Measuring chlorophyll content in corn leaves at soil salinity conditions by using spectrophotometer and its correlation with plant yield.

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Abstract: Salinity is one of the environmental limiting factors in agricultural product producing. So the investigation of the plants and finding some method to resist the plants against salinity stress is very important. Considering Iran and Azerbaijan as origin countries in Astara region, and in order to study the effects of salt stress (NACL) on, leaf relative water content (LRWC), Chlorophyll a, Chlorophyll b Content and yield of 8 maize cultivars were experimented in three replications on the basis of randomized complete block design in three years (2007-2009). Results from the experiment showed that, between locations (normal and saline) in all traits, significant differences were seen. Between varieties in all traits, significant differences were seen. The interaction between years and varieties, years and varieties and locations for all traits was not significant. Comparison traits in different salinities showed that in most traits, there are significant differences between genotypes. The highest amount of chlorophyll a, in normal condition was observed in S.C704 with 1.873 mg/g fresh weight of leaves, which was no significant difference with B73. Maximum LRWC in B73 was measured in normal conditions which were no significant difference with K3653.2, S.C704 and Waxy at 5% level. Between chlorophyll a and chlorophyll b, total chlorophyll and Ratio of Chlorophyll a/b significant positive correlations were observed in non-stress condition.

[Davar Molazem, Jafar Azimi, Marefat Ghasemi, Mohsen Hanifi and Ali Khatami. **Measuring chlorophyll content in corn leaves at soil salinity conditions by using spectrophotometer and its correlation with plant yield.** *Life Sci J* 2012;9(4):5615-5619] (ISSN:1097-8135). http://www.lifesciencesite.com. 836

Key words: Salinity, Maize, spectrophotometer

INTRODUCTION

Salinity is one of the major environmental threats for agriculture and affects approximately 7% of the world's total land area (Ben-Salah et al, 2011) nearly 40% of the world land surface can be categorized as suffering from potential salinity problem(Payakapong et al,2006). After wheat and rice, maize (Zea mays L.) is the third most important cereal crop grown all over the world in a wide range of climatic condition. Maize, being highly cross pollinated, has become highly polymorphic through the course of natural and domesticated evolution and thus contains enormous variability (Paternian, 1990) in which salinity tolerance may exist. Maize is considered as moderately salt sensitive (Mass and Hofffman, 1977; Katerji et al., 1994; Ouda et al., 2008; Carpici et al., 2009).

Maize (Zea mays L.) is considered as one of the most important cereal crops used in human consumption, animal feeding and starch industry and oil production (Amin et al, 2007). According to Mass and Hoffman (Mass and Hoffman, 1977), maize is generally regarded as a highly salt sensitive species. The world population is expanding rapidly and is expected to be around 8 billion by the year 2025 (Andersen et al., 1999). This represents an addition of nearly 80 million people to the present population every year. It is forecast that the increase in world population will occur almost exclusively in developing countries, where serious nutritional problems exist at present, and population pressure on agricultural soils is already very high. Many arid and semi-arid regions in the world contain soils and water resources that are too saline for most of the common economic crops, which affect plants through osmotic effects, ion specific effects and oxidative stress (Munns, 2002; Pitman and Lauchli, 2002).

MATERIALS AND METHODS

Considering Iran and Azerbaijan as origin countries in Astara region, and in order to study the effects of salt stress (NACL) on, leaf relative water content (LRWC), Chlorophyll a, Chlorophyll b Content and yield of 8 maize cultivars were experimented in three replications on the basis of randomized complete block design in three years (2007-2009). Cultivars included K3615/1, S.C704, B73, S.C302, Waxy, K3546/6, K3653/2, and Zaqatala and they were cultivated in two pieces of land in Astara: one with normal soil and the other with salty soil. During the experiment, before dealing amount of leaf relative water content (LRWC), chlorophyll a and Chlorophyll b Content were measured in the laboratory. Photosynthetic pigments (chlorophyll a and b) were measured in fresh leaf samples, a week before the

harvest. One plant per replicate was used for chlorophyll determination. Prior to extraction, fresh leaf samples were cleaned with deionized water to remove any surface contamination. Leaf samples (0.5 g) were homogenized with acetone (80% v/v), filtered and make up to a final volume of 5 mL. Then the solution for 10 minutes away in 3000 (rpm) centrifuged. Pigment concentrations were calculated from the absorbance of extract at 663 and 645 nm using the formula given below :

Chlorophyll a (mg/g FW)=[12.7× (A663) _ 2.69× (A645)]×0.5

Chlorophyll b (mg/g FW)=[22.9× (A645) _ 4.69× (A663)]×0.5

Chlorophyll a+b (mg/g FW)= [20.2× (A645) _ 8.02× (A663)]×0.5

leaf relative water content (LRWC) was calculated on the basis of Yamasaki & Dillenburg method (1999). Two leafs were randomly chosen from middle parts of the plants in each repetition. At first, leafs were separated from the stems and their fresh masses (FM) were calculated. In order to measure the saturation mass (TM), they were placed into the distilled water in closed containers for 24 hours under the air condition of 22° C, for the purpose of being reached to their greatest amount of saturation mass and then, they were weighed. Then leafs were placed inside the electrical oven for 48 hours under the air condition of 80° C and the dry mass of the leafs (DM) were obtained (DM). All of the measurements were done by scales with 0.001g accuracy and were placed into the following formula and into the following formula:

LRWC (%)= [(FM-DM)(TM –DM)] ×100

Statistical analysis of the data was done on the basis of randomized complete block design. The average of attendances was calculated on the basis of Duncan method at 5% probability level.

RESULT AND DISCUSSION

Analysis of Variance

Results from the experiment showed that, regarding the most of the characteristics, there were significant differences among cultivars and that, compared to normal conditions; saltiness had caused reduction in their values. Results from the analysis of variance showed that there were no significant differences between different years (Table 1 and 2). Between locations (normal and saline) in all traits, significant differences were seen. Between varieties in all traits, significant differences were seen. The interaction between years and varieties, years and varieties and locations for all traits was not significant. The interaction between varieties and locations for all traits showed significant differences at 1% level.

Comparison of mean

Comparison traits in different salinities showed that in most traits, there are significant differences between genotypes. The highest amount of chlorophyll a, in normal condition was observed in S.C704 with 1.873 mg/g fresh weight of leaves, which was no significant difference with B73. Lowest chlorophyll a, in condition of salt, was measured in Waxy. Maximum chlorophyll b in B73 was measured in normal conditions which was significant difference with all varieties at 5% level. Lowest chlorophyll b, in condition of salt, was measured in Waxy. Similar results were also reported by Iqbal *et al.,* (2006), Ashraf *et al.,* (2005), Khan *et al.,* (2009), Oncel and Keles (2002) and Almodares *et al.,* (2008). The highest amount of ratio of chlorophyll, in normal condition was observed in K3545/6, which was no significant difference with Zaqatala, S.C302, S.C704 and Waxy. Lowest chlorophyll b, in condition of salt, was measured in Waxy. Tuna et al (2008) in The study of gibberellic acid and salinity on plant growth parameters and antioxidants of maize showed that With increasing salt concentration, significant reduction in dry weight, chlorophyll content and leaf relative water content was observed. Maximum LRWC in B73 was measured in normal condition which was no significant difference with K3653.2, S.C704 and Waxy at 5% level. Lowest LRWC, in condition of salt, was measured in S.C302. The highest yield per plant, in S.C704 obtained in normal conditions, that with all the varieties in normal and saline conditions was significant differences.

Simple correlation for normal condition was calculated (Tables 3). Between chlorophyll a and chlorophyll b, total chlorophyll and Ratio of Chlorophyll a/b significant positive correlations were observed in non-stress condition. Between chlorophyll b and total chlorophyll were positively correlated. But Between chlorophyll b and Ratio of Chlorophyll a/b A Negative correlation was observed in 1% level.

Table 1 - Analysis of variance for maize varieties

Source	DF	Mean Square						
		Chlorophyll a	Chlorophyll b	Total Chlorophyll	Ratio of Chlorophyll a/b	LRWC	Yield Per plant	Grain yield
Year	2	0.0001ns	0.0001ns	0.008ns	0.001ns	0.001ns	0.0001ns	0.0001ns
Location	1	1.025**	1.449**	26.643**	0.024ns	17.851ns	6.76**	16889.972**
YL	2	0.001ns	0.001ns	0.018ns	0.001ns	0.0001ns	0.0001ns	0.0002ns
R(LY)	12	0.061	0.148	1.589	0.093	280.255	0.043	108.598
Variety	7	0.101**	0.222**	3.295**	0.087**	76.171**	0.161**	403.136**
YA	14	0.000ns	0.0001ns	0.003ns	0.001ns	0.0001ns	0.0002ns	0.0001ns
LA	7	0.074 **	0.136**	2.005**	0.083**	41.653**	0.104**	259.220**
YLA	14	0.000ns	0.000ns	0.002ns	0.001ns	0.0002ns	0.0001ns	0.0003ns
Error	84	0.023	0.031	0.396	0.024	24.681	0.029	72.088
	CV %	14.53%	15.49%	25.58%	17.74%	8.10%	16.03%	16.03%

ns. Non-significant, * significant at 5% **, significant at 1%

Table 2- Comparing the average of understudy characteristics in eight cultivars of the maize in combined analysis

condition	cultivars	Chlorophyll a mg/g FW	Chlorophyll b mg/g FW	Total Chlorophyll mg/g FW	Ratio of Chlorophyll a/b	LRWC (%)	Yield Per plant (kg/ (plot	Grain yield (Kg/ha)
Normal	1-Zaqatala	1.107 d	1.091 def	2.196 ef	1.003 ab	61.02 bc	1.796 bc	4489.167 bc
	2-S.C302	1.474 bc	1.519 cd	2.996 cd	0.9644 abc	57.88 c	1.479 bcde	3698.333 bcde
	3-K3653.2	1.192 cd	1.996 b	3.188 bc	0.6767 e	63.78 ab	1.372 de	3429.167 de
	4-B73	1.616 ab	2.492 a	4.108 a	0.7667 de	67.15 a	1.433 cde	3583.333 cde
	5-S.C704	1.837 a	1.840 bc	3.677 ab	1.003 ab	62.57 abc	2.347 a	5866.667 a
	6-Waxy	1.114 cd	1.279 def	2.393 de	0.9089 abcd	61.89 abc	1.228 ef	3069.167 ef
	7-K3615.1	1.024 de	1.494 cde	2.519 de	0.7667 de	61.07 bc	1.908 ab	4770.833 ab
	8-K3545.6	1.038 de	1.016 ef	2.056 ef	1.032 a	58.43 bc	1.736 bcd	4339.167 bcd
salty	1-Zaqatala	0.9267 de	1.098 def	2.024 ef	0.8678 abcd	61.47 bc	0.8650 gh	2162.500 gh
	2-S.C302	0.8956 de	1.036 def	1.931 ef	0.8611 abcd	57.27 c	0.9583 fg	2395.833 fg
	3-K3653.2	1.030 de	1.193 def	2.226 ef	0.8822 abcd	61.70 bc	0.6133 hi	1533.333 hi
	4-B73	0.9778 de	1.142 def	2.118 ef	0.8778 abcd	61.68 bc	0.6950 ghi	1737.500 ghi
	5-S.C704	0.9989 de	1.174 def	2.171 ef	0.8567 bcd	62.36 abc	1.042 fg	2604.167 fg
	6-Waxy	0.7378 e	0.8556 f	1.588 f	0.8856 abcd	61.60 bc	0.7367 ghi	1841.667 ghi
	7-K3615.1	1.016 de	1.278 def	2.293 e	0.8122 cde	58.44 bc	0.4967 i	1241.667 i
	8-K3545.6	0.8867 de	1.012 ef	1.898 ef	0.8711 abcd	63.62 ab	0.5250 i	1312.500 i

Table 3 – Simple Correlation between traits in normal conditions

traits	Chlorophyll b	Total Chlorophyll	Ratio of Chlorophyll a/b	LRWC	Yield Per plant	Grain yield
Chlorophyll a	.344**	.712**	.325**	.171	.146	.146
Chlorophyll b	1	.904**	-.732**	.231	-.215	-.215
Total Chlorophyll		1	-.40**	.250*	-.094	-.094
Ratio of Chlorophyll a/b			1	-.045	.252*	.252*
LRWC				1	-.115	-.115
Yield Per plant					1	1.000**

**. Correlation is significant at the 0.01 level.

*. Correlation is significant at the 0.05 level.

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