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EVALUATION OF THE PHYSIOLOGICAL STATE OF FEIJOA (FEIJOA SELLOWIANA BERG) IN SUBTROPICAL RUSSIA

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ABSTRACT

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The article presents the results of research examining varietal diversity with respect the activity of oxidative enzymes (EC 1.11.1.6) and the dry matter and Proline accumulation of leaves under optimal and stressful conditions. For feijoa, the most stressful period in the subtropics of Russia, with respect to hydrothermal conditions, occurs between July and September. Studies have shown that the highest degree of enzymatic activity is observed in August in the 'Superba' variety of feijoa, which was used as a control in this study, and the lowest level of activity was observed in the 'Sentjabrskaja' variety. The long-term water deficit experienced in September coincides with fruiting in feijoa. This causes a change in catalase activity in leaves, which is maintained until it is inhibited. Form ShV-1 of feijoa is characterised by its metabolic stability. In fact, the activity of oxidative enzymes in leaves of the variety is stable. Dry matter content per unit area increases as the leaf grows. During the drought period, which coincides with active fruiting, the leaves of the 'Dachnaja' variety and the ShV-1 form accumulate significantly less dry matter than other varieties. In the 'Dagomysskaja' variety, the intensity of organic matter consumption via respiration and outflow exceeds visible photosynthesis, which is expressed as a negative value (average = 1.96 g.dm⁻² h). To fully characterise the physiological state of feijoa plants under the influence of abiotic factors and catalase activity in the humid subtropics of Russia, indicators of dry matter accumulation and true photosynthesis intensity can be used.

Keywords: feijoa; enzymes; catalase; photosynthesis; stability; stress factors

INTRODUCTION

According to the modern Botanical classification, feijoa belongs to the Myrtaceae family of the order Myrtales (Bose, Mitra and Sanyal, 2001; Omarova and Kulyan, 2019; Ryndin, 2019). The family includes 72 genera and about 100 species that grow in the tropics and subtropics, mainly throughout America, North-West Africa and Australia. Feijoa is native to the subtropical zone of South America. In its wild form, it grows over a large area consisting of shrubby and mixed forests in southern Brazil, Paraguay, Uruguay, and Northern Argentina. These areas lack sharp fluctuations in air temperature and precipitation (Belous, Omarov and Omarova, 2014; Kedelidze et al., 2015; Phan et al., 2019). The genus Feijoa includes three species, F. obovata Berg, F. shenkiana, and F. sellowiana Berg. In culture, F. obovata and F. shenkiana do not occur. Since its use is advantageous relative to the other varieties of feijoa, only F. sellowiana (synonyms: Acca sellowiana Berg, Orthostemon sellowiana Berg) is widely known and industrially significant.

Feijoa Zellova (*F. sellowiana*) is an evergreen tree/shrub that grows to a height of 3 to 5 meters or more. The crown of a young plant is compact, but with age, usually after entering the fruiting season, it spreads out. Before fruiting,

bushes tend to increase in height. Afterward, apical shoots slow growth, and side shoots contribute to increases in the diameter of each plant. When grown industrially, different forms of feijoa plants are used that consist of erect plants with a pronounced stem, vigorously growing types that are structurally similar to trees, squat types with dense branching and a low-growing, compact and leafy type.

Feijoa is one of the most valuable crops, since its fruits are made up of 11.4 - 12.4% dry substances, 7.92 - 9.16%total sugars, 1.56 - 1.84% sucrose, 6.20 - 7.41% invert sugars, 1.60 - 2.45% glucose and fructose 3.36 - 5.24%. The dry weight of feijoa is also, 0.94 - 1.43% acids, producing a sugar acid index is of 6.09 - 9.06 units. Other components include vitamin C (26.0 - 32.4 mg), starch (0.92 - 1.16%), pectin (1.28 - 1.91%) and hemicellulose (3.63 - 4.25%) (Bontempo et al., 2007; Belous, Omarov and Omarova, 2014; Aoyama, Sakagami and Hatano, 2018; Kedelidze et al., 2015; Montoro et al., 2020).

Plants of different varieties can be characterised by their metabolic features and considering what type of physiological and biochemical processes occur in a plant can facilitate the elucidation of adaptation mechanisms that are used to maximise plant fitness under changing environmental conditions. The purpose of our study was to examine metabolic characteristics of a number of varieties and forms of feijoa. For example, the main indicators of the water status, and features of enzymatic activity, characteristics of the pigment apparatus and accumulation of free Proline were assessed. These indicators were selected because the photosynthetic apparatus is highly sensitive to environmental factors, and the content of plastid pigment has traditionally been used as a diagnostic indicator of the plant health (Belous, Klemeshova and Malyarovskaya, 2018; Horčinová Sedláčková et al., 2018).

The enzymatic activity of catalase (CAT), an oxidative enzyme, varies in plants as they react to adverse factors (Hodges et al., 1997; Apel and Hirt, 2004; Belous, 2012; Abilfazova and Belous, 2018), and the accumulation of damage can inactivate the enzyme. The strength of CAT inhibition depends on many factors (plant age, varietal characteristics, etc.). The accumulation of free Proline occurs as a response to various damaging factors, since Proline helps to reduce the magnitude of damage (Nilsen and Orcutt, 1996). Finally, the intensity index of true is used to characterise photosynthesis overall photosynthesis, and is directly related to the functional state of the plant. The intensity of true photosynthesis is associated with the accumulation of assimilates within the plant (Osmond et al., 1987; Gaspar et al., 2002) and can be calculated by assessing changes in the amount of dry matter within leaves over a defined period.

In this article, we have assessed CAT activity in feijoa leaves, which is a diagnostic characteristic of the functional state of the plant. Also, we have determined levels of protein and dry matter accumulation, and have measured the intensity of true photosynthesis, which determines assimilation levels within the plants assessed.

Scientific hypothesis

Determination of CAT activity is a marker of the adaptive potential of plants and can be used to identify crop resistance to hydrothermal stress.

Active defense mechanisms in feijoa plants allow plant leaves to accumulate a large amount of dry matter.

Evaluation Proline accumulation in leaf is necessary for fully characterizes the physiological status of feijoa plants.

MATERIAL AND METHODOLOGY

Plants used in the study were a one-zoned 'Superba' variety of feijoa, three varieties from the institution ('Dagomysskaja', 'Dachnaja' and 'Sentjabrskaja') and well as a promising (ShV-1), which was grown in the collection garden of the Russian Research Institute of Floriculture and Subtropical Crops. The determination of physiological parameters was carried out at the laboratory of plant physiology and biochemistry of the Institute. Leaf samples consisting of 52 - 60 leaf pieces were collected dynamically during both the most optimal and highly stressful dry growing season. Experiments were conducted using three field and three laboratory replicates.

The analysis of CAT activity in leaves was performed using by gasometrical method (**Tretyakov**, **1990**). The principle of the method for determining CAT activity is based measuring the quantity of oxygen released throughout the decomposition of hydrogen peroxide. CAT activity was expressed in mL oxygen released per g of raw plant tissue.

Percentage dry substance values were calculated by drying samples at a temperature of 105 °C (Erzhanov, 2016). The process is designed to remove both hygroscopic and external moisture. To calculate the content of components within the dry substance, the mass of the determined component was expressed as a percentage of the mass of the dry sample.

The intensity of true photosynthesis was calculated based on measured quantities of accumulated dry matter (Marakaev, 2005). The intensity of true photosynthesis is equal to the sum of the intensity of visible photosynthesis and the intensity of the consumption of organic substances for respiration and outflow to other organs (since the fluctuation of water in the leaf masks change in dry matter mass, leaves were cut when fully saturated with water).

To determine free Proline content, the Bates method was used (Bates, Waldren and Teare, 1973). The method is based on the interaction between free proline and ninhydrin reagent, which can be measured colourimetrically as a pink-red colour is produced. Free Proline content was determined using a calibration curve constructed using Proline solutions ranging from 50 to 150 mgL⁻¹, and values were expressed in micrograms Proline per 1 g of raw mass.

Statistical analysis

Statistical processing of experimental data was carried out using the ANOVA package in STATGRAPHICS Centurion XV (version 15.1.02, StatPoint Technologies) and MS Excel 2007. Statistical analyses included univariate analysis of variance, which is a method for comparing averages using variance analysis and a t-test) and variance analysis (ANOVA). Differences between means compared using least significant difference (LSD) were considered significant when p < 0.05. All experiments were performed in triplicate and values were expressed as mean \pm standard deviation (SD). Differences between the samples were assessed using unpaired t-tests.

RESULTS AND DISCUSSION

The resistance of plants to adverse environmental factors is largely determined by the activation of the antioxidant enzyme system, which inhibit the damaging effects of oxidative stress. CAT is a key enzyme involved in protection against oxidants, and the enzyme catalyzes a number of metabolic reactions. In particular, it catalyzes the decomposition of hydrogen peroxide to water and molecular oxygen. CAT activity is a marker of the adaptive potential of plants and can be used to identify the resistance of crops to hydrothermal stress, thus establishing their adaptive potential (Belous, 2012; Apel and Hirt, 2004; Hodges et al., 1997).

The effect of factors such as drought on plants includes the suppression of many physiological processes. Simultaneously, drought stress activates protective mechanisms. In recent years there has been a significant increase in temperature throughout the summer period during which has prolonged drought periods for active vegetation. There ideal conditions were determined to study the effect of hydrothermal stressors on the metabolic activities of subtropical crops, in particular, feijoa. If May precipitation of 70 - 100 mm exceeds the norm (on average 110 mm), then in other periods will experience a stable water deficit. Precipitation is reduced from 82% (June) to 21 - 26% (August) of the average annual precipitation rate. This is accompanied by increased air temperatures, which reach daytime maximum values of 34.1 - 37.0 °C, which is 4 - 9 °C higher than long-term parameters. This causes not only early attenuation of growth processes, but also fruiting in many crops, which affects the crop quality and quantity.

Changes in enzymatic activities were measured during a period of peak hydrothermal stress, which occurred as the crop was fruiting. According to a number of authors, when plants get into water deficit conditions, there is an increased regulation of certain AOS-producing enzymes (for example, catalase, ascorbate peroxidase, guaiacol peroxidase. etc.) (Arbind, Sheela Devi and Sundareswaran, 2014; Hodges et al., 1997). An increase in the activity of a number of enzymes during the progression of water deficiency in plants has shown that a direct consequence of water deficiency is damage to the cell membrane. The results highlight the important role of certain antioxidant enzymes and compounds in protecting against drought stress (Sofo, Dichio and Xiloyann, 2010). Our studies have shown that, in general, the enzymatic activity of CAT ranges from 104.4 to 170.4 mL O2.g⁻¹ of raw weight. The highest CAT index is expected in August (the most stressful period of vegetation) was observed in the 'Superba' variety, which is a control, and the lowest activity observed was determined using the 'Sentjabrskaja' variety (Figure 1). Long-term water deficiency provoked a change in the CAT activity of feijoa leaves. This was especially true in September, when plants were in the fruit loading phase, which enhanced plant stress in nearly all varieties. This was reflected by the inhibition of CAT activity, which was observed in all varieties with the exception of the 'Sentjabrskaja', whose enzymatic activity was 1.3 times higher in September than its annual average value. It must be noted that form ShV-1 did not experience noticeable changes in CAT activity, which suggests that metabolic processes on the form are stable, despite changes observed regarding the strength and duration stress experienced.

The dynamics of dry matter accumulation in feijoa leaves revealed that dry matter content per unit area increased with leaf area (Figure 2). Water stress can limit the accumulation of dry matter in the leaves, so it is important to select varieties resistant to water deficiency (de Lacerda et al., 2003; Apel and Hirt, 2004; Amirjani, 2010; Boussadia, Ben Hassine and Braham, 2018). Moreover, during the drought period, which coincided with the processes of active fruiting, leaves of the 'Dachnaja' variety and the ShV-1 form accumulated dry matter significantly less than other varieties. The most active protective mechanisms observed were in plants of the 'Sentjabrskaja' variety. The continuing drought allowed leaves, despite the active formation of fruits, to accumulate greater quantities of dry matter than other varieties ('Sentiabraskaja' averaged up to 53.90 g dry matter, while other varieties averaged 49.85 – 52.91 g).

Proline levels in the leaves of varieties and forms in July ranged from 93.75 to 201.79 mg.g⁻¹, and depend on the genotypic characteristics of plants (Figure 3). Throughout the period of high stress (August), we observed a 1.5 -2.8 fold increase in Proline levels in feijoa leaves. The greatest numbers observed were determined in 'Dachnaja', 'Dagomysskaja' varieties and form ShV-1 (266 – 270 mg.g⁻¹). Low concentrations of Prolin in 'Sentjabrskaja' and 'Superba' varieties confirmed their resistance to abiotic stressors. These data in which Proline levels increasing under stressful conditions is consistent with studies by other authors (de Lacerda et al., 2003; Ashraf and Foolad, 2007; Amirjani, 2010), who reported that increasing Proline content is a common physiological response to drought, mineral nutrition deficiency, and other adverse effects in plants. Determination of free Proline content can serve as an express method for determining the level of plant resistance, which will allow at the early stages of the selection process to reject less than stable breeding material (Bates, Waldren and Teare, 1973; Nilsen and Orcutt, 1996; Amirjani, 2010).

The activity of photosynthetic processes, determined by measuring increases in the amount of dry matter present within cells, is a key indicator of plant resistance, since the active accumulation of photosynthetic products in conditions of stress is associated with high levels of plant resistance (Marakaev, 2005, Horčinová Sedláčková et al., 2018). Calculations of the intensity of true photosynthesis during this period confirmed the physiological state of feijoa plants. Thus, the 'Superba' variety (4.82 – 13.56 g.dm⁻² h dry matter) is characterised as being highly resistant to stress. It is known that not all dry matter that is formed accumulates in leaves. Some is consumed in the process of respiration. Thus, in the 'Dagomysskaja' variety, the intensity of organic matter consumption for respiration and outflow exceeded visible photosynthesis, which was expressed as a negative average value (-1.96 g.dm⁻² h). The result was in accordance with the small difference observed in the dynamics of dry matter accumulation of the variety (Figure 2).

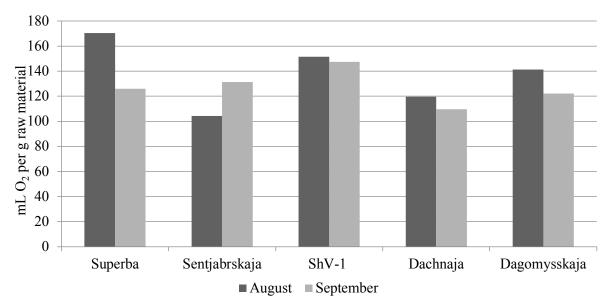


Figure 1 Catalase activity in feijoa leaves. Note: LSD ($p \le 0.05$) = 12.16 (August) and 5.54* (September); * – NS.

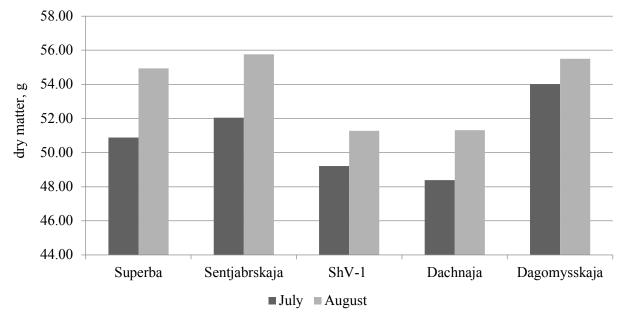


Figure 2 Dynamics of dry matter accumulation (%) in feijoa leaves. Note: LSD ($p \le 0.05$) = 3.02 (August) and 2.15 (September); all differences were significant.

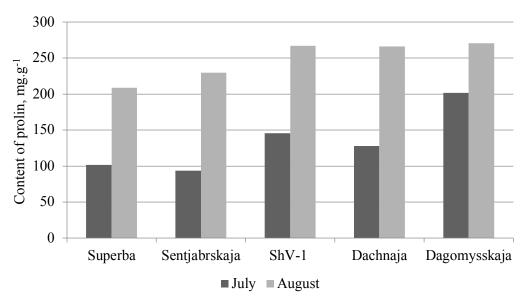


Figure 3 Dynamics of Proline accumulation (mg.g⁻¹) in feijoa leaves. Note: LSD ($p \le 0.05$), NS.

CONCLUSION

The study has shown that varieties and forms of feijoa subjected to stress factors (drought and high air temperatures during active vegetation) altered CAT activities in their leaves. It should be noted that form ShV-1 did not significantly alter CAT activity indicators as its stress level increased, which indicates that it is a form that is highly resistant to the stress factor. To fully characterise the physiological state of feijoa plants under the influence of abiotic factors in the humid subtropics of Russia, in addition to the activity of the oxidative enzyme CAT, it is necessary to use indicators of dry matter accumulation and the intensity of true photosynthesis.

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