Vol. 19(9), pp.602-612, September, 2020
DOI: 10.5897/AJB2019.17029
Article Number: 3A22E1C64737
ISSN: 1684-5315

http://www.academicjournals.org/AJB

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African Journal of Biotechnology

Full Length Research Paper

Evaluation of sugar beet monogerm O-type lines for salinity tolerance at vegetative stage

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Received 16 December, 2019; Accepted 10 April, 2020

Increased production of sugar beet under rainfed conditions on saline–sodic soils in the Iranian areas highlights the importance of salt tolerant varieties. Screening of genotypes for salinity tolerance is difficult in field due to heterogeneity of physical and chemical properties of soil. In order to evaluate the salinity tolerance of 21 sugar beet monogerm O-types lines, a pot experiment was conducted using a split plot design. The evaluation of plants was performed using 11 morphological and physiological traits at vegetative growth stage under severe salt stress (~16 dS m^{-1}) and control (0.3 dS m^{-1}) for 8 weeks. Salinity stress significantly reduced weight related traits. The response of genotypes for total weights and stem weights was very similar under both conditions. But, ranking of O-type lines for root weights under normal and stress condition was different. Indeed, there was high significant genotype × treat interaction for two these traits. Cluster analysis by using STI index of all traits allowed the identification of tolerant, moderate tolerant and sensitive genotypes toward salinity. The four salt-tolerant genotypes, O-type 9669, O-type 1609, O-type 463-2, and O-type 463-5 identified in this study, could be used in the development of salt-tolerant sugar beet varieties. In the second part of this study in order to assess a simple, rapid, and nondestructive method to estimate chlorophyll content, the chlorophyll meter (SPAD 502) readings were recorded and the relation was determined. Regression analysis indicated that there was a significant linear regression between chlorophyll content and chlorophyll meter and about 74% of changes in chlorophyll meter based on chlorophyll content were predicted.

Key words: Sugar beet (*beta vulgaris* L.), salt tolerant index, screening, hybrid.

INTRODUCTION

Threats to the 21st Century include depletion of water resources, environmental contamination, and excessive salinity of soil and water. It has been estimated that 20% of the world's lands and almost twice as much of the irrigated lands are affected by salinity. By 2050, the worldwide 50% of total cultivated land will be salinized (Rozema and Flowers, 2008; Jamil et al., 2011; Zhang et al., 2014). The increased production of sugar beet under

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rainfed conditions on saline–sodic soils highlights the importance of salt tolerant varieties. Fortunately, compared to other crops, sugar beet is comparatively tolerant to abiotic stress conditions, owing to *Beta vulgaris* sp. *maritima* as the wild progenitor of sugar beet, which prospered insuch harsh conditions (Ober and Rajabi, 2010). In sugar beet, the selection for improving stress tolerance in seedling stage ameliorates plant establishment in the field (McGrath et al., 2008). Thus, it is necessary for the majority of sugar beet breeding programs to focus on increasing germination and establishment in saline environments in order to maintain crop productivity. So far, in many researches, different agronomy and physiological traits have been used to evaluate salinity tolerance in crop species, but due to the complexity of the tolerance mechanism and the lack of suitable technique, limited improvement has been made (Munns and James, 2003). Most studies screening genetic sources under salt conditions have been accomplished in controlled environments with a single level of salt stress and no validation of the results under field conditions.

Sugar beet is a salt tolerant plant that shows a great potential for cultivation in salt-affected areas (Wang et al., 2017; Tahjib-UI-Arif et al., 2019) so that it has exhibited better growth status under 3mMNaCl than 0mMNaCl (Peng et al., 2014). The previous report revealed the ability of sugar beet growth at low to moderate (75–100 mM NaCl) salinity in soil culture test (Tahjib-UI-Arif et al., 2019) and to a higher degree in soil containing 85–140 mM salt (Li et al., 2007). The salt tolerance of sugar beet is a complex trait determined by many physiological and metabolic response mechanisms, including: accumulation of the Na^+ and Cl^- in old leaves and petioles, increased accumulation of compatible solutes such as betaine and free amino acids, increased activity of antioxidant enzymes and enhanced activity of photosynthesis relate enzymes under moderate salt stress (Wang et al., 2017).

The most effects of abiotic stress, such as drought and salinity, on the chlorophyll content leads to reduction in growth and photosynthesis (Dadkhah and Rassam, 2017). The measurement of the chlorophyll content is expensive, laborious and time consuming. Thus, a quick and straightway approach, as alternative, can be very effective for estimating leaf chlorophyll concentration. A Chlorophyll Meter SPAD-502 is used for measuring the absorbance of the leaf in two regions, a red 650 nm and an infrared 940 nm (Minolta, 1989). The SPAD Chlorophyll Meter Reading (SCMR) has been positively correlated with chlorophyll content in rice (Turner and Jund, 1991), wheat (Uddling et al., 2007) and sugarcane (Jangpromma et al., 2010). The growth stage, genotype and environmental conditions affects the regression equations of chlorophyll content on the chlorophyll meter (Campbell et al., 1990; Peng et al., 1993; Smeal and Zhang, 1994; Balasubramanian et al., 2000; Esfahani et al., 2008). Due to the involvement of nitrogen in chlorophyll-producing enzymes in plants (Chapman and Barreto, 1997), the researchers have also used chlorophyll reading to predict leaf nitrogen concentration (Peng et al., 1995b; Esfahani et al., 2008).

The detection of cytoplasmic-gene male-sterility (CMS) system has contributed to the practical production of hybrid seed in sugar beet. Propagation and maintenance of CMS plants is feasible with near isogenetic pollen-fertile lines that has normal cytoplasm (N) and two recessive loci ([N]xxzz) in nucleolus (Moritani et al., 2013). This system of genetic fertility restoration was first identified by Owen (1945) and Owen-type (O-type) source was known as maintainer line for CMS line. Therefore, hybrid cultivars in sugar beet are produced by male sterile lines, O-type lines and pollinator (Bosemak, 2006). Studies have been carried out on tolerance to salinity of pollinators; but, there was no information on salinity tolerance of O-type lines in sugar beet. Recently, one study on resistance against rhizoctonia crown and root rot (Rcrr) disease in these lines has been reported (Hassani et al., 2019). Thus, the objectives of the present study were: 1) the evaluation of salt tolerance in sugar beet monogerm O-type lines from Iran at vegetative growth stage based on morphological and physiological parameters in order to select tolerant and sensitive genotypes for use in breeding programs. As regards this, male sterile lines equivalent to salt tolerant O-type lines derived from this study were used in factorial design for genetic study of sugar beet salinity (Abbasi et al., 2019). 2) The determination of the best relationship between SPAD readings with Net CO_2 assimilation rate (A) and Transpiration rate (T) in sugar beet plant for prediction of chlorophyll content using SPAD.

MATERIALS AND METHODS

Plant materials

Twenty one sugar beet monogerm O-type lines provided at Sugar Beet Seed Institute (SBSI) of Iran, were assessed for salinity stress at germination and early seedling growth stages (Table 1) by eleven traits (Table 2). O-type 231 and 7233.P.29, were used as susceptible and tolerant controls, respectively, in greenhouse experiment. The population of 7233-P.29 as a broad open pollinated population, was improved after some cycles of simple recurrent selections using selected roots for salinity tolerance under saline field conditions.

Greenhouse experiment

Due to drip irrigation system, the split plot experiment with two factors of genotypes (nineteen O-type lines along with two controls) and salinity with two levels (0.3 dS m^{-1} and 16 dS m^{-1} (~175 mMNaCl)) were used. Salt water for experiment was prepared from the Agricultural Research Experiment Station located at Rodasht (65 km east of Isfahan, 328290 N and 528100 E, 1560 m asl).in a natural manner. In a previous experiment, EC= 16 dS m^{-1} was identified as critical electrical conductivity to differentiate between sugar beet genotypes (Khayamim et al., 2014). The experiment was

Table 1. Sugar beet O-types lines evaluated in greenhouse.

No.	Pedigree
1	O-type 9621
2	O-type 9669
3	O-type 445
4	O-type 9590
5	O-type 1609
6	O-type 7173
7	O-type 8090
8	O-type 7617
9	O-type 463-1
10	O-type 463-2
11	O-type 463-3
12	O-type 463-4
13	O-type 463-5
14	O-type 419
15	O-type 463-6
16	O-type 474
17	O-type 452
18	O-type 419
19	O-type 428
20	O-type 231- susceptible control
21	7233-P.29 – tolerant control

Table 2. Abbreviations and units of measurement for the measured traits of sugar beet in greenhouse.

Trait	Abbreviation	Unit of measurement
Germination percentage	GP	%
Mean daily germination	MDG	day
Mean time to germination	MTG	day
Establishment percentage	EP	%
Relative water content	RWC	-
Total fresh weight	TFW	g
Shoot fresh weight	SFW	g
Root fresh weight	RFW	g
Total dry weight	TDW	g
Shoot dry weight	SDW	g
Root dry weight	RDW	g
SPAD chlorophyll meter reading	SCMR	-
Chlorophyll content	ChlC	µmol m^{-2}
Net CO_2 assimilation rate	A	µmol CO_2 m^{-2} s^{-1}
Transpiration rate	E	mmol H_2O m^{-2} s^{-1}
Stress tolerance index	STI	-
Field emergence potential	FEP	-

conducted at Isfahan Agriculture and Natural Resources Research Center, Iran in October 2012. The electrical conductivity (EC) of the NaCl solutions was measured directly using a conductivity meter (Model 1481-50, Cole-Parmer Instrument Company, Chicago). The treatment combinations were replicated three times and arranged in a completely randomized design (CRD). Each experimental unit consisted of 24 seeds/pot planted in a circular pattern (at a depth of 1.5 cm) in plastic pots (18cm diameter and 20 cm depth) filled with perlite. Salt stress was imposed from planting time and lasted for two months. The control and saline irrigation solutions were separatelypreparedintwo100-Lreservoirscontainingahalfstrength Hoagland's solution (Table 1S) (Hoagland and Arnon, 1959), and

drip irrigation system was applied. Overflow irrigation was returned through drainage to the reservoirs. The drip irrigation was performed once a day for 30 min. Some control (not planted) pots were placed among the pots to control the EC in perlite. The experiment was conducted under day/night temperatures of 23-34°C/15-20°C, day length of 13–13.5 h and humidity range from 40 to 85%. The number of germinated seeds was recorded daily. Germination percentage (GP) was recorded 24 days after sowing. Plants were harvested after two months. Seedling establishment percentage (EP) was recorded at the end of experiment. Mean daily germination (MDG) that is 'the average number of seeds germinated per day of the actual test period' was calculated as follow (Gidner et al., 2005) (Equation 1):

$$MDG = \frac{FGP}{D}$$

where FGP is the final germination percentage and D is the number of days to the end of the test.

Mean time to germination (MTG) is the index of germination rate calculated as follow (Lein et al., 2008) (Equation 2):

$$MTG = \frac{\sum(nd)}{\sum n}$$

where*n* is the number of germinated seeds in *d*th day and $\sum n$ is the total number of germinated seeds.

Indexes

Field emergence potential (FEP) (McGrath et al., 2000) for all traits was determined as:the ratio of stress to non-stress seedling characteristics represents the salt tolerance during vegetative growth.

Stress tolerance index (STI) was calculated for seedling characteristics using the following equation as example (Fernandez, 1991) (Equation 3):

$$\text{STI (GP)} = \frac{GP_S \times GP_N}{\overline{GP}_N}$$

where GP_S and GP_N represent germination percentage under stress and non-stress conditions, respectively for each genotype and $\overline{GP}_N$ represents the mean of germination percentage in non-stress conditions for all genotypes.

Field emergence potential (FEP) (McGrath et al., 2000) for germination was determined as follow: number of germinated seeds in stress treatment/number of germinated seeds in control treatment. Similarly, this index was calculated for other traits.

Physiological measurements

Biomass

Biomass was determined from control and salt stressed plants. At harvest times, the roots and shoots of plants from each replication were separated. The fresh weight was measured for shoot (SFW), root (RFW) and total fresh weight plant (TFW). After being dried at 70°C in an oven until the samples reached a constant weight, the dry weight of roots (RDW) and shoots (SDW) per plant were measured.

Leaf relative water content (RWC)

Leaf relative water content (RWC) was determined by using the method described by Ghoulam et al. (2002) in fully expanded leaves. Leaf discs were excised from the interveinal areas of each plant. For each plot, discs were pooled and their fresh weight (FW) determined. They were floated on distilled water in Petri dishes for 4 h to regain turgidity, then thawed and re-weighed as turgid weight (TW). The leaf samples were dried at 80°C for 24 h to determine dry weight (DW). RWC was defined as follows:

RWC (%)=[(FW−DW)/(TW−DW)]×100

Percentage variation (increase/decrease) in comparison to control for each trait was calculated as below:

Percentage variation (%)=[(Control - Stress)/(Control)]×100

Photosynthetic parameters

Leaf gas exchange parameters) net CO_2 assimilation rate (A) and transpiration rate (E)) were measured using a Li-Cor 6400 gas-exchange portable photosynthesis system (Li-Cor, Lincoln, Nebraska, USA). The chlorophyll content were measured using the method mentioned in Jamil et al. (2007).

A chlorophyll meter [SPAD-502, Soil and plant analysis development (SPAD), Minolta Camera Co. Osaka, Japan] was used for chlorophyll measurement on fully expanded leaves.Three SPAD readings were taken around the midpoint of each leaf blade averaged to represent the mean SPAD readings of each plot.

Data analysis

Data were assessed by SAS software version 9.2 (SAS Inc., Cary, NC, USA) as the split plot experiment. The comparison of means was determined using LSD test among genotypes for each measurement, under stress and normal condition as separately (Steel and Torrie, 1984). In order to discriminate 21 sugar beet O-type lines for salt tolerance, cluster analysis was performed using STI of traits by Ward's method. Linear regression was used to determine the relationship between SCMR with chlorophyll content, photosynthesis and transpiration.

RESULTS AND DISCUSSION

In this study, the response of 21 sugar beet O-type lines under salinity and normal conditions were assessed by eleven morphological and physiological traits and two index (Table 2).

Morpho-physiological response under stress and normal conditions

The variance analysis revealed significant ($P \leq 0.01$) effects of genotype, treatment and their interaction for germination and establishment percentage, relative water content and weight related traits (data not shown). Salinity showed the negative effect by reducing the value of all traits except MGT and MTG. The percentage variation (decrease/increase) of traits under salinity

Table 3. Mean comparison, mean, percentage decrease and relation between STI and EFP indices for eleven different traits of sugar beet investigated at seedling growth stage.

No.	Genotype	Germination percentage (GP)		Mean daily germination (MGT)		Mean time to germination (MTG)		Establishment percentage (EP)		Relative water content (RWC)		Total fresh weight (TFW)	
		Normal	Saline	Normal	Saline	Normal	Saline	Normal	Saline	Normal	Saline	Normal	Saline
1	Otype 9621	75.00	80.55	5.36	6.71	7.65	8.78	75.00	61.11	90.77	83.81	32.41	10.95
2	Otype 9669	95.83	80.56	6.85	6.71	7.72	8.22	93.75	73.61	88.67	85.12	33.60	10.98
3	Otype 445	81.25	80.55	5.80	6.71	8.01	8.15	81.25	52.78	89.89	86.75	28.93	6.69
4	Otype 9590	70.83	83.33	5.06	6.94	6.58	7.85	77.83	76.39	91.28	85.67	34.17	12.31
5	Otype 1609	91.67	90.28	6.55	7.52	7.40	8.49	91.67	63.89	89.22	86.46	32.34	10.24
6	Otype 7173	83.33	76.39	5.95	6.36	8.18	8.49	81.25	61.11	88.01	86.48	32.45	8.72
7	Otype 8090	77.08	72.22	5.51	6.02	7.78	8.75	77.08	61.11	90.47	86.08	31.07	7.64
8	Otype 7617	72.92	62.50	5.21	5.21	6.87	8.71	72.92	47.22	90.39	85.55	34.09	11.24
9	Otype 463-1	91.67	65.28	6.55	5.44	7.39	7.92	91.67	50.00	87.94	86.27	38.38	7.27
10	Otype 463-2	85.42	88.89	6.10	7.41	7.09	8.79	85.42	75.00	89.49	87.04	29.02	10.48
11	Otype 463-3	97.92	79.17	6.99	6.60	7.58	8.68	97.92	51.39	86.47	86.60	20.08	6.52
12	Otype 463-4	77.08	84.72	5.51	7.06	6.47	8.59	77.08	65.28	90.46	89.93	23.11	8.10
13	Otype 463-5	91.67	84.72	6.55	7.06	7.59	8.26	91.67	61.11	90.74	86.42	30.36	10.51
14	Otype 419	81.25	87.50	5.80	7.29	7.02	8.22	81.25	52.78	89.56	87.04	32.33	7.40
15	Otype 463-6	93.75	73.61	6.70	6.13	7.56	8.13	93.75	52.78	91.50	86.66	35.35	7.73
16	Otype 474	89.58	84.72	6.40	7.06	7.62	8.60	89.58	48.61	89.83	86.05	30.13	6.19
17	Otype 452	81.25	81.94	5.80	6.83	6.85	8.56	79.17	51.39	90.24	85.37	26.27	5.57
18	Otype 419 bulk	72.92	75.00	5.21	6.25	7.11	9.15	72.92	55.55	89.20	86.82	31.52	8.34
19	Otype 428	89.58	80.56	6.40	6.71	7.52	8.49	89.58	54.17	91.40	85.35	33.22	7.41
20	Otype 231	66.67	56.94	4.76	4.74	7.38	7.01	60.42	27.78	88.64	86.90	22.21	3.90
21	7233-P.29	93.75	98.61	6.70	8.22	6.43	8.07	93.75	90.28	89.40	86.72	33.75	18.88
LSD (5%)		15.296	15.377	1.0936	1.2811	1.7811	2.2126	15.038	18.593	1.5052	2.919	1.1211	0.6019
Mean		83.83	79.43	5.98	6.61	8.22	8.34	83.23	58.73	89.69	86.34	30.7	8.91
% decrease		5.25		10.54		1.95		29.44*		3.74		70.99**	
R^2 (STI, EFP) (%)		25.2		21.7		22.34		58.4*		31.2		53.8*	

No.	Genotype	Shoot fresh weight (SFW)		Root fresh weight (RFW)		Total dry weight (TDW)		Shoot dry weight (SDW)		Root dry weight (RDW)		
		Normal	Saline	Normal	Saline	Normal	Saline	Normal	Saline	Normal	Saline	
1	Otype 9621	28.45	9.42	3.96	1.53	3.74	1.99	2.63	1.53	1.11	0.45	
2	Otype 9669	30.83	9.36	2.77	1.62	4.13	1.82	3.43	1.39	0.70	0.43	
3	Otype 445	25.62	5.94	3.31	0.75	3.49	0.97	2.60	0.79	0.89	0.18	
4	Otype 9590	30.00	10.79	4.17	1.51	3.85	1.92	2.69	1.55	1.17	0.37	

Table 3. Contd.

5	Otype 1609	29.74	9.10	2.60	1.14	3.96	1.51	3.22	1.24	0.74	0.27
6	Otype 7173	28.28	7.55	4.17	1.17	5.22	1.28	3.40	1.02	1.82	0.26
7	Otype 8090	27.86	6.64	3.21	1.00	3.64	1.21	2.66	0.92	0.99	0.28
8	Otype 7617	31.55	9.65	2.54	1.59	3.62	1.79	3.03	1.38	0.59	0.41
9	Otype 463-1	35.86	6.40	2.52	0.87	5.04	1.10	4.33	0.87	0.71	0.23
10	Otype 463-2	26.96	8.99	2.06	1.49	3.42	1.51	2.85	1.16	0.58	0.35
11	Otype 463-3	17.30	5.90	2.78	0.62	3.62	0.93	2.34	0.79	1.28	0.14
12	Otype 463-4	20.57	7.41	2.54	0.68	2.64	0.97	1.89	0.79	0.75	0.18
13	Otype 463-5	27.21	8.94	3.15	1.56	3.42	1.53	2.52	1.23	0.90	0.30
14	Otype 419	29.81	6.56	2.52	0.84	3.80	1.04	3.09	0.87	0.71	0.17
15	Otype 463-6	33.09	6.73	2.27	1.00	3.38	1.14	2.80	0.90	0.59	0.23
16	Otype 474	26.36	5.23	3.77	0.95	3.59	0.97	2.69	0.74	0.90	0.23
17	Otype 452	24.42	4.82	1.85	0.75	2.94	0.90	2.42	0.70	0.52	0.19
18	Otype 419 bulk	26.81	7.15	4.71	1.19	4.22	1.20	2.86	0.94	1.37	0.26
19	Otype 428	31.39	6.49	1.83	0.92	3.21	1.15	2.70	0.95	0.51	0.20
20	Otype 231	20.04	3.51	2.17	0.40	2.83	0.54	2.27	0.45	0.56	0.10
21	7233-P.29	30.23	16.34	3.52	2.54	4.85	2.42	3.20	1.91	1.65	0.52
LSD (5%)		1.1211	0.6019	1.1211	0.6019	1.1211	0.6019	0.7052	0.4723	1.7310	0.5711
Mean		27.73	7.76	2.97	1.15	3.74	1.33	2.84	1.05	0.90	0.27
% decrease		72.02**		61.34**		64.44**		63.03**		69.65**	
R^2 (STI, EFP) (%)		52.88*		50.9*		53.3*		53.12*		51.8*	

stress ranged from the increase of 10.54% for mean daily germination (MGT) to the decrease of 72.02% for shoot fresh weight (SFW) (Table 3). Indeed, the most percentage decreases were owned to weight related traits with the difference between genotypes. The genotypes showing the highest percentage decrease are considered as the most sensitive to salt stress.

According to LSD test, significant differences were detected between the analyzed genotype that shows the effect of salinity varied among genotypes (Table 3). For instance, germination percentage ranged from 66.67% “Otype 231“genotype to 97.92 “Otype 463-3“ genotype under normal condition and ranged from 56.94% “Otype 231“genotype to 98.61 “7233-P.29“ genotype under stress condition. The establishment percentage for normal conditions was approximately equal to the germination percentage under the same conditions, but the establishment percentage under salinity stress ranged between 27.78 and 90.28 with 58.73 mean. Seed establishment appears to be more important than seed germination, meaning that the salt tolerant genotypes are those that have survival potential after germination. The present data shows that genotypes#5 and 10 were good for two traits and genotypes#8 and 9 were bad for two traits, but genotype#14 with high germination was not able to overcome salt stress and survive. On the contrary, genotypes#2 and 4 with germination percent about 80% show high survival. These results corroborate those obtained by Chikha et al. (2016).

For weight related traits, significant differences were observed between genotypes (Table 3). The genotypes showed almost the same ranking for TFW and SFW and also for TDW and SDW under both conditions. This result showed that salt

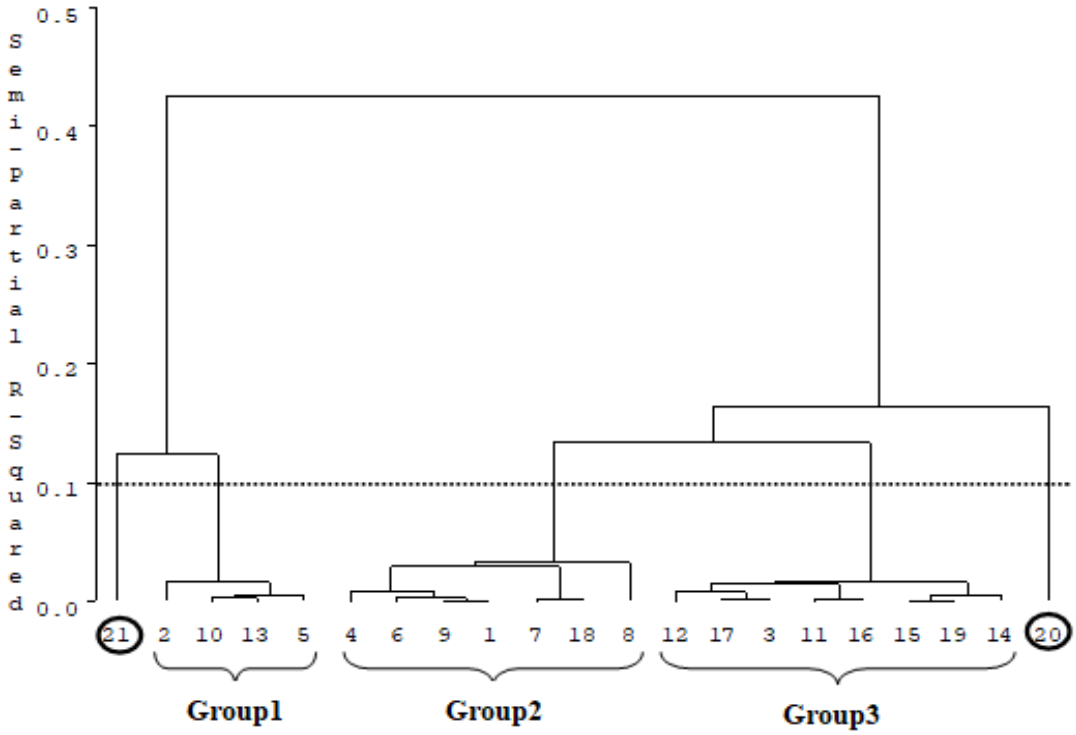

Figure 1. Cluster analysis of 21 sugar beet O-types lines investigated at seedling growth stage using STI of traits by Ward's method.

stress causes more damage to plant aerial part than plant roots that was confirmed by previous studies (Eschie et al., 2002; Wang et al., 2017). Ranking of O-type lines for SFW and SDW under normal and stress condition was different. Indeed, there was high significant genotype×treat interaction for two these traits. Weight loss under stress is a surefire occurrence in all plants. Under salt stress, the phenomenon of necrotic appeared in plant leaves, but only salt tolerant genotypes were able to maintain their biomass and photosynthesis and hence able to overcome salt stress.

These results indicated the existence of genetic potential for salt tolerance among this sugar beet O-type lines that could maintain a good growth status in plant aerial part under salt stress and also show that stress intensity (16 dS/m) used in our study, was appropriate which was able to differentiate between susceptible and tolerant controls, and to differentiate genotypes for different traits. This goes in pair with many other studies (Khayamim et al., 2014; Chikha et al., 2016; Abbasi et al., 2018), which illustrate that severe saline stress, could be used as a rapid method to identify visible phenotypic differences among salt tolerant and sensitive genotypes.

This study documented that the vegetative stage as a very important stage in sugar beet (McGrath et al., 2000) was well able to evaluate genotypes response towards salinity. Several findings in sugar beet indicated that screening at vegetative stage in controlled conditions was accompanied with improving field emergence of sugar beet (Durrant and Gummerson, 1990; McGrath et al., 2000; De los Reyes and McGrath, 2003; McGrath et al., 2008).

Cluster analysis based on the STI values for salt tolerance

The ward's cluster analysis (Figure1) showed that the most sensitive (#20) and resistant (#21) controls were completely separated, indicating that the experiment was performed carefully. According to the dendrogram (Figure 1) and based on the STI of traits, the studied genotypes exhibited different responses toward salt treatment and three distinct groups were identified. The first group with four genotypes #2, 5, 10 and 13 was defined as salt-tolerant genotypes due to high STI value for the traits related to germination and establishment under stress and normal conditions. The second group consisting of seven genotypes were dedicated to moderately tolerant to salinity and the remaining eight O-type lines with low amount of weight related traits, were classified as sensitive to salinity. In many researches, Ward's clustering technique based on STI values was able to distinguish genotypes with contrasting demeanor toward salinity (tolerant/sensitive) (Win et al., 2011; Mini et al., 2015; Kim et al., 2016; Sakina et al., 2016; Abbasi et al.,2018).

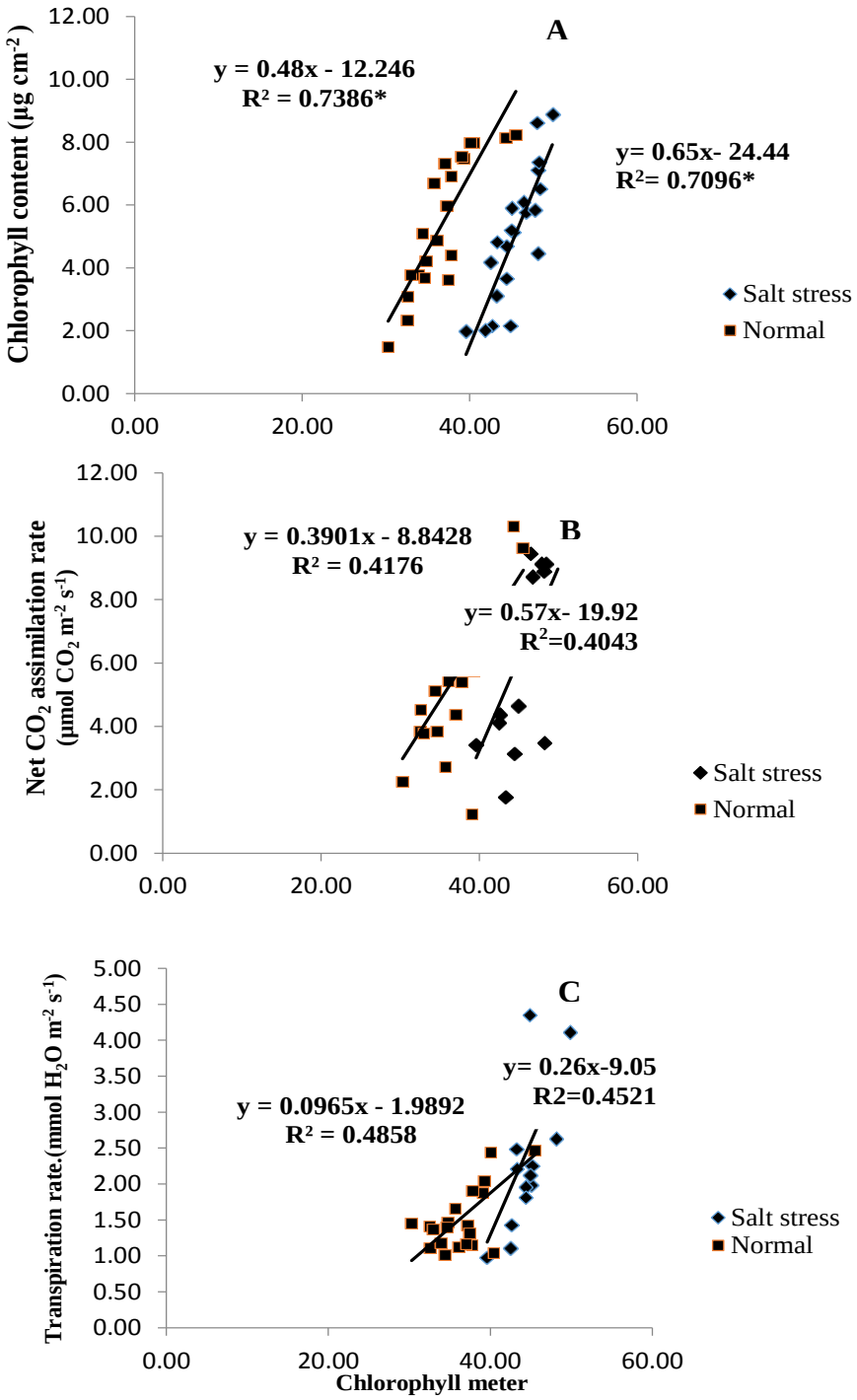

Figure 2. Relationship between (A) total chlorophyll content (µg cm^{-2}), (B) Net CO_2 assimilation rate (µmol CO_2 m^{-2} s^{-1}) and (C) Transpiration rate) mmol H_2O m^{-2} s^{-1} (with chlorophyll meter reading at final establishment of sugar beet (n=21) under salt stress and normal condition. * Significant at p≤0.05.

Relation between SCMR with chlorophyll content, photosynthesis and transpiration

Relationships between total chlorophyll content, net CO_2 assimilation rate and transpiration rate with chlorophyll meter reading (SCMR) at final establishment of sugar beet were shown in Figure 2 (A, B, C). Regression analysis indicated that there was a significant linear regression between chlorophyll content and SCMR and about 74% of changes in SCMR based on chlorophyll

content were predicted (Figure 2A). These results showed that chlorophyll content affected the chlorophyll meter readings; in fact, the accuracy of chlorophyll content prediction is related to SCMR.

Regression analysis showed that there was no significant correlation between net CO_2 assimilation rate (A) and SPAD readings and only about 42% of variation in A was explained by chlorophyll meter reading(Figure 2B). Relationship between transpiration rate (E) and SPAD readings was poor and non-significant (R^2 = 48%) and showed only about half of the changes in E was justified by SCMR (Figure 2C). So, the SPAD chlorophyll meter reading as a simple, low-cost, fast and non-destructive method for prediction of chlorophyll content in salinity research could be used. Since, this relationship is influenced by on growth stage, genotype and environmental conditions, an individual calibration for different cultivars grown under specific growth conditions can increase the accurate prediction. In a study, the relationship between SPAD readings and nitrogen concentration for different rice cultivars increased by an individual calibration (Peng et al., 1995b). Esfahani et al. (2008) presented that adjusting the SPAD readings for specific leaf weight (SLW) improved the estimation of N concentration from 23 up to 88%.

Conclusion

The STI index used in this research could classify sugar beet O-type lines into different categories of sensitive, moderately tolerant and tolerant to salinity; so that the four salt-tolerant genotypes #2, 5, 10 and 13 obtained, were well incorporated in the breeding program after evaluation in the field (in another study). The association between total chlorophyll content with chlorophyll meter reading showed that chlorophyll content of sugar beet leaves can be achieved without cost and time, only by using the chlorophyll meter reading. For different plant species and different growth conditions, the process of testing and calibration may be required.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Table 1. Supplement. Compounds and amount of ingredients used in Hoagland nutrient solution

No	Name	Amount in stock solution (g/lit)	Amount in 100 liters (ml)
			Solution A
1	H_3BO_3	2.8	100
2	$ZnSO_4$	0.22	
3	$MnSO_4$	4.3	
4	$CuSO_4$	0.1	
5	$(NH_4)_6Mo_7O_{24}$	0.01	
			Solution B
6	H2SO4	5 CC	
			Solution C
7	Na2-EDTA	6.72	
8	Fe- SO4	5.58	
			Solution D
	$NH_4H_2PO_4$	1.2	100
	KNO_3	6.6	
	$Ca(No_3)_2$	9.4	
	$MgSO_4$	5.2	