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Compared salt tolerance of five local wheat (*Triticum aestivum* L.) cultivars of Albania based on morphology, pigment synthesis and glutathione content

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Abstract

Stressful saline concentrations in soils affect photosynthesis by damaging pigments, photosystems, components of electron transport system, and enzymes involved in the process. Plants respond through very complex stress adaptation mechanisms including proteome modulation, alterations in pigment content, cell osmotic adjustment and control of ion and water homeostasis mechanisms, which stabilize cytosolic glutathione redox potential, etc. The level of plant sensitivity depends on salt toxicity levels, growth stage, physiological and genetic factors. With aim the investigation of the salinity tolerant cultivars, and for the elucidation of mechanisms underlying this complex biological process, here we analyze the impact of four NaCl concentrations (0-50-100-200mM) in growth parameters (root, shoot and leaves length), pigment content (chla, chlb, carotenoids), and GSH content, during seedling of five bread wheat (*Triticum aestivum* L.) cultivars in modified Hoagland solutions. Based on biometric parameters, pigment synthesis and GSH content cultivar Nogal is salt-sensitive (growth and pigments reduced); cultivar Viktoria is medium-tolerant (growth partially impaired, pigments constant), cultivar Toborzo and cultivar Suba are medium-tolerant (growth partially impaired, pigments increased), cultivar Dajti salt-tolerant (growth partially impaired/ leaves developed, pigments increased). Quantity of GSH in response to different levels of salinity is cultivar specific, and time of exposure to salinity is in negative correlation to GSH content for all investigated cultivars.

Keywords: *Triticum aestivum* L., photosynthetic pigments, GSH, Chl, PSII

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Introduction

The fact that salinity impairs crop growth and productivity has driven the engagement of institutions related to crop agriculture in long-term researches (1, 2, 3). Even though there are several strategies to increase wheat production in the salt-affected areas, the cultivation of tolerant genotypes is recognized as the most effective way to overcome this limitation (4).

The results on the impact of salinity and level of sensitivity displayed by wheat cultivars have brought to the conclusion that, tolerance to salt stress is a complex biological phenomenon governed by several physiological and genetic factors, and it is growth stage specific (5). However, the extent of the damage to crops is also reported to depend on the concurrent salt toxicity levels (6-8).

As already reported, the salinity stress hampers the process of photosynthesis in most plants by altering the ultrastructure of the organelles and concentration of various pigments and metabolites (9) by inhibiting many physiological and metabolic processes, in particular nitrate uptake, translocation, and assimilation (8); by inhibiting plant growth mainly due to osmotic stress and ion toxicity (8, 10, 11) by inhibiting photosystem II (PSII) activity and destruction of chlorophyll pigments due to the accumulation of toxic ions (4), etc.

On the other side, photosynthetic rate is highly correlated with the amount of Rubisco (12), which is highly regulated in response to short-term fluctuations in the environment

(13). The extent to which the continuous process of Rubisco protein turnover (a function of synthesis, maintenance, and degradation) represents a drain on cellular resources is uncertain, but on account of its abundance could be considerable, particularly in the presence of a relatively oxidizing environment induced by stress (13).

Different stressful environments have been reported to reduce the contents of photosynthetic pigments (9). The salt induced alterations in a leaf Chl content could be due to impaired biosynthesis or accelerated pigment degradation. However, during the process of Chl degradation, Chlb may be converted into Chla, thus resulting in the increased content of Chla (14). In salt tolerant species, Chl content increases, whereas it decreases in salt-sensitive species under saline regimes (15, 16). In some other studies, Chl accumulation under saline stress is not always associated with salt tolerance.

Carotenoids are necessary for photoprotection of photosynthesis and they play an important role as a precursor in signaling during the plant development under biotic/abiotic stress. The role of carotenoids in scavenging ROS has been well studied (9). Growth improvement in plants under stressful environment has been widely reported to be due to the significant role of zeaxanthin in alleviating oxidative damage of membranes (17, 18).

According to previous reports, plants have developed sophisticated mechanisms to minimize stress damage. Thus, the exposure to salinity triggers specific strategies for cell osmotic adjustment and control of ion and water homeostasis to minimize stress damage and to re-establish growth (8, 19, 20). Nitrogen availability is reported to be important for plants under salinity, not only for growth, but also for the synthesis of the organic solutes involved in osmoprotection (8, 21). The chloroplast as a key site for photosynthesis, is highly sensitive to different stressful environments such as salinity, etc, and plays a premier role in the modulation of stress responses (9). According to previous reports, the ROS salt stress signaling pathway was determined to have a pivotal regulatory function in salt tolerance, fundamental of which is the control of ion homeostasis (22, 23), and the molecular identities of key ion transport systems that are fundamental to plant salt tolerance are now known (25).

Glutathione is one of metabolites with complex roles in biosynthetic pathways, detoxification, antioxidant biochemistry and redox homeostasis. Oxidant production and oxidative stress can exert a strong influence over glutathione status, and is governed by the rates of photosynthesis, photorespiration and respiration, as well as NADPH oxidases and other ROS-producing systems (26). Mechanisms appear to have evolved to stabilize cytosolic glutathione redox potential during increased ROS production, and these include increases in total glutathione and accumulation of GSSG in compartments that may be less sensitive to redox perturbation (26).

It is clear that, the identification of signaling components involved in stress adaptation in plants is a meaningful approach to identify transcriptional activators of adaptive mechanisms to stressful environments that are promising for improvement of crop tolerance (9), and so does the accurate isolation and characterization of stress responsive proteins (27).

With aim the investigation of the salinity tolerant cultivars, and for the elucidation of mechanisms underlying this complex biological process, here we report on the impact of three saline treatments (50-100-200mM NaCl) on seedling of five local bread wheat cultivars of region of Vlora, Albania, taking into consideration biometric parameters, photosynthetic pigments (chla, chlb, carotenoids and xantophylls), and GSH content.

Materials and Methods

Germination conditions

One hundred seeds from each of five bread wheat (*T. aestivum* L.) cultivars (Suba, Nogal, Dajti, Toborzo, Viktoria) donated from the Public Directory of Agriculture of Vlora, Albania, were kept at 4°C for 24hrs, and later in running water for 30 min following (28).

After, seeds (25 seeds / plate) were sown in Petri dishes containing filter paper soaked in distilled water, and kept in vegetative room under the photoperiod 16/8 day-night at 25°C for four days to germinate.

Seedling

Thereafter, plantlets were transferred to cultures with modified Hoagland solution (29). Four different solutions containing respectively 0-50mM-100mM-200mM NaCl were used to develop plantlets during three consecutive weeks. Altogether, 4 experiments (controls plus three treatments) with four replications each, for each of the five cultivars were conducted in the growth room under a photoperiod 16/8 day-night, at 25°C.

Measurement of biometric parameters

To measure the length of leaves, shoots and roots were chosen plantlets (3 paralels/each culture) 3 weeks after the application of the saline stress.

Pigments extraction and measurement

Photosynthetic pigments (chla, chlb, carotenods and xantophylls) were extracted from leaves of plantlets 2 and 3 weeks after saline treatment using a non-destructive method, by mixing dimethyl sulfoxide solution with plants tissues following the standard DMSO protocol (30) adopted by (31). For each cultivar were used 3 replicates x 4 categories of cultures (1 control and three treatments). Absorbance of the pigments extracts was measured at 470nm, 645nm and 663nm, and quantities were calculated following Arnon's equations (32).

Thiols extraction and measurement

Plantlets aged two and three weeks after treatment with salinity were used to extract total thiols following (33) and GSH content was evaluated following Ellman's test (34). The so-called Ellman's reagent enters the reaction with free sulfhydryl groups to form mixed disulfides and 2-nitro-5-thiobenzoic acid. The last one has a yellowish color and its absorbance was measured at 412 nm

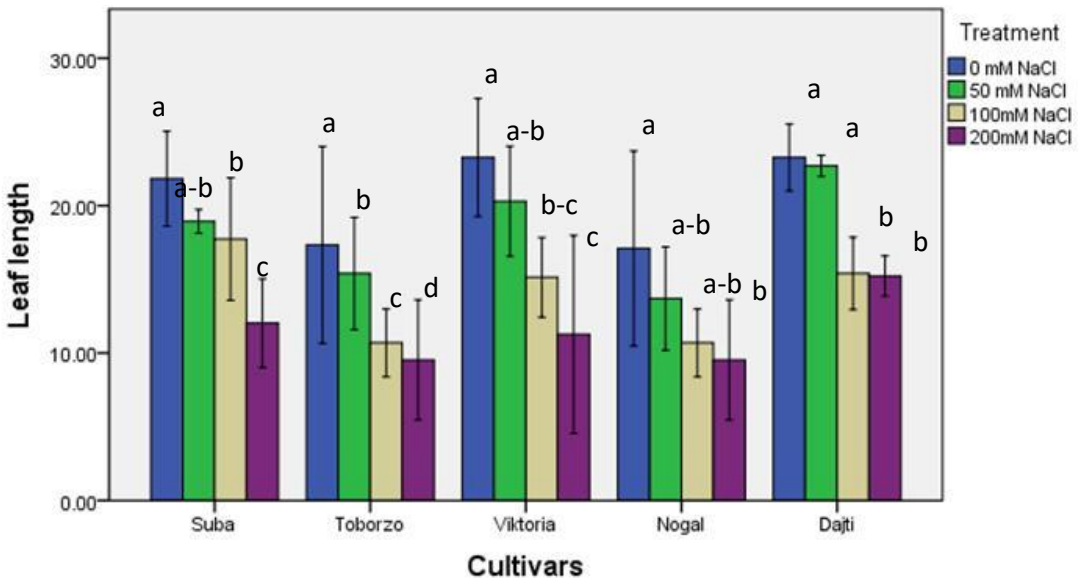

Figure 1. Impact of different concentrations of salinity on leaf lengths (cm) for five wheat cultivars. Data represent mean values of leaves length (cm) and standard deviation (error bars) for three repeated measurements. Mean values followed by different letters within the same cultivar are statistically different ($p<0.05$) according to Tukey Test.

in spectrophotometer. For each cultivar were used 3 replicates x 4 categories of cultures (1 control and three treatments).

Statistical analysis

Statistical analysis of biometric parameters, pigments and thiol concentrations were carried out using the IBM SPSS Statistics 20 program. It was used to verify the impact of different saline concentrations on biometric parameters, pigments, and thiols for each cultivar, as well as to compare GSH content among cultivars, and the impact of the time of exposure to treatment.

Results

Impact of salinity on biometric parameters

Three biometric parameters (length of leaf, shoot and root) were measured three weeks after treatment with saline stress. Leafs length displayed a progressive reduction, in negative correlation to the increase in salt concentration, for all cultivars except Dajti, whose leaves kept the same length under the saline concentrations 100-200mM (**Table 1**, **Fig 1**).

The biometric parameter length of roots displayed a cultivar specific trend. All cultivars had slower root growth at samples

Table 1. Impact of salinity on morphological parameters (length of leaf, root and shoot)

Parameters measured (in cm)	Treatment (mMNaCl)	Wheat cultivars				
		Suba	Toborzo	Viktoria	Nogal	Dajti
Root	0	11.6±0.87 a	10.76±1.78 a	24.13±0.32 a	10.37±1.98 a	15.43±0.55 a
	50	11.0±0.45 a	8.43±3.10 a	15.03±0.15bc	8.63±2.030 a	13.47±0.42ab
	100	11.4±1.32 a	7.27±0.85 a	15.15±0.5 cd	7.23±1.096 a	11.60±0.79bcd
	200	12.37±0.51 a	8.36±2.45 a	8.97±0.25 d	7.43±2.57 a	10.03±1.86 cd
Leaf	0	21.83±1.61 a	17.33±3.34 a	23.27±2.0 a	17.10±3.3 a	23.27±1.14 a
	50	18.93±0.40ab	15.4±1.9 b	20.3±1.87ab	13.7±1.75ab	22.70±0.40 a
	100	17.73±2.08 b	10.7±1.15 c	15.13±1.35bc	10.70±1.15ab	15.4±1.22 b
	200	12.03±1.50 c	9.53±1.15 d	11.27±3.35 c	9.53±2.04 b	15.23±0.10 b
Shoot	0	8.20±0.20 a	11.07±0.21 a	6.88±0.11 a	9.63±2.4 a	7.27±0.25 a
	50	7.17±0.31bc	7.97±0.41 b	6.03±0.15 b	7.96±0.42abc	6.80±0.26 a
	100	6.87±0.32 cd	5.23±0.25 c	6.10±0.2 b	6.03±0.15bc	6.07±0.31 b
	200	4.02±0.18 d	6.03±0.15 d	6.0±0.18 b	5.40±0.15 c	5.00±0.2 c

Values present the mean of three repeated measurements (±*DS*).

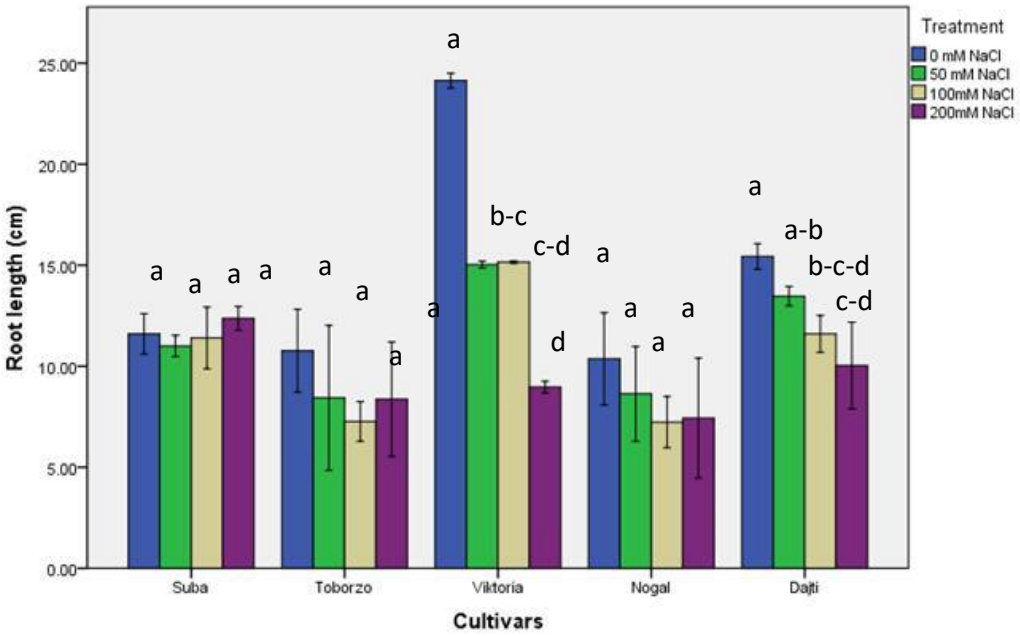

Figure 2. Impact of different concentrations of salinity on root lengths (cm) for five wheat cultivars.
Data represent mean values of leaves length (cm) and standard deviation (error bars) for three repeated measurements. Mean values followed by different letters within the same cultivar are statistically different ($p<0.05$) according to Tukey Test.

treated with 50mM NaCl compared to controls; Suba had an almost stabilized root growth under all treatments; Dajti had a progressive reduction of root length with the increase on salt concentration; Toborzo and Nogal recuperated root growth from treatment 100mM to 200mM; Viktoria displayed the biggest reduction in root length (control plants compared to those treated with the highest salt concentration) (**Table 1**, **Fig. 2**).

The highest shoot length was measured at control plants for the five cultivars, and this biometric parameter followed a cultivar specific trend. Thus, Suba, Nogal and Dajti displayed progressive reduction of shoot length with the increase of salt concentration; Viktoria had a stabilized shoot length independently of treatment with salinity; Toborzo had higher shoot length under treatment 200mM compared to 100mM (**Fig. 3**).

Impact of saline stress on pigments content

Results on photosynthetic pigments Chla, Chlb, carotenoids and xantophylls extracted and measured at wheat plant material under saline treatment, two and three weeks after the application are presented at **Table 2** and **Figure 4, 5, 6, 7**.

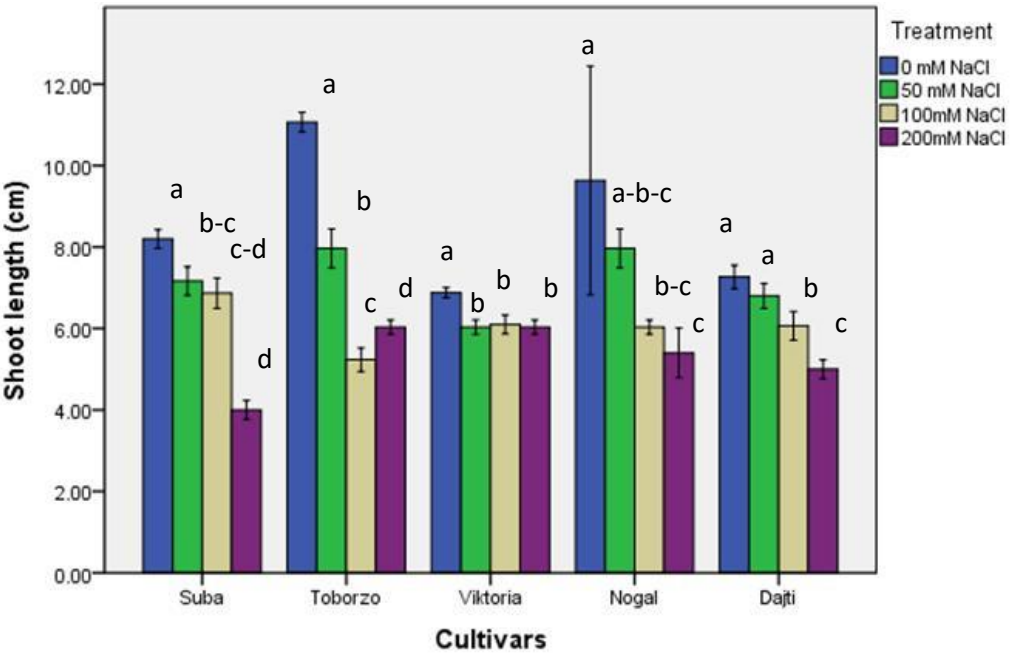

Figure 3. Impact of saline treatments on mean shoot length (cm).
Mean values followed by different letters within the same cultivar are statistically different ($p<0.05$) according to Tukey Test.

Table 2. Chlorophyll concentration (chla, chlb, tot chl, carotenoids and xanthophylls) of different wheat cultivars grown under normal and saline environments. Data is displayed as means±standart deviation (n=3). Different letters, for each cultivar, indicate significant differences ($p<0.05$) among NaCl treatments by Tukey test.

Cultivar	Treatment (NaCl)	2nd week				3rd week			
		chla in mg/g fresh weight	chlb in mg/g fresh weight	tot chl	carotenoids + xanthophylls in mg/g fresh weight	chla in mg/g fresh weight	chlb in mg/g fresh weight	tot chl	carotenoids + xanthophylls in mg/g fresh weight
Suba	0 mM	0.60±0.25 a	0.147±0.5 a	0.75±0.29 a	42.8±15.8 a	0.59±0.01 a	0.12±0.01 a	1.18±0.05 a	46.9±0.1 a
	50 mM	0.78±0.27 a	0.21±0.08 a	0.98±0.35 a	55.4±15.9 a	1.097±0.15 b	0.17±0.05 b	0.74±0.03 b	58.22±0.56 b
	100 mM	1.01±0.07 a	0.255±0.02 a	1.26±0.09 a	65.2±10.4 a	1.06±0.01 c	0.24±0.14 c	1.34±0.013 b	68.5±0.55 c
	200 mM	0.77±0.26 a	0.21±0.07 a	0.97±0.32 a	55.2±10.6 a	0.47±0.01 d	0.27±0.02 d	1.33±0.04 c	65.8±0.15 d
Toborzo	0 mM	0.51±0.26 a	0.13±0.7 a	0.63±0.33 a	35.47±19.7 a	0.94±0.001 a	0.21±0.01 a	0.48±0.6 a	56.9±0.15 a
	50 mM	0.74±0.23 a	0.17±0.6 a	0.91±0.29 a	48.4±12.8 a	0.72±0.02 b	0.19±0.003 a	0.92±0.01 a	51.7±0.03 b
	100 mM	0.82±0.18 a	0.21±0.05 a	1.03±0.23 a	56.2±13.1 a	0.53±0.01 c	0.14±0.001 b	0.67±0.002 a	40.9±0.02 c
	200 mM	0.8±0.39 a	0.22±0.1 a	1.07±0.48 a	57.4±21.5 a	0.66±0.002 d	0.2±0.01 a	0.84±0.001 a	50.0±0.5 d
Viktoria	0 mM	0.55±0.1 a	0.14±0.03 b	0.68±0.13 a	39.6±6.95 a	0.84±0.001 a	0.20±0.005 a	1.02±0.02 a	52.1±0.27 a
	50 mM	0.77±0.07 ab	0.21±0.02 b	0.99±0.09 ab	54.9±4.54 ab	0.97±0.01 b	0.21±0.003 b	1.18±0.01 b	63±0.087 b
	100 mM	0.72±0.17 ab	0.17±0.04 b	0.9±0.21 ab	49.9±10.7 ab	0.65±0.01 c	0.13±0.005 c	0.80±0.005 b	41.7±0.006 c
	200 mM	0.9±0.13 b	0.23±0.04 b	1.1±0.17 b	63.1±7.25 b	0.64± 0.02 d	0.16±0.001 d	0.80±0.008 c	49.1±0.04 d
Nogal	0 mM	1.08±0.3 ab	0.31±0.01 a	1.38±0.04 a	74.5±1.8 a	0.71±0.03 a	0.19±0.002 a	0.9±0.01 a	51.4±0.01 a
	50 mM	0.9±0.05 a	0.24±0.02 b	1.14±0.7 b	63.7±1.44 a	0.5±0.01 b	0.13±0.001 b	0.63±0.02 b	35±0.005 b
	100 mM	1.0±0.09 ab	0.24±0.01 b	1.26±0.1 ab	70.4±8.54 a	0.71±0.01 a	0.16±0.006 c	0.87±0.002 c	39.1±0.2 c
	200 mM	1.0±0.1 b	0.25±0.04 b	1.38±0.13 a	74.7±8.54 a	0.69±0.01 c	0.122±0.01 d	0.81±0.002 d	38.1±0.17 d
Dajti	0 mM	0.82±0.11 a	0.22±0.04 a	1.04±0.15 a	57.3±7.46 a	0.42±0.01 a	0.17±0.04 a	0.53±0.4 a	32.6±0.01 a
	50 mM	0.91±0.08 a	0.26±0.02 a	1.17±0.1 a	66.9±3.9 a	0.74±0.03 b	0.21±0.02 a	0.95±0.01 b	56.85±0.05 b
	100 mM	0.86±0.17 a	0.02±0.01 a	0.97±0.1 a	56.78±4.6 a	0.66±0.01 c	0.31±0.25 a	0.82±0.08 c	46.27±0.03 c
	200 mM	0.8±0.11 a	0.04±0.02 a	1.0±0.14 a	57.1±9.0 a	0.40±0.02d	0.09±0.007 a	0.49±0.03 d	29.4±0.02 d

Pigments at cultivar Suba

The pigment content for cultivar Suba displayed increase in Chla in correlation with the time of exposure to salinity; increase under treatment with 50-100mM NaCl, and lowering under treatment with 200mM NaCl. The quantity of chla versus chlb varies from 2,6 – 4; Carotenoids and xantophylls increased by the time of exposure to treatment.

Pigments at cultivar Toborzo

At plants after the 2nd week of treatment the chla and chlb increased in positive correlation with the concentration of salinity; after the 3rd week chla is decreased in negative correlation with salinity, while the chlb is decreased in second treatment (100mM NaCl), and kept almost the same concentration during the first and third treatment (50-200mM).

Pigments at cultivar Viktoria

Chla and Chlb increased under first treatment (50mM) and decreased under the effect of 100-200mM NaCl in both 2nd and 3rd week. It is clear that the Chla/Chlb remain more stable during the 2nd week after treatment; increase during 50-100mM treatments, and decrease under the highest saline concentration after the 3nd week. It seems that the possible conversion of Chlb to Chla, and the reduction of Chlb synthesis is more active during the prolonged effect of salinity. Carotenoids and xantophylls content reach highest concentration at cultivar Viktoria under 200mM NaCl / 2nd week and at 50mM salinity / 3rd week.

Pigments at cultivar Nogal

From **Figures 4, 5** can be depicted that Chla and Chlb quantities decrease from 2nd to 3rd week of treatment with salinity; A sharp decrease was evidenced at plants with 50mM NaCl treatment, followed by the gradual increase under the rest of salinity conditions.

Ratios Chla/Chlb reach maximum (for cultivar Nogal) values at plants treated with 50mM NaCl, decrease for the rest of treatments, while values remain higher for the 3rd week (**Fig. 8**). It seems that, either the synthesis of Chla increases, or conversion of Chlb is favored under stress conditions.

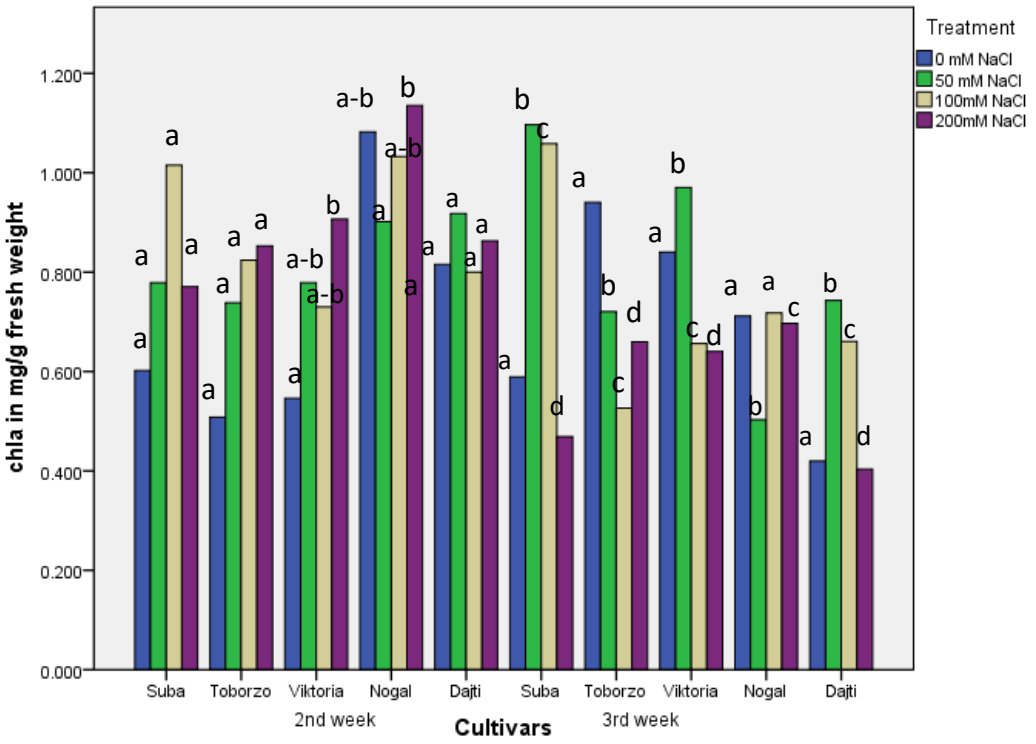

Figure 4. Differences among mean chla content (mg/g fresh weight) for five wheat cultivars under saline treatments. Mean values followed by different letters within the same cultivar are statistically different ($p<0.05$) according to Post Hoc Tukey Test.

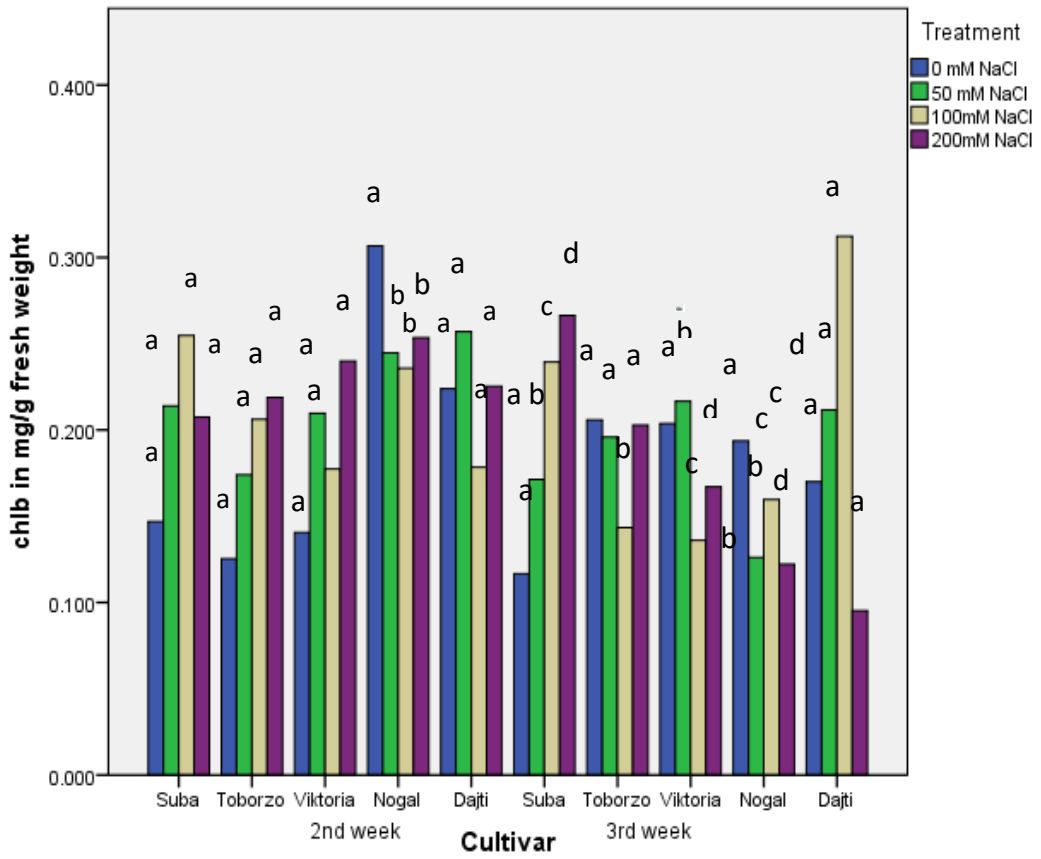

Figure 5. Differences among mean chlb content (mg/g fresh weight) for five wheat cultivars under saline treatments. Mean values followed by different letters within the same cultivar are statistically different ($p<0.05$) according to Tukey Test.

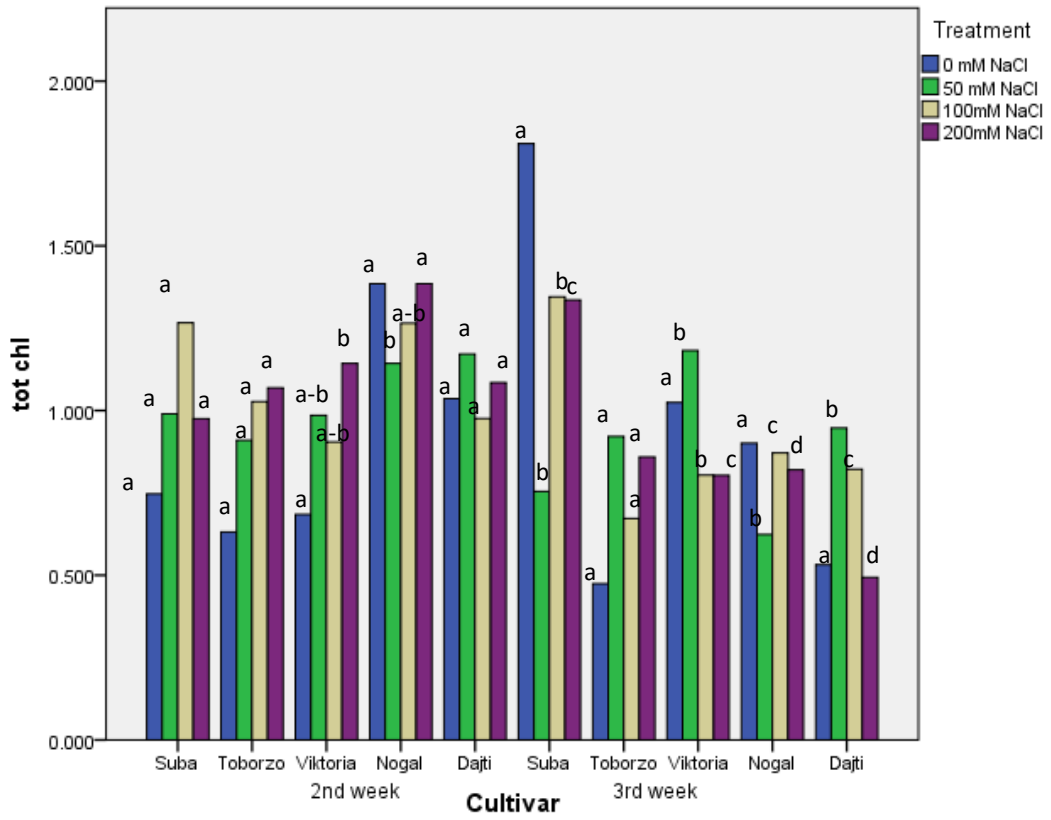

Figure 6. Differences among mean total chl content (mg/g fresh weight) for five wheat cultivars under saline treatments. Mean values followed by different letters within the same cultivar are statistically different ($p<0.05$) according to Tukey Test. Values are means from three independent experiments. Different letters indicates statistically significant difference between treatments of the same cultivar.

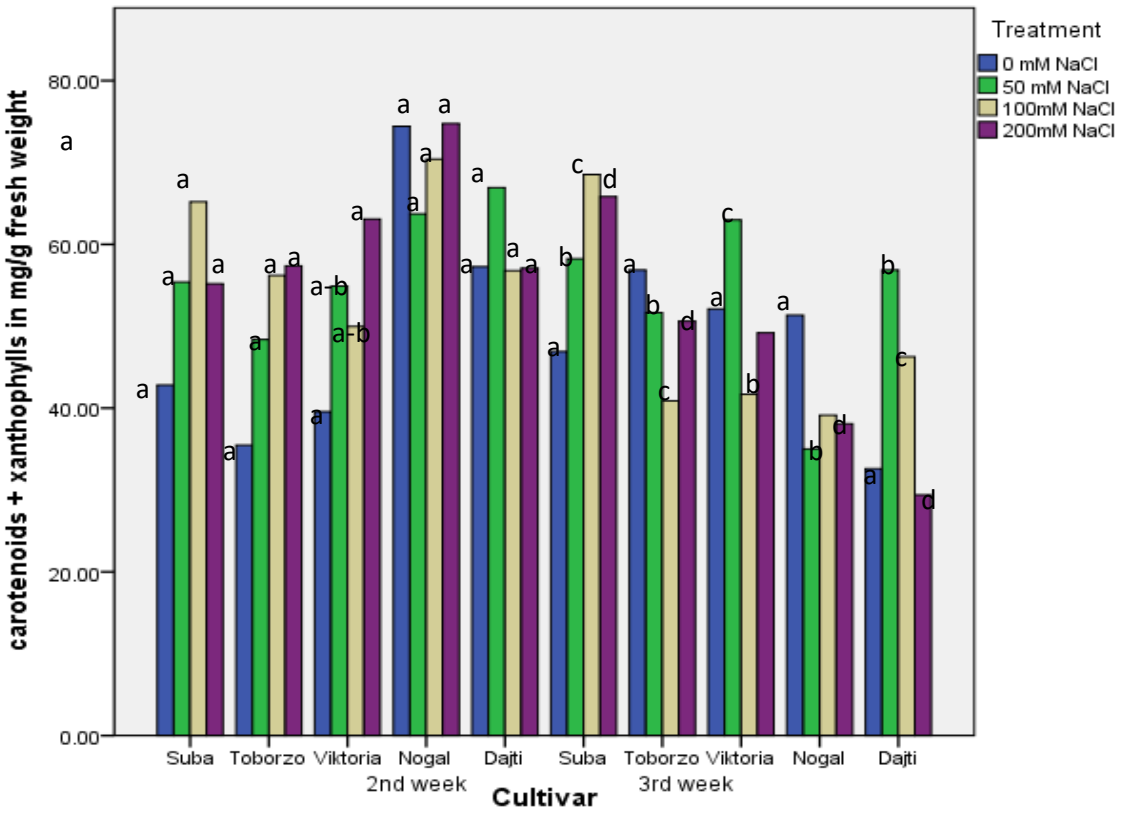

Figure 7. Differences among mean carotenoids+xantophylls content (mg/g fresh weight), for five wheat cultivars under saline treatments. Mean values followed by different letters within the same cultivar are statistically different ($p<0.05$) according to Tukey Test.

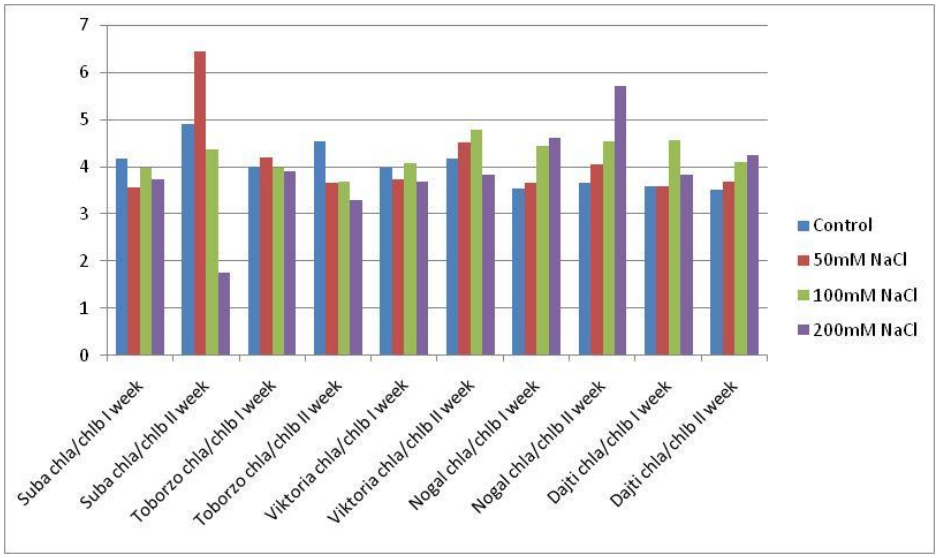

Figure 8. Ratio Chla / Chlb at wheat cultivars two and three weeks after saline treatment.

Pigments at cultivar Dajti

Chla content decreases from 2nd to 3rd week of treatment; Chlb on the opposite increases with time of exposure; Both Chla and Chlb reach highest values at treatment with 50mM NaCl.

Ratio chla/chlb decreased slightly from the 2nd to 3rd week; Peak values were measured at plants under treatment with 100mM NaCl.

Results show a sharp decrease of carotenoids and xantophylls from the 2nd to 3rd week of treatment; the lowest values are reached for plants under 50mM NaCl treatment (**Fig. 7**).

Their concentration after the 2nd week of treatment reaches highest values at 50mM NaCl and remains the same as controls for the rest of salinities applied; Three weeks after treatment concentration decreases for plants grown in 50-100mM and grows to the values of control plants at 200mM NaCl.

GSH content

Table 3 describes results on GSH content at five cultivars under treatment with modified Hoagland solutions.

Table 3. GSH (mM) for wheat cultivars Suba, Toborzo,Viktoria, Nogal and Dajti treated with modified Hoagland solution (0-50-100-200 mM NaCl), 2 and 3 weeks after treatment

Wheat cultivar	Week of measurement	Treatment with salinity (NaCl)				Value of test p=0.05
		Controls (0 NaCl)	50 mM	100 mM	200 mM	Week/ treatment
Suba	2nd	0.64 a	0.86 a	0.31 b	0.14 b	p=0.002
	3rd	0.31 a	0.78 b	0.38 a	0.09 a	p=0.003
Toborzo	2nd	0.11 a	0.37 a	0.52 a	0.50 a	p=0.085
	3rd	0.19 a	0.39 ab	0.54 b	0.48 ab	p=0.042
Viktoria	2nd	0.58 c	0.72 c	0.71 c	0.83 c	p=0.408
	3rd	0.43 c	0.77 c	0.39 c	0.36 c	p=0.055
Nogal	2nd	0.72 ab	1.06 b	0.93 b	0.58 a	p=0.016
	3rd	0.21 ab	0.15 a	0.51 ab	0.67 b	p=0.026
Dajti	2nd	0.36 a	0.59 ab	0.96 b	0.23 a	p=0.005
	3rd	0.45 a	0.51a	0.167 a	0.27 a	p=0.467

Values are means of three replicates, mean values followed by different letters within the same cultivar are statistically different ($p<0.05$), generated by Tukey post hoc test.

Discussion

Effects of salinity on biometric parameters

Soil salinity is a major constraint to food production because it limits crop yield, even though most halophytes and glycophytes tolerate salinity (24). Previous reports have summarized research on plant ion homeostasis in saline environments trying to integrate current understanding of salt stress sensing, which leads to the activation of the ROS pathway and the regulation of ion transport systems that facilitate homeostasis (8, 24, 26, 35).

Fundamentally, plants cope by either avoiding or tolerating salt stress (24). According to (4) the cultivation of tolerant genotypes is recognized as the most effective way to overcome limitations in wheat production in salt-affected areas. Albania is a Mediterranean country, which faces environmental stresses such as high temperatures, drought, and salinity in different regions.

In this study, five bread wheat local cultivars of Vlora were analyzed to compare their salt-related tolerance during seedling in Hoagland solutions, by investigating biometric parameters, pigment synthesis and glutathione metabolism under the effect of 50mM-100mM-200mM NaCl. **Table 4** summarizes the effect of salinity concentration in root, shoot and leaf development three weeks after growth in modified Hoagland solutions.

From **Table 4** can easily be depicted that cultivar Nogal experiences growth reduction for the three parameters measured; Roots development is impaired at 3/5 cultivars; Shoots development is impaired at 4/5 cultivars; Leaf development is reduced at 4/5 cultivars. As previously reported (36) the chemical potential of the saline solution initially establishes a water potential imbalance between the apoplast and symplast that leads to turgor decrease, which if severe enough can cause growth reduction. Our results prove that the effect of salinity is displayed not only in the areas exposed to salinity (roots). Following (8), plant operates as an integrated system in which metabolic stress-induced signals spread in the plant and change the metabolism even in areas in which the stress conditions are not present. Furthermore, different parts of plant root system may behave as physiologically autonomous units with different responses to environmental signals and preserving their own capability to supply shoots with water, nutrients or assimilates (8). In accordance to (10) shoot growth is more sensitive to salt stress than the root growth for the five investigated cultivars. In conclusion, the increase in salinity concentration from 50-200mM has affected growth parameters for the five cultivars; cv Nogal is the most sensitive; cv Dajti is the only which continues an unimpaired leaf development. Suba and Toborzo have a stable root development.

Effects of salinity on pigment synthesis

A summary of the results on the impact of saline stress on photosynthetic pigments for five investigated is presented at **Table 5**. For 3 out of 5 cultivars total chlorophylls increased; for 1 out of 5 cultivars Tot Chl decreased, and 1 out of 5 cultivars was not impacted by the exposure to salinity. According to (9), the increase in Chl content happens in salt-tolerant species, and following our results cultivars Dajti, Suba and Toborzo could be classified as such. The decrease of the ratio chla/chlb can be explained by the possible conversion of Chlb into Chla (for Dajti and Toborzo), while in the case of Suba the variation of the ratio could be explained by the degradation of Chla. Nogal seems to be the most impacted cultivar (tot chl and carotenoids synthesis decrease) meaning the most sensitive to salinity; Cultivar Viktoria displayed unchanged total Chl, and carotenoids+xantophylls meaning a stable tot chl content, which might be explained by the the ratio of Chla / Chlb (**Fig. 8**).

Carotenoids and xantophylls in 4 out 5 cultivars remain in constant concentrations; Nogal is the only who experiences reduction. As previously reported, these pigments are necessary for photoprotection of photosynthesis and they play an important role as precursors in signaling during the plant development under abiotic/biotic stress (9). In conclusion, the reduction of carotenoids, Chla and Chlb for cultivar Nogal,

Table 4. Summary of the effect of salinity concentration on root, shoot and leaf development of five bread wheat cultivars during seedling in modified Hoagland solution (three weeks after treatment).
Arrows directions show: ↘ reduced length; → unchanged length

Cultivars	Roots development	Shoots development	Leaf development
Dajti	↘	↘	→
Suba	→	↘	↘
Toborzo	→	↘	↘
Viktoria	↘	→	↘
Nogal	↘	↘	↘

Table 5. Impact of saline treatment (from 2nd to 3rd week) on pigment synthesis (Chla, Chlb, Tot Chl, Carotenoids+xantophylls) at five bread wheat cultivars of Vlora, Albania

Cultivars	Chla synthesis	Chlb synthesis	Total Chl	Carotenoids & xantophylls	Ratio Chla/Chlb
Dajti	↘	↗	↗	→	↘
Suba	↗	↘	↗	→	↘ ↗
Toborzo	↘	↗	↗	→	↘
Viktoria	↗	↘	→	→	↗
Nogal	↘	↘	↘	↘	↗

speak for an impaired pigment synthesis and lead us to classify it as salt-sensitive. The rest of cultivars could be classified as salt-tolerant cultivars because they kept an almost constant carotenoids+xantophylls content, and increased total Chl. The cultivar specific reductions of Chla or Chlb (Table 4) have been previously reported in *Triticum aestivu*m by (9, 14). Following (14) the salt-induced alterations of leaf Chl content could be due to impaired biosynthesis or accelerated pigment degradation, Chlb may be converted into Chla, thus resulting in the increased content of Chla.

Effects of salinity on GSH content

According to (26) known functions of glutathione include roles in biosynthetic pathways, detoxification, antioxidant biochemistry and redox homeostasis. Based on (27, 38) most important factors affecting synthesis of GSH are γ-ECS activity and cysteine availability. So far, the induction of the transcripts of GSH1 and GSH2 genes is reported to be caused by a few substances such as jasmonic acid, heavy metals, some special light conditions, draught, and certain pathogens. High salinity decreases the capacity of roots to extract water from soil, and high concentrations of salts within the plant itself can be toxic, resulting in nutritional imbalance and oxidative stress (7, 10).

Five cultivars under study have a decreased amount of GSH from 2nd to 3rd week after treatment with modified salinity; Cultivar Suba experiences progressive reduction with the increase of NaCl concentration applied; Toborzo has higher GSH content with increase of salt cc; Viktoria has lower GSH content compared to control, but almost equal cc among treatments; Nogal and Dajti reach peak GSH content at plants treated with 50mM NaCl.

As previously reported (26), mechanisms appear to have evolved to stabilize cytosolic glutathione redox potential during increased ROS production, and these include increases in total glutathione and accumulation of GSSG in compartments that may be less sensitive to redox perturbation. Based on our results, the quantity of GSH in response to different levels of salinity is cultivar specific, but the time of exposure to salinity is in negative correlation to GSH content for all investigated cultivars. As already known, glutathione accumulation, turnover and transport involve a number of enzymatic pathways many of which not elucidated, however it is well known that at cereals GSH1 and GSH2 genes regulate its biosynthesis in plastids (γ-ECS synthesis) and cytosol (26, 37). Either of the modes of regulation of glutathione involve γ-ECS, which seems to contribute for the glutathione homeostasis via a feedback inhibition mechanism. Based on (38), local wheat cultivars, grown in the absence of biotic or abiotic conditions, display typical tissue and cultivar specific accumulation of GSH.

In conclusion, based on biometric parameters, pigment synthesis and GSH content: wheat cultivar Nogal is salt-sensitive (growth and pigments reduced), cultivar Viktoria medium-tolerant (growth partially impaired, pigments constant), Toborzo and Suba medium-tolerant (growth partially impaired, pigments increased), Dajti salt-tolerant (growth partially impaired / leaves developed, pigments increased). Mechanism used to stabilize GSH content is cultivar specific.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Ethical Compliance

This article does not contain any studies involving human participants or animals performed by any of the authors.

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