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DYNAMIC OF PROLINE, PIGMENT CONTENTS, WATER FRACTIONS IN APPLE (*Malus domestica* Borkh.) FOLIAGE UNDER TEMPERATURE DROUGHT STRESS AND PROTECTION MEASURES

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Abstract

The hydrothermal factors and the high summer solar radiation in the South of Russia actualize the experimental studies and search for objective expressly determined quantitative indicators to evaluate functional state of fruit plants and their seasonal variability in agrocenoses. The main purpose of this work was to confirm the hypothesis of the possibility to use the amino acid proline content as a criterion for rapid assessing effects of abiotic stress intensity (soil and air drought, intensity of solar radiation) on perennial fruit plants in agrocenosis. On the example of apples (*Malus domestica* Borkh.) varieties Prikubanskoe and Aidared we identified the regulatory functions of special fertilizers in combination with growth regulator Novosil to improve adaptive properties of the apple trees. For the rapid determination of the free amino acid proline content in apple leaves we used capillary electrophoresis in the Kapel 103P device (Lumeks, Russia). To analyze tolerance of the apple trees against the summer period stressors, we used the weight method, determined the content of free and bound water in leaves, and also assessed content of chlorophyll (a + b) and carotenoids in leaves using a UNICO 2800 spectrophotometer (United Products & Instruments, USA). In the course of 4 year-studying (2009-2012) from May to August, it was shown that the leaf level of bound water increased while free forms of water declined. The leaf treatment with fertilizers together with growth regulator Novosil led to an increase in the content of the bound water in the leaves as compared to the control, which indicates an increase in the resistance of the apple tree to stress factors of the summer time. In July and August, at maximum adverse hydrothermal factors, the free proline content in the shoot leaves of apple plants became higher compared to the values under normal environmental conditions (decade III of May). Proline contents annually increased 1.4-2.9 times in July compared to May which is due to a lack of moisture, extremely high air temperatures and excessive insolation. Our data showed that the use of aqueous solutions of special fertilizers in combination with the growth regulator Novosil contributed to a decrease in the proline amount in apple leaves in July and August as compared to the control and led to an increase in plant resistance to stressors of the summer period, that is also consistent with the dynamics of the bound water we found. The conducted researches attest the possibility of using expressly estimated free proline content in apple shoots leaves as an important criterion for assessing resistance to drought and anthropogenic factors in perennial fruit plants in commercial orchards.

Keywords: *Malus domestica* Borkh., apple, environmental stress factors, the dynamics of proline content, pigments, fertilizer, growth regulator, adaptation

Ensuring sustainability of bio-technological systems is closely connected with water and temperature stress intensity in the cycle of seasonal development of plants [1-3]. The importance of such studies in the south of Russia is due to extremely high air temperatures against the background of dry hot winds and lack of precipitation during the summer period [4, 5]. In perennial fruit crops, this leads to disrupted bud set, prefloration, differentiation of flower parts and,

as a result, the productivity losses [6]. Therefore, it is important to quickly identify physiological and metabolic changes in plants at the intensity of the extreme factor and to use the agro-engineering measures necessary to stabilize biological processes [7, 8]. This requires objective quantitative indicators of the crops condition and their seasonal variability in conditions of fruit agrocoenosis.

Different aspects of diagnosing functional status of agricultural plants influenced by abiotic factors are regularly covered in scientific publications of domestic [9-11] and foreign authors [12-14]. The effect of temperature stress, mineral nutrition deficiency, water and salt stresses on citrus fruit crops in the conditions of Israel and the Republic of South Africa was evaluated in accordance with changes in the content of amino acids and other biochemical indicators [12, 13]. Data are published on the effect of drought on the accumulation of proline in the leaves of 3-year-old apple trees [14], on the enzymatic activity of the leaves of young apple trees as an indicator of drought resistance [15], on the diagnosis of changes in the activity of the oxidoreductase enzyme in apple skin at high-temperature summer stress and excess of solar radiation [16]. An increase in the amount of proline and soluble sugars with a decrease in the content of soluble protein in the leaves of alycha under soil drought is shown [17]. Pot trials under soil drought conditions showed a decrease in the amount of chlorophyll and water in leaves of plum seedlings and an increase in proline content [18]. The dynamics of proline and chlorophyll content in the European olive leaves under irrigation with industrial wastewater in Jordan [19] and the effect of rhizospheric drought on the accumulation of proline and primary metabolites in apple leaves in vitro were reported [20].

Our paper is the first to report data of field trials which show seasonal changes in the leaf level of proline, an osmoprotector increasing cell resistance to dehydration, in apple shoots under the soil climatic conditions of southern Russia. In preliminary studies, the effect of aqueous solutions of fertilizers and growth regulating chemicals on the productivity of apple plants in the region was determined [21]. It is shown that changes in the ratio of water fractions and in the content of photosynthetic pigments of leaves serve as the estimates of plant physiological state under the effect of physical stress.

The purpose of the study was to confirm the hypothesis of the possibility to use proline amino acid for rapid assessment of the resistance of perennial fruit plants to soil and air drought, as well as identify the regulatory effect of leaf treatment with fertilizers in combination with the biologically active substance Novosil.

Techniques. Apple tree (*Malus domestica* Borkh.) plants of Prikubanskoe and Aidared winter ripening varieties (commercial plantations of the experimental farm Tsentralnoye, Krasnodar, 2009-2012) were studied. During the experiment, weather observation was conducted to describe in detail the conditions of the seasonal development of plants in the summer during the differentiation of reproductive buds (III-IV stages of organogenesis), as well as after reduction of excessive productivity due to the fall of flowers, ovaries and fruits (X-XII stages of organogenesis). Trees were treated with 0.5% aqueous solutions of complex nutrient salts of the Aquarin series (OAO Buysky Chemical Plant, Russia). Fertilizers $N_{18}P_{18}K_{18}Mg_1S_{1.5}$ were used during the first half of the vegetation period, $N_{12}P_{12}K_{35}Mg_2S_{0.7}$ during the second half. The composition included trace elements Fe, Cu, Zn, Mn, Mo, B in the chelate form. A natural polyfunctional growth regulator Novosil (Biokhimzashchita, Russia) at a concentration of 0.2% was introduced into the aqueous solutions of fertilizers. The active ingredient is triterpenic acids produced from needles of Siberian fir. In the control (without fertilizers and the growth regulator) and experimental variants,

there were 6 trees. The analysis was conducted in 3 analytical replications.

The content of proline free amino acid in leaves was determined using the method of capillary electrophoresis in a modification for the analysis of plant samples [22, 23]. The method is based on the separation of charged components of complex mixtures, which allows analyzing ionic and neutral components in the plant material with high speed and accuracy. Super high frequency (SHF) extraction of proline from the plant material, free from external contaminants, was performed on an SHF extractor-mineralizer Minotavr (Lumeks, Russia). An average sample (not less than 15 healthy leaves, 1.0 g of cuttings) was placed in the SHF mineralizer container with the addition of 25 ml of a 10% ethyl alcohol aqueous solution; then extraction was carried out for 10 min in the decomposition mode without pressure, after this the container was removed and cooled. The resulting extract was quantitatively transferred to a 25 ml measuring flask using a 10% aqueous solution of alcohol. The extract was analyzed in a capillary electrophoresis system Capel-103R (Lumeks, Russia) at a voltage of 17 kV, applied current of 30±5 A, analysis time of 12 min, when the sample was injected under a pressure of 30 mbar for 5 s. With the help of the instrument software, using an electropherogram, the mass concentration of the components was calculated according to the established calibration characteristics.

The content of chlorophylls (a + b) and carotenoids in the leaves was determined with a spectral method using a Unico 2800 spectrophotometer (United Products & Instruments, USA) [24]. The water content in the leaves and the content of free and bound forms of water were analyzed by the weight method [25]. All parameters were studied in May and July-August.

Statistical analysis was carried by F.A. Volkov [26]. The calculations were performed using the Microsoft Office 2010 software package. Each year, the significance of the difference between the analyzed indicators at 5% significance level was estimated, with calculation of the arithmetic mean (M), variance (σ^2), standard deviation ($\pm$SD), the coefficient of variation (Cv) and sampling error (μ).

Results. In 2010, the greatest intensity of hydrothermal factors was observed during the summer months. In different years, the maximum air temperatures in July and August were 37.3-38.5 °C, and the temperature on the surface of the soil reached 63.2-64.5 °C. The minimum values of the relative humidity of air were 26-42%.

Leaves of apple trees shoots are not only a source of nutrient substrates and plastic equivalents but also the center of active regulation of vital processes of the perennial woody plant, the metabolite composition of which varies depending on environmental conditions. By analyzing the dynamics of the photosynthetic pigments content, it was determined that the effect of high air temperatures during the summer period against the background of a decrease in the amount of atmospheric precipitation in July and August compared with May caused a decrease in the content of chlorophyll (a + b) by 10-16%, carotenoids by 4-18%. The use of aqueous solutions of special fertilizers reduced the loss of chlorophyll (a + b) by 3.0-7.0%, carotenoids by 1.5-5.0% at an average. The decrease in chlorophyll losses at the use of fertilizers was significant in 2010 and 2011: LSD_{05} was 0.02 mg/g of dry matter; deviation from the standard 0.01 mg/g of dry matter and the experiment accuracy ($S_{x\%}$) was 0.54 and 2.45%, respectively. The decrease in the content of carotenoids was significant (in the years of observation, LSD_{05} was 0.03, 0.02, 0.03 mg/g of dry matter), deviation from the standard, respectively, was 0.01; 0.02; 0.01 mg/g of dry matter and the experiment accuracy was 1.42; 4.73; 4.62%.

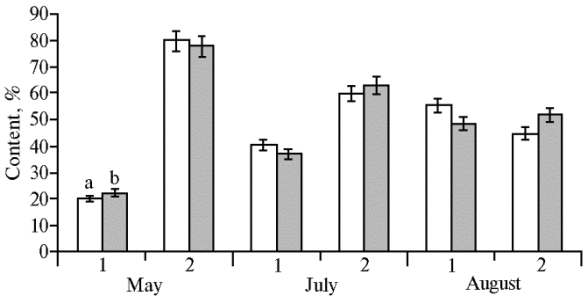

Fig. 1. Dynamics of free (1) **and bound** (2) **water content in leaves of apple (*Malus domestica* Borkh.) tree shoots on average for the Prikubanskoe and Aidared varieties:** a — control (without treatment), b — leaves fertilizing using aqueous solutions of fertilizers with the addition of the growth regulator Novosil (experimental farm Tsentralnoye, Krasnodar, 2009-2012).

The observed changes in the content of pigments at maximum intensity of hydrothermal factors are associated with the dynamics of the water fractional composition in the leaves. Such functional shifts in apple plants, typical for the state of stress, were noted by other researchers [8, 18].

From May to August, at a decrease in the total content of moisture in the leaves by 7-9%, the amount of free water increased 2.0-2.8 times and the amount of its bound form decreased. Under the effect of aqueous solutions of fertilizers and the growth regulator Novosil, the water-retaining capacity of apple tree leaves in summer slightly increased in comparison with the control (without treatment) (Fig. 1).

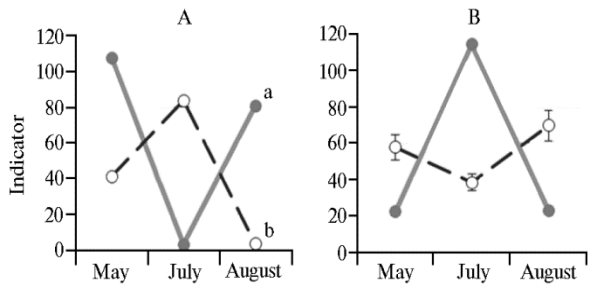

Fig. 2. Precipitations (A) **and the proline content** (B) **in leaves of apple (*Malus domestica* Borkh.) tree shoots on average for the Prikubanskoe and Aidared varieties:** a — 2011, b — 2012 (experimental farm Tsentralnoye, Krasnodar, 2009-2012).

When changing the humidity and air temperature, an increase in the free proline content was observed, which agrees with the previously published data [9, 11, 14]. In July 2011, in comparison with May, the proline content in the leaves of the studied apple varieties increased by 5 times, and in August 2012 increased by 20.8% and was inversely proportional to the amount of precipitation (Fig. 2). The proline content in leaves in May at an average air temperature of 13.8-24.8 °C and precipitation of 36.9-67.2 mm did not exceed 22.6-57.6 mg/kg.

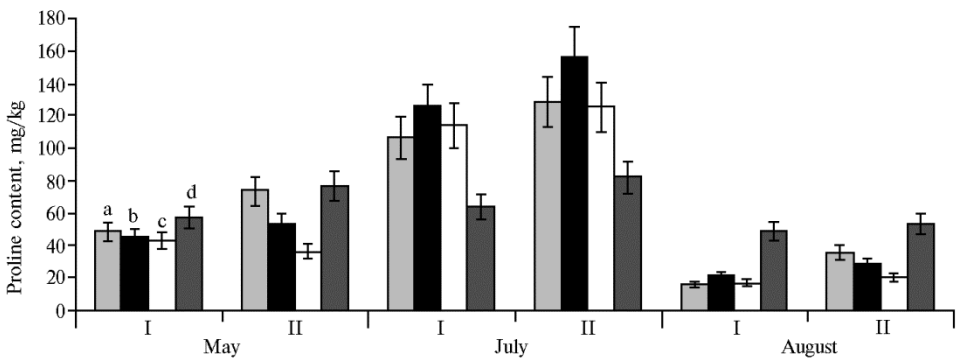

Fig. 3. The proline content in leaves of apple (*Malus domestica* Borkh.) tree shoots in the control (without treatment) (I) **and with the use of aqueous solutions of fertilizers with the addition of the growth regulator Novosil** (II) **on average for the Prikubanskoe and Aidared varieties:** a — 2009, b — 2010, c — 2011, d — 2012 (experimental farm Tsentralnoye, Krasnodar, 2009-2012).

The change in the free proline content in plants (including apple trees) under the conditions of hydrothermal factors intensity is consistent with the data of the studies of other authors [27-29].

The effect of aqueous solutions of special fertilizers used in the summer allowed reducing the content of proline slightly, which, perhaps, is associated with a weakening of stress (Fig. 3).

Thus, a decrease in the pigments content in apple tree leaves and a change in the water regime during the summer period indicated a significant effect of external conditions on the functional stability of perennial fruit plants in the seasonal development cycle. At stress (lack of moisture, extremely high air temperatures), an increase in the free proline content as an indicator of resistance to drought is observed in leaves of shoots. The use of aqueous solutions of special fertilizers in combination with the growth regulator Novosil partially contributes to a decrease in the intensity of stress, which is confirmed by a decrease in the proline accumulation in the leaves. At moderate air temperatures and regular precipitation, treatment of leaves does not have a significant effect on the proline content. As per the reduction of this indicator, the third decade of August can be considered as a period of plant reparation after stress. Our study indicates the dynamics of free proline content to be considered as an important criterion for assessing drought resistance and anthropogenic impact on perennial fruit plants in anagrocenosis.

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