# The effect of salinity on chlorophyll levels in 20 sensitive and tolerant genotypes of bread wheat

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#### Abstract

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In order to investigate the concentration of chlorophyll in 20 salinity susceptible and tolerant genotypes of wheat in 2019 an experiment was repeated three in two places as a randomized complete block design. The first place of the farm of the research center was located in khoy (EC of soil and water, 3.5 and 4.5 ds / m) respectively and the second place of the research farm of Urmia was located in the city of Urmia (EC water and soil, 10.2 ds/m and 11.9 ds/m) respectively. The treatments consisted of 12 genotypes, roshan, Sorkh tokhm, Karchya, Mahuti, Gaspard, moghan 3, Arg, Arta, shole, Sistan, Shotordndan, Bolani and 8 lines omidbakhsh KRL-4, Mv-17, N-83-3, 107 -PR-87, S-78-11, 139-PR-87, SNH-9 and 140-PR-87. The results of data combined the analysis of variance for all different traits were significant. The comparison of the mean for different traits showed that the highest concentration of chlorophyll a in Arg cultivar and chlorophyll b in Gaspard cultivar and the lowest concentration of chlorophyll a and b were related to MV-17. The highest a / b ratio was 139-PR-87 and the lowest ratio was Gaspard and Moghan-3. As a result, the a / b ratio due to salinity in susceptible genotypes is less than resistant. The results obtained from the application of salinity stress showed that there is a very significant positive correlation between chlorophyll a and b. The chlorophyll a / b ratio was negatively correlated with chlorophyll a but was not significant, while the correlation of this ratio with b was very significant and the result of increasing this ratio due to salinity was due to a significant decrease in chlorophyll b. Chlorophyll meter had no significant correlation with any of the chlorophyll concentrations. In the comparison of the percentage of changes in genotypes, it was observed that the tolerant cultivar Arg had the highest percentage of changes in the amount of chlorophyll a, b and total chlorophyll., The result is that due to salinity, the concentration of chlorophyll increased and this increase was more in tolerant cultivars than in sensitive cultivars.

Keywords: salinity; chlorophyll; wheat

### Introduction

Plant growth is the result of coordinated and regular physiological processes. Physiological processes are influenced by various factors and are decisive in the plant response to stress. Photosynthesis is one of the most important physiological processes in plants during which air carbon enters plant metabolism. The product obtained from photosynthesis constitutes about 90% of the dry matter of the plant, so with the least change in the amount of photosynthesis, many changes occur in the yield of crops. The intensity of photosynthesis can be affected by various environmental factors. Salinity is one of the factors that affect photosynthesis and its side processes. This effect varies according to the type of crop and its cultivar and climatic conditions. In weak salinities, the reduction of photosynthesis is due to the closure of pores and in strong salinities it is due to destructive and biochemical reactions, Hasan et al., 2015). In studies so far, reduced vegetative growth hasa definite effect of salinity on non-saline plants such as wheat.

Undoubtedly, this effect includes a decrease in leaf area, which has been considered by some researchers as the main cause of reduced photosynthesis, and thus they recognize the reduction of photosynthesis as a secondary effect to reduced growth, but according to some other authors, Photosynthetic efficiency is also a factor that can be independently affected by salinity. This theory can be deduced from the results of a number of researchers, (Azizpour et al., 2010; Gerona et al., 2019; & Ibrahimova et al., 2021). Although there are reports of photosynthesis being prevented under salt (salinity) stress (Chaudhuri & Choudhuri ,1997; Ali-Dinar, 1999), there are also reports that photosynthesis is reduced under salinity stress. Not found and increased even at low salt concentrations (Al-Salihy et al., 2018). Qaseem et al, (2019) reported that under salinity stress, leaf pigments decreased in 9 rice genotypes but high levels of pigment were observed in six genotypes. According to the research of many researchers, salinity reduces the amount of chlorophyll in plants, (Kiani-Pouya & Rasouli, 2014; Aycan et al., 2021) . Decreased chlorophyll content in salinity conditions has been reported due to higher chlorophyll activity, (Saqib et al., 2012). Also, some growth regulators such as ABA, ethylene and heteroxin stimulate the activity of this enzyme, (Cuin et al., 2010). Decreased chlorophyll content may also be due to changes in nitrogen metabolism in relation to the production of compounds such as proline, which is used in osmotic regulation (Shannon, 1997). According to (Munns et al., 2006), salinity stress in wheat growth medium reduces the amounts of chlorophyll a, b, total and carotenoids, and this decrease is associated with greater activity of chlorophylase enzyme under salinity stress (Singh et al., 2020). Kumar et al., (2017) observed that salinity reduced wheat chlorophyll content of Karchia cultivar by 16.8%. However (Bai et al., 2011) reported, that the amount of chlorophyll in Crownaceae plant increases under saline conditions., Hasan et al., (2015) by examining susceptible and resistant varieties to wheat showed that the chlorophyll a / b ratio in susceptible varieties due to salinity stress is more than resistant varieties, Aycan et al. (2021) studied the effect of salinity on rice and observed that the content of chlorophyll and carotenoids in leaves did not decrease significantly due to water salinity and the chlorophyll a / b ratio increased significantly due to salinity. This indicates that b is more sensitive than a by studying the effect of salinity on chlorophyll a and carotenoids in beans, (Turki et al, 2012; Zahra et al.,;2020 & Saddig et al., 2020) reported that with the increase in salinity, its amount increases in beans. In their research, Dhyani et al., (2013) concluded that under salinity conditions, the balance of photosynthetic pigments is disturbed and pointed out that in very sensitive plants, chlorophylls are damaged by salinity, while in tolerant plants, the amount of chlorophyll increases and this increase is probably due to the accumulation of chlorophyll a or b, In sensitive plants the decrease in chlorophyll content is mainly due to the degradation of chlorophyll a, which is more degradable, Płażek et al., (2013). Zeeshan et al., (2020) reported a decrease in chlorophyll content in tomatoes with increasing salinity and stated that the concentration of a in this plant increases with increasing salinity. Naz et al., (2019) also reported an increase in chlorophyll and carotenoid concentrations due to salinity in beans. However, (Munir et al., 2018; Al-Ashkar et al.,2020; Khayatnezhad & Gholamin, 2021) stated that salinity stress had no effect on chlorophyll.

The aim of this experiment was to investigate the concentration of chlorophyll in 20 susceptible and tolerant genotypes of wheat to salinity.

#### Materials and methods

This experiment was performed in the fall of 2019 as a randomized complete block design with 3 replications in two locations. The first place of the farm of Agricultural Research Center located in Khoy city (EC water and soil 3.5 and 4.5 ds / m respectively) and the second place of Urmia research farm located in Urmia city (EC water and soil respectively 2.2ds / m and was 11.9 ds / m). Due to the fact that the two places have the same climate, so the difference in the reaction of plants can only be attributed to the difference in salinity. Experimental treatments include 12 genotypes Roshan, Sorkh tokhm, Karchya, Mahuti, Gaspard, Moghan 3, Arg, Arta, shole, Sistan, Shotordndan, Bolani and 8 lines omidbakhsh KRL-4, Mv-17, N-83-3, 107 -PR-87, S-78-11, 139-PR-87, SNH-9 and 140-PR-87. Chlorophyll content was determined in two ways. The first method, which was a non-destructive method, was the use of a chlorophyll-meter SPAD 502 )from Konica Minolta, Japan), which calculated the relative chlorophyll content, and the second method was a destructive method, which was based on the Arnon method, chlorophyll a, b and total using a cary300 spectrophotometer. Was measured. In the second method, 3 flag leaves from each plot were sampled in the field and wrapped in aluminum foil and frozen in liquid nitrogen. In Karaj Agricultural Biotechnology Research Institute, frozen samples were dried with a freezer and the chlorophyll content was measured by Arnon method with a spectrophotometer with wavelengths of 663, 652 and 645.

#### Statistical analysis

The experiment was performed as a randomized complete block design with 3 replications in two locations. The experiment was performed as a randomized complete block design with 3 replications in two locations. The data of the analysis were combined. The data were analyzed using SAS software, and LSD test was used to compare the mean.

#### **Results and discussion**

Due to the salinity stress, a very significant increase was observed in chlorophyll a and b and total chlorophyll a and b, and a very significant decrease was observed in chlorophyll a / b ratio (Table 1). Also, wheat genotypes had a significant difference in terms of the above traits and also the interaction of genotype in the environment was very significant. The comparison of the mean of interactions (Table 2) showed that the highest concentration of chlorophyll a in Arg cultivar and chlorophyll b in Gaspard cultivar and the lowest concentration of chlorophyll a and b were related to MV-17. The highest concentration of total chlorophyll a and b was observed in Arg cultivar and the lowest concentration was observed in MV-17. The highest a / b ratio was 139-PR-87 and the lowest ratio was Gaspard and Moghan-3. As a result, the a / b ratio due to the salinity in susceptible genotypes is less than resistant. In fact, chlorophyll a appears to be more vulnerable in susceptible cultivars. The comparison of the mean interaction of cultivar in salinity showed that with the increase in the salinity level, the leaf chlorophyll content of cultivars increased but the amplitude of increase was different between cultivars. Due to the salinity stress, a very significant increase in relative chlorophyll content was observed (Table 1). Also, the genotypes of wheat were significantly different in terms of relative chlorophyll content. The interaction was also very significant. The highest relative chlorophyll concentration was observed in Mahouti cultivar which was not significantly different from ostrich tooth cultivar and the lowest concentration was related to Karchia cultivar. The results obtained from this experiment were consistent with the results of other researchers.

Mansour et al. (2020) in investigating the effects of salinity stress on chlorophyll a and b in 30 bread wheat cultivars reported that the salinity in all cultivars reduced the grain yield and increased the concentration of chlorophyll a and b and total chlorophyll content and according to the test results, the increase in chlorophyll content in tolerant cultivars was more than intolerant cultivars. The reason for this seems to be that at high salinity levels, the leaf area decreases and in fact the concentration of remaining molecules increases per unit area of leaf area (Aycan et al., 2021). A very significant positive correlation was observed between chlorophyll a and b (Table 3). The chlorophyll a / b ratio was negatively correlated with chlorophyll a but was not significant, while the correlation of this ratio with b was very significant and it is concluded that the increase in this ratio due to salinity was due to a significant decrease in chlorophyll b.

Comparing the percentage increase of genotypes, showed that the tolerant cultivar Arg had the highest percentage of changes in the amount of chlorophyll a, b and total chlorophyll. Finally, we concluded that due to the salinity, chlorophyll concentration increased and this increase was more in tolerant cultivars than sensitive cultivars.

S.O.V	df	Chlorophyll a (mg/100g)	Chlorophyll b (mg/100g)	a / b	a + b	SPAD
Location (L)	1	7.7 **	1.46**	2.16**	37.4**	272.85**
Genotype (G)	19	1.47**	0.28 **	2.27 **	1.24**	78.97 **
$G \times L$	19	1.42 **	0.18**	1.3 **	1.42 **	27.36 **
Erorr	76	0.02	0.002	0.02	0.07	4.902
C.V	-	5.58	5.77	4.93	7.91	4.76

Table 1. Results of combined analysis of variance of the effect of salinity on chlorophyll content

S.O.V – Sources of variation, C.V., % – Coefficient of variation, Chlorophyll-meter SPAD 502

\*\*The mean squares are significant at the 0.01 level according to results of combined analysis of variance

Table 2. Comparison of the average effect of environment on chlorophyll content

	Treatment	Chlorophyll a (mg/100g)	Chlorophyll b (mg/100g)	a / b	a + b	SPAD
Normal	Roshan	1.87 ab	1.07 b	1.74	2.94	44.96 abcde
	Sorkh tokhm	1.55 ab	1.45 a	1.06	3	43.75 bcdefg
	Karchya	1.63 abc	0.83 bcde	1.96	2.46	42.56 defg
	Mahuti	1.62 abc	0.79 bcde	2.05	2.41	43.21 cdefg
	Gaspard	1.19c	0.78bcde	1.52	1.97	44.75 abcdefg

# Table 2. Continued

	Moghan 3	1.70 abc	0.88 bcde	1.93	2.58	42.56 defg
	Arg	1.95 ab	0.79 bcde	2.46	2.74	45.65 abcd
	Arta	1.91 ab	1.03 bc	1.85	2.94	43.84 bcdefg
	shole	1.64abc	0.75bcde	2.18	2.39	42.45 defg
	Sistan	1.59ab	1.42a	1.11	3.01	41.26 g
	Shotordndan	1.61abc	1.05bc	1.53	2.66	43.75 bcdefg
	Bolani	1.94ab	1.62bcd	1.19	3.56	42.51 defg
	Omidbakhsh KRL-4	1.58ab	1.42a	1.11	3	41.30 fg
	Mv-17	1.60abc	0.77bcde	2.07	2.37	43.32 bcdefg
	N-83-3	1.55ab	1.41a	1.09	2.96	42.85 defg
	107 -PR-87	1.02d	0.72bcde	1.41	1.74	44.65 abcdefg
	S-78-11	1.57ab	1.40a	1.12	2.97	41.26 fg
	139-PR-87	1.65abc	0.74bcde	2.22	2.39	43.41b bcdefg
	SNH-9	1.89ab	1.05bc	1.8	2.94	41.39 efg
	140-PR-87	1.60abc	1.06bc	1.50	2.66	44.65 abcdefg
Salinity	Roshan	1.44 b	0.97 bcd	1.48	2.41	46.91 ab
	Sorkh tokhm	1.56 b	0.77 bcde	2.02	2.33	45.65 abcd
	Karchya	1.65 abc	0.77 bcde	2.14	2.42	44.54 abcdefg
	Mahuti	1.49 bc	0.73 bcde	2.04	2.22	45.24 abcd
	Gaspard	1.76 abc	1.60 bcd	1.1	3.36	46.91 ab
	Moghan 3	1.57b	1.46a	1.07	3.03	44.52 abcdefg
	Arg	2.15 a	1.58 a	1.36	3.75	47.97 a
	Arta	1.61 abc	0.82 bcde	1.96	2.43	45.84 abcd
	Shole	1.52b	0.79bcde	1.92	2.29	44.43 abcdefg
	Sistan	1.62abc	0.75bcde	2.16	2.37	43.26 cdefg
	Shotordndan	1.41b	0.95bcd	1.48	2.36	45.72 abcd
	Bolani	1.54b	0.76bcde	2.02	2.3	44.50 abcdefg
	Omidbakhsh KRL-4	1.15c	0.52e	2.21	1.67	43.28 cdefg
	Mv-17	0.99d	0.76bcde	1.3	1.75	45.30 abcd
	N-83-3	1.46b	0.98bcd	1.48	2.44	44.82 abcdef
	107 -PR-87	1.72abc	1.62bcd	1.06	3.34	46.62 abc
	S-78-11	1.50bc	0.74bcde	2.02	2.24	43.24 cdefg
	139-PR-87	1.17 c	0.50 e	2.34	1.67	45.42 abcd
	SNH-9	1.17c	0.54e	2.16	1.71	43.36 bcdefg
	140-PR-87	1.64abc	0.8bcde	2.05	2.44	46.62 abc

The mean difference is significant at the (p < 0.05) according to LSD test

# Table 3. Correlation between chlorophyll a, b, a / b and total chlorophyll

	Chlorophyll a (mg/100g)	Chlorophyll b (mg/100g)	a / b	a + b	SPAD
Chlorophyll a	1				
Chlorophyll b	0.809**	1			
a / b	-0.187	-0.713**	1		
a + b	0.899**	0.739**	-0.25	1	
SPAD	-0.106	-0.139	0.121	-0.136	1

\*\* Correlation is significant at the 0.01 level. SPAD – Chlorophyll-meter SPAD 502

#### Conclusion

In the effect salinity, the concentration of chlorophyll increased, and this increase was more in tolerant cultivars than in sensitive cultivars. Comparison of the mean for different traits showed that the highest concentration of chlorophyll a in Arg cultivar and chlorophyll b in Gaspard cultivar and the lowest concentration of chlorophyll a and b were related to MV-17. The highest concentration of total chlorophyll a and b was observed in Arg cultivar and the lowest concentration was observed in MV-17. The highest a / b ratio was 139-PR-87 and the lowest ratio was Gaspard and Moghan-3. As a result, the a / b ratio due to salinity in susceptible genotypes is less than resistant.

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