Biochemical Strategy of Drought Resistance of Dry Habitat Plants of Georgia

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Abstract**—***The climatic cataclysms taking place in different corners of the world are clear confirmation of the climate negative change in our planet. Especially disturbing is the temperature rise, accompanied by hot waves, forest fires, intensive melting of the ice cover, and other undesirable aftereffects. Under these conditions chances of dying of many plant species significantly increases. Drought resistant plants have the highest potential of adaptation to increased temperature and water deficiency. Thus, knowledge of their biology will be especially important in deserted regions restoration. The nonspecific mechanisms of resistance, especially antioxidant system, are concerned as one of the leading in plants drought resistance. The presented study aimed the comparative study of the indices of antioxidant system of drought resistant species* - *Astragalus microcephalus Willd. (Astracantha microcephala (Willd.) Podlech)* - *goat's thorn, Theucrium polium L. – felty germander, Euphorbia seguieriana Neck.* - *spurge, Capparis spinosa L. – caper bush, Paliurus spina-christi Mill. – Christ's thorn, growing in different arid habitats of the East Georgia (Iagludja and Kvernaqi hills). The defence mechanisms of the antioxidant system appeared to be partially different in one and the same species of various habitats, as well as in different species of the same habitats. Activation of phenolic substances and anthocyanins synthesis against extreme conditions of both habitats (water deficiency, high temperature and intensive irradiation) was common for all tested species. Additionally, activation of peroxidase in Kvernaqi species and intensive accumulation of soluble carbohydrates in Iagludja plants was mentioned.*

Keywords—antioxidants, drought resistance, Georgia.

I. INTRODUCTION

The latest scientific documentations demonstrating evident negative changes all over the world has been presented in the preliminary report of the world meteorological organization on the climate global situation in 2021. Especially disturbing is the temperature rise, accompanied by hot waves, forest fires, intensive melting of the ice cover, and other undesirable aftereffects. According to data last seven years were regarded as the hottest through the whole history of climate observation (WHO report, 2021).

Climate warming significantly rises the risk of plants dying off under the increased stresses (Allen et al., 2015; Overpeck and Udall, 2010; McDowell et al., 2008). Presumably the area of distribution of many plant species will change. The migration rate will depend on species features, competition, climate conditions, etc. (Garamvolgyi and Hufnagel, 2013) Drought resistant species will have the highest potential of adaptation to increased temperatures and accompanying water deficiency. Thus, the knowledge of their biology will be very important in the restoration of deserted areas; moreover, most of them are used in medicine

Drought resistant plants possess evolutionary developed physiological and biochemical mechanisms of stability against water deficiency and high temperature. The nonspecific mechanisms of resistance are regarded as one of the principle under stress conditions; the antioxidant system is of special importance among them (Li, and Liu, 2016; Laxa et al., 2019).

Characteristics of the antioxidant system of drought resistant plants of the arid territories of Georgia are practically unexplored.

The presented work aimed comparative studying of some characteristics of drought resistant species growing at different arid habitats of east Georgia (Yagludja and Kvernaqi hills**).** The study may be regarded as the continuation of previous year's investigations (Badridze et al., 2021).

Content of ascorbic acid, tocopherol, carotenoids, anthocyanins, soluble phenols, proline, total proteins and soluble carbohydrates, as well as the activity of catalase, peroxidase, nitrate reductase and the total antioxidant activity in percents of inhibition have been studied in leaves of experimental plants,

II. MATERIALS AND METHODS

2.1 Research area

Experimental species were collected in July of 2020-21, at two different arid habitats of East Georgia – Iagludja hill (Gardabani municipality) and Kvernaqi hill (Kaspi municipality).

Iagludja hill is situated in Kvemo Kartli (lower Kartli), on the north-east of Marneuli plane, near the city Rustavi. The climate here is continental, with mild winter and hot, dry summer. The mean annual temperature is 12^o-13^oC; in the coldest month – January the mean temperature is 0.3^o-0^oC. Especially hot is July and August. Absolute minimal temperature is minus 20^o-25^oC and maximal – 40^o-41^oC. The mean annual amount of precipitations is 350-500mm (Kordzakhia and Djavakhishvili, 1971).

Most part of Marneuli municipality soils are degraded to various degrees; this is clear from the worsening of their physical and mechanical, chemical and microbiological properties and fertility decrease. Degradation of a plant cover resulted in a formation of clay-rich, low-humic, calcareous grey-brown soils (Official web-page of Marneuli municipality).

Kvernaqi hill is situated in Shida Kartli (inner Kartli), near the city Kaspi. Climate here is transitional from subtropical to humid. The winter is moderately cold, summer is dry and hot. The mean annual temperature of air is 11.4^oC, in January - minus 0.5^oC, in August – 23^oC. The absolute minimal temperature is minus 27^oC, and absolute maximal – 40^oC. Annual amount of precipitations makes 450mm. On the south slope of Kvernaqi hill (where the material was collected) the radiation is intensive in summer; that is why the air temperature here is higher compared to the northern slope. In spite of comparatively high precipitations in Kvernaqi, compared to central regions of Shida Kartli plane, their efficiency here is low; because of it the soil is dryer. Kvernaqi hill is constructed of Neogenic conglomerates, sandy-gravel shales. In Kotsakhura gorge, where the material was picked, soils are alluvial, here and there rocky and gravel (Kordzakhia and Djavakhishvili, 1971; Ukleba, 2018).

2.2 Experimental plants

Middle age, mature, healthy leaves were collected at least from 5 different individuals of each experimental species: *Astragalus microcephalus* Willd. (*Astracantha microcephala* (Willd.) Podlech) - goat's thorn, *Theucrium polium* L. – felty germander, *Euphorbia seguieriana* Neck. - spurge, *Capparis spinosa* L. – caper bush, *Paliurus spina* Mill. – Christ's thorn. Material was taken at 530-395m above sea level; in fruit bearing phase, during the hottest period for these locations (38^o-40^oC). Analyses were performed both on raw and dry material, with 3-fold repetitions.

2.3 Biochemical assays

2.3.1 Antioxidant enzymes assay

Peroxidase activity was determined spectrophotometrically: optical density of the products of guaiacol oxidation was measured at the wave length of 470nm by the spectrophotometer (SPEKOL 11, KARL ZEISS, Germany) (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).

2.3.2 Nitrate reductase assay

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).

2.3.3 Ascorbic acid

A titration method was used to measure the content of ascorbic acid in plant material. 2 g of fresh leaves were 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).

2.3.4 Tocopherol

Two grams of ground leaves were extracted with 20-25ml of pure ethanol (three-fold). The combined extract was mixed with 20 ml of 60% potassium hydroxide, and saponificated on water bath for 2h. Tocopherol was extracted from the obtained hydrolyzate using diethyl-ether (3-fold extraction). The combined extract was washed with distilled water until a complete removal of alkaline residuals. Water was removed with Na_2SO_4; the obtained solution was evaporated on the water bath, cooled, mixed with alcohol-nitric acid (1 ml of concentrated HNO_3:5ml of 96^o alcohol), and boiled during 3 min till the color became dark red. Extinction of the extract was measured at 470nm by the spectrophotometer (SPEKOL 11, KARL ZEISS, Germany) (Filippovich et al., 1982).

2.3.5 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).

2.3.6 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 carotenoides was calculated by the formula of Wintermanns (Gavrilenko et al., 1975).

2.3.7 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).

2.3.8 Total protein assay

Content of proteins was determined after Lowry (1951).

2.3.9 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 funnel. Optical density of upper layer was measured on a spectrophotometer (SPEKOL 11, KARL ZEISS, Germany) at 520 nm (Bates et al., 1973).

2.3.10 Soluble carbohydrates

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

2.3.11 Nitrates

After the water-extraction of 500g of plant material (homogenized for 30min at room temperature), it was filtered. Hydrogene peroxide was added to 10ml of the filtrate and evaporated. disulphophenolic acid was added to the obtained sediment and optical density was determined at 410nm (SPEKOL 11, KARL ZEISS, Germany) (Danilova, 1963; Pleshkov, 1985).

2.3.12 Total antioxidant activity

This index was measured by modified method using diphenyl-picryl-hydrazyl (DPPH) (Koleva *et al.,* 2002). 200 mg of experimental powder was extracted with 96^o ethanol (two-fold). The obtained extract was evaporated on a water bath and the sediment was dissolved in 10ml of water-alcohol mixture. The 0.01ml of the received solution was added with 4ml of 40µM DPPH solution and after 30 minutes of incubation in the dark, the optical density was measured at 515nm by the spectrophotometer (SPEKOL 11, KARL ZEISS, Germany). The percent of inhibition was calculated.

2.3.13 Statistical processing of data

One way ANOVA and Tukey's multiple comparison tests were used to test differences between the means. All calculations were performed using statistical software Sigma Plot 14.5.

III. RESULTS AND DISCUSSION

As it was mentioned above, the experimental plants were collected in July, which is the hottest and driest month of the studied habitats. Also the fact that the last seven years are regarded as the hottest through the whole history of climate observation must be taken into account (WHO report, 2021). Thus, the tested species had to survive under combined stress of high temperature, water deficiency and intensive irradiation simultaneously.

3.1 Nitrate reductase and nitrates

Nitrate reductase **(**EC 1.6.6.1.) plays an essencial role in plant's physiological and metabolic condition as it is a key enzyme in synthesis of amino acids, proteins, chlorophylls and other nitrogen-containing substances; it may serve as a marker of drought stress as well (Ananthi, Vijayaraghavan, 2012; McCarthy et al., 2017; Sinay, Suripatty, 2019). It is established that the activity of nitrate reductase decreases under the water deficiency; which is associated with photosynthesis inhibition (Kapoor et al., 2020). Moreover, as the nitrate reductase is the substrate-dependent enzyme, and its activity is regulated by the concentration of nitrates and ammonium, the decline of enzyme's activity may be caused by reduction of nitrates in leaves (Nicodemus et al. 2008; Dias et al., 2011). As the absorbtion of water and minerals, among them of nitrates, is reduced during the drought, this will be reflected on enzyme's activity as well (Correia *et al.*, 2005; Kapoor et al., 2020)

According to the experimental results it is clear that the one and the same species of two habitats showed statistically different activity of nitrate reductase ($p \leq 0.002$); indices of goat's thorn and felty germander growing at Iagludja were respectively 3.6 and 1.6-times higher, compared to Kvernaqi results ($p \leq 0.002$). In other tested species on the contrary – Kvernaqi data of nitrate reductase activity were higher: in caper bush – 2-times, in spurge – 2.5 times, and in Christ's thorn – 1.5-times (Fig. 1).

Although the activity of nitrate reductase has been associated with nitrates content in leaves (Nicodemus et al. 2008; Dias et al., 2011), in our experimental plants this correlation was not clear. Generally, the content of nitrates in Kvernaqi species was higher that of Iagludja ones ($p<0,05$) (Fig. 5). Moreover, in Iagludja experimental species content of nitrates was low and statistically similar, while the same species of Kvernaqi statistically differed by this index ($p<0,05$) (Fig. 1).

If we consider the content of chlorophylls as the indicator of photosynthetic activity, there may be assumed some relationship between nitrate reductase activity and chlorophylls content in leaves; as the decrease of the enzyme's activity is associated with photosynthesis inhibition under the water deficiency (Kapoor et al., 2020).

Comparision of nitrate reductase activity and chlorophylls content in tested plants revealed the coincidence of the decrease of these two indices (Fig. 1, 2).

As the nitrate reductase gives some idea on the general physiological condition of a plant, according to the obtained results it may be concluded that Iagludja conditions were more stressful for experimental plants, than Kvernaqi. Equally low data of nitrates in Iagludja species is demonstration of this (Fig. 1).

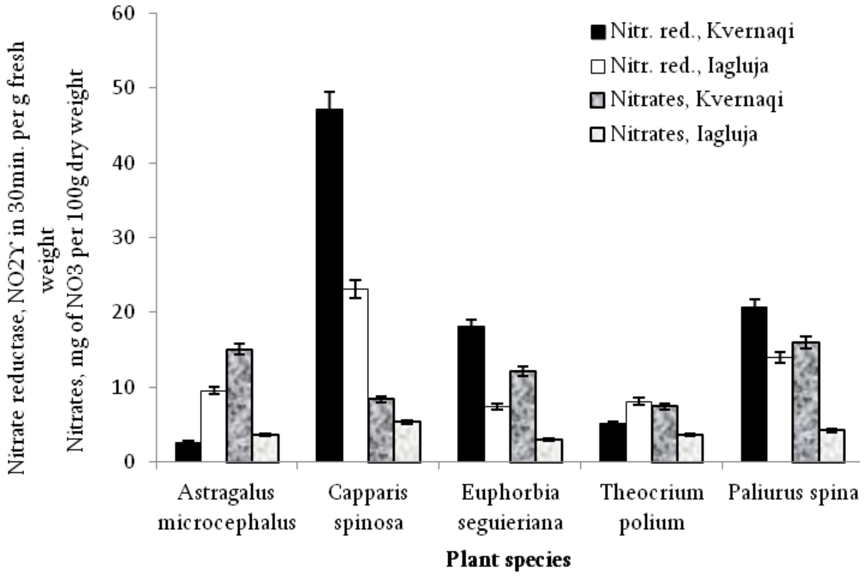

FIGURE 1: Nitrate reductase activity and content of nitrates in leaves of Kvernaqi and Iagluja (East Georgia) plants

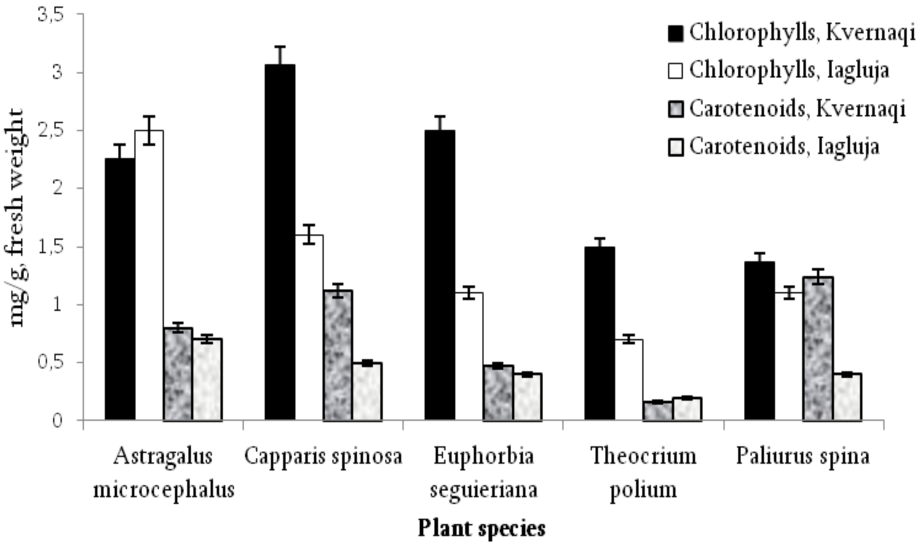

FIGURE 2: Content of chlorophylls and carotenoids in leaves of Kvernaqi and Iagluja (East Georgia) plants

3.2 Catalase and peroxidase

Generally the high activity of antioxidant enzymes – catalases and peroxidases has been mentioned in drought resistant plants (Laxa et al., 2019; Kapoor et al., 2020). Although the high specificity of the activity is characteristic for enzymatic antioxidants – they affect particular type of radicals, with specific cellular and organ localization (Chupakhina et al., 2011). Catalases of the photosynthesizing tissue mainly are localized in peroxisomes (CAT, EC 1.11.1.6), where they distruct the hydrohen peroxide produced during photorespiration (Noctor et al., 2014); Moreover, proliferation of peroxisomes and diffusion additional hydrogen peroxide from the cytosol may take place under stress, which is actively neutralized by catalases as well (Lopez-Huertas et al., 2000); Althogh, in some plants decrease of catalases activity is possible, which is regared as a demonstration of a significant role of peroxidases and ascorbate-glutathione cycle, as of principal oxygen-scavengers. (Harinasut et al., 2003).

So called classic, or non-specific peroxidases (EC 1.11.1.7) are multifunctional enzymes, which like catalases, neutralize the active forms of oxygen. For this purpose they use different reducing agents, often phenolic substances. They are concentrated mainly in a vacuole and cytosol (Kolupaev and Kokorev, 2019).

In studied species the activity of catalase was generally low (Fig. 3). Comparison of results of one and the same species of two habitats revealed their statistical identity (p>0,05).

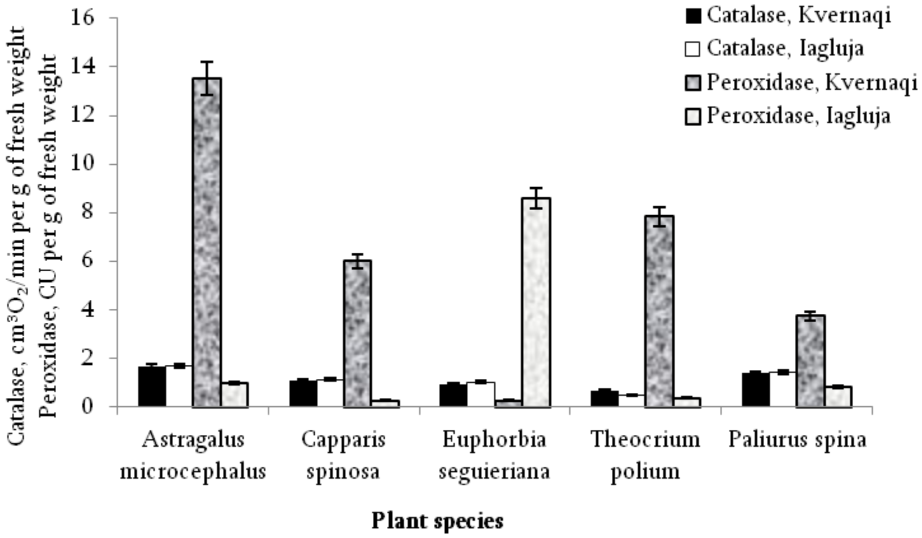

FIGURE 3: Activity of catalase and peroxidase in leaves of Kvernaqi and Iagluja (East Georgia) plants

Among Kvernaqi plants statistically similar activity of catalase was found in goat's thorn and Christ's thorn (p=0,09), caper bush and Christ's thorn (p=0,4), caper bush and spurge (p=0,2). Among Iaglidja species statistically similar activity of catalase was revealed in caper bush, spurge and Christ's thorn (p = 0,5) (Fig. 3).

The peroxidase activity of same species from different habitats was statistically different (P≤0,001). Generally the peroxidase activity of Kvernaqi experimental species was significantly higher, compared to Iagludja data (except spurge) (Fig. 3): in goat's thorn leaves it was 14-times higher, in caper bush – 26-times, in felty germander – 22-times, and in Christ's thorn – 4.5-times higher

While comparing peroxidase activity of different species, among Iagludja plants spurge and Christ's thorn results were similar (p=0,5), in other species there was statistical difference (p<0,05) (Fig. 3). In Kvernaqi plants all data on peroxidase activity were statistically diverse (p<0,01) (Fig. 3).

Analyzing the experimental results it may be supposed that low catalase activity in plants of both habitats indicates to the low concentration of hydrogen peroxide in leaves. Since catalase has a low affinity to hydrogen peroxide, compared to peroxidase, it is active under high concentrations of the substrate (Mhamdi et al., 2010; Chakraborty et al., 2016).

Decreased activity of catalase may be caused by its inactivation because of intensive radiation as well (Chupakhina et al., 2011).

High activity of peroxidase in experimental plants of Kvernaqi hill may be explained if we take into account the fact that peroxidases are active in those systems, where the primary transformation of derivatives of hydrohen peroxide – organic peroxides takes place by means of appropriate reducing agents (Mhamdi et al., 2010).

High peroxidase activity of spurge individuals at Iagludja, as exception, may be linked with the diverse from other species C4 way of CO2 assimilation in this species.

Thus, more extreme conditions of Iagludja hill compared to Kvernaqi, cause diverse biochemical changes in experimental species.

It may be supposed that the enzymatic antioxidant system of Iagludja experimental plants is not leading in adaptation to the intensive irradiation, high temperature and water deficiency conditions of this habitat; while under Kvernaqi conditions peroxidase was distinguished from this point of view.

3.3 Chlorophylls and carodinoids

It is known that water deficiency, together with intensive radiation and high temperature inhibits photosynthesis. One of the reasons is chloroplasts damage and chlorophylls decrease, regarded as some kind of protective reaction towards stresses (Ommen et al., 1999; Herbinger et al., 2002; Ma et al., 2020).

Comparative study of chlorophylls and carotenoids in one and the same species of the studied habitats has revealed the statistical similarity of chlorophylls in leaves of goat's thorn felty germander and Christ's thorn ($p=0,4$, $p=0,2$ and $p=0,7$ respectively), while in other species it was different ($p<0,05$). Moreover, Kvernaqi data were higher than Iagludja ones: in caper bush - 1,9-times, in spurge – 2,3-times (Fig. 2).

The between-species comparison of results revealed statistical similarity of chlorophylls in spurge and goat's thorn ($p=0,6$), caper bush and spurge ($p=0,1$), felty germander and Christ's thorn ($p=0,5$) growing at Kvernaqi hill; while among Iagludja species chlorophylls content of leaves was statistically similar in all tested species ($p<0,05$), except goat's thorn (Fig. 2).

It must be mentioned that in spite of stressful conditions in leaves of both habitat plants content of chlorophylls was not low; which indicates to reliable protection of the photosynthetic apparatus (Fig. 2).

As for carotenoids, between-habitat differences were revealed in caper bush ($p=0,003$) and Christ's thorn ($p=0,006$) individuals (Fig. 2).

Between-species comparison cleared statistical similarity of carotenoids in caper bush and Christ's thorn leaves ($p=0,4$) growing at Kvernaqi; while at Iagludja statistically same were indices of goat's thorn and caper bush ($p=0,06$), as well as of all other species ($p>0,05$) (Fig. 2).

3.4 Ascorbic acid and tocopherol

Ascorbic acid is regarded as one of the leading substances for protection of the photosynthetic apparatus against the oxidative stress (Venkatesh, et al., 2014). Under the intensive irradiation, as well as drought, content of ascorbic acid in plant increases (Yang et al., 2008). High content of the substance is one of the features of plant stress-resistance (Singh et al., 2012).

In a number of plants the increase of tocopherol, together with ascorbic acid, has been indicated as one of the primary reactions against drought or intensive radiation stress (Abbasi et al., 2007; Giacomelli et al., 2007).

Among one and the same species of different habitats the content of ascorbic acid was statistically similar in leaves of spurge and felty germander ($p=0,1$ and $p=0,8$ respectively), while in other species it was different ($p<0,05$); in particular, Kvernaqi indices were higher compared to Iagludja: in caper bush by 28%, in spurge – by 6%, in Christ's thorn – by 66,5%. In leaves of goat's thorn in contrary – it was by 22,5% lower (Fig. 4).

According to obtained results it may be supposed that in leaves of caper bush, spurge and Christ's thorn, growing in Kvernaqi conditions, ascorbate system is one of the leading mechanisms in leaves protection against stress; while under Iagludja conditions this mechanism was clearly expressed only in caper bush and spurge leaves.

It must be mentioned that chloroplast membrane contains high amount of α-tocopherol, which protects it against photo oxidation. By well-known ascorbate-tocopherol cycle the oxidized tocopherol is reduced by means ascorbic acid (Mullineaux et al., 2006; Gill, Tuteja, 2010).

Among the same species of studied habitats content of tocopherol was statistical identical only in goat's thorn leaves ($p=0,109$) (Fig. 4); in other experimental species Iagludja data were higher compared to Kvernaqi ones ($p<0,05$): in caper bush - 9-times, in spurge - 2,8-times, in felty germander – 2 times; in Christ's thorn – in contrary Kvernaqi index was 2,5-times higher (Fig. 4); According to results it may be supposed that increase of the tocopherol synthesis in leaves of caper bush, spurge and felty germander is one of the stress-protective mechanisms under Iagludja conditions, while in Kvernaqi this mechanism was essential only in Christ's thorn (Fig. 4).

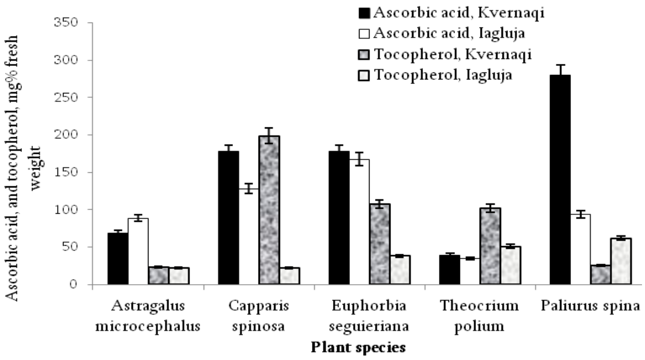

FIGURE 4: Content of ascorbic acid and tocopherol in leaves of Kvernaqi and Iagluja (East Georgia) plants

3.5 Soluble phenols and anthocyanins

Among the low-molecular antioxidans phenolic substances are distinguished by the active interaction with free radicals. Accordingly, their role in the defence of cell membrane against the oxidative stress is very high (Winkel-Shirley, 2002; Cesar, Fraga, 2010). Some papers deal with the phenolics accumulation in plant cell under water deficiency (Sharma et al., 2019).

It must be mentioned that generally high content of phenols was revealed in plants of both habitats; although in same species of the two habitats statistical similar results were received only in goat's thorn and felty germander ($p=0,08$) (Fig. 5). As for other species, Kvernaqi results of spurge and Christ's thorn were 2-times higher compared to Iagludja data ($p<0,05$), while in caper bush in contrary – Iagludja results were 2-times higher (Fig. 5).

According to results it is clear that high content of phenolics in both habitats is one of the leading stress-protective mechanisms in tested species.

One of the groups of phenolic substances – anthocyanins, which are partially concentrated in leaf epidermis, plays a role of light reflector, protecting chlorophyll from excess radiation (Gould et al., 2018). It has been established that accumulation of anthocyanins in vegetative tissues increases under various stresses (drought, intensive radiation, etc.) (Kovinich et al.; 2015; Gould et al., 2018; Kamjad et al., 2021). It is supposed that they take part in diminution of cell osmotic potential and thus retain water and turgor in the cell (Chalker-Scott, 2002).

High content of anthocyanins was mentioned in experimental plants of both studied habitats. Although, while comparing data of one and the same species growing at different locations, it is evident that in Kvernaqi individuals anthocyanins were higher, compared to Iagludja ($p\leq0.001$); in particular results of goat's thorn were 3-times higher, that of caper bush and Christ's thorn – 2-times, and of spurge – 1,5-times higher. (Fig. 5).

According to experimental results it may be assumed that accumulation of anthocyanins, especially in Kvernaqi plants demonstrates the leading stress-protective role of these substances.

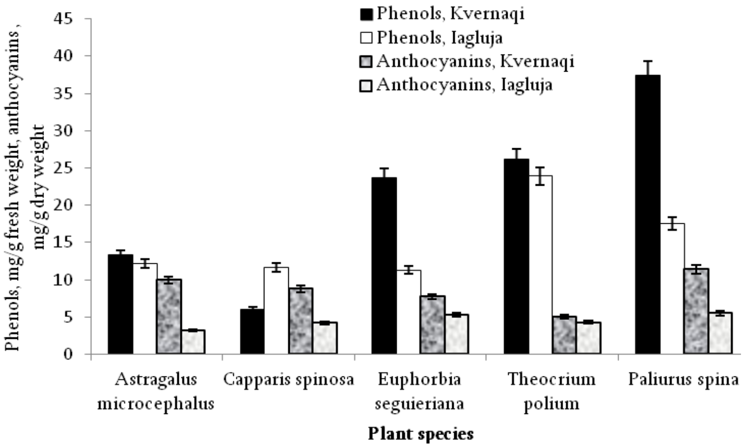

FIGURE 5: Content of polyphenols and anthocyanins in leaves of Kvernaqi and Iagluja (East Georgia) plants

3.6 Proline, total proteins and soluble carbohydrates

Other metabolites may also take part in plant protection against oxidative stress, together with antioxidants. These substances (amino acids, soluble carbohydrates, proteins) - so called osmoprotectants, are not antioxidants, but reveal similar properties and reliably protect lipo-protein components of membranes against damage (Szabados, Savory, 2010; Meng, 2014; Iqbal et al., 2020).

Many authors point to a positive role of amino acid proline in drought-resistance of plant (Kaur, Asthir, 2015; Ashraf et al., 2018). It holds water in the cell and retains its turgor (Kartashov, 2013; Joseph et al., 2015).

Individuals of one and the same species, growing at different habitats had statistically different content of proline in leaves ($p \leq 0.01$); moreover, in Iagludja species the content of amino acid was lower compared to Kvernaqi: in goat's thorn – 2,7-times lower, in caper bush – 2,5-times, in spurge – 3,3-times, and in Christ's thorn – 5,6-times lower. Only data of felty germander coincided in both habitats ($p=0,3$) (Fig. 6). Generally the content of proline in plants of both habitats was not high. Exception was Christ's thorn; especially Kvernaqi individuals of this plant were distinguished with high content of proline in leaves. Thus, it may be concluded that the stress-protective function of proline was not principal in tested plants, except Christ's thorn.

Adaptation of plants to unfavorable conditions accounts for the qualitative and quantitative changes of the composition of cell proteins (Parida et al., 2007; Mohammadkhani and Heidari, 2008). It is well-known that the synthesis of so called stress-proteins – dehydrins is activated under stress; they reveal osmolyt-type activity and take part in the stabilization of membrane proteins and cell osmotic regulation; thus protecting the cell structures against the oxidative stress (Iqbal et al., 2020; Mohammadkhani and Heidari, 2008).

Content of total proteins, like proline, was higher in Kvernaqi plants compared to the same species of Iagludja ($p \leq 0.03$): in goat's thorn it was 2,5-times higher, in caper bush – 3,2-times, in spurge – 2,2-times, and in Christ's thorn – 1,6-times higher. Again, the exception was felty germander. Proteins content in this species appeared to be higher in Iadludja individuals by 30%, compared to Kvernaqi data (Fig. 6).

It has been established that decrease of the amount of total proteins takes place under various stresses. This is explained by the outflow of nitrogenous substances from the leaf and their synthesis decrease (Sorkheh et al., 2012). At the same time the inhibition of photosynthesis and proteins proteolysis may become the reason of total proteins decline (Mohammadkhani and Heidari, 2008; Taiz and Zeiger, 2016).

Significant decrease of total proteins in Iagludja experimental species may be explained by the decline of nitrates and nitrate reductase activity in their leaves (Fig. 1). The increase of total proteins content in felty germander leaves under Iagludja conditions, which we consider to be more extreme than Kvernaqi, may be one of the individual mechanisms of protection against the severe stress.

Many authors have mentioned the accumulation of soluble carbohydrates in plant cells in response to various stresses (Prado et al., 2000; Finkelstein and Gibson, 2001; Mohammadkhani and Heidari, 2008). These substances decrease the water potential of cells and support the water retention. At the same time they protect proteins from denaturation and account for membrane stability (Couee et al., 2006; Laxa et al., 2019)

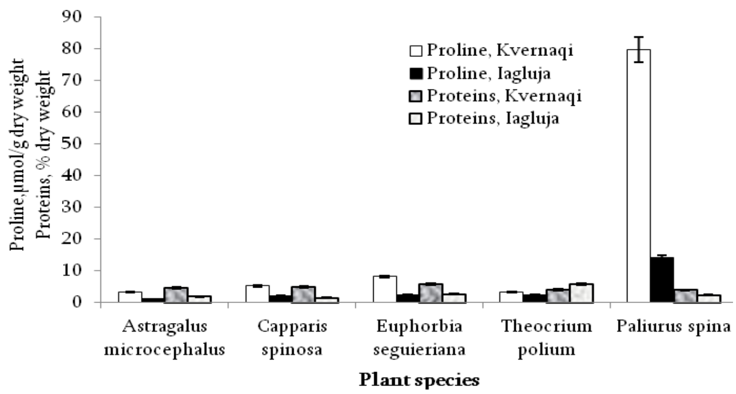

FIGURE 6: Content of proline and total proteins in leaves of Kvernaqi and Iagluja (East Georgia) plants

Evidently higher concentration of soluble carbohydrates was established in Iagludja experimental plants, compared to the same species of Kvernaqi hills ($p \leq 0.001$) (Fig. 7). The results were 6-times higher in goat's thorn, 20-times higher in caper bush, 5-times – in spurge and 1,2-times higher in Christ's thorn. It is clear that accumulation of such a big amount of soluble carbohydrates under the severe conditions of Iadlugja hill is indication to the leading stress-protective function of these substances, while in Kvernaqi hill the protective mechanism of carbohydrates was evident only in Christ's thorn (Fig. 7).

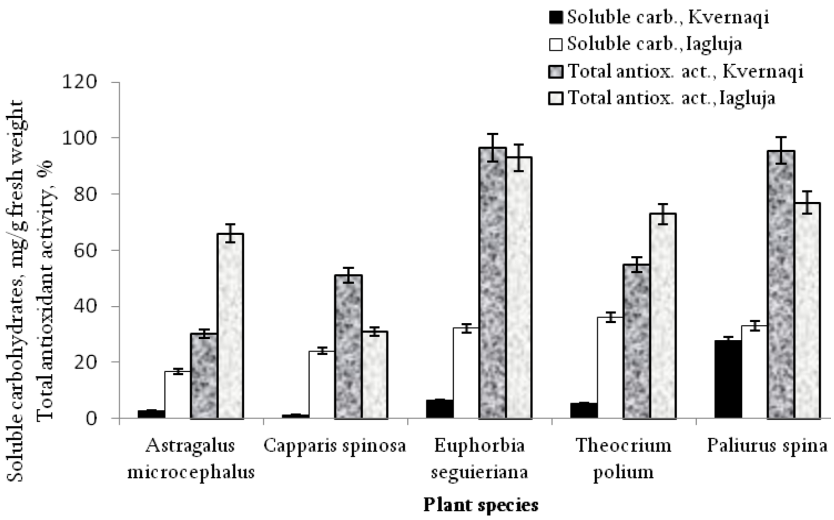

FIGURE 7: Content of soluble carbohydrates and total antioxidant activity in leaves of Kvernaqi and Iagluja (East Georgia) plants

3.7 Total antioxidant activity

Is a significant characteristic which may be used as a criterion for the evaluation of plant's stress-resistance.

The antioxidant activity of the leaves of one and the same plant species appeared to be different by habitats (except the spurge) (Fig. 7). In goat's thorn and felty germander the Iagludja data prevailed over Kvernaqi ones (2 and 1,3-times respectively), while in caper bush and Christ's thorn in contrary – the Kvernaqi results were higher (1,8 and 1,2-times respectively) (Fig. 7).

According to obtained results the antioxidant activity of caper bush leaves from Iagludja may be regarded as low, that of goat's thorn – moderate, and of spurge, felty germander and Christ's thorn - high (Luzia and Jorge, 2014). Among Kvernaqi species the low antioxidant activity had goat's thorn, moderate – caper bush and felty germander; the high antioxidant activity was revealed in spurge and Christ's thorn (Fig. 7).

Soil conditions are one of the important factors for normal development of plant. It has been established that the soil microflora significantly accounts for plant's stress-resistance by means of suitable mechanisms, and facilitates their existence under stress conditions (Bardi and Malusa, 2012; Hossain et al., 2022). Accordingly it may be supposed that local soil conditions play a significant role in experimental plants' adaptation to stress-conditions of the studied habitats as well. By our opinion, this fact must be taken into account in further observation.

IV. CONCLUSIONS

1. Inspite of similarity of climatic conditions of Kvernaqi and Iagludja hills, obtained results let assume that Iagludja conditions are more extreme for plants; which may be linked with soil conditions as well.
2. The antioxidant defence mechanisms of experimental species appeared to be partly diverse following habitats.
3. It is evident that stress-adaptive mechanisms have specific peculiarities; athough these mechanisms are determined by those factors which plant has to adapt.
4. Activation of the phenol-anthocyanin antioxidant defence mechanism against the stress conditions of both studied habitats was common in all tested plants.
5. Together with the phenol-anthocyanin mechanism of stress-protection, activation of peroxidase was expressed in all tested species of Kvernaqi hill; while in Iagludja accumulation of soluble carbohydrates was evident.

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