# Study of Antioxidants in Leaves of Xerophytes of Georgia

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***Abstract—*** *Climate warming may appear fatal for many plant species. Thanks to evolutionary formed mechanisms of resistance xerophytes possess high ability of adaptation to elevated temperature and water deficiency. That is why the knowledge of biology of drought resistant species seems very important for possible recovering of deserted regions in future. Antioxidant system is regarded to play an essential role in plant resistance against water deficiency. On the base of analysis of antioxidant system, stress-adaptation strategies of xerophytes, growing at two arid habitats of Georgia – v. Udabno (Iori plateau) and Kotsakhura gorge (Kvernaqi hill), has been investigated. It was established that several mechanisms of antioxidant system are involved in stress-resistance of studied xerophytes. In some cases these mechanisms were similar in species of the same habitat and may be linked with environmental conditions; while in some cases the specific peculiarities of plants were revealed. In particular, ascorbate-tocopherol system was activated in most experimental species. Protective mechanism of phenolic substances accumulation was active in all species as well. Udabno plants were distinguished by the accumulation of osmoprotective soluble carbohydrates; while accumulation of another osmoprotectant - proline was common in both habitat plants.*

***Keywords— Antioxidants, Georgia, water deficiency, xerophytes.***

## I. INTRODUCTION

Recent scientific investigations offer a strong evidence of climate global change to undesirable direction. Moreover, last seven years are regarded as the hottest during the whole history of climate observation (WMO provisional report, 2021).

Climate warming may appear fatal for many plant species. Presumably the geographic range and borders of a great number of species will change (Garamvolgyi and Hufnagel, 2013; Allen et al., 2015). Thanks to evolutionary formed nonspecific biological mechanisms of resistance xerophytes possess high ability of adaptation to elevated temperature, water deficiency, intensive irradiation, etc.; especially the antioxidant system plays an essential role (Laxa et al., 2019). That is why the knowledge of biology of drought resistant species seems very important for future forecasting and possible recovering of deserted regions.

The investigation of stress-adaptation strategies of xerophytes, growing at two arid habitats of Georgia – v. Udabno (Iori plateau) and Kotsakhura gorge (Kvernaqi hill), on the base of analysis of antioxidant system, was the purpose of the presented study. Demonstrated data may be regarded as the part of long-term investigations, which aim to explore the drought resistance mechanisms of plant species occupying dry habitats of Georgia (Badridze et al., 2021; Badridze et al., 2022).

Content of ascorbic acid, tocopherol, carotenoids, anthocyanins, soluble phenols, proline, total proteins, soluble carbohydrates and activity of catalase and peroxidase were studied in leaves of experimental plants.

# II. MATERIALS AND METHODS

## 2.1 Research area

Iori plateau (Sagarejo municipality, East Georgia) is distinguished by lack of water and poor vegetation. Its landscape varies from desert to steppe and forest-steppe. Climate is dry subtropical; annual amount of precipitations is 499-600mm; in village Udabno, where the plant material was picked – 434mm (Kordzakhia and Djavakhishvili, 1971).

Kvernaqi hill is situated near the city Kaspi (East Georgia). Its climate is transitional from subtropical to humid. Annual amount of precipitations is 450mm (Kordzakhia and Djavakhishvili, 1971; Ukleba, 2018).

## 2.2 Experimental plants

Experimental plant species collected in v. Udabno were: *Pyrus* sp. L., *Peucedanum ruthenicum* M. Bieb., *Galium humifusum* M. Bieb.; in Kvernaqi hills - *Berberis iberica* Stev & Fisch., *Rhamnus pallasii* Fisch & C.A.May, *Peganum harmala* L., *Cotinus coggygria* Scop., *Elaeagnus angustifolia* L. Middle age, mature, healthy leaves were picked at least from 5 different individuals of each experimental species in post-flowering phase, in July - 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

Biochemical analysis of studied antioxidans was based on spectrophotometrical methods described in our early work (Badridze et al., 2022). That is why we give here their short description.

Tocopherol was studied spectrophotometrically at 470 nm (SPEKOL 11, KARL ZEISS, Germany) (Fillipovich et al., 1982).

Anthocyanins were studied spectrophotometrically. Optical density of the leaves extract in 96% ethanol with 1% HCL was measured at 540nm (Ermakov, 1987).

Proline was investigated after Bates at al. spectrophotometerically at 520 nm (Bates et al., 1973).

Soluble phenols were determined using Folin-Ciocalteu reagent. Optical density was measured at 765 nm (Ferraris et al., 1987).

The content of proteins was studied after Lowry (Lowry et al., 1951).

Soluble carbohydrates were tested with anthrone reagent at 620 nm with a spectrophotometer (SPECOL 11, KARL ZEISS, Germany) (Turkina and Sokolova, 1971).

Carotenoids were determined spectrophotometricaly. Optical density of the extract of fresh leaves in ethanol was measured (spectrophotometer SPEKOL 11, KARL ZEISS, Germany). Calculations were done by Wettstein formula (Ermakov, 1987).

Activity of peroxidase was determined spectrophotometrically, using guaiacole. Optical density of guaiacole oxidized products was measured at wavelength of 470 nm over a period of 2 min (Ermakov, 1987). Results are given in conditional units per one gram of fresh weight.

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

## 2.4 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

Water deficiency combined with high temperature and intensive irradiation is the main stress, affecting the species which inhabit the studied habitats. Correspondingly, the characteristics of antioxidant system of experimental species has been analyzed on the background of mentioned stressors.

### 3.1 Ascorbic acid and tocopherol

According to observations content of ascorbic acid and tocopherol in leaves of tested plants was mainly high (Fig.1). *Rhamnus* was especially distinguished by ascorbate content. Statistically similar amount of the vitamin was found in *Galium, Eleagnus and Cotinus* (p>0.05). The lowest value of ascorbate was mentioned in *Berberis* leaves (Fig. 1).

Content of tocopherol in leaves of experimental species was high as well (Fig. 1). Statistically similar and higher (p>0.05) amounts of the vitamin was discovered in Udabno plants, compared to Kvernaqi species. Among Kvernaqi plants statistically similar (p>0.05) was tocopherol content in leaves of *Rhamnus, Elaeagnus* and *Cotinus*; the lowest value of the vitamin was revealed in *Berberis* (Fig. 1).

Both ascorbate and tocopherol are important protectors of the cell against different stresses (Hasanuzzaman et al., 2014; Kolupaev and Kokorev, 2019). Their increase is one of the primary responses to drought and intensive irradiation (Giacomelli et al., 2007; Yang et al., 2008). Thus, high content of ascorbate and tocopherol in tested plants may be regarded as one of the protective mechanisms against the stress conditions of the studied habitats. Moreover, in case of necessity these species may become a natural alternative source of the mentioned vitamins.

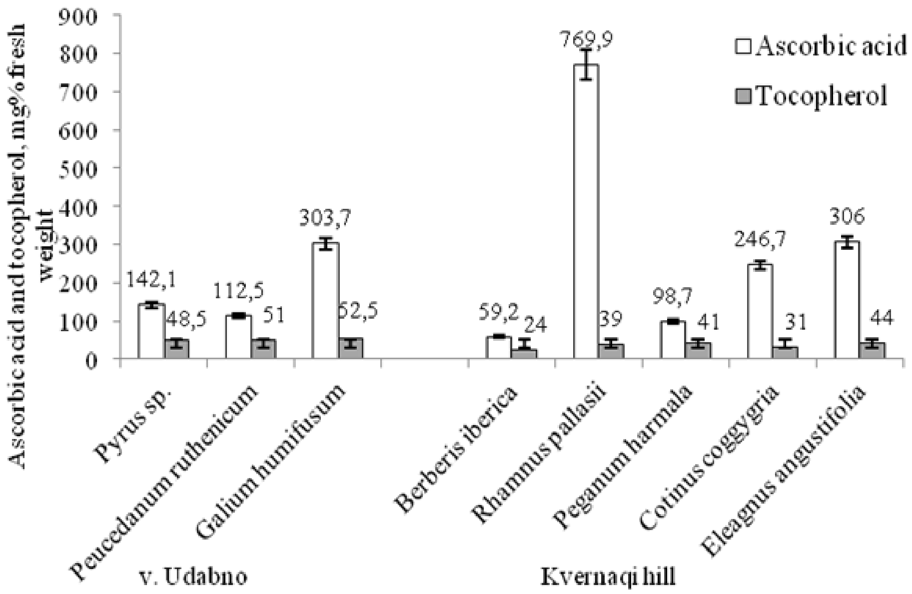


**FIGURE 1: Content of ascorbic acid and tocopherol in leaves of xerophytes from v. Udabno and Kvernaqi hill**

### 3.2 Carotenoids and anthocyanns

The highest, but statistically different (p<0.05) values of carotenoids were revealed in leaves of *Rhamnus* and *Pyrus*; while in *Peganum* and *Berveris* the results were minimal among the tested plants (Fig. 2). Any regularity of carotenoids content among the experimental plants following the habitats has not been revealed.

By the value of carotenoids one can judge about stress affecting a plant as well as about its resistance (Strzalka et al., 2003). From this point of view species with high content of carotenoids possess one additional mechanism of stress resistance.

Content of anthocyanins appeared to be higher in Kvernaqi plants, compared to Udabno ones (except *Peganum*). The highest and statistically similar (p>0.05) results were received in *Rhamnus* and *Elaeagnus* (Fig. 2).

It is established that accumulation of anthocyanins in vegetative tissues of plants increases under the drought and intensive irradiation (Kamjad et al., 2021). These substances are regarded to decrease the osmotic potential of the cell as well, supporting its turgor and water retention (Chalker-Scott, 2002).

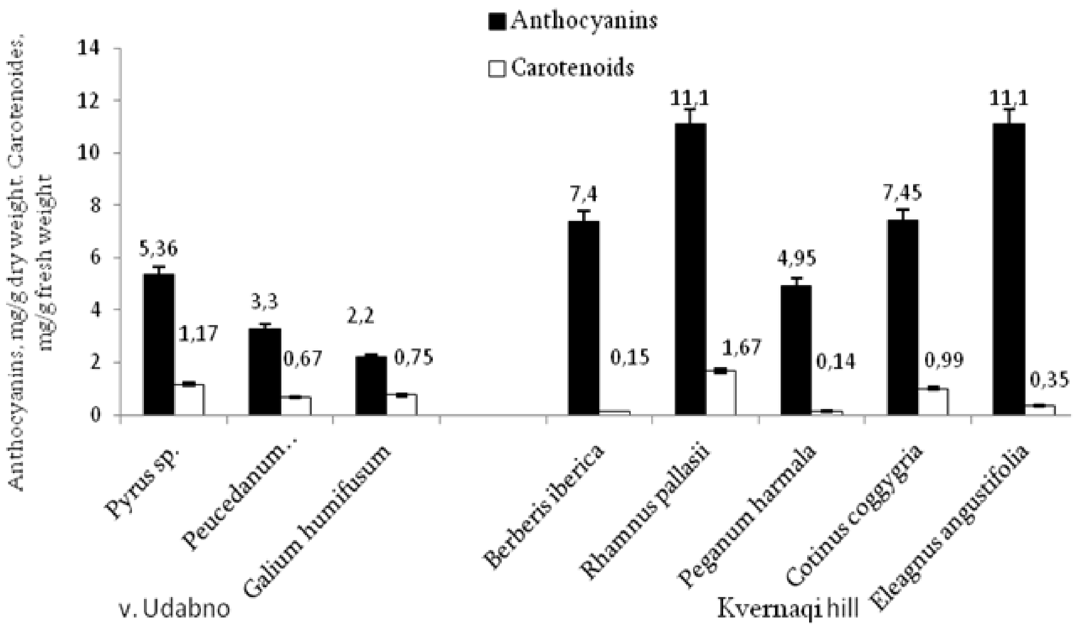


**FIGURE 2: Content of Carotenoids and anthocyanins in leaves of xerophytes from v. Udabno and Kvernaqi hill**

According to obtained results it may be supposed that anthocyanins accumulation under Kvernaqi conditions is one additional stress-protective mechanism of studied xerophytes.

### 3.3 Soluble phenols, carbohydrates, proteins and proline

Amount of soluble phenols was high in all tested plants (except *Peganum*). By this index especially were distinguished *Cotinus, Berberis* and *Peucedanum* (Fig. 3).

Among low-molecular antioxidants the role of phenolic substances in protection of cell membrane against stressors is very significant (Cesar and Fraga, 2010). Different authors mention increase of phenolic substances under water deficiency (Sharma et al., 2019).

Experimental results clearly demonstrate the leading role of phenolic substances in protection of tested plants from existing stresses.

It has been established that together with antioxidants so called osmoprotectants like free amino acids, soluble carbohydrates, proteins, etc. may take an active part in protection of plants from different stresses (Iqbal et al., 2020).

High content of total proteins was revealed in leaves of studied species (except *Galium*). Especially high, but statistically diverse results ($p<0.05$) were revealed in *Pyrus* and *Peganum*. Other plant species demonstrated similar, but statistically different results as well ($p<0.05$) (Fig. 3).

The qualitative and quantitative content of plant proteins changes under unfavorable conditions. The synthesis of stress-proteins – dehydrins is activated, which reveal osmolite-like effect and take part in membrane proteins stabilization and cell osmotic regulation (Ashraf et al., 2018; Iqbal et al., 2020).

High content of proteins in experimental plants may be one of the stress-protective reactions.

The content of soluble carbohydrates in tested species clearly differed by habitats. The index was two and more-times higher in Udabno species (Fig. 3). Among Kvernaqi species only in *Peganum* was mentioned comparatively high result.

Accumulation of soluble carbohydrates as a response to stress has been mentioned in many plants. These substances support decrease of the cell water potential and its water retention. At the same time they protect membrane proteins from denaturation and stabilize the membrane (Couee et al., 2006; Mohammadkhani and Heidari, 2008; Laxa, 2019).

From the obtained results is clear that accumulation of soluble sugars in Udabno plants is one of the stress-adaptive mechanisms in this habitat.

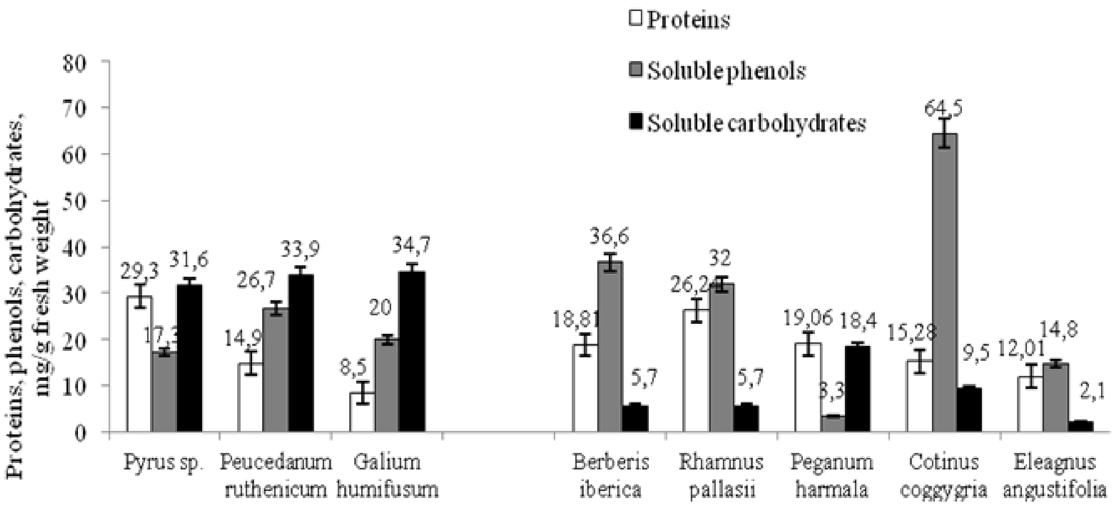


**FIGURE 3: Content of total proteins, soluble phenols and soluble carbohydrates in leaves of xerophytes from v. Udabno and Kvernaqi hill**

Especially high content of another osmoprotectant - amino acid proline was revealed in leaves of *Peganum* and *Cotinus*. The minimal amount of this amino acid was mentioned in *Pyrus*. In other tested species the content of proline was also high, but statistically differed ($p<0.05$) (Fig. 4).

Many authors mention the role of proline in drought resistance of plants. It retains water in the cell under deficiency conditions and supports its turgor (Ashraf et al., 2018). It is evident that proline accumulation in leaves of experimental plants is one of the stress-protective mechanisms under existing conditions.

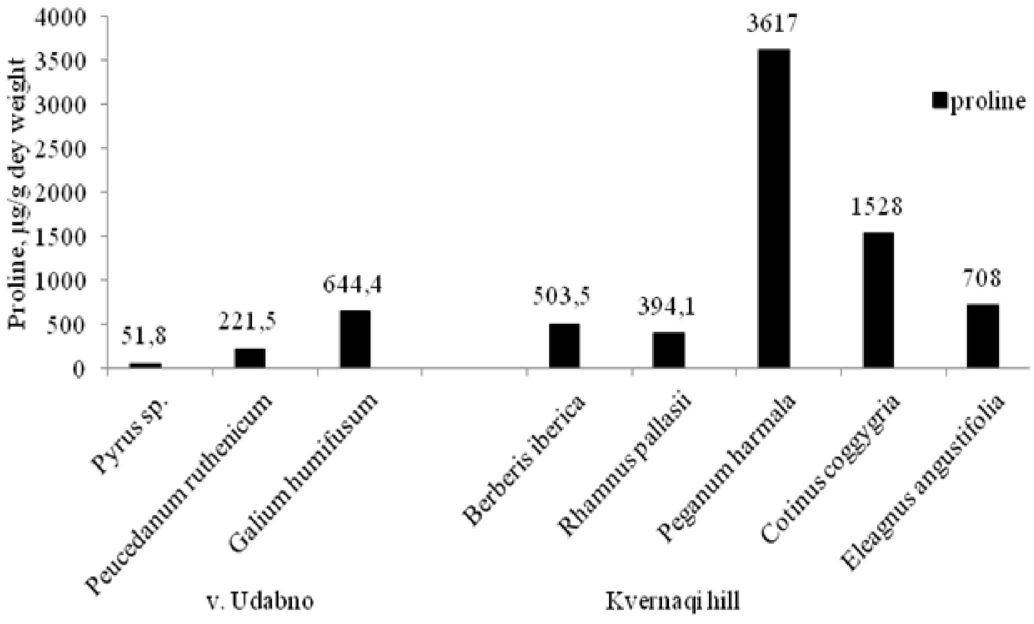


**FIGURE 4: Content of amino acid proline in leaves of xerophytes from v. Udabno and Kvernaqi hill**

### 3.4 Catalase and peroxidase

In drought resistant species the activity of antioxidant enzymes – catalase and peroxidase is high (Laxa, 2019; Kapoor et al., 2020). Though, enzymatic antioxidants have high specificity of activity. They affect a particular type of radicals, with specific cell- and organ localization. Moreover, decrease of catalase activity is possible under stress conditions (Chupakhina, 2011). As it is supposed, this fact is linked with an important role of peroxidases and ascorbate-glutathione cycle in oxygen binding (Harinasut. 2003).

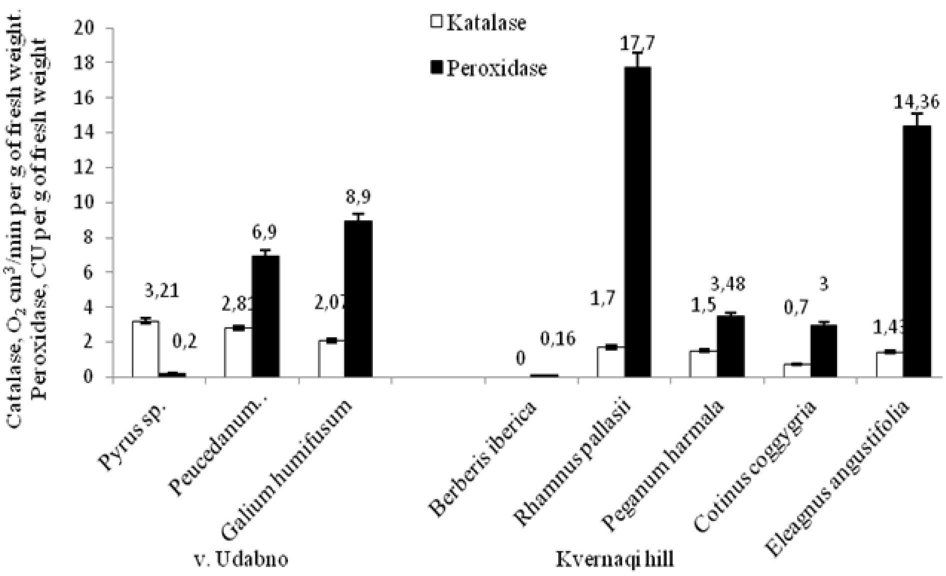


**FIGURE 5: Activity of catalase and peroxidase in leaves of xerophytes from v. Udabno and Kvernaqi hill**

Udabno plants revealted two- and more times higher activity of catalase, compared to Kvernaqi species (Fig. 5); while the activity of peroxidase demonstrated species individual peculiarities rather than habitat influence. In particular, the highest activity of peroxidase was mentioned in leaves of *Rhamnu*s and *Elaeagnus*; the minimal – in *Pyru*s and *Berberis* leaves (Fig. 5). It is known that catalase has low affinity to hydrogen peroxide, compared to peroxidase. That is why it is more active under the high concentration of peroxide (Chakrabarty, 2016). High catalase activity of Udabno plants may be indication to high concentration of hydrogen peroxide there; this points to more stressful conditions of Udabno habitat, compared to Kvernaqi.

## IV. CONCLUSION

Analyzing the experimental results it may be concluded that several mechanisms of the antioxidant system are involved in stress-resistance of studied xerophytes. In some cases these mechanisms were similar in species of the same habitat and may be linked with environmental conditions; while in some cases the specific peculiarities of plants were revealed. In particular: 1. ascorbate-tocopherol system was activated in all experimental species (except *Berberis*). 2. Protective mechanism of phenolic substances accumulation was active in all species as well (except *Peganum*). 3. Udabno plants were distinguished by the accumulation of soluble carbohydrates; while accumulation of another osmoprotectant - proline was common in both habitat plants (except *Pyrus*)

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