate in Sepideh than Omidbakhsh, but at 23.1 dS m^{-1} no significant differences was observed between CVS (Table 5). Effect of silicon on transpiration rate of cultivars showed that, transpiration rate in Omidbakhsh due to silicon application was higher than Sepideh (Table 7). Interaction effects of salinity and time after salinization on transpiration rates were significant (P≤0.01). A week after salt application, plants under 10.5 and 5.2 dS m^{-1} salinity levels showed the highest (5.3 mmol $m^{-2}s^{-1}$) and lowest (3.2 mmol $m^{-2}s^{-1}$) transpiration rate, respectively, but 21 to 49 days after salt application, plants under 5.2 dS m^{-1} salinity showed highest transpiration rate and 63 days after salinization all levels of salinity showed the equal transpiration rates (Table 6). Interaction effects of Si and time on transpiration rates were significant (P≤0.01) (Table 8). Transpiration rate in both CVs and in the first week after Si application was maximal compare to later sampling. During the experiment, salt tolerant CV showed higher transpiration

Table 5. Interaction between different levels of salinity and two sorghum varieties on the photosynthetic rate (A) (µmol $m^{-2}s^{-1}$), transpiration rate (E) (mmol $m^{-2}s^{-1}$), intercellular CO_2 concentration (Ci) (ppm), chlorophyll fluorescence yield (Yq) and chlorophyll content (SPAD value)..

	Salinity(dS m^{-1})						
	5.2		10.5		23.1		
	Cultivar						LSD† 0.05
Parameters	Omidbakhsh	Sepideh	Omidbakhsh	Sepideh	Omidbakhsh	Sepideh	
A(µmol $m^{-2}s^{-1}$)	20.2	15.9	19.1	13.3	17.6	14.1	1.33
E(mmol $m^{-2}s^{-1}$)	2.6	2.1	2 .7	2.4	2.5	2.5	0.13
Ci (ppm)	316.7	311.2	302.1	312.1	310.4	311.6	5.45
F0	277.0	286.8	298.3	321.1	284.7	311.1	36.16
Fm	474.7	552.9	492.7	534.5	452.4	544.3	64.84
Fv	197.7	266.1	194.5	213.4	167.7	233.2	53.64
Yq	0.366	0.423	0.369	0.374	0.340	0.378	0.07
SPAD	44.2	44.6	44.8	44.9	44.2	44.2	2.08

†Least significant different

Table 6. Effect of different levels of salinity on photosynthetic rate (A) (µmol $m^{-2}s^{-1}$), transpiration rate (E) (mmol $m^{-2}s^{-1}$), chlorophyll fluorescence yield (Yq) and chlorophyll content (SPAD value) in sorghum at different sampling from 7 to 63 days after salinization.

	Salinity(dS m^{-1})															
	5.2					10.5					23.1					
	Days after treatment															LSD†
Parameters	7	21	35	49	63	7	21	35	49	63	7	21	35	49	63	0.05
A(µmol $m^{-2}s^{-1}$)	23.7	22.5	18.9	14.4	10.8	24.3	19.8	14.5	11.1	11.2	24.5	17.8	15.5	10.7	10.8	2.10
E(mmol $m^{-2}s^{-1}$)	3.2	3.2	2.6	1.7	1.1	5.3	2.8	2.2	1.4	1.1	4.1	2.7	2.3	1.4	1.2	0.21
Ci(ppm)	275.3	300.1	301.8	353.3	339.4	253.3	295.1	301.0	346.5	339.8	256.5	297.0	296.3	358.1	347.1	8.62
F0	238.4	163.5	268.6	365.3	373.8	242.3	175.4	275.5	500.4	354.8	247.7	178.4	279.5	394.7	389.4	57.18
Fm	330.1	243.9	483.9	840.7	670.3	345.3	270.2	502.2	759.3	691.1	366.2	237.2	472.6	719.5	696.3	102.5
Fv	91.8	80.4	215.3	475.4	296.5	103.0	94.8	226.7	258.9	336.3	118.5	58.8	193.1	324.8	306.9	2.68
Yq	0.269	0.306	0.423	0.548	0.427	0.292	0.332	0.439	0.344	0.449	0.293	0.238	0.395	0.440	0.429	0.11
SPAD	46.9	44.8	45.5	43.5	41.4	46.0	46.3	47.3	44.1	40.8	45.2	46.2	46.8	43.2	39.5	3.29

†Least significant different

Table 7. Interaction between different levels of Si and two sorghum varieties on photosynthetic rate (A) (µmol $m^{-2}s^{-1}$), transpiration rate (E) (mmol $m^{-2}s^{-1}$), intercellular CO_2 concentration (Ci) (ppm), chlorophyll fluorescence yield (Yq) and chlorophyll content (SPAD value).

	Silicon(g.kg^{-1}soil)						
	0		1.44		1.92		
	Cultivar						LSD†
Parameters	Omidbakhsh	Sepideh	Omidbakhs	Sepide	Omidbakhs	Sepideh	0.05
A (µmol $m^{-2}s^{-1}$)	20.1	14.9	17.7	14.3	19.1	14.1	1.33
E (mmol $m^{-2}s^{-1}$)	2.7	2.2	2.5	2.6	2.7	2.3	0.13
Ci (ppm)	308.9	314.2	314.0	310.6	306.3	310.1	5.45
F0	277.0	290.8	290.2	331.4	292.8	296.8	36.1
Fm	467.3	500.1	464.6	573.7	487.9	557.9	64.8
Fv	190.3	209.3	174.4	242.3	195.1	261.1	53.6
Yq	0.372	0.376	0.337	0.374	0.365	0.426	0.07
SPAD	45.7	44.6	44.4	43.9	43.0	45.3	2.08

†Least significant different

Table 8. Effect of different levels of Si on photosynthetic rate (A) (µmol $m^{-2}s^{-1}$), transpiration rate (E) (mmol $m^{-2}s^{-1}$), intercellular CO_2 concentration (Ci) (ppm), chlorophyll fluorescence yield (Yq) and chlorophyll content (SPAD value) in sorghum from 7 to 63 days after salinization.

	Silicon(g.kg-1soil)															
	0					1.44					1.92					
	Days after treatment															LSD†
Parameters	7	21	35	49	63	7	21	35	49	63	7	21	35	49	63	0.05
A (µmol $m^{-2}s^{-1}$)	24.9	19.8	17.6	12.6	12.6	22.9	19.6	14.7	12.3	10.6	24.6	20.8	16.7	11.4	9.6	2.11
E (mmol $m^{-2}s^{-}$	4.0	3.0	2.4	1.5	1.3	4.1	2.8	2.3	1.5	1.0	4.6	3.0	2.4	1.4	1.1	0.21
Ci (ppm)	264.6	297.9	300.8	351.7	342.9	264.6	298.7	301.5	356.9	339.8	255.9	295.6	296.7	349.4	343.6	8.62
F0	276.6	171.0	266.9	380.2	324.9	231.4	178.1	297.6	463.4	383.4	220.4	168.1	259.1	416.8	409.7	57.18
Fm	383.9	243.2	472.8	695.4	623.3	311.4	248.4	498.0	804.3	733.7	346.3	259.7	487.9	819.8	700.8	102.50
Fv	107.3	72.2	205.9	315.2	298.4	80.1	70.2	200.4	340.9	350.2	125.9	91.6	228.9	403.0	291.1	84.81
Yq	0.277	0.279	0.427	0.446	0.441	0.246	0.272	0.385	0.405	0.470	0.331	0.325	0.446	0.481	0.394	0.11
SPAD	46.7	45.9	47.4	44.4	41.3	46.0	45.9	47.0	43.0	39.0	45.3	45.5	45.2	43.3	41.4	3.29

†Least significant different

Table 9. Mean comparison of chlorophyll a (Ch a) ($mg.gdw^{-1}$), chlorophyll b (Ch b) ($mg.gdw^{-1}$), Ch a/ Ch b ratio and dry matter (DM) ($ton.h^{-1}$) accumulation, in different salinity and Si levels of two sorghum cultivars.

	Salinity(dS m^{-1})				Silicon(g.kg^{-1}soil)				cultivar		
Parameters	5.2	10.5	23.1	LSD	0	1.44	1.92	LSD	Omidbakhsh	Sepideh	LSD†
Ch a ($mg.gdw^{-1}$)	3.50	2.52	3.38	0.73	3.29	2.92	3.19	0.55	2.76	3.51	1.03
Ch b ($mg.gdw^{-1}$)	3.11	2.25	2.92	0.49	2.76	2.64	2.88	0.48	2.46	3.06	1.30
Ch a/ Ch b	1.13	1.12	1.19	0.20	1.22	1.10	1.12	0.13	1.11	1.18	0.27
DM ($ton.h^{-1}$)	10.31	5.93	5.67	2.59	7.82	6.55	7.54	1.71	7.80	6.81	2.50

†Least significant different

Table 10. Interaction between levels of salinity and Si treatments on chlorophyll a (Ch a) ($mg.gdw^{-1}$), chlorophyll b (Ch b) ($mg.gdw^{-1}$), Ch a/ Ch b ratio and dry matter (DM) ($ton.h^{-1}$), in sorghum.

	Salinity(dS m^{-1})									
	5.2			10.5			23.1			
	Silicon (g.kg^{-1}soil)									
Parameters	0	1.44	1.92	0	1.44	1.92	0	1.44	1.92	LSD†
Ch a ($mg.gdw^{-1}$)	3.32	3.42	3.77	2.51	2.51	2.54	4.04	2.85	3.25	0.96
Ch b ($mg.gdw^{-1}$)	2.96	3.07	3.31	2.22	2.29	2.23	3.11	2.55	3.10	0.84
Ch a/ Ch b	1.13	1.12	1.14	1.12	1.09	1.14	1.39	1.11	1.07	0.24
DM ($ton.h^{-1}$)	10.9	10.62	9.40	5.70	5.20	6.89	6.85	3.83	6.33	2.97

†Least significant different

rate than the salt sensitive one.

Intercellular CO_2 concentration

There was no significant ($P \le 0.01$) effect of salinity on intercellular CO_2 concentration (Ci) but Ci decreased due to Si application significantly ($P \le 0.05$) (Table 2). Application of 1.92 g.kg^{-1}soil Si on Ci caused more dramatic decrease than in the no Si and 1.44 g.kg^{-1}soil Si application (Table 2). Interaction between salinity levels and Si application showed that in the 5.2 and 10.5 dS m^{-1}, Si application imposed a negative effect on Ci but at 23.1 dS m^{-1} salinity, effect of Si application on Ci was positive (Table 4). There were no significant difference ($P \le 0.05$) in the Ci between salt sensitive CV and salt tolerant CV in different levels of salinity (Table 2). Ci gradually and significantly ($P \le 0.01$), increased with increasing age of leaves (Table 3).

Table 11. Correlation matrix of photosynthetic rate (A), transpiration rate (E), intercellular CO_2 concentration (Ci), chlorophyll fluorescence yield (Yq) and chlorophyll content (SPAD value) in two sorghum varieties grown on varied of salinity and silicon levels.

Parameters	A	E	Ci	SPAD	F0	Fm	Fv	Y
A	1.00	0.83**	-0.56**	0.33**	-0.50**	-0.53**	-0.40**	-0.20**
E		1.00	-0.69**	0.35**	-0.49**	-0.57**	-0.47**	-0.27**
Ci			1.00	-0.30**	0.48**	0.61**	0.53**	0.36**
SPAD				1.00	-0.24**	-0.28**	-0.23**	-0.11ns
F0					1.00	0.79**	0.38**	-0.01ns
Fm						1.00	0.87**	0.55**
Fv							1.00	0.84**
Y								1.00

Significant difference at the 0.01 (**) and not significant (ns)

Table 12. Correlation matrix of chlorophyll a (Ch a), chlorophyll b (Ch b), Ch a/ Ch b ratio, and dry matter (DM), in the average of different salinity, Si levels and sorghum cultivars.

Parameters	Ch a	Ch b	Ch a/Ch b	DM
Cha	1.00	0.84**	0.40**	0.20ns
Chb		1.00	-0.14ns	0.19ns
Cha/Chb			1.00	0.00ns
Dm				1.00

(**) refer to significant difference at the 0.01 and ns refer to not significant

Chlorophyll fluorescence

There were no significant differences (P≤0.05) in quantum yield in different levels of salinity, silicon treatments and CVs. Quantum yield at 10.5 and 23.1 dS m^{-1} was lowered by 8.1, 8.5 %, respectively, compare with that in the 5.2 dS m^{-1} (Table 2). The value of Fm increased with increasing salinity levels but this increasing was no significant (Table 2).

Relative chlorophyll content (SPAD value)

As shown in Table 2, chlorophyll content in salinity treatments was not significant (P≤0.05). From Table 2, it is clear that Si application reduced leaf chlorophyll content significantly (P≤0.05). SPAD unit was markedly higher in the absence of Si than Si treatments (Table 2). Interaction of salinity and Si showed that in low level of salinity (5.2 dS m^{-1}) with increased rate of Si application, SPAD unit was significantly (P≤0.01) decreased (Table 4). Chlorophyll content in both cultivars (P≤0.01) decreased significantly by increased Si concentration in the soil (Table 2). With accumulation of ions in the leaves during the time, chlorophyll content decreased gradually (Table 3).

Chlorophyll concentration

Chlorophyll concentration in leaves was also significantly affected by salinity and cultivars, and this effect depends on the levels of salinity. Increased salinity level up to 10.5 dS m^{-1} caused a decrease in leaf Ch a and Ch b concentrations but at 23.1 dS m^{-1} chlorophyll concentration was increased (Table 9). There were no significant difference (P≤0.05) among Si application treatments on Ch a, Ch b and Cha/ Chb (Table 9). Interaction of salinity and Si application had shown no significant (P≤0.05) but remarkable difference in concentrations of Cha (Table 10). Salt sensitive CV Sepideh showed significantly more Ch a and Ch b than Omidbakhsh. Chlorophyll a:b ratio was not significantly (P≤0.05) affected by increasing salinity and Si treatments (Table 9). Interaction between salinity and CV and, S and CV shown no significant difference (P≤0.05) in Ch a, Ch b and chlorophyll a:b ratio.

Dry Matter accumulation

Biomass production showed significant difference in salinity (P≤0.01), silicon (P≤0.05) levels and sorghum cultivars (P≤0.05) (Table 9). Sorghum irrigated with 5.2 and 23.1 dS m^{-1} saline water showed the highest (10.31

ton.h^{-1}) and lowest (5.67 ton. h^{-1}) biomass production, respectively (Table 9). Biomass production was highest (7.82 ton.h^{-1}) in the absent of silicon and lowest at 1.44g.kg^{-1}soil (6.55 ton.h^{-1}), respectively (Table 9). Salt tolerant CV (Omidbakhsh) accumulated 12.62% higher dry matter than salt sensitive (Sepideh) CV (Table 9). Interaction of salinity and silicon showed that increase in silicon rate at saline water 5.2 dS m^{-1} imposed a negative effect on dry matter production but at 10.5 dS m^{-1} this effect was positive. However, lowest dry matter production was observed at 23.1 dS m^{-1} and 1.44g.kg^{-1}soil silicon (Table 10).

DISCUSSION

Salinity caused a dramatic decline in growth of sorghum. Effects of salinity on photosynthesis rate at 10.5 and 23.1 dS m^{-1} showed that sorghum is not tolerant enough to high levels of salinity. In spite of large decrease in the initial slope of the photosynthetic response to salinity observed in the 10.5 dS m^{-1} but after that there was no significant decrease in 23.1 dS m^{-1}. According to pervious study by Maas et al. (1986) sorghum is moderately tolerant to salinity. Base on this result, photosynthetic reduction threshold of sorghum to salinity is higher than 5.2 dS m^{-1} and lower than 10.5 dS m^{-1}. Munns and Tester (2008) with review the mechanisms of salinity tolerance reported that, salts can build up in leaves to excessive levels at high salinity. Photosynthetic organelles in higher plants have an important involvement with silicon (Raven, 2003) and satisfactory results of Si supply to alleviate NaCl stress showed in different experiments (Liang et al., 1996,; Liang 1998; Liang 1999; Matoh et al., 1986; Ahmad et al., 1992; Liang and Ding 2002). The gas exchange could have been the primary factors for Si induced growth improvement under saline and non-saline conditions.

Plant growth is the result of integrated physiological processes (Parida and Das, 2005). Previous studies reported different transpiration rate in salinity conditions. Both reduction (Dudley et al., 2009) and increase in transpiration by high levels of salinity (Radwan et al., 2000) was reported. Radwan et al. (2000) reported that increased salinity from 0 to 24 dS m^{-1} in *Balanites aegyptiaca* caused a reduction in transpiration rate. Transpiration is usually strongly correlated with stomatal conductance. Therefore, it is assumed that salinity reduced transpiration rates mainly by effects on stomatal opening (Radwan et al., 2000). In some cases, reduction of transpiration rate was due to decrease in water uptake by root. In our experiment increase in transpiration observed a week after treatments application in high level of salinity and at the rest of plant life transpiration decreased by increasing salinity. Si application against NaCl stress showed satisfactory results in barley (Liang et al., 1996; Liang 1998; Liang 1999; Liang and Ding 2002), rice (Matoh et al., 1986) and wheat (Ahmad et al., 1992). Probable explanations proposed for this results include limiting the transpiration by accumulation of silicon in leaves (Matoh et al., 1986), formation of complexes with Na in roots (Ahmad et al., 1992), protection of cell membranes (Liang et al., 1996, Liang 1998), increase activity of antioxidative enzymes (Liang 1999, Liang et al., 2003). In our experiment, transpiration rate in the Si application treatments was higher than Si absence a week after treatments application, but as age of plants increased effect of Si, cause decreased transpiration rate. Relative salt tolerant CV showed more transpiration rate at all levels of salinity and Si application.

Chlorophyll fluorescence is the possible tool for salinity tolerance screening in crops (Belkhodja et al., 1994). Measuring light utilization efficiency of the photosynthetic machinery is a way to detect levels of stress effects on plants (Roháek 2002). There are some reports that quantum yield in unaffected by salt stress (Belkhodja et al., 1994; Dionisio-Sese, and Tobita, 2000) but in other hand, some reports indicated that salt stress affected quantum yield (Yamane et al., 2008). In the present study quantum yield was decreased in high levels of salinity (Table 2). Theoretically, the level of F0 is the fluorescence emission when all reaction centers are open and photochemical quenching is maximal. Therefore, increase value of F0 is characteristic of damage to the PSII or inhibition of transfer of excitation energy from the antennae to the reaction centers (Bolhar Nordenkampf et al., 1989). Plants that exposed to salinity stress had thickened leaves compare to normal plants (Munns and Tester, 2008). Thickened leaves under salinity condition might be because of swelling of thylakoids that caused lipid peroxidation by reactive oxygen species (Yamane et al., 2008). Reactive oxygen species are related to damage of reaction centers (Nishiyama et al., 2001). Present study showed that F0 value was increased as age of leaves increased and in the other hand Fm was decreased in high level of salinity that caused by increase in nonphotochemical quenching (Bolhar Nordenkampf et al., 1989). This result showed that F0 was increased in the presence of salinity several weeks after salt application that is probably because of the effect of salinity and age on thickness of leaves. The quantum yield was improved in the salt stressed leaves by Si addition (Table 4). Previous study have shown that in salt stress condition supply Si can improve photochemical efficiency of PSII by increased chlorophyll content and detoxify ROS induced in tomato (Al- aghabary et al., 2004). In the present study, salt sensitive sorghum showed the higher quantum yield compare salt tolerance CV.

Chlorophyll content is an important factor in plant productivity because it is directly proportional to the photosynthesis rate of plant for biomass production (Wang et al., 2002). The previous study proved that halophyte and non-halophyte plants that subject to salinity stress, showed difference chlorophyll content behavior (Wang et al., 2002; Kafi et al., 2010). Kafi et al. (2010) reported that the salinity treatment did not significantly disturb the chlorophyll contents in halophyte plant kochia. On the other hand, studies on non-halophyte plants showed that decrease of the chlorophyll contents under saline condition (Wang et al., 2002). In the present study chlorophyll content in the sorghum were not affected by saline water but supply of Si showed markedly lower value of chlorophyll content compare to control. It seems that Si cause a decrease in stay green of the leaves. The chlorophyll content decreased with plant age that showed the senescence of leaves.

Effect of salinity on sorghum chlorophyll concentration at 10.5 dS m^{-1} salt level was more negative than 23.1 dS m^{-1} salinity. This result showed that decrease in Ch a and Ch b in the 10.5 dS m^{-1} salinity was 27.1% and 27.7% compare to 5.2 dS m^{-1} and at 23.1 dS m^{-1} salinity they reduced 3.7% and 6.1 compare to 5.2 dS m^{-1}, respectively (Table 9). Correlation between shoot dry weight and Ch a and Ch b concentrations was no significant (P≤0.05) (Table 12). This result is in agreement with Lee et al. (2004) who reported no correlation between shoot dry weight and Ch a and Ch b concentrations. In the previous study by Netondo et al. (2004b), negative effect of salinity on chlorophyll concentration in sorghum was reported. The chlorophyll a:b ratio significantly increased with salinity therefore Ch b was more sensitive than Ch a (Sultana et al., 1999). Parida and Das (2005) reported that in general under salt stress chlorophyll contents of leaves was decreased. The chlorosis of the oldest leaves start to develop and fall with prolonged period of salt stress. However, in some cases such as Amaranthus, chlorophyll content was increased under salinity. A decrease in chlorophyll concentration in saline condition could be attributed to increased activity of the chlorophyll-degrading enzyme chlorophyllase (Liang, 1998.). Added 1.92g.kg^{-1}soil Si application increased Ch a and Ch b under salt stress after 49 days of treatment (Table 9). Increased Ch a and Ch b in tomato plants by Si application after 10 days of treatment was reported but after 27 days of treatment, this effect was negligible (Al-aghabary et al., 2004).

The beneficial effects of Si application are more significant when plants were grown under stressful environments. For example, dry matter in the highest-Si treatment was increased at 10.5 and 23.1 dS m^{-1} compares with 5.2 dS m^{-1} which is in accordance to Liang et al. (2006). Review by Liang et al. (2007) showed the positive effects of Si on mitigating salinity in rice, mesquite, wheat, barley, cucumber and tomato in recent investigations. There was a positive significant correlation (p<0.001) between photosynthesis and transpiration obtained and transpiration rate was increased but not significantly at high level of Si application (Table 11). Matoh et al. (1986) reported that Si-induced reduction in transpiration rate and partial blockage of the transpiration bypass flow. Therefore, Na concentration in the shoots of plant was decreased by reduction in transpiration rate but there were contradictory reports (Savant et al., 1997). Netondo et al. (2004a) reported that chlorophyll concentration of the leaves of sorghum grown at high NaCl concentrations reduced. The reduction in dry matter in saline condition might be through inhibition of current photo assimilation because salinity reduces the contents of photosynthetic pigments. However, the highest level of Si application increased Cha and Chb concentrations.

Conclusion

In conclusion, the assessment of the effect of salinity and Si on the photosynthetic parameters in two sorghum cultivars allows us to conclude that all of the studied parameters were affected by salinity with a varietal difference. At highest level of salinity, Si application alleviated negative effects of salinity on net CO_2 uptake rate. Omidbakhsh showed higher photosynthesis rate than the Sepideh. However, transpiration rate in Sepideh was lower than Omidbakhsh. Decline of transpiration rate was occurred at 1.44 g.kg^{-1}soil Si. Si application decreased Ci and leaf chlorophyll content. Ch a, Ch b and Cha/Chb were not affected by Si application. Considering the photosynthetic parameters supply these rates of Si cannot improve biomass production in sorghum under 5.2 dS m^{-1} salinity but at 10.5 dS m^{-1} this effect was positive. However, lowest dry matter production was observed at 23.1 dS m^{-1} and 1.44g.kg^{-1}soil Si.

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