

RELATIONSHIP BETWEEN THE ACTIVITY OF GUAIACOL PEROXIDASE AND THE CONTENT OF PHOTOSYNTHETIC PIGMENTS IN TEA LEAVES

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ABSTRACT

The dynamics of guaiacol peroxidase and photosynthetic pigments in 3-leaf sprouts (flushes) of tea plants were studied. The presence of declines and peaks in the activity of the enzyme associated with the meteorological conditions of each month was noted. It is shown that there is a direct relationship between the increase in enzyme activity and hydrothermal factors. The most significant correlation was found between the activity of GPO in a 3-leaf tea flush and the amount of precipitation ($r = 0.86$). The highest activity of guaiacol peroxidase during the entire vegetation period is distinguished by the Sochi variety and form 582. The lowest activity was observed in forms 3823 and 2264, which indicates a low intensity of redox reactions in these plants in stressful situations. Determining the dynamics of the pigment complex revealed its dependence on hydrothermal factors. Studies have shown that precipitation is a significant factor affecting the pigment complex of tea plants. It was found that the largest amount of green pigments is synthesized by leaves at the beginning of active vegetation (May). The participation of the pigment apparatus in the adaptation of the tea plant is directly related to carotenoids, the increase in the number of carotenoids coincides with the period of drought. In the content of photosynthetic pigments and the activity of guaiacol peroxidase manifest genotypic features. The revealed patterns are common to all tea plants.

Keywords: tea; chlorophyll; carotenoid; guaiacol peroxidase; air temperature; precipitation; correlation; stability

INTRODUCTION

One of the most important problems of phytophysiology is the study of the mechanisms of plant resistance to adverse environmental factors (Foyer and Noctor, 2000; Kareska, 2009; Belous and Platonova, 2018a). One of the consequences of stress is the formation of reactive oxygen species (ROS). In a comfortable state of the cell, the ROS content is maintained at a low level, thanks to the work of special antioxidant enzyme systems that utilize reactive oxygen species. The amount of ROS that is not eliminated by enzymes, accumulated in the cell, and causing damage to it, is called oxidative stress. Oxidative stress is a non-specific reaction of plants to the action of stress factors (Kareska, 2009; Belous and Platonova, 2018c). Under the action of stressors, the content of ROS in cells increases significantly, especially in heat-loving plants, which include all subtropical crops, such as tea.

Several authors noted an increase in the activity of peroxidases, especially when inactivating certain antioxidant enzymes, such as catalase (Krasensky and Jonak, 2012; Eremchenko, Kusakina and Luzina, 2014; Kaur and Asthir, 2017; Belous, Klemeshova and Malyarovskaya, 2018; Belous and Platonova, 2019). It is no accident that recently special interest has been directed to the study of peroxidases – enzymes of the class of

oxidoreductases that catalyze the oxidation of various substances with the help of hydrogen peroxide. The level of peroxidase activity in plant organs is used to characterize their functional state in response to extreme environmental factors (Bukhov et al., 2001; Kapchina-Toteva et al., 2004; Ladygin and Shirshikova, 2006). An increase in peroxidase activity in the photosynthetic organs of plants indicates active photosynthesis during stress.

An important question for us is related to the fact that the peroxidase substrates are phenolic compounds, ascorbic acid, a number of aromatic acids, carotenes, etc. (Kasote et al., 2015). All these compounds are significant components of the antioxidant system of tea and determine its nutritional value (Fedotova, 2009; Mulgund, Doshi and Agarwal, 2015; Belous and Platonova, 2018b). But the main substrate is phenols, which under the action of the enzyme are oxidized to polyphenols and quinones, which are strong oxidizers (Rogozhin, 2004). Quinones are capable of polymerization, resulting in dark-colored compounds. This process is especially active at the stage of enzymatic oxidation of raw materials (3-leaf sprout) in the production of black tea.

The effectiveness of the photosynthetic apparatus in stressful conditions is due to the peculiarities of the

pigment complex. This is one of the most important indicators of the adaptive potential of plants since carotenoids are important components of the antioxidant system and play an important role in protecting plants from photo-oxidative processes. Carotenoids take part in redox processes; normalize oxygen consumption by plant tissues. These pigments are effective antioxidants because they absorb singlet molecular oxygenized radicals.

The composition of the antioxidant system of tea includes the main components that determine the taste of the finished product, the importance of these substances for humans is undeniable and the study of the patterns of their accumulation is necessary (Belous and Platonova, 2018b). Tea plants in the conditions of subtropical Russia are often exposed to stress factors during the active vegetation period; the study of the regularities of the formation of components of the antioxidant system of tea is undoubtedly relevant.

The picture of tea sprout is presented in Figure 1, black tea from Krasnodar sprout is presented in Figure 2 and the tea harvesting on the experimental plantation is presented in Figure 3.

Scientific hypothesis

There is a relationship between the activity of guaiacol peroxidase and hydrothermal factors.

A decrease in the activity of guaiacol peroxidase is unfavorable for the stability and quality of tea plants.

Hydrothermal factors influence the content of photosynthetic pigments.

The ratio of the amount of chlorophyll to carotenoids indicates the degree of adaptation of tea plants to adverse conditions.

MATERIAL AND METHODOLOGY

Objects of research – 3-leaf sprouts (flushes) of tea plants (*Camellia sinensis* (L.) O. Kuntze) varieties of Sochi and forms of Institute selection 3823, 582, 855, and 2264, grown on the experimental collection and breeding site of the Russian Institute of Floriculture and Subtropical Crops in the village Uch-Dere (Lazarevsky district, Sochi, Russia). Control – Colchida variety. The plantations are located at an altitude of 280 m above the sea. Soils in the Krasnodar region where tea plantations are located are brown forest slightly unsaturated. In the humid subtropical zone of Russia, the driest period coincides with the period of active vegetation – June and August. Weather conditions in recent years differ significantly from the long-term norm, both in terms of precipitation and air temperature. Thus, the precipitation deficit in May – August is on average 28.5 – 87.0 mm; the absolute maximum temperature is in the range of 35.5 (in June) – 32.3 (in August) °C, at that time, an average monthly average temperature of 22.3 ± 1.2 °C. The average duration of sunshine is 244 hours per month (May – August), which corresponds to almost completely cloudless days in summer.

The selection of flushes was carried out in 3-fold field repetition in the period from May to August 2017 – 2019. The determination of laboratory analyses was performed in 3-fold repetition in the laboratory of plant physiology and

biochemistry. All chemicals were analytical grade and obtained from LENREACTIV (Russia).

Determination of pigments

We used a spectrophotometric method for determining the content of chlorophyll and carotenoids with the extraction of pigments with 96% ethanol and using the calculated formulas of Smith and Benitez (Shlyk, 1971). The optical density of extracted pigments was measured using a PE-5400VI spectrophotometer, manufactured by EKROSHIM LLC (Russia) at a wavelength of 665 and 649 nm for chlorophylls a and b, and 440.5 nm for carotenoids in cuvettes with a layer thickness of 10 mm.

Determination of the activity of guaiacol peroxidase

The activity of guaiacol peroxidase (GPO) was determined by the spectrophotometric method, taking into account the rate of utilization of hydrogen peroxide in the reaction mixture, into which the plant material is introduced. The intensity of utilization of hydrogen peroxide was judged by the rate of extinction reduction at a wavelength of 440 nm against the phosphate buffer (pH 6.7) (Vorobyov et al., 2013).

Statistical analysis

The arithmetic mean values of the measured values and their standard deviations are shown in Figures and Tables. The correlation between the samples was estimated by calculating the Spearman rank correlation. To check the significance of correlation and estimate statistical values, an analysis was performed using the ANOVA package in STATGRAPHICS Centurion XV (version 15.1.02, StatPoint Technologies) and MS Excel 2007. Statistical analysis included a one-dimensional analysis of variance (a method for comparing averages using variance analysis, t-test). The significance of the difference between the average values at $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

When determining the activity of guaiacol peroxidase in freshly harvested 3-leaf tea flushes during the active vegetation period, the presence of declines and peaks in the activity of the enzyme associated with the meteorological conditions of each month was noted (Figure 4 and Figure 5). At the beginning of the growing season (May), the activity of the enzyme was low – in the range of 0.363 to 0.607 unit.g⁻¹.sec.

In June, there is a decrease in activity, which, however, is not significant and is due to the biological characteristics of the tea culture. After the May surge in growth processes in tea plants, there is a period of rest, in which the metabolic processes are somewhat slowed down. As a rule, in the future, in our zone there is a stressful period under hydrothermal conditions, which affects the functional state of plants; in particular, we can note an acute water deficit. This leads to the manifestation of oxidative stress, which is expressed in an increase in the activity of GPO, as a non-specific reaction to a stress factor (Birben et al., 2012; Keshari et al., 2015; Ognik et al., 2016).



Figure 1 Tea sprout.



Figure 2 Black tea from Krasnodar sprout.

We performed a correlation analysis that showed a direct relationship between increased enzyme activity and hydrothermal factors (Table 2). At the same time, the most significant correlation was found between the activity of GPO in freshly harvested 3-leaf tea flushes and the amount of precipitation ($r = 0.86$).

However, the dynamics of enzyme activity in a 3-leaf sprout is variety-specific (see Table 1). The highest activity of guaiacol peroxidase during the entire vegetation period is characterized by the Sochi variety and form 582 (about $0.56 \text{ unit} \cdot \text{g}^{-1} \cdot \text{sec}$).

The lowest activity was observed in forms 3823 and 2264, which indicates a low intensity of redox reactions in stressful situations, under the influence of changing environmental factors on plants. Tea sprouts are used to prepare a drink whose nutritional value is made up of substances formed both in the process of photosynthetic reactions and in the process of processing raw materials, the basis of which is redox enzyme reactions (Chen and Asada, 1989; Pandey et al., 2017; Skhalyakhov et al., 2019). Given this fact, a lower level of activity of guaiacol peroxidase is a negative phenomenon, both for the stability of the plant itself and for the quality of the finished tea.

One of the indicators of plant response to changes in hydrothermal conditions is the quantitative content of chlorophyll and carotenoids – the main photoreceptors of the photosynthetic cell (Fedotova, 2009; Eremchenko, Kusakina and Luzina, 2014). Stress factors, including lack of precipitation and high positive temperatures, significantly increase the probability of photo-oxidative damage in chloroplasts (Foyer and Noctor, 2000; Mittler, 2002; Kapchina-Toteva et al., 2004; Ladygin and Shirshikova, 2006; Kareska, 2009; Nikolaeva et al., 2010). Only by studying the pigment system of plants can we fully identify the biological and adaptive capabilities of the culture. As shown by research, a significant factor that affects the pigment complex of tea plants is the amount of precipitation.

Determination of the dynamics of the pigment complex showed its dependence on hydrothermal factors: the largest amount of green pigments is synthesized by leaves at the beginning of active vegetation (May). In July, after short rains of a stormy nature that do not cover the water deficit in the soil, growth processes in tea resume, but less actively than in May. There is an increase in new sprout; green pigments which are intensively synthesized in leaf blades, which manifest itself in an increase in their number. In August, the drought increases, which affects not only the inhibition of growth processes but also a significantly lower accumulation of the green group of pigments ($\text{LSD } (p \leq 0.05) = 0.12$).



Figure 3 Tea harvesting on an experimental plantation (Sochi, Russia).

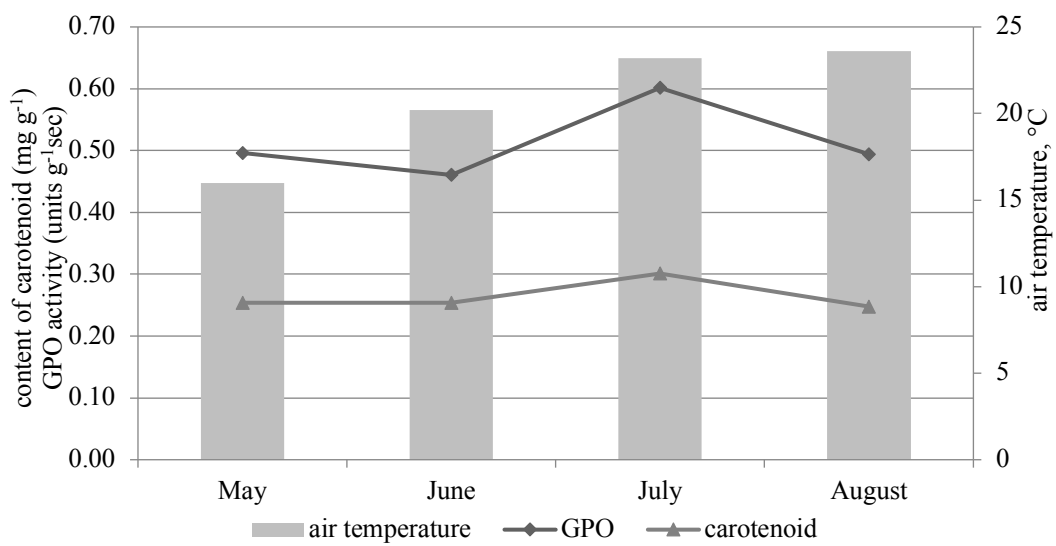


Figure 4 Dynamics of AOS components in a 3-leaf sprout *Camellia sinensis* depending on temperature conditions of vegetation.

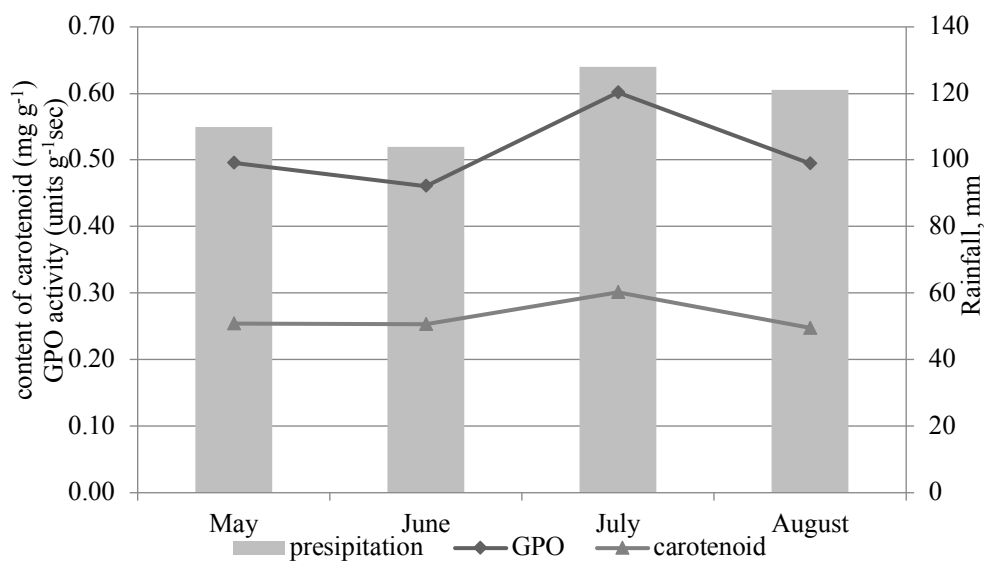


Figure 5 Dynamics of AOS components in a 3-leaf sprout *Camellia sinensis* depending on the amount of precipitation during the growing season.

Table 1 Varietal features of accumulation of components of AOS by 3-leaf *Camellia sinensis* sprout.

Variety/form	The activity of GPO, unit.g ⁻¹ .sec	V,%	Sum of chlorophylls, mg. g ⁻¹	V,%	Sum of carotenoids, mg. g ⁻¹	V,%
cv. 'Colchida'	0.52 ±0.10	8	1.09 ±0.07	7	0.29 ±0.02	9
cv. 'Sochi'	0.56 ±0.11	17	1.04 ±0.07	7	0.29 ±0.02	6
f. 3823	0.56 ±0.17	20	0.86 ±0.09	11	0.24 ±0.03	14
f. 582	0.45 ±0.05	39	0.97 ±0.10	10	0.26 ±0.03	11
f. 855	0.54 ±0.05	10	0.92 ±0.09	9	0.25 ±0.03	13
f. 2264	0.46 ±0.01	11	0.95 ±0.11	11	0.26 ±0.03	13
HCP (<i>p</i> ≤0.05)	0.05		0.05		0.01	

Table 2 Coefficient of pair correlation between the studied parameters and hydrothermal factors.

Parameters	GPO, unit.g ⁻¹ .sec	Sum of chlorophylls, mg. g ⁻¹	Sum of carotenoids, mg. g ⁻¹
GPO, unit.g ⁻¹ .sec	1.00	-	-
Sum of chlorophylls, mg. g ⁻¹	-0.26	1.00	-
Sum of carotenoids, mg. g ⁻¹	0.94	-0.48	1.00
Air temperature, °C	0.42	0.54	0.38
Amount of precipitation, mm	0.86	0.27	0.68

The participation of the pigment apparatus in the adaptation of the tea plant is directly related to carotenoids (Figure 5); as can be seen from Figure 5, a slight increase in the number of carotenoids coincides with the period of drought and the natural June decay of growth processes. In August, with significant water scarcity there has been a sharp increase in the synthesis of carotenoids, because of the continuous drought period, followed by increasing the temperature to 30 degrees or more, and reduced atmospheric humidity of 50 – 60%, what is the tea plant even bigger stressor than lack of soil moisture. Increasing the synthesis of carotenoids leads to a significant decrease in the ratio of the amount of chlorophyll to the number of carotenoids.

The identified patterns are common to all tea plants. However, the content of photosynthetic pigments also shows genotypic features (see Table 1).

The main photosynthetic pigment is chlorophyll-a (Ognik et al., 2016; Potoroko et al., 2017; Platonova and Belous, 2019). Significant accumulation of chlorophyll in the leaves is characteristic of the control cv. Colchida. While the studied mutant forms contain chlorophyll a significantly lower (Table 1). The content of chlorophyll b indicates the level of adaptation of plants to low light (Ognik et al., 2016; Potoroko et al., 2017; Steinman et al., 2017; Platonova and Belous, 2019). For tea plants, this is not relevant, since the crop is grown in open spaces and pruning the trellis stimulates the growth of leaves on the upper part of it. But often a tightly closed trellis restricts the space open to sunlight, and many of the leaves of the side surfaces are in shadow. In this case, increased content of chlorophyll b is preferable for the photosynthetic

activity of leaves of this tier (Beneragama and Goto, 2010; Biswal et al., 2012). We noted the same pattern as with chlorophyll a: more chlorophyll b accumulates in the control variety; and the differences are significant or at the border of materiality, as in the case of forms 3823 and 2264. Important is not only the content of pigments but also their ratio. In all the tea plants studied, the a-b ratio ranges from 2.81 mg. g⁻¹ to 3.36 mg. g⁻¹ and the differences between the forms are insignificant (Table 1).

The ratio of the amount of chlorophylls to carotenoids is a more informative sign since it indicates the degree of adaptation of plants to adverse conditions (Thongsook and Barrett, 2005; Potoroko et al., 2017). The stability of the variety is higher than the ratio is lower. According to this indicator, all new forms of tea can be classified as fairly stable, in their flushes, the ratio of the amount of chlorophyll to carotenoids is significantly lower than in the cv. Colchida (see Table 1).

CONCLUSION

Thus, we determined the change in the activity of GPO and photosynthetic pigments in a freshly collected 3-leaf sprout. The presence of declines and peaks in the activity of the enzyme, which are associated with meteorological conditions of vegetation, is noted.

The existence of a close correlation between increased enzyme activity and precipitation is shown. The stressful period under hydrothermal conditions affected the functional state of plants.

The acute water deficit led to the manifestation of oxidative stress, which is expressed in an increase in the

activity of GPO, as a non-specific reaction to a stress factor.

The dynamics of GPO activity in a 3-leaf sprout is determined by the genotypic features of the variety: the lowest activity was observed in forms 3823 and 2264, which indicates a low intensity of redox reactions in stressful situations in these plants.

The participation of the pigment apparatus in the adaptation of the tea plant is directly related to the content of carotenoids. With a significant water deficit, there is a sharp increase in their synthesis. The ratio "chlorophylls/carotenoids" is an informative sign of the degree of adaptation of tea plants to adverse conditions. The revealed patterns are common to all tea plants.

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