The Biochemical Indices of Drought Resistant Species of Iori Plateau (East Georgia)

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Abstract— *Existing forecasts of climate change predict significant warming, seasonal precipitation change, and strong and frequent droughts in the coming decades. Drought resistant plant species have more chance to survive. Predictions make the study of the biology of drought-resistant species especially relevant today. Antioxidant system, which plays an important role in plant stress resistance, is of special interest. Moreover, antioxidant substances are characterized by healing properties as well.* ***Mechanisms*** *of drought resistance of plants growing on arid territories of Georgia are practically unexplored. Presented study aimed to investigate the characteristics of antioxidant system of leaves of drought resistant species (Euphorbia falcata L. (sickle spurge), Lycopsis orientalis L. (small bugloss), Cotinus coggygria Scop. (smoke tree), Elaeagnus angustifolia L. (Russian olive) and Amygdalus communis L. (almond)) growing at one of the most arid regions of Georgia - Iori plateau (East Georgia). Analyses were made in two vegetative phases – flowering and fruit-bearing. From the obtained results, it is clear that the studied species have more or less different biochemical stress-adaptive mechanisms, which include certain enzymatic and non-enzymatic components of antioxidant system. In small bugloss in response to stress catalase was activated and synthesis of ascorbate-tocopherol and anthocyanins was enhanced; especially high amount of proline accumulation was noted. Phenols, anthocyanins and proline should be actively involved in stress resistance of sickle spurge. Russian olive was distinguished with high levels of ascorbate-tocopherol and anthocyanins, as well as proline; in addition the enzymatic antioxidants - catalase and peroxidase were activated, and soluble carbohydrates were accumulated. The protective systems of ascorbic acid and tocopherol, as well as phenolic compounds were active in smoke tree. From osmolytes content of proline increased, while the level of soluble carbohydrates was already the highest, compared to all tested species. Catalase was activated in response to stress in almonds; protective systems of ascorbate-tocopherol, phenols, and anthocyanins were active as well; among osmolytes content of carbohydrates was increased. The stability of carotenoids protective system of all studied species under stress conditions presumably indicates that experimental plants are less sensitive to radiation stress, and water deficiency is the main stress factor for them.*

Keywords— antioxidants, drought resistance, osmolytes.

I. INTRODUCTION

Existing forecasts of climate change predict significant warming, seasonal precipitation change, and strong and frequent droughts in the coming decades (IPCC, 2007). It is likely that the combination of drought and unusually high temperatures that accompany global warming will cause vegetation change on Earth (IPCC, 2012; Jentsch *et al.*, 2007; Smith, 2011). The mentioned combination of drought and temperature forms the so-called "Global change type drought", also known as "hot drought". Under such drought conditions, the risk of severe plant stress and death is significantly increased (Allen *et al.*, 2015; McDowell *et al.*, 2008; Overpeck and Udall, 2010). Drought resistant plant species have more chance to survive.

Drought resistance is based on evolutionarily established physiological-biochemical mechanisms, many of which are studied today. Resistant plants are characterized by the high content of antioxidants and osmolytes. They possess the ability to alter metabolism in order to strengthen the antioxidant system, which helps to overcome the lack of water, as well as to recover from dehydration. (Aslam *et al.*, 2015; Laxa *et al.*, 2019).

Humans have been using plants for medicinal purposes since time immemorial. According to statistics more than 60% of the world's population today prefers medicinal plants and natural products. Moreover, 25% of the commercial preparations produced in the world are of plant origin. In recent decades, interest in herbal remedies has grown in both Europe and America. This is due to the fact that phytopreparations are more available; furthermore, they are thought to have fewer side effects than synthetic drugs (Benzie and Wachtel-Galor, 2011; Harvey, 2000).

Against the background of expected climate change it may be assumed that the resources of plant raw material for medical use will be significantly reduced and probably replaced by drought-resistant species. All abovementioned predictions make the study of the biology of drought-resistant species especially relevant today. Characteristics of the antioxidant system, which play an important role in plant stress resistance, including drought, are of special interest. Moreover most antioxidant substances are characterized by healing properties as well (Pisoschi and Negulescu, 2011).

The physiological and biochemical indices of drought resistant plants growing on arid territories of Georgia are practically unexplored.

Based on the above, the aim of the study was to investigate the biochemical characteristics of leaves of the drought-resistant plants of one of the most arid regions of Georgia - Iori plateau (east Georgia). Mainly the elements of the antioxidant system have been studied: plastic pigments, ascorbic acid, tocopherol, anthocyanins, soluble phenols, total proteins, soluble carbohydrates, proline; as well as enzymes'- catalase, peroxidase and nitrate reductase activity, and total antioxidant activity, expressed in percents of inhibition.

Such studies give an idea of the adaptive mechanisms that either plant uses under stressful conditions and deepen our knowledge of the biology of drought-resistant plant species. This type of information under variable climate conditions allows to select plant species, that may be used against soil erosion and desertification, on the one hand, and as raw material for the preparation of natural remedies, on the other.

II. MATERIALS AND METHODS

2.1 Investigated species

Five species of plants have been studied: herbaceous - *Euphorbia falcata* L. (sickle spurge) and *Lycopsis orientalis* L. (small bugloss), woody plants - *Cotinus coggygria* Scop. (smoke tree), *Elaeagnus angustifolia* L. (Russian olive) and *Amygdalus communis* L. (almond). Healthy middle-aged leaves were collected for analysis from at least 5 different plants. The material was taken at an altitude of 828 m above sea level, at the territory of v. Udabno (Iori plateau, east Georgia), in two terms - in June (during the flowering phase) and in July (during the fruiting phase). Analyses were performed on raw material, with 3-fold repetition.

Sickle spurge (*Euphorbia falcata* L.) is one of the species of genus *Euforbia*. It is an annual herbaceous plant from the family Euphorbiaceae; is distributed in semi-deserts, steppes and forest-steppes, is xeromesophyte. In general, sickle spurge grows in many parts of the world as a crop and ruderal weed (USDA, 2016). It is found on dry slopes and foothills in the Caucasus as well. The plant is used in folk medicine as a medicinal herb. Phytochemistry of members of the Euphorbiaceae family is currently being actively studied for the use of their compounds in medicine (Kemboi *et al*., 2020).

Small bugloss (*Lycopsis orientalis* L.) is an annual herbaceous plant of the family Boraginaceae. The plant is considered as drought tolerant. It is found in the southern drought zone, where it grows in steppes, dry shrubs, roadsides, pastures. Small bugloss is a ruderal and agricultural weed; is used as food and in folk medicine. Currently its phytochemical composition is being studied more actively (Ertaş *et al*., 2014).

Russian olive (*Elaeagnus angustifolia* L.) - a perennial shrub, or low-stemmed tree from Elaeagnaceae family. According to the literature, it can withstand both, very low (-30°C) and very high (+ 46°C) temperatures. Russian olive is a drought- and saline-resistant plant and plays an important role in protecting arid areas from erosion. It develops a strong root system that far exceeds the aboveground parts of the plant and is a strong water-absorbing mean. The lanceolate leaves of the plant are covered with scales and have a xeromorphic structure, which is an indicator of economic water consumption. During prolonged droughts, Russian olive loses 70% of its leaf cover and thus avoids water loss (Bartha and Csiszar, 2008). The plant is used in traditional medicine for healing (Tehranizadeh *et al*., 2016).

Smoke tree (*Cotinus coggygria* Scop.) is a perennial shrub, from the family Anacardiaceae. The plant is less demanding to the soil - adapts to its different types and acidity in the range of 3.7-6.3. Smoke tree grows on dry, stony slopes, shrubs and forests; is light-requiring and drought-resistant; is used to plant and strengthen slopes (Global Invasive Species Database, 2020). The common area of smoke tree distribution includes the Caucasus, Central Asia, Central and Southern Europe, Asia Minor, Iran. The plant is widespread in Georgia, especially in its eastern part. It has long been used in folk medicine as a remedy (Matić *et al*., 2016).

Almond (*Amygdalus communis* L.**)** is a tree from the Rosaceae family. It is accustomed to the climatic conditions of the Mediterranean, steppes and deserts, which are characterized by mild, humid winters and hot, dry summers. Almonds are light-requiring and drought-resistant, which is supported by a well-developed root system and economical transpiration (Kester *et al.,* 2003). The plant has long been used as a remedy for various diseases (Abdullah *et al*., 2017).

2.2 Research area

The landscape of the Iori plateau varies from semi-desert to steppe and forest steppe. It is characterized by lack of water and scarce vegetation. The climate is dry, subtropical; the average temperature in January varies from 0 to -2°C, in July is 23°-24°C. The absolute minimum of temperature recorded in January was -13°C - -16°C, and the absolute maximum - in August was +37°C - +39°C. Precipitations of the plateau reach 499-600 mm per year, while in v. Udabno, where the experimental material was collected - 434 mm. Amount of precipitations increase from January and reaches its maximum in May, and then decreases (Apkhazava, 1975).

2.3 Biochemical assays

2.3.1 Antioxidant enzymes

Peroxidase activity was determined spectrophotometricaly: 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

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 g 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 was detected by indicator paper. 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 proteins

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 12.5.

III. RESULTS AND DISCUSSION

As mentioned above, the study of biochemical characteristics in the leaves of experimental plants was carried out in June (flowering phase) and July (fruiting phase). In June, the soil on the Iori plateau is not very dry, as it still retains traces of May precipitations; Plants therefore experience less drought pressure, despite strong insolation and high temperature. While in July, when the plants are in fruiting phase, the soil becomes very dry due to lack of precipitations; the high temperature and intensive irradiation combined with water deficiency establish a very stressful environment for existence. Nevertheless, the experimental species continue to vegetate, that is indication to their resistance to existing stress conditions.

Investigation of the studied characteristics in June and July allowed following their change under water deficit conditions and in two different phases of vegetation. The results obtained according to the vegetation phases within one species are compared with each other, as well as the data of different species in each phase.

3.1 Catalase and peroxidase

Catalase and peroxidase, along with other antioxidant enzymes, catalyze the detoxification of superoxide radicals and hydrogen peroxide in plants (Garifzyanov et al., 2011). It has been established that in drought tolerant species mostly peroxidase, ascorbate peroxidase and catalase are activated to neutralize active forms of oxygen (Kapoor et al., 2020; Laxa et al., 2019).

As catalase is mainly concentrated in peroxisomes, it actively contributes to the neutralization of hydrogen peroxide generated during photosynthesis, which is synthesized in large quantities under water-deficient conditions (Noctor *et al.*, 2014). It has been established that more than 70% of hydrogen peroxide generated under drought conditions is the result of photorespiration (Cruz, and de Carvalho, 2008).

From the obtained results it is clear that the activity of catalase in fruiting phase increased 4.9 times in small bugloss leaves and 1.2 times in Russian olive and almond leaves, compared to flowering phase ($p \leq 0.001$, $p=0.01$ and $p=0.03$, respectively); while in smoke tree leaves it decreased by 1.6 times ($p=0.02$), and remained statistically same in sickle spurge ($p=0.7$) (Fig. 1).

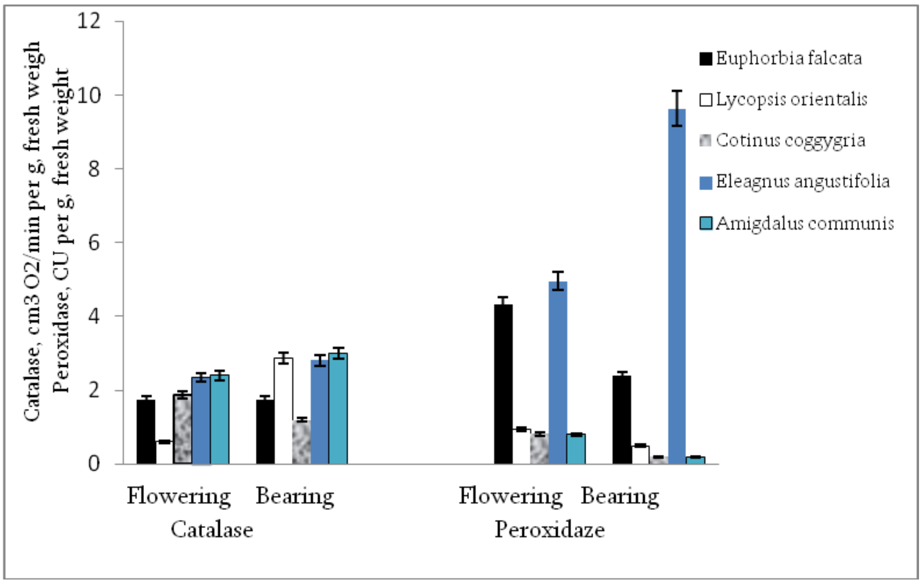

FIGURE 1: Activity of catalase and peroxidase in leaves of drought resistant species of Iori plateau (East Georgia) plants in two vegetative (flowering and fruit-bearing) phases

Comparison the results between species has revealed that the catalase activity in flowering phase in sickle spurge and smoke tree ($p=0.8$) as well as in Russian olive and almond ($p=0.9$) was statistically similar. In fruiting phase, statistically similar results were found in small bugloss, Russian olive and almond leaves ($p \geq 0.06$) (Fig. 1).

Catalase is believed to work more actively under conditions of high concentrations of hydrogen peroxide (Nyathi, and Baker, 2006). Taking this fact into account, it can be assumed that one of the consequences of stressors aggravation in July was the high concentrations of hydrogen peroxide in small bugloss, Russian olive and almond leaves, which induced the activation of catalase antioxidant mechanism in these plants.

Peroxidases are multifunctional enzymes that use various reducing agents, most commonly phenolic compounds, to neutralize hydrogen peroxide. The involvement of peroxidases in antioxidant protection of various plants under the drought has been shown (Kolupaev, Kokorev, 2019).

Peroxidase activity statistically reduced in leaves of all studied species during the fruiting phase, (1.8 times in sickle spurge, 1.9 times in small bugloss, 4 times in smoke tree and almonds ($p \leq 0.003$)), except Russian olive (the index in leaves of the latter increased 1.9-times, $p \leq 0.001$) (Fig. 1).

Significant increase in peroxidase activity in Russian olive leaves, where by less intensity but increase in catalase activity also has been observed, suggests that the enzymatic antioxidant system in this species is one of the most effective means of protection against drought and other stressors.

Peroxidase activity in the flowering phase was found to be statistically similar in small bugloss, smoke tree and almond leaves ($p \geq 0.3$); while in the fruiting phase statistically similar and the lowest data were observed in smoke tree and almond leaves ($p > 0.05$).

Thus, based on the results obtained, it may be concluded that the enhanced water deficiency in July, accompanied by the intensive insolation and high temperature, activates the catalase-peroxidase defense enzymatic mechanism in Russian olive, while in small bugloss and almond the catalase protective mechanism is more active.

It is believed that despite the important role of enzymes in the detoxification of active forms of oxygen, the enzymatic antioxidant system cannot provide complete protection of the cell from damage, which is due to a number of factors (Polesskaya *et al*., 2006). Therefore, it has been suggested that low-molecular-weight antioxidants, such as ascorbic acid, tocopherol, anthocyanins, phenolic compounds, etc., often more effectively protect metabolism from active forms of oxygen (Blokhina *et al*., 2003; Radyukina *et al*. 2012).

3.2 Ascorbic acid and tocopherol

Ascorbic acid is one of the most important low molecular weight antioxidants synthesized in the plant. It affects many physiological processes. However, its main role is to protect plant metabolic processes from free radicals. Ascorbic acid is involved in the recovery of tocopherols and in the xanthophyll cycle; Thus, it also participates in the protection of the photosynthetic apparatus and membranes in general (Shao *et al*., 2007; Smirnoff, 2000). Ascorbic acid is found in various organelles and apoplasts of plant cells. It is most abundant in chloroplasts (20–300 mmol) (Horemans *et al*., 2000; Smirnoff, 2000).

During the fruiting phase the ascorbic acid content in small bugloss and smoke tree leaves increased 2.2 and 1.6 times, respectively ($p \leq 0.001$) (compared to flowering); in sickle spurge leaves - decreased by 8.5% ($p=0.012$), while in Russian olive and almond leaves the data of both phases were statistically similar ($p=0.6$ and $p=0.25$, respectively) (Fig. 2).

Comparing the data of experimental species have revealed that in the flowering phase the content of ascorbic acid in sickle spurge and smoke tree leaves was statistically similar ($p=0.09$), while in other species it was statistically different ($p<0.05$). The maximal content of vitamin was determined in leaves of Russian olive and the minimal - in leaves of small bugloss (Fig. 2).

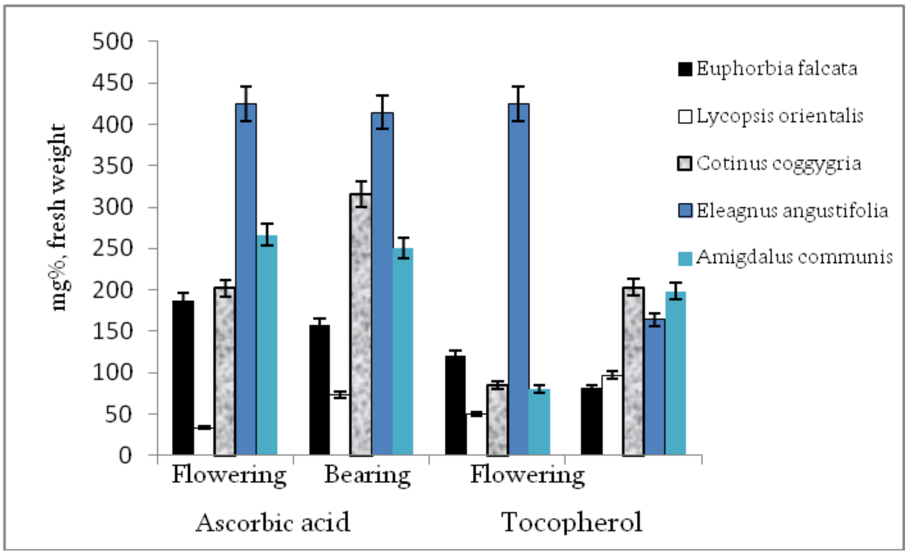

FIGURE 2: Content of ascorbic acid and tocopherol in leaves of drought resistant species of Iori plateau (East Georgia) plants in two vegetative (flowering and fruit-bearing) phases

The ascorbic acid content in leaves of all experimental plants was statistically different during the fruiting phase ($p < 0.001$).

Increase and maintenance of the ascorbate content in the cell is considered as one of the indicators of plant resistance to stress (Kolupaev and Kokorev, 2019). Enhancement of the ascorbic acid under the conditions of intensive radiation and drought has been established (Yang *et al*., 2008). It affects the activity of many enzymes and reduces oxidative stress-induced damage through synergistic interactions with other antioxidants. It is believed that ascorbate-dependent protective system is activated in drought-resistant plants (Pourcel *et al*., 2007).

According to significant increase of ascorbate in small bugloss and smoke tree leaves under the stress conditions, it may be suggested that it is one of the leading antioxidants in the drought resistance of these plants.

Tocopherol content increased 1.9 times in small bugloss leaves, 2.4 times in smoke tree and 2.5 times in almond leaves (compared to flowering) ($p=0.008$, and $p \leq 0.001$) during the fruiting phase; while decreased in sickle spurge and Russian olive leaves 1.5 and 2.6 times, respectively ($p=0.031$ and $p \leq 0.001$) (Fig. 2).

In June (flowering phase) the maximal amount of tocopherol was found in Russian olive compared to other studied species. The vitamin content measured in the leaves of smoke tree was statistically similar to the data established in leaves of sickle spurge, small bugloss and almond ($p>0.05$). Different results were obtained in sickle spurge and small bugloss leaves ($p=0.003$) (Fig. 2).

In July (fruiting phase) statistically similar results of tocopherol content were obtained in leaves of sickle spurge and small bugloss ($p=0.5$), smoke tree and Russian olive ($p=0.06$), smoke tree and almond ($p=0.9$), Russian olive and almond ($p=0.05$) (Fig. 2)

Tocopherols are antioxidant compounds found in all parts of the plant (Srivalli *et al.*, 2003). They are the most active and dominant antioxidants in the chloroplast membrane and mainly serve as protectants against photoxidation. (Ledford and Niyogi, 2005; Mullineaux *et al.*, 2006). Especially active is α-tocopherol, the content of which increases in the photosynthetic tissue in response to various abiotic stresses (Abbasi *et al.*, 2008; Giacomelli *et al.*, 2007; Noctor, 2006). Increase of tocopherols in combination with ascorbic acid is one of the plant's primary responses to water deficiency (Hasanuzzaman *et al.*, 2014; Pourcel *et al.*, 2007; Wu, *et al.*, 2007). Therefore, it can be concluded that tocopherols, along with ascorbic acid, are an important factors in drought resistance of small bugloss, smoke tree and almonds. Since the content of ascorbic acid in leaves of Russian olive did not change during the fruiting phase, and the amount of tocopherol even decreased, the pool of these compounds in leaves was still higher compared to all the studied species, which is the indication to the important role of these compounds in the drought resistance of Russian olive as well.

3.3 Soluble phenols

Phenolic compounds are a large group of secondary metabolites that play an essential role in vital processes of a plant. These compounds have a strong ability to bind free radicals and effectively protect the cell membrane from damage caused by oxidative stress (Winkel-Shirley, 2002). Under conditions of abiotic stress, including water deficiency, the biosynthesis of phenolic compounds in the plant increases. A number of literary data demonstrate the accumulation of phenolic compounds in plants during drought; which significantly increased their drought resistance (Sharma *et al.*, 2019).

The content of soluble phenols in leaves of the studied species was not high, except smoke tree, which is known for its particularly high content of these compounds (Matić *et al.*, 2016).

From the results obtained, it is clear that the response to the enhancement of stressors in experimental plants in July was different in terms of the content of phenolic compounds; including those species that demonstrated statistically similar content of phenolic compounds in the flowering phase (sickle spurge, Russian olive, almond) (Fig. 3). During the fruiting phase, the content of phenolics in sickle spurge and almonds increased 3-fold and 1.6-fold, respectively (compared to the flowering phase ($p\leq0.001$)), while in smoke tree and Russian olive decreased by 14% and 20%, respectively ($p<0.05$); In small bugloss, which was distinguished by the minimal content of phenols compared to other species, the index did not change statistically ($p>0.05$) (Fig. 3).

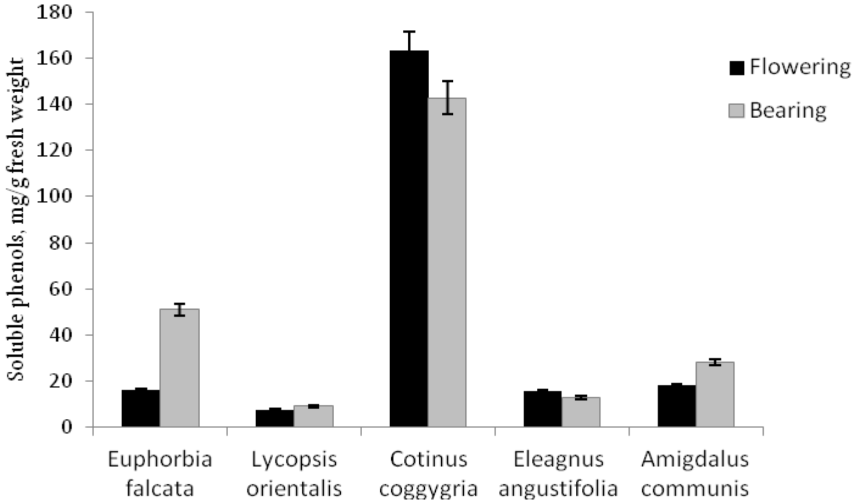

FIGURE 3: Content of soluble phenolic substances in leaves of drought resistant species of Iori plateau (East Georgia) plants in two vegetative (flowering and fruit-bearing) phases

While comparing species, in June statistically similar results were revealed in sickle spurge, Russian olive and almonds; results of all species in July were statistically different (Fig. 3).

Thus, the antioxidant system of sickle spurge and almonds responded to the enhanced water shortage and elevated temperature in June by the activation of phenylpropanoid pathway. Taking into account the abatement of ascorbate-tocopherol pool in sickle spurge under the enhanced stresses, the pronounced activation of phenolic synthesis may be considered as one of the leading mechanisms of stress adaptation in this species. Despite a slight decrease in phenolics content in smoke tree in July, its especially high pool of these compounds evidently plays an essential role in resistance to drought and temperature stresses. Here must be added the fortified ascorbate-tocopherol system, which "protects" smoke tree, as well as it happens in small bugloss.

3.4 Plastic pigments and anthocyanins

The functional state of the photosynthetic apparatus is assessed by the content of chlorophylls and carotenoids (Lichtenthaller and Buschmann, 2001). At the same time it is a good indicator to evaluate the performance of the antioxidant system.

The content of chlorophylls in leaves of the experimental plants changed with different regularities in June-July. The results demonstrate that chlorophylls content in the fruiting phase increased by 25% in sickle spurge (p=0.01), in almonds - did not change statistically (p=0.8), while in other species - decreased ($p<0.05$): in small bugloss – by 25.5%, in smoke tree - by 28%, and in Russian olive - by 11% (compared to the flowering phase). Smoke tree and Russian olive leaves were distinguished by high chlorophyll content among the studied species (Fig. 4).

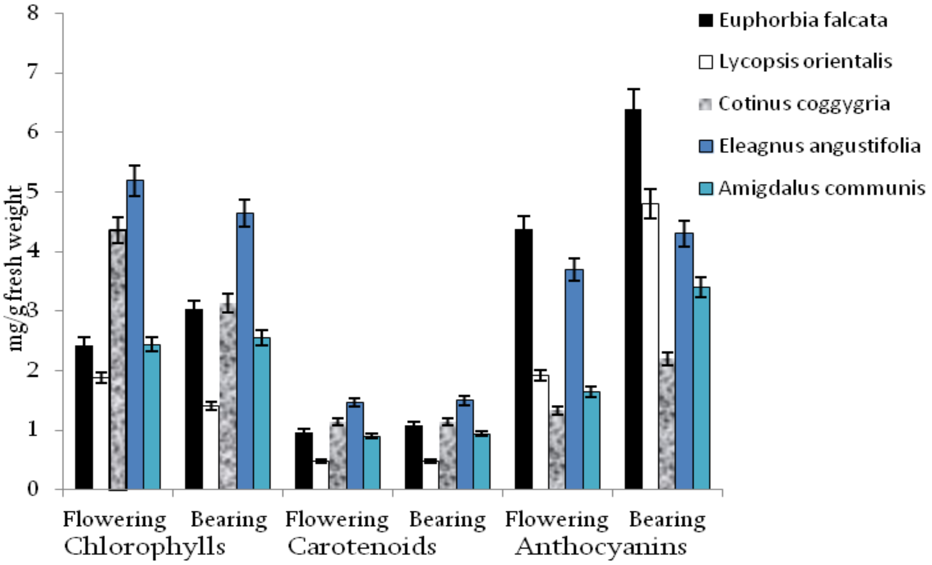

FIGURE 4: Content of chlorophylls, carotenoids and anthocyanins in leaves of drought resistant species of Iori plateau (East Georgia) plants in two vegetative (flowering and fruit-bearing) phases

Comparison of species by chlorophylls content, revealed statistically similar results in sickle spurge and almonds in the flowering phase (p=0.4), and in sickle spurge and smoke tree in the fruiting phase (p=0.9). At the same time, by the maximal content of both chlorophylls and carotenoids were distinguished leaves of Russian olive, and by the minimal - those of small bugloss (Fig. 4).

Chlorophyll reduction is a common event under drought conditions; which may be caused by inhibition of pigment biosynthesis as a result of stress, or breakdown of pigments (Batra *et al.*, 2014). It has an adaptive purpose and relieves excess energy of the plant photosynthetic apparatus. In particular, under conditions of water shortage and high temperature, when the plant is forced to close its stomata to prevent desiccation, the efficiency of photosynthesis decreases and besides the energy absorbed by the plant remains unused; it also causes the accumulation of active forms of oxygen. The last of its side poses a risk of oxidative stress in the plant (Noctor *et al.*, 2014). Avoidance of this phenomenon is achieved by the destruction of pigments, i.e. by reducing their number (Mafakheri *et al.*, 2010). Chorophyll is constantly synthesized and decomposed under light conditions; but under intensive illumination, accompanied by high temperatures, the rate of its decomposition exceeds the synthesis and the equilibrium shifts towards low pigment concentrations (Morais *et al.*, 2007).

Thus, the reduction of chlorophylls in small bugloss, Russian olive and smoke tree should be considered as an expression of adaptation to stressful conditions along with the intensification of drought. It may be assumed that the above mentioned equilibrium between the synthesis and decomposition of chlorophyll under stress conditions should not fall below a certain minimal level of chlorophyll content for the plant to continue and complete its vegetation cycle. At the same time, no "excess" amount of chlorophyll is needed, because the plant not only "is not able to use" it, but it is also "harmful" to it. Indeed, if we observe the chlorophyll content in the studied plants, by its highest content in the flowering phase were distinguished Russian olive and smoke tree. Such "excess" pigments posed a "threat" to these plants in the face of drought. Consequently, they decreased during the fruiting phase, although less in the leaves of Russian olive. The last can be related to the anatomical features of the leaves of this plant: they are covered with scales, which protect them from excess light and overheating (Fig. 4).

After decrease in July, the chlorophyll content in smoke tree leaves became statistically equal to the increased content of the pigment in sickle spurge leaves. It may be assumed that this amount of chlorophyll was within the "safe limits" for plants under those light, temperature, and water supply conditions, which were on the studied area in July; so it became "permissible" for sickle spurge to increase its chlorophyll content despite stress enhancement. Here should also be noted one advantage of the sickle spurge photosynthetic apparatus in its resistance to stress: it is established that the species of *Euforbia* genus have C_3 photosynthesis under the conditions of better water supply and relatively low temperature, while under high temperature and water deficiency they “switch to” C_4 or CAM metabolism, which allows to fix more carbon through reduced water loss and thus they are better adapted to hot and dry conditions (Batanouny *et al*., 1991; Davies *et al*., 2018).

It may be assumed that such an event occurs in the species of studied sickle spurge, which corroborates its greater adaptation to the conditions of increased stress.

As for almonds, the chlorophylls data in its leaves were statistically similar in June and July and quantitatively approached the June rate of sickle spurge. It seems that for almonds this number of chlorophylls, as for the tree plant, was "sufficient" to survive under stress (Fig. 4).

In small bugloss, which revealed the lowest chlorophyll content in leaves, the pigment amount fell by another 25% in July; however, this did not prevent him, as an annual herb, to complete his life cycle successfully. It turns out that the reduced level of chlorophylls was "enough" for him.

While discussing about the content of chlorophylls, of course, it should not be forgotten their protective and auxiliary pigments - carotenoids. These compounds with antioxidant properties belong to different groups of terpenoids, which play an important role in protecting the photosynthetic apparatus from excess light and are responsible for its structural integrity in the thylakoid membrane (Guidi *et al.*, 2017). However, there is another group of carotenoids called secondary carotenoids, which are not structurally related to the photosynthetic apparatus and accumulate outside the thylakoids. They play an active role in protecting cell structures from photo-damage as potent antioxidants, by neutralizing active forms of oxygen and enhancing the sink ability of photoassimilates. Thus, the photoprotective mechanism based on secondary carotenoids does not require additional energy and metabolites and is favorable for the plant to adapt to long-term stress, will it be an excessive radiation, drought, extreme temperatures, or salinization (Solovchenko and Neverov, 2017).

Carotenoids content in experimental species did not change statistically by phases ($p>0.05$) (Fig. 4). Comparison of species with each other revealed that both in June and July statistically similar carotenoids content was in sickle spurge, smoke tree and almond leaves ($p> 0.05$). It should be noted that the difference in carotenoid content between the studied species was similar to the chlorophyll content, i.e. the higher was the carotenoid content in leaf, the higher was iths chlorophyll content, which should be related to the protective function of carotenoids; This may explain the low chlorophyll content of small bugloss, the similar chlorophyll values of almond, sickle spurge, and smoke tree leaves, or even the maximal chlorophyll content of Russian olive leaves (Fig. 4).

Another group of pigments with antioxidant properties that accumulate in the vegetative tissues of plant in response to various abiotic stresses are anthocyanins - compounds of the flavonoid group (Gould, 2004). In July, during the fruiting phase, when the plants were exposed to stronger drought, solar irradiation, and high temperatures, the anthocyanins content in leaves of most experimental plants increased significantly, compared to June data. Sickle spurge, which had the highest content of anthocyanins in June, showed a 46% increase in pigments ($p=0.006$); the content of anthocyanins increased 2.5 times ($p=0.009$) in small bugloss leaves and 2 times - in almond leaves ($p=0.001$). No statistical difference was found between the two-phase data of smoke tree and Russian olive leaves ($p=0.07$ and 0.15, respectively) (Fig. 4).

Between-species comparison of anthocyanins content it was found that in the flowering phase this data of sickle spurge and smoke tree did not differ statistically ($p=0.3$) and were maximal. Statistical differences between other species were also not established ($p> 0.05$) (Fig. 4).

In the fruiting phase, sickle spurge was again mentioned with the highest content of anthocyanins compared to other experimental species. The minimal data were revealed in smoke tree, while in small bugloss, Russian olive and Almond the data were statistically similar ($p>0.05$) (Fig. 4).

The synthesis of anthocyanins in vegetative tissues enhances in response to various abiotic stresses, including drought, excessive ligh, and unfavorable temperatures. They bind active forms of oxygen and reveal photoprotective, and signaling function under stress (Gould *et al.*, 2018; Kovinich *et al.*, 2015). Significant increase in anthocyanins in sickle spurge, small bugloss and almond leaves against the background of increased stress makes to suggest that these pigments make an important contribution to the protection of the studied plants from oxidative stress, including their photosynthetic apparatus.

3.5 Amino acid Proline, total proteins and soluble carbohydrates

In addition to hydrogen peroxide converting enzyme systems, many metabolites may be involved in its neutralization, which reveal antioxidant properties but are not antioxidants. In particular, synthesis and accumulation of osmoprotectants, such as free amino acids, proteins, and soluble sugars, has been established under stress in plants. They protect membrane' protein-lipid components from denaturation. (Franco and Melo, 2000; Iqbal *et al*., 2020; Meng, 2014; Szabados and Savory, 2010).

The role of proline as an osmoregulator in plant adaptation to drought is well known. Numerous papers have shown the positive impact of proline accumulation on drought resistance (Ashraf *et al.*, 2018; Kaur and Asthir, 2015). It increases the cell osmolarity which in turn attracts water to the cell, or reduces its outflow; thus providing the water potential to maintain turgor in the cell under conditions of water deficiency (Anjum *et al*., 2000; Joseph *et al*., 2015; Kartashov, 2013). The last decade has focused on the antioxidant effect of proline. A model is proposed that demonstrates the ability of proline to neutralize hydroxyl radicals (Signorelli *et al.*, 2014).

Among the studied species small bugloss and Russian olive were distinguished with high content of proline in the flowering phase. During the fruiting phase, the index increased by 15% in sickle spurge and smoke tree leaves ($p=0.004$ and $p=0.009$, respectively), while it decreased in leaves of small bugloss, Russian olive and almond, by 11.4%, 11.7% and 38.4% respectively ($p\leq0.001$, $p=0.01$, and $p=0.004$) (Fig. 5).

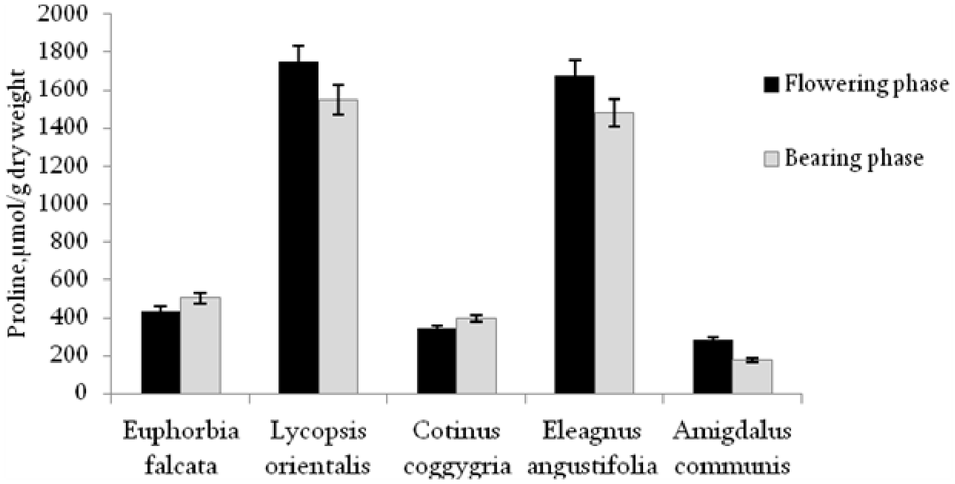

FIGURE 5: Content of proline in leaves of drought resistant species of Iori plateau (East Georgia) plants in two vegetative (flowering and fruit-bearing) phases

According to June results, the proline content in the experimental species was statistically different ($p<0.05$), while in July only in small bugloss and Russian olive leaves were statistically similar ($p=0.07$) (Fig. 5).

At first glance, it may seem that in most of the species studied, the proline accumulation strategy is not the leading one against water deficiency. A slight increase in this compound in sickle spurge and smoke tree leaves makes to suggest that it plays some role in protecting these species from stress; but if we observe how high the proline content is in leaves of small bugloss and Russian olive, despite its slight decrease in July, we must assume that this compound plays one of the leading roles in the drought resistance of these species.

Thus, in sickle spurge, smoke tree and especially small bugloss and Russian olive leaves proline should be considered as one of the means of protection from drought.

It is known from the literature that adaptation to unfavorable environmental conditions is accompanied by changes in the qualitative and quantitative composition of proteins (Mohammadkhani and Heidari, 2008; Parida *et al.*, 2007).

The quantitative study of total proteins showed that in the fruiting phase their content decreased significantly in all experimental species ($p \leq 0.001$) (compared to the flowering phase): in sickle spurge and small bugloss - 2 times, in smoke tree - 1.6 times, in Russian olive - 1.4, 1.5 times (Fig. 6).

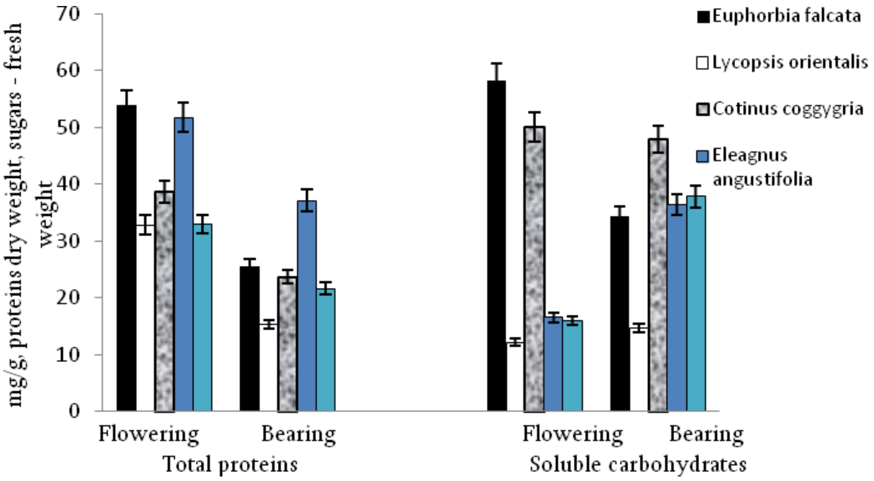

FIGURE 6: Content of total proteins and soluble carbohydrates in leaves of drought resistant species of Iori plateau (East Georgia) plants in two vegetative (flowering and fruit-bearing) phases

When comparing the results between species, the highest content of total proteins was found in sickle spurge and Russian olive leaves. In June the index was statistically similar in small bugloss - almonds (p=0.5), and sickle spurge –Russian olive (p=0.4), in July - in sickle spurge and smoke tree (p=0.05) (Fig. 6).

Decrease in total proteins under various stresses has been shown, which may be related to the outflow of soluble nitrogen-containing compounds from the leaves or their reduced synthesis (Sorkheh *et al.*, 2012). At the same time, the reduction of total proteins under drought conditions is explained by reduced photosynthesis, on the one hand and proteolysis of proteins, on the other (Mohammadkhani and Heidari, 2008; Taiz *et al*., 2016). In the case of tested species none of the above mentioned reasons is excluded. In most experimental plants a decrease in chlorophylls occurred during the fruiting phase, which implies a decrease in photosynthetic activity, while an increase in the content of the amino acid proline, as an osmoprotectant, in some species may be the result of proteolysis.

The decrease in total protein content in leaves of experimental plants may be partially related to the developmental phase as well. In the fruiting phase, when the fruit is growing and forming, the flow of assimilates towards it increases, as to an important acceptor. This is reflected on the leaf composition as a donor as well (Tegeder and Masclaux - Daubresse, 2018).

It has been established that synthesis of so called stress proteins are activated in plant cells under various stress conditions, including drought. These proteins, known as dehydrins and exerting an osmolite-like effect, are involved in the stabilization and osmotic regulation of membrane proteins. At the same time they regulate the concentration of solutions in the cytoplasm and protect cell structures from oxidative stress (Iqbal *et al.*, 2020; Mohammadkhani and Heidari, 2008). Although dehydrins have not been the subject of our study, we must assume that they would inevitably be in the leaves of experimental plants as in drought-resistant species. Their study is the subject of further research.

Although soluble carbohydrates are involved in the metabolic pathways that lead to the formation of active forms of oxygen, they also play an important role in neutralizing oxygen. The high sugar content protects proteins from denaturation and promotes membrane stabilization (Couee *et al.*, 2006; Laxa *et a*l., 2019).

A number of papers demonstrates the accumulation of soluble carbohydrates in plants in response to various stresses (Finkelstein and Gibson, 2001; Mohammadkhani and Heidari, 2008; Prado *et al*., 2000). Carbohydrates create a low water potential in the cell, which is necessary for the absorption of water from a highly mineralized soil solution (Eryomchenko *et al*., 2013).

High carbohydrate content in the flowering phase was found in sickle spurge and smoke tree leaves, compared to other experimental species. In the fruiting phase the index decreased 1.7 times in sickle spurge (p=0.001), in Russian olive and almond increased 2.2 times and 2.4 times (p=0.002 and p<0.05) respectively, while in small bugloss and smoke tree it was statistically similar to the previous phase (p=0.09 and p=0.7) (Fig. 6).

Comparing the results between species clears that all data in June statistically differed from each other (p <0.001). Statistically similar results were obtained in July in Russian olive and almonds (p=0.05) (Fig. 6).

Significant increase in soluble sugars under stress conditions in Russian olive and almond leaves makes to suggest that these osmolytes should play a leading role in protecting these plants from drought stress. Generally high content of soluble sugars in smoke tree leaves also demonstrates a stress-protective function of these compounds.

3.6 Nitrate reductase and nitrates

Nitrogen metabolism, growth and productivity of the plant can be discussed according to nitrate reductase (NR, E.C. 1.6.6.1) activity, as it plays an important role in the regulation of these processes (Garg and Singla, 2005). During the flowering phase, a high rate of nitrate reductase activity was observed in small bugloss, while in smoke tree and almond the enzyme's activity was not detected at all (Fig. 7).

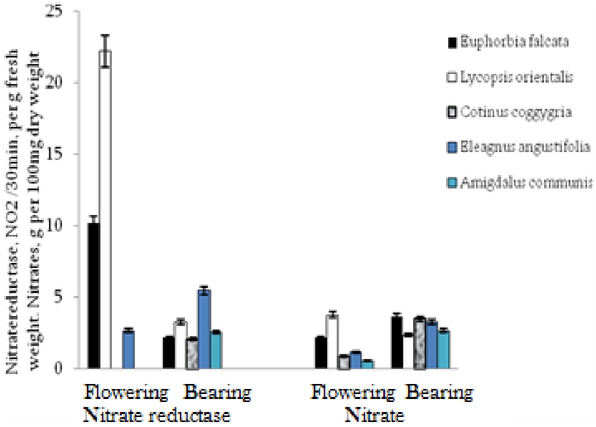

FIGURE 7: Nitrate reductase activity and nitrates content in leaves of drought resistant species of Iori plateau (East Georgia) plants in two vegetative (flowering and fruit-bearing) phases

In the fruit bearing phase, a sharp decrease in nitrate reductase activity was observed in sickle spurge and small bugloss (4.6 and 6.7 times, respectively; p≤0.001), while in smoke tree and almonds, on the contrary - nitrate reductase activity exceeded zero. Enzyme' 2-fold activation was bserved in Russian olive leaves (p=0.002) as well (Fig. 7).

Comparison of species revealed that during the flowering phase enzyme; activity statistically differed in all tested variants (p <0.001); while in the fruit bearing phase only Russian olive was distinguished by statistically different (p <0.001) results (Fig. 7).

Decrease in the activity of nitrate reductase under drought conditions has been established, which is associated with a decline in photosynthetic activity (Kapoor *et al.*, 2020).

Experimental results on nitrate reductase activity demonstrate fairly a strange picture, which may be related to the life-forms of the experimental species: sickle spurge and small bugloss are therophytes, while smoke tree, Russian live and almond are phanerophytes. The conversion of nitrates in herbaceous plants with the participation of nitrate reductase actively takes place in leaves. While it is known that in a number of woody plants the reduction of nitrates occurs mainly in roots (Cruz *et al.*, 1993; Thomas and Hilker, 2000). Accordingly, the enzyme was active in sickle spurge and small bugloss leaves during the flowering phase, although during the fruiting phase, its activity sharply reduced under stress conditions (Fig. 7). The fact that nitrate reductase is a substrate-dependent enzyme should be taken into account as well. This means that its synthesis and activity are regulated by the concentration of nitrates and intracellular ammonium (Dias *et al.*, 2011; Nicodemus *et al.*, 2008). Nitrates content in smoke tree and almond leaves was particularly low during the flowering phase, which probably led to a delay in nitrate ductase activity (Fig. 7).

Nitrate reductase activity is also inhibited by light, if carbon fixation is impaired (Kaiser and Forster, 1989). Such conditions are created by water shortage. In July, plants were exposed to such stressful conditions, which were reflected on enzyme' decreased activity (Fig. 7).

Regulation of nitrogen metabolism plays an important role in plant stress resistance, as many physiological processes are associated with it. Drought inhibits nitrogen uptake and movement to aboveground parts of the plant, leading to reduced transpiration and cell membrane permeability. Due to the soil drying it is also difficult to dissolve nitrogen and ammonium salts in water. It turned out that the activity of antioxidant enzymes significantly depends on the nitrogen concentration. Therefore, under conditions of poor nitrogen supply, the effect of oxidative stress on the plant enhances (Iqbal *et al*., 2020).

The highest content of nitrates was detected in small bugloss leaves in the flowering phase. However, during the fruit-bearing phase, this index decreased 1.6 times ($p=0.014$). In small bugloss in the flowering phase in terms of the high content of nitrates the activity of nitrate reductase was highest; which decreased in the fruiting phase in parallel with the decrease in nitrates content. In the fruiting phase, the content of nitrates in leaves of analytical plants significantly increased: in sickle spurge - 1.9 times, smoke tree - 3.9 times, Russian olive - 2.7 times, in almonds - 2.5 times ($p<0.05$). This accordingly, caused an increase in nitrate reductase activity in woody species (Fig. 7).

Comparison of nitrates content between species showed that in the fruit-bearing phase the index was statistically similar in all studied species ($p>0.1$) (Fig. 7).

3.7 Total antioxidant activity

This is an integrated characteristic of the ability of hydrophilic antioxidants to bind free radicals, without specifying compounds (Arnao *et al*., 1999). Investigation of the total antioxidant activity of tested leaves has shown that during the fruiting phase the index increased in sickle spurge and small bugloss 1.9 and 1.3 times respectively. In smoke tree and Russian olive it stayed unchanged, while in almond - decreased by 1.3 times (Fig. 8).

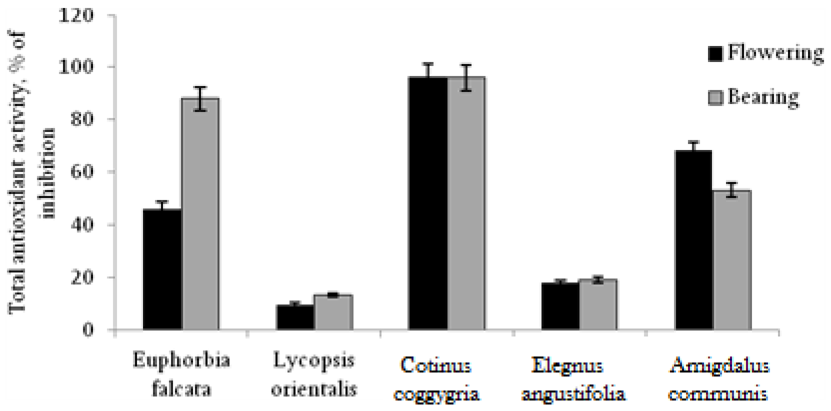

FIGURE 8: Total antioxidant activity of leaves of Iori plateau (East Georgia) drought resistant species in two vegetative (flowering and fruit-bearing) phases

As the studied plants are of medical interest along with drought resistance, the characteristic of total antioxidant activity can serve as a kind of preliminary test in terms of their use as a source of antioxidants. However, this index does not give a complete picture of plants antioxidant capacity and medical value.

According to some authors, an extract with more than 70% of total antioxidant activity is considered as effective; If the index of a sample is 60-70%, it has moderate activity; and data below 60% indicate a weak antioxidant capacity (Luzia and Jorge, 2014). Based on this criterion, smoke tree has demonstrated high antioxidant activity among the tested plants. In sickle spurge this characteristic was weak during the flowering phase and increased in the fruiting phase. The moderate antioxidant activity was revealed in almond, and low – in small bugloss and Russian olive (Fig. 8).

As it was mentioned, this characteristic does not give a complete picture of the antioxidant capacity of the test-objects because it comprises only hydrophilic antioxidants. While among plant antioxidants there are lipophilic compounds as well.

The enhancement of antioxidant activity in the fruit-bearing phase in sickle spurge may be associated with an increase in anthocyanins and phenolic compounds; in small bugloss – with ascorbic acid and anthocyanins. In smoke tree the increase in ascorbic acid is likely to be offset by a decrease in phenols in the fruit-bearing phase; therefore, the overall level of antioxidant activity has not changed. It is incomprehensible the slight decrease of the antioxidant activity in almond leaves; as its rise on the expense of anthocyanins and phenols enhancement was presumable.

IV. CONCLUSIONS

From the obtained results, it is clear that the studied drought-resistant species have more or less different biochemical stress-adaptive mechanisms. This is not surprising, as such differences may exist not only at species but at the genotypic level within a single species as well (Abuelsoud and Papenbrock, 2019).

For each studied species, it may be identified a stress coping strategy that includes certain enzymatic and non-enzymatic components of the antioxidant system.

Catalase was activated in small bugloss in response to stress enhancement, while from low-molecular-weight antioxidants synthesis of ascorbate-tocopherol and anthocyanins was enhanced. An especially large amount of proline accumulation was noted from osmolytes in its leaves.

Phenols, anthocyanins and proline should be actively involved in drought and high temperature stress in sickle spurge. Russian olive was distinguished by the maximal level of almost all studied indices, compared to other species. Apparently, this is its specific feature. Since some of these characteristics were reduced under increased stress, they still retained their maximal value and obviously played an active role in stress protection of the plant. In particular, high levels of ascorbate, tocopherol and anthocyanins, as well as proline, were found in Russian olive leaves. In addition, the enzymatic antioxidants catalase and peroxidase were activated, and carbohydrates were accumulated.

The protective systems of ascorbic acid and tocopherol, as well as phenolic compounds were active in smoke tree. From osmolytes content of proline increased, while the sugars level was already the highest, compared to all other species. Catalase was activated in response to stress in almonds; protective systems of ascorbate-tocopherol, phenols, and anthocyanins were active from the low-molecular compounds; among osmolytes content of carbohydrates was increased.

The stability of carotenoids protective system of all studied species under stress-enhancing conditions suggests that experimental plants were less sensitive to radiation stress, and water deficiency was the main stress factor for them. All tested species can be recommended as drought resistant, which may be cultivated under water deficiency conditions.

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