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Enzymes activity and content of antioxidants in leaves of halophytes from saline soils of Kumisi lake

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Abstract

The purpose of the given study was to investigate characteristics of antioxidant system and other biochemical indices of some salt resistans species growing on saline soils of Georgia. Activity of antioxidant enzymes (peroxidase and catalase) and nitrate reductase, also low molecular antioxidants (proline, ascorbic acid, soluble phenols, anthocyanins and carotenoids), and of content of total proteins, chlorophylls, and soluble carbohydrates has been investigated in leaves of salt resistnt plants – *Salsola soda* L. – opposite-leaved saltworth, *Tamarix ramosissima* Ledeb. – salt cedar, *Chenopodium album* L. – goosefoot, *Artemisia lerchiana* (Web.) – sagebrush, *Achillea biebersteinii* (Afan.)- allheal and *Adonis bienertii* (Butkov ex Riedl.) – pheasant's eye – growing coastwise and in surroundings of Kumisi Lake (East Georgia, lower Kartli), in order to study the influence of salinization level on the studied parameters. Spectrophotometrical, gazometrical and titration methods has been used for investigations. Increase of salinity induced activation of peroxidase, rise of proline and total proteins content in leaves of eu- and crynohalophytes (saltworth, goosefoot, salt cedar). Activation of catalase and peroxidase, also increase of the content of anthocyanins, phenols, total proteins and soluble carbohydrates was mentioned in leaves of glycohalophytes (sagebrush, allheal, peasant's eye) under the same conditions. Activation of peroxidase and increase of the content of total proteins seemed to be the uniting mechanism for adaptation to high level salinization among the studied species..

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Introduction

Global climate change and increase of man impact turned soil salinization into an economical and ecological problem. Amount of species growing on saline soils is not high and is presented by typical halophytes and salt resistant species.

Though the halophylic plants make only 2% of the total terrestrial plant species, their investigation becomes more and more popular, because the natural resources of halophytes may serve as a source of medicinal and oil raw material and as ammelioratiors of the arid soils (Dajic, 2006; Atia *et al*, 2011). According to the literary data halophytes may cultivataed for nutritional purposes, also for phytoremediation of alkali or polluted with heavy metals soils (Manousaki, Kalogerakis, 2011; Ventura, Sagi, 2013).

Since halophytes possess a wide spectrum of mechanisms of morphological, physiological and biochemical adaptations, corresponding to their degree of salt resistance, investigation of the mechanisms of adaptivity to salt stress is one of the approaches for their perception.

Nowadays it is generally accepted that formation of active oxygen species (AOS) is an usual reaction of plant to abiotic stress (Bartels, Sunkar, 2005). The antioxidative system, which consists of enzymes and low-molecular antioxidants, is responsible for the protection of plants from the oxidative stress (Sharma *et al*., 2012).

The total area of saline soils in Georgia is about 112 thousand ha (Urushadze, Blum, 2014). Inspite of dimentions the physiology and biochemistry of plants inhabiting those territories hasve not been studied yet. Thus, investigation of halophylic vegetation of Georgia is necessary and in some extent even obligative. Obtained knowledge may be used for their popularization and application in future, taking into account the fact that some of halophytes have been used as food and for medical purposes since ancient time (Lieth, 2000; Ventura, Sagi, 2013).

The purpose of the given study was to investigate characteristics of antioxidant system and other biochemical indices of some salt resistans species growing on saline soils of Georgia.

Obtained data demonstrate the general picture of adaptation to salt stress of investigated species and enrich the knowledge about their nutritional and medicinal value as well, which may be taken into account for cultivation of these plants for agricultural and soil amelioration purposes in future.

Materials and methods

Activity of antioxidant enzymes (peroxidase and catalase) and low-molecular antioxidants (proline, ascorbic acid, soluble phenols, antocyanins, carotenoids), also activity of nitrate reductase, content of total proteins, chlorophylls and soluble carbohydrates has been investigated in leaves of salt resistant plants growing coastwise and in surroundings of Kumisi Lake. This saline lake is situated in East Georgia, on the Soth-East from the village Kumisi, at 475m above sea level. Soils in surroundings of the lake are gray-brown, brackish, with chloride-sulfate and sulfate-cloride-sodium salinity (Urushadze, Blum, 2014).

Plant species with different salt exchange mechanisms were taken as test objects: salt accumulating halophyte *Salsola soda* L. - opposite-leaved saltworth, salt releazing halophytes: *Tamarix ramosissima* Ledeb. – salt cedar and *Chenopodium album* L. – goosefoot, also salt unpermeable halophytes: *Artemisia lerchiana* (Web .) – sagebrush, *Achillea biebersteinii* (Afan.) – allheal and *Adonis bienertii* (Butkov ex Riedl.) – peasant's eye. Plant material was picked at the intensive growth phase – the end of May and in June. For comparative analysis one and the same plant species were collected at coastwise and in surroundings of Kumisi Lake. In particular, intensively (I), moderately (II) and weakly (III) salinated zones were separated, where the experimental species were taken. Intensively salinated soils were situated within a radius of 20m from the coast line (total content of soluble salts 4.8%).

Moderately salinated zone was with in a radius of 50-60m (total content of salts 3.5%). Weakly salinated soils were with in a radius of 80-100m (total content of salts 0.8%). Soil acidity was slightly alkaline (pH7-7.5). For analysis were picked fully expanded leaves from 5-7 plants. Analyses were made in three biological replicates.

Total concentration of water-soluble salts in soil

Soil samples of 20cm height were dig out from the surface layers of Kumisi Lake experimental sites. Tested samples of soil were mixed with distilled water, filtered and after evaporation of the filtrate, residue of water soluble salts was determined gravimetrically (Radov *et al.*, 1978).

Enzymes

Peroxidase activity was determined spectrophotometricaly: optical density of the products of guaiacol oxidation was measured at the wave length of 470nm (Ermakov, 1987).

Catalase activity was studied gasometricaly: volume of the oxygen released in the process of reaction between hydrogen peroxide and enzyme was measured (Pleshkov, 1985).

Method of determining the nitrate reductase activity was based on measurement of nitrites amount, which were formed as a result of nitrate reductase reaction with the infiltrated nitrates (Ermakov, 1987).

Ascorbic acid

A titration method was used to measure the content of ascorbic acid. 2 g of fresh leaf material was mashed in 15 ml of 2% hydrochloric acid and 10 ml of 2% metaphosphoric acid, and filtered. One ml of the filtrate was added to 25 ml of distilled water and titrated with a 0.001 M solution of dichlorphenolindophenole (Ermakov, 1987).

Proline

0.5 g of dry leaves were mashed in 10ml of 3% sulphosalicylic acid and filtered. 2 ml of the filtrate was added to 2 ml of acid ninhydrin and 2 ml of ice acetic acid. After 1 h exposition on a water bath the extract was cooled and added with 4 ml of toluene and divided in a separating unnel. Optical density of upper layer was measured on a spectrophotometer (SPEKOL 11, KARL ZEISS, Germany) at 520 nm (Bates *et al.*, 1973).

Anthocyanins

100mg of grinded leaves were added with 20 ml of 96% acidified (with 1% HCl) ethanol (99:1). After 24h retention in dark the optical density at 540nm was measured (spectrophotometer SPEKOL 11, KARL ZEISS, Germany) (Ermakov, 1987).

Plastid pigments

Chlorophyls and carotenoids were determined spectrophotometricaly. Fresh leaves (100-200mg) were mashed with sand and $CaCO_3$ and washed with ethanol. Optical density of the filtrate was measured (spectrophotometer SPEKOL 11, KARL ZEISS, Germany). Concentration of chlorophylls a and b, also carotinoides was calculated by the formula of Wintermanns (Gavrilenko *et al.*, 1975).

Total phenols

A 0.5 g of fresh leaves was boiled in 80% ethanol for 15 min. After centrifugation the supernatant was saved, and residues of leaves were mashed in 60% ethanol and boiled for 10 min. Obtained extract was added to the first supernatant and evaporated. The sediment was dissolved in distilled water. One ml of the received solution was added with the Folin-Ciocalteu reagent and optical density was measured at 765 nm. The chlorogenic acid served as control (Ferraris *et al.*, 1987).

Total protein assay

Content of proteins was determined after Lowry (1951).

Soluble carbohydrates

Content of soluble carbohydrates was tested with anthrone reagent (Turkina, Sokolova, 1971). To 100mg of air-dry leaf material was added 96° alcohol for extraction (3-fold extraction).

The total amount of the obtained extract was evaporated on a water bath and dissolved in 5ml of distilled water. To 0.5ml of the tested water extract was added 2ml of anthrone reagent and heated in a water bath for 10min. After this procedure the test-tubes were placed in a cold water bath and 15min later the optical density of the solution was measured at 620nm with a spectrophotometer (SPECOL 11, KARL ZEISS, Germ-any).

Statistical analysis

One way ANOVA and Tukey's multiple comparison tests were used to analyse differences between the means. All calculations were performed using statistical software Sigma Plot 12.5. Mean values and their standard deviations are given in tables.

Results and discussion

Catalase and peroxidase

Main substrates for antioxidant enzymes – catalase and peroxidase are superoxide radical and hydrogen peroxide (Garifzyanov *et al.*, 2011). Hydrogen peroxide is formed in several subcellular components like chloroplasts, peroxisomes and mitochondria Correspondingly, beside the main locality of catalase (CAT, EC 1.11.1.6) in peroxisomes and glioxisomes (in relation with photorespiration), its special isoforms were discovered in mitochondria and chloroplasts as well (Mhamdi *et al.*, 2010).

Peroxidases (EC 1.11.1) represent a big group of enzymes, which are met in all parts of the cell and have different functions in plant metabolism (Passardi *et al.*, 2005). They take an active part in detoxication of hydrogen peroxide, which was formd as a result of stress; regulate amount of auxins and phenols during plant growth (Cevahir, 2004; Graskova, 2010). More over, peroxidases of cell wall take part in formation of reactive oxygen species which play a signaling function under some stresses by activation the protective mechanisms of plant (Mika *et al.*, 2004).

Incease of the activity of peroxidase and catalase under the high concentrations of salt is one of the demonstrations of adaptivity to salt stress (Zhang *et al.*, 2013; Bagheri, 2014).

Among the experimental plants growing on the intensively salinated zone (I) comparatively high ativity of peroxidase was mentioned in leaves of salt unpermeable sagebrush, also in crinohalophyte goosefoot and euhalophyte saltworth (table 1). Increase of soil salinity caused activation of peroxidase almost in all studied species (by 53% in saltworth, 41% - salt cedar, 8% - goosefoot, 44% - sagebrush, and 45% - peasant's eye, $P<0.001$) (table 1).

Salinization caused activation of catalase in leaves of all experimental species except saltworth and goosefoot. In the last ones the activity of the enzyme incontrary decreased (27%, $P<0.001$ and 10.6%, $P=0.001$ respectively). The result may be connected with the C4 type of photosynthesis of these plants. It is known that photorespiration in C4 plants is significantly lower compared with C3 plants (Edwards *et al.*, 2001). Accordingly, activity of enzymes, among them of catalase, associated with the process, is lower in C4 plants (Li *et al.*, 2001; Ueno *et al.*, 2005). Decrease of catalase activity in leaves of saltworth and goosefoot may be the demonstration of additional abation of photorespiration in these C4 plants, which is directed to save photosyntates. More over, it is established that among the biochemical subtypes of C4 plants (NADP-malic enzyme, NAD-malic enzyme and PhEP-carboxsycinase) there exist differences by the number of mitochondria and peroxisomes in bundle sheath cells. In NADP-malic enzyme type both organelles exist in small amount, while grans in chloroplasts in this biochemical subtype are weakly developed and photorespiration is reduced. This is explained by depressed release of O2 (weak grans means weak PS2, i.e. reduced release of O2), that from its side means depression of the oxigenase activity of Rubisco (Ueno *et al.*, 2005). Thus, it may be supposed that high concentration of salt depresses oxigenase activity of Rubisco in leaves of the mentioned C4 plants. As a result, photorespiration and correspondingly catalase activity decrease.

According to our observations it may be supposed that activation of enzymes of antioxidant system (peroxidase and catalase) is one of the mechanisms of adaptation to salt stress in experimental plants.

Nitrate reductase (NR, E.C. 1.6.6.1) is a significant enzyme of nitrogen metabolism and plant development. Accordingly, by its activity one can judge about plant growth and development, and productivity as well (Garg, Singla, 2005). High level of salinity because of osmotic stress causes ion toxicity and nutritional disbalance in plants. It negatively affects nitrogen metabolism as well. The reason for it may be disorder in NO_3^- anions uptake, or their reduction delay, connected with nitrate reductase activity (Sergeichik, Sergeichik, 1988). Inhibitory effect of salinization on nitrate reductase activity was documented by many authors (Khan, 1996; Garg *et al.*, 1997). In our observations with the rise of salinization level activity of the enzyme increased almost in all experimental palnts (by 6-38%) (Table1). Facultative halophytes allheal and peasant's eye were exclusion. The last grew only on moderately and weakly salinated places and evidently soil salinization negatively influenced its nitrogen metabolism. Nitrate reductase activity of peasant's eye diminished by 37% (compared to weakly salinated zone) while the enzyme activity of allheal leaves from intensively salinated places decreased slightly (by 8%) (compared with weakly salinated zone) (Table1).

Table 1. Activity of catalase, peroxidase and nitrate reductase in leaves of halophytes from saline soils of Kumisi Lake.

Plant	Peroxidase activity, conditional unit per g fresh weight			KCatalase activity, см³ O_2 /min per g fresh weight			Nitrate reductase activity, γ NO2 in 30min per g fresh weight		
	I zone	II zone	III zone	I zone	II zone	III zone	I zone	II zone	III zone
Salsola soda L.	9.11±0.14^a	5.92±0.14^b	---------	10.16±0.29^a	14.01±0.04^b	---------	14.27±0 .31^a	10.30 ± 0.30^b	----------
Tamarix ramosissima Ledeb.	7.34±0.06^a	5.19±0.04^b	---------	19.01±0.04^a	17.00±0.05^b	---------	16.73± 0.20^a	16.26 ±0.20^a	---------
Chenopodium album L.	10.08±0.09^a	9.31±0.04^b	---------	13.67±0.15^a	15.30±0.30^b	---------	22.53± 0.31^a	20.68 ± 0.34^b	---------
Artemisia lerchiana (Web.)	12.63±0.03^a	8.79±0.03^b	6.70±0.02^c	15.09±0.18^a	14.05±0.13^b	11.17±0.15^c	12.77± 0.17^a	10.63 ± 0.31^b	6.74± 0.38^c
Achillea biebersteinii (Afan)	7.13±0.07^a	7.83±0.03^b	4.04±0.02^c	17.17±0.15^a	16.33±0.31^b	16.60±0.20^{ab}	12.30± 0.30^a	13.37 ± 0.25^b	15.18± 0.36^c
Adonis bienertii Butkov ex Riedl.	--------	0.64±0.02^a	0.44±0.03^b	-------	20.03±0.25^a	19.05±0.13^b	--------	4.27 ± 0.31^a	6.77 ±0.42^b

Different letters indicate to significant differences between mean values of a particular index of the given species $p<0.05$ (according to Tukey's test).

According to experimental data it may be concluded that those experimental species, where the activation of nitrate reductase with the growth of salinization level was mentioned, seem to be well adaped to salinity stress and regulate nitrogen metabolism respectively to normal growth and development.

Inspite of the central role of enzymes in detoxication of ROS, it must be mentoned that the enzymatic antioxidant system is not able to avoid the cell damadge fully (Polesskaya *et al.,* 2006). That's why it is supposed that low molecular antioxidants often appear to be more effective in protection of metabolism against ROS (Blokhina *et al.*, 2003; Radiukina *et al.,* 2012).

Ascorbic acid

L-ascorbic acid (AA) or vitamin C is an important metabolite both, in plants and animals. According to the latest data becides the signal function AA protects plants against different stressors (heavy metals, salinization, temperature, UV-irradiation etc.) by switching on corresponding genes (Vwioko *et al.*, 2008; Zhang *et al.*, 2012).

Positive role of AA in adaptation to the salt stress has been proved by many authors (Khan *et al.,* 2010; Abou-Leila *et al.*, 2012). According to experimental data increase of resistance to salt stress is due to rise of AA content in plants (Hemavathi *et al.*, 2010; Zhang *et al.,* 2012).

From the obtained results is clear that the ascorbate pool of experimental species was not high, except peasant's eye and goosefoot. Content of AA in most species significantly decreased (almost two fold) with the growth of soil salinization level. Only salt cedar and peasant's eye made exception (increased 1.5 fold) (Table 2).

Table 2. Content of ascorbic acid, proline and soluble carbohydrates in leaves of halophytes from saline soils of Kumisi Lake.

Plant	Proline, μmol/g dry weight			Ascorbic acid, mg% fresh weight			Soluble carbohydrates, mg/g fresh weight		
	I zone	II zone	III zone	I zone	II zone	III zone	I zone	II zone	III zone
Salsola soda	7.30±0.44a	2.34±0.04b	----------	30.34±0.36a	75.60±0.40b	----------	4.82±0.31a	9.71±0.14b	----------
Tamarix ramosissima	8.86±0.09a	1.75±0.04b	----------	91.22±0.50a	63.40±0.62b	----------	10.22±0.03a	10.87±0.16b	----------
Chenopodium album	6.47±0.15a	6.60±0.10a	----------	49.36±0.40a	105.36±0.50b	----------	8.66±0.14a	15.51±0.25b	----------
Artemisia lerchiana	65.70±0.32a	76.60±0.46b	74.13±0.15c	29.64±0.10a	58.23±0.32b	51.43±0.50c	16.36±0.27a	17.71±0.14b	15.43±0.39c
Achillea biebersteinii	66.90±0.15a	75.60±0.36b	135.60±1.81c	50.33±0.31a	57.30±0.44b	62.46±0.45c	22.22±0.30a	23.98±0.22b	15.45±0.13b
Adonis bienertii	----------	21.27±0.38a	2.48±0.09b	----------	257.9±1.02a	175.5±0.46b	---------	24.67±0.11a	5.27±0.13b

Different letters indicate to significant differences between mean values of a particular index of the given species $p<0.05$ (according to Tukey's test).

Since AA plays the role of the main antioxidant in the process of photosynthesis (Foyer, 1993), its content ablation in the studied species may be explained by the synergic and intensive affect of several stressors on the photosynthetic apparatus, like intensive solar irradiation, high temperature, and soil salinization (under these environmental conditions did the experimental plants grow). Such conditions would undoubtly induce increase of AOS in leaves, and first of all, in the photosynthetic apparatus.

If we remember the significant role of AA in the process of photosynthesis (photoprotective (co-substrate in zeaxanthin synthesis), substrate of ascorbate peroxidase and electron sink in ETC through the Mehler reaction), it becomes clear that polygonal loading on the AA could diminish its pool among the studied species. Presumably, the compensatory mechanism of AA degradation must exist in studied plants (the enzymatic antioxidant system may be considered here), otherwise the investigated species would not be so effectively adapted to saline conditions (Davey *et al.*, 2000).

According to the obtained data it may be supposed that in two investigated species – salt cedar and peasant's eye – one of the mechanisms of adaptation to saline conditions is ascorbate-gluthathione cycle. This explains the increase of AA synthesis in the named species with the growth of soil salinity.

Proline and soluble carbohydrates

Accumulation of ptoline and water soluble carbohydrates in cell is one of the effective mechanisms of physiological adaptation to salinity (Kafi *et al.*, 2003). Simple sugars and proline, which accumulate in plants under the influence of different stressors, are widely spread universal osmotics. They protect the protein-lipid components of the membrane against denaturation in case of dehydration (Franko and Melo, 2000; Szabados, Savoure, 2010).

Free proline reveals polyfunctional effect under stress conditions. Becides osmoregulatory function it has antioxidant, energetical, protein-stubilizing and other functions, responsible for the support of cell homeostasis (Kuznetsov *et al.*, 1999; Anjum *et al.,* 2000; Kartashov, 2013). It is considered that proline plays the role of a metabolic signal, which regulates the metabolite pool, gene expression and influences plant growth and development (Szabados and Savoure, 2009).

Carbohydrates stimulate water absorbing capacity of cells from the saline soil by reducing their water potential (Eriomchenko *et al.*, 2013).

Though soluble carbohydrates are associated with those metabolic pathways, which induce formation of AOS, they play a siginificant role in neutralization of AOS as well (Couee *et al.*, 2006). Some authors mention accumulation of soluble carbohydrates in a various parts of the plant in respond to different stresses (Prado *et al.*, 2000; Finkelstein and Gibson, 2001; Mohammadkhani and Heidari, 2008).

It is established that metabolism of soluble carbohydrates under stress conditions is a dynamic process combining both, reactions of synthesis and degradation (Hilal *et al.*, 2004).

Changes in salinizaton degree diversly influenced content of proline and soluble carbohydtares in leaves of tested plants. Differences were revealed between glyco-, eu- and crynohalophytes by these indices.

In glycohalophytes growth of salinization level stimulated accumulation of soluble carbohydrates, while in leaves of cryno- and euhalophytes proline accumulation was evident. The highest level of carbohydrates was revealed in allheal, peasant's eye, and sagebrush (Table2). Content of proline in leaves of saltworth and salt cedar on the first (intensively salinated) zone significantly exceeded the results obtained for the second (moderately salinated) zone (3 and 8 times respectively).

Increase of proline content and degradation of soluble carbohydrates level in leaves of saltworth in response to salinization was demonstrated by other authors as well (Teimouri *et al.*, 2009). Significant growth of proline content was revealed also in leaves of peasant's eye (10 times) (Table 2). Decrease of proline amount by the salinization gradient was evident in glycohalophytes – allheal and sagebrush.

It must be mentioned that inspite of significant gaining of proline content in leaves of above mentioned plants, its pool was evidently smaller compared with glykohalophytes. This may be the indication to different protective mechanisms against salinization.

It is well known that glycohalophytes impair uptake of extra salt already by root cells, while euhalophytes concentrate the absorbed salts in vacuole, and crynohalophytes let them pass freely (Eriomchenko *et al.*, 2013). Thus, glycohalophytes need high concentration of osmolits for water uptake.

This is reached by high concen-trations of proline and increased synthesis of soluble carbohydrates. Proline accumulation in case of salin-ization may be associated not only with osmore-gulation, but also with its antioxidant and protein-stabilizing function (Ben Ahmed *et al.,* 2010; Kartashov, 2013).

Thus, accumulation of proline in leaves of saltworth and salt cedar and increase of soluble carbohydrates in studied glycohlophytes is one of the mechanisms of adaptation to salt stress.

Anthocyanions

These substances of phlavonoid group are concen-trated in vacuole and reveal strong antioxidative properties (Kahkonen & Heinonen, 2003).

Accumulation of anthocyanins in vacuole blocks their direct contact with the localities where the AOS are formed. Nevertheless, accumulation of anthocyanins in cases of different stresses has been established (Mobin & Khan, 2007). Anthocyanins play a sugnificant protective role in adaptation of plants to salt stress, by means of neutralization of free radicals (Gould, 2004).

Since most of unfavorable conditions are somehow associated with water stress, some authors express an opinion about the osmoregualtive function of anthocyanins in plant cell (Chalker-Scott, 2002).

According to our observations, soil salinizaition (I and II zones) stimulated synthesis of anthocyanins in glycohalophytes (Table 3). Intensive salinization had no effect upon anthocyanins content in leaves of saltworth and salt cedar (Table 3).

Table 3. Content of soluble phenols, asnthocyanins and total proteins in leaves of halophytes from saline soils of Kumisi Lake.

Plant	Soluble phenols, mg/g fresh weight			Anthocyanins, mg/g fresh weight			Total proteins, mg/g fresh weight		
	I zone	II zone	III zone	I zone	II zone	III zone	I zone	II zone	III zone
Salsola soda	5.17±0.04a	3.40±0.02b	----------	3.36±0.05a	3.21±0.05b	----------	51.25±0.33a	39.69±0.09b	----------
Tamarix ramosissima	7.07±0.04a	8.53±0.06b	----------	5.85±0.05a	5.91±0.05a	----------	69.05±0.06a	44.76±0.03b	----------
Chenopodium album	4.06±0.03a	4.81±0.15b	----------	6.65±0.04a	8.06±0.03b	----------	57.21±0.04a	47.20±0.05b	----------
Artemisia lerchiana	10.77±0.06a	7.98±0.03b	7.77±0.03c	11.67±0.04a	15.14±0.03b	8.84±0.03c	88.26±0.07a	77.61±0.03b	74.39±0.03c
Achillea biebersteinii	8.98±0.02a	8.63±0.05b	4.75±0.10c	10.09±0.08a	19.32±0.03b	8.12±0.03c	72.29±0.27a	71.33±0.04b	59.20±0.06c
Adonis bierentii	----------	7.86±0.05a	6.05±0.04b	----------	16.77±0.07a	8.25±0.06b	--------	85.60±0.03[a]	63.73±0.03b

Different letters indicate to significant differences between mean values of a particular index of the given species $p<0.05$ (according to Tukey's test).

According to literary data ascorbic acid is the cofactor of the enzymes taking part in synthesis of anthocyanins (Gallie, 2013). Though, evident relations between content of ascorbic acid and synthesis of anthocyanins in our observations have not been revealed.

Experimental results have once again cleared the difference between glycohalophytes and eu- and crynohalophytes, for this time expressed in variation of anthocyanins content in reply to stress. Increase of anthocyanins synthesis in glycohalophytes may be associated with the physiological mechanisms of protection from high concentration of salts. Presumably, glycohalophytes are more sensitive to water deficiency under saline conditions, because of hard accessibility of water from the soil, compared to eu- and crynohalophytes. Stimulation of the synthesis of anthocyanins could serve as one of the means of protection of the photosynthesis apparatus against stress.

Total phenols

Phenolic compounds are considered to be the most active metabolites in plants. They are components of ETC, connected with photosynthesis and respiration, play an important role in regulation of growth and development, in particular in synthesis of lignins and pigments. By their antioxidative properties most of phenolic substances overpass even ascorbic acid and tocopherols. (Cesar, Fraga, 2010; Bhattacharya *et al.*, 2010). Phenols neutralize the AOS earlier than the last manage to damage the cell (Lovdal *et al.*, 2010). Significant rise of phenolic compounds in reply to different stress conditions, among them to salinization, has been established (Winkel-Shirley, 2002; Ksouri *et al.*, 2007; Rezazadeh *et al.*, 2012). In particular, phenols affect membrane permeability, absorbtion and transport of ions, synthesis of proteins and DNA. Moreover, phenols protect the membrane lipids from oxidation i.e. reveal protective function in case of salt stress (Kusakina *et al.*, 2011).

Investigation of soluble phenols in leaves of experimental plants has shown that intensive salinization induced activation of the synthesis of phenolic substances in all studied glycohalophytes and in euhalophyte saltworth (Table 3). Decrease of the index was observed only in salt cedar and goosefoot (by 13-14%, $P<0.001$).

Presumably, the clear picture of accumulation of the phenolic substances in glycohalophytes is associated with different from other plants mechanism of salt exchange. High concentration of salts serves as a signal for activation of genes responsible for synthesis of osmoregulators (Kartashov *et al.,* 2008; Kosova *et al.*, 2013). Low water potential of cells in salt unpermeable glycohalophytes is reached by accumulation of soluble carbohydrates. Taking into account the relationship between fructose and synthesis of phenolic compounds (Hilal *et al.,* 2004), it may be supposed that the phenilpropanoid way, responsible for increase of the total phenols content, is activated in glycohalophytes. In salt cedar and goosefoot, which freely conduct salt ions,

their concentration appeared to be toxic for the synthesis of phenols. This from its side must be associated with the activity of photosynthesis apparatus.

Chlorophylls and carotenoides

Content of chlorophylls and carotenoids may be used as one of the criteria for evaluation of the activity of photosynthetic apparatus (Lichtenthaller, Busch-mann, 2001). It is established that high concentration of salt ions changes functioning of the photosynthetic apparatus, tightly connected with the activity of pigment-protein-lipid complex and organization of the thylacoid membrane (Orlova, 2009; Rozentsvet *et al.,* 2013; Kuznetsova *et al.,* 2014).

Opinions about the influence of salinity on the content of chlorophylls are controversial (Agrawal, Shaheen, 2007; Munns and Tester, 2008; Orlova, 2009; Boestfleisch *et al.*, 2014).

Quantitative studies of chlorophylls and carotenoids in leaves of experimental plants revealed differences by the content and ratio of photosynthetic pigments between halophytes with different mechanisms of salt regulation (Table 4). In particular, in leaves of obligate halophyte – saltworth and crynohalophytes – salt cedar and goosefoor content of chlorophylls significantly exceeded the results obtained for glycohalophytes (Table 4). Moreover, with enhancement of salinity, amount of chlorophylls increased in leaves of crynohalophytes (salt cedar – 34%, goosefoot – 11%, $P<0.001$). Though in saltworth this index decreased (22%, $P<0.001$), it significantly overpassed the results of glycohalophytes. In leaves of sagebrush, growing in intensively salinated zone total amount of chlorophylls increased (by 12%, $P<0.001$, compared with middle salinated zone), while in leaves of allheal and peasant's eye – it decreased by 49% and 9% ($P<0.001$) respectively (Table 4).

Table 4. Content of chlorophylls and carotenoids in leaves of halophytes from saline soils of Kumisi Lake.

Plant	Chlorophylls, mg/g fresh weight			Carotenoids, mg/g fresh weight			Carotenoids/Chlorophylls		
	I zone	II zone	III zone	I zone	II zone	III zone	I zone	II zone	III zone
Salsola soda	3.15±0.03a	4.06±0.03 b	----------	0.87±0.03a	1.11±0.02b	----------	0.27±0.01	0.27±0.01	----------
Tamarix ramosissima	5.15±0.05a	3.83±0.03b	----------	1.21±0.02a	1.05±0.02b	----------	0.23±0.02	0.27±0.02	----------
Chenopodium album	4.94±0.06a	4.44±0.02b	----------	1.22±0.03a	1.12±0.02b	----------	0.24±0.01	0.23±0.01	----------
Artemisia Lerchiana	2.72±0.04a	2.81±0.03b	2.43±0.03c	1.12±0.02a	1.12±0.02b	0.98±0.03c	0.40±0.0	0.40±0.01	0.41±0.01
Achillea biebersteinii	1.75±0.02a	2.75±0.05b	2.97±0.03c	0.73±0.03a	1.15±0.03b	1.22±0.03b	0.42±0.01	0.41±0.01	0.41±0.01
Adonis bienertii	----------	2.04±0.03a	2.23±0.02b	----------	0.84±0.02a	0.89±0.03a	----------	0.41±0.01	0.40±0.01

Different letters indicate to significant differences between mean values of a particular index of the given species $p<0.05$ (according to Tukey's test).

Content of carotenoids in leaves of all studied species did not differ significantly. With the growth of salinization degree their amount decreased in saltworth and allheal (by 21.5% and 36.5% respectively, $P<0.001$), while in salt cedar and goosefoot - slightly increased (by 15% and 9% respectively, $P<0.001$ and $P=0.01$) (Table 4).

Ratio of chloropylls to carotenoids (which reflects the proportion of the reactive center of the photosynthetic apparatus to the light harvesting complex) is a significant index as well, giving some information about the functional condition of the photosynthetic apparatus (Kartashov, 2013; Rozentsvet *et al.*, 2013; Kuznetsova *et al.*, 2014).

From the obtained results it is clear that in studied glycohalophytes this ratio was two times higher compared to the same index of eu- and crynohalophytes (Table 4). It may be supposed that increase of carotenoids concentration in the pigment complex contributes to protection of chloroplasts from photooxidation and negative effect of free radicals (Fiedor, Burda, 2014).

Experimental results make possible to conclude that the pigment system of eu- and crynohalophytes is stronger compared to glycohalophytes. Partialy this may be connected with their type of photosynthesis. Mighty pigment system is indication to the intensive photochemical processes,

implying active accumulation of energy. Presumably, significant part of the energy, stored in light reactions, is spent for retention of the ion exchange and detoxication, subjected to the type of salt balance.

Total proteins

Metabolism of nitrogen-containing substances, and first of all proteins plays a key role in halophytes metabolism (Orlova *et al.*, 2007).

High content of proteins was mentioned in leaves of studied glycohalophytes (sagebrush, allheal, peasant's eye), in comparision with salt-accumulating saltworth and salt-extracting halophytes (salt cedar, goosefoot). Increase of soil salinization degree played a role of additional stimulus for proteins accumulation in leaves of the studied plants (Table 3) (following the order in the table by 29%, 54%, 21%, 19%, 22% and 34% respectively, $P<0.001$).

Presumebly this is one of the adaptive reactions of glycohalophytes to salinization. Water soluble proteins increase protoplasm resistance and thank to their hydrophilic properties, increase cell water-holding ability and content of bound water (Orlova *et al.,* 2007).

According to literary data in the process of adaptation to unfavorable environmental conditions synthesis of characteristic for the cell proteins is swiched to activation of stress-proteins formation. The last ones protect cell structures from oxidation stress (Gill, Tuteja, 2010; Suzuki *et al.*, 2012). It may be supposed that with the rise of soil salinization together with other adaptive processes, taking place in studied plants, synthesis of stress proteins occurs, which plays a protective role.

Conclusions

Obtained data clear that halophytes growing on saline soils of Kumisi lake surroundings use diverse ways for adaptation to soil salinization.

Differences between halophyte species with various type of salt exchange were revealed while studing the influence of salinization degree on idices of antioxidant system.

In particular, activation of catalase and peroxidase, increase of ptoline and total proteins content in leaves of eu- and cryno-halophytes (saltworth, goosefoot, salt cedar – all are C_4 plants) with the rise of salinization level was mentioned. Together with activation of catalase and peroxidase, increase of anthocyanins, soluble phenols, total proteins and soluble carbohydrates was evealed in leaves of glycohalophytes (sagebrush, allheal, peasant's eye) under the same conditions.

Experimental results prove the fact that existence of various antioxidants in plants and interchangebility of their functions rise the reliability of the total defence system and makes possible to compensate any disorders in functioning of its components. Functioning of the compensatory mechanism between low molecular antioxidants and enzymes of the antioxidants system is acceptable (Kartashov *et al.*, 2008). For e.g. in most studied species activity of the glutathione-ascorbate cycle was inhibited, in some species the pool of phenolic compounds was small (salt cedar, goosefoot), in others catalase activity was inhibited (saltworth, goosefoot), etc. In spite of this all plants demonstrated active metabolism under the given conditions and successfully ended their life cycle.

Increase of peroxidase activity and content of total proteins in leaves of the experimental plants may be considered as the uniting mechanism of adaptation to salinization of the studied species.

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