

BIOLOGICALLY ACTIVE SUBSTANCES OF *CAMELLIA SINENSIS* IN A HUMID SUBTROPICAL CLIMATE OF RUSSIA

Oksana Belous, Natalia Platonova

ABSTRACT

We did a comparative analysis of tea and raw tea materials. There is an increase in the content of carotenoids and flavonoids (thearubigins and theaflavins) in June, a decline in July, and August, and consequently a slight increase again in other months. The increase is due to unfavorable conditions – drought during these periods. In raw new variety forms No. 855 and No. 582, as well as in black tea variety form No. 582 (0.09 mg.g⁻¹), we determined the high value of theaflavins (0.10; 0.11 and 0.09 mg.g⁻¹, respectively). The highest content of thearubigins was found in variety forms No. 582 and No. 3823 (1.33 mg.g⁻¹ and 1.17 mg.g⁻¹). Ascorbic acid is significantly disintegrated (on average 96 – 97%) in the production of black tea. In green tea, ascorbic acid disintegrates to a lesser extent, leaving about 13% of its initial amount in the raw material. The dynamics of GPOD activity in a 3-leaf sprout are variety-specific. At the beginning of the growing season (May), the activity of the enzyme was low – in the range of 0.363 to 0.607 g-unit in sec. In June, there is a decrease in activity, which, however, is not significant ($p < 0.05$) and is due to the biological characteristics of the tea culture. In green tea, the ruthine is on average 3 times more than in black tea (on average about 38.09 and 12.12 mg.100g⁻¹, respectively). We have identified 11 amino acids; the highest percentage accounted for proline (from 30 to 70%), valine (17 – 30%), and serine (about 10%). We have identified 11 amino acids in Krasnodar tea, a large proportion of these amino acids has proline, valine, and serine. There was a variation in the content of biologically active substances depending on genotype characteristics. Studies have identified some controversial issues that require explanation and further study.

Keywords: fresh sprout; biologically active substances; plant antioxidative system; growing and processing conditions; genotype characteristics; *Camellia sinensis*

INTRODUCTION

The antioxidant system of tea contains components that determine the taste of the finished product, and the importance of these substances for humans is undeniable. The composition of these components, as in the raw tea materials and the finished product is rather well studied: we studied the influence of environmental factors on biochemical components, considered the effect on the quality of the tea micro-, and macro, raised the issues of changes of biochemical characteristics with the different methods of collecting leaves and the timing of rejuvenating pruning (Willson, 1975; Marimuthu and Kumar, 2001; Wright, 2005; Khan and Mukhtar, 2007; Wang et al., 2014).

The essence of black tea technology is to accelerate the transformation of substances by activating the action of enzymes and the oxidation process. This causes changes in biologically active substances (BAS) that are part of the tea raw materials (Wright, 2005; Skhalyakhov et al., 2019). In the production of green tea, enzyme activity is suppressed, which is achieved by steaming flushes. Therefore, in green tea, retains almost the entire volume of

vitamins contained in the raw material (tea leaf sprout), and it's number of several times more than in black tea.

The composition of the tea leaf includes no less important components – ascorbic acid (AA) and Ruthin (Wright, 2005; Konnov et al., 2019; Zhang et al., 2020). AA is one of the water-soluble antioxidants, participates in all types of metabolism, in the regulation of cell division and growth, protects biomolecules from free radical oxidation, and serves as a cofactor of many enzymes. Ruthin is the main vitamin of tea, in combination with vitamin C, it enhances its effectiveness, promotes the accumulation and retention of ascorbic acid in the body.

The composition of tea contains about 17 amino acids, which together with proteins is about 16 – 25% soluble extractives (Belous, 2013; Gai et al., 2019). Tea amino acids contribute to the formation of the bouquet and aroma of the beverage since the interaction with the tannin and catechin in high temperatures in the tea production process to form aldehydes.

For many decades based on the Subtropical Scientific Centre of the Russian Academy of Sciences the control of quality indicators of tea was conducted, which revealed a significant variation in the basic biochemical parameters

(the content of tannin and extractive substances) depending on the area where tea plants were grown, weather conditions, varieties, agriculture, leaf maturity, processing, storage, and many other factors (Argunova et al., 1994; Prokopenko and Tuov, 1994; Belous and Pritula, 2010; Belous, 2013; Platonova et al., 2017; Belous and Platonova, 2017; Belous and Platonova, 2018; Konnov et al., 2019; Platonova et al., 2019; Gvasaliya, 2019). However, complex fundamental studies of the biochemical characteristics of raw tea materials, in particular, the finished tea (with an emphasis on the antioxidant complex) at the present world level has not been previously carried out. The purpose of our research is to study regularities within the formation of antioxidant components in the tea system that was grown on the Krasnodar region plantations. One of many tasks is to conduct a comparative biochemical analysis of tea and raw materials with the establishment of varying the content of substances of the antioxidant group depending on the varietal characteristics and to determine the changes in the main biochemical components in the processing of raw materials in the finished black and green tea.

Scientific hypothesis

The content of photosynthetic pigments has genotypic features.

There is a direct relationship between the quality of raw materials and the content of flavonoids in tea.

There are significant losses of ascorbic acids during processing raw materials to finished tea, despite the equal conditions of collection.

The dynamics of GPOD activity in a 3-leaf sprout are variety-specific. There is a direct relationship between increased enzyme activity and hydrothermal factors.

The largest number of amino acids is synthesized in May, and in the future, AAs are included in the synthesis, the processes of transformations, and mutual conversions to other compounds.

MATERIAL AND METHODOLOGY

Samples

The finished product of tea was made in the plant physiology and biochemistry laboratory of the Federal

Research Centre the Subtropical Scientific Centre of the Russian Academy of Sciences using classical technologies.

Black tea (Figure 1) – in the production of black tea, the raw material passed the stages of withering, twisting (moisture loss up to 55%), fermentation (oxidation of polyphenols with the formation of theaflavins and thearubigins), drying (at a temperature of about 95 °C to a final water content of not more than 5 – 6%).

Green tea: steaming (temperature about 95 – 100 °C), twisting, drying (at a temperature of about 95 – 105 °C to the final water content of no more than 3 – 5%).

Chemicals

Ethanol (96% ethyl alcohol), U.S.P. – JSC LenReactiv (St. – Petersburg, Russia).

Potassium iodide (KJ), Reagent – JSC LenReactiv (St. – Petersburg, Russia).

Hydrochloric acid (HCl), U.S.P. - JSC LenReactiv (St. – Petersburg, Russia).

2,6-dichlorophenol indophenol (C₁₂H₆Cl₂NNaO₂xH₂O), Reagent – LLC TD "Himmed" (LLC Sigma-Aldrich, USA).

Hydrogen peroxide (H₂O₂), N.F. - JSC Reachim (Moscow, Russia).

Guaiaicol (C₇H₈O₂), Reagent – LLC TD "Himmed" (LLC Sigma-Aldrich, USA).

Ninhydrin (C₉H₄O₃*H₂O), Reagent – JSC Interhim (St. – Petersburg, Russia).

Animals and Biological Material:

The research began in 2016, the targets are samples from raw material (3-leaf fresh sprout) of cultivars Kolkhida and Sochi, and forms 3823, 582, 855, and 2264 (Sochi, Lazarevsky district, Krasnodar region, Russia) (Figure 2a, b).

Instruments

We using a PE-5400VI spectrophotometer, manufactured by EKROSCHEM (Russia) and capillary electrophoresis system «Kapel 105-M», manufactured by LUMEX (Russia).

Laboratory Methods

The main component determination of the antioxidant system was carried out in the plant physiology and biochemistry laboratory of Subtropical Scientific Centre. Contents of photosynthetic pigments using method invented by Shlyk and the calculated formulas of Smith and Benitez (Shlyk, 1971).

The determination of ascorbic acid was performed using the classical iodometric method (Vorobyov et al., 2013).



Figure 1 Black tea from experimental plants.



Figure 2 Experimental tea plantation (a), and raw materials (b), Sochi, Russia.

Flavonoid compounds with P-vitamin activity were determined by titration following the method of vitamin analysis (Voskresenskaya et al., 2006).

The content of free amino acids amount in samples was determined by the ninhydrin dyeing method and then use a high-performance liquid chromatography method (Speckman et al., 1958).

The activity of guaiacol peroxidase (GPOD) was determined by the spectrophotometric method (Vorobyov et al., 2013).

Description of the Experiment

Sample preparation:

The content of chlorophyll and carotenoids: The exact weight (100 mg) of the crushed raw material was placed in a mortar and ground with 96% ethyl alcohol. The resulting extract was drained through a glass filter no. 3. The extract and was transferred to a 25 mL volumetric flask and brought to the required volume with pure 96% ethyl alcohol. The optical density of extracted pigments was measured 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.

The determination of ascorbic acid: The raw material sample (2 g) was ground with 4 mL of 2% hydrochloric acid, transferred to a 25 mL flask and 0.001 n is titrated with a solution of 2,6-dichlorophenolindophenol to a persistent blue staining.

Flavonoid compounds: Standard solutions of the studied flavonoids in 95% ethyl alcohol with a concentration of 1 mg.mL⁻¹ were prepared according to an exact suspension. Working alcohol solutions were prepared by diluting the standard ones and stored in the refrigerator for no more than 7 days. To determine the TFs and the TRs, the method of spectrophotometry was at a wavelength range of 665 nm for TFs and 825 nm for TRs in contrast to water used as a blind sample.

Determination of the activity of guaiacol peroxidase: The tissue sample after extraction of weakly bound peroxidases was placed in 5 mL of cold citrate-phosphate buffer, and rubbed. The resulting homogenate was

centrifuged at 3 thousand rpm for 15 minutes. The activity of peroxidases in leaves was determined in a reaction mixture of the following composition: 0.5 mL of 0.1 M citrate-phosphate buffer (pH 6.7), 0.5 mL of 0.3% hydrogen peroxide, 0.5 mL of 0.05% guaiacol, and 0.5 mL of the sample (1 g of the sample was ground in 10 mL of a pH 5.5 citrate-phosphate buffer). The peroxidase activity was determined at 25 °C immediately after the isolation of the enzymes from the samples. 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).

The content of free amino acids: conducted using the capillary electrophoresis system «Kapel 105-M». The raw material of the leaves (100 mg) was dried, 1 mL of water was added, and placed in a thermostat (-20 °C) for 2 – 3 hours. Centrifuged (13,000 rpm) for 20 min. The eluate was evaporated at 65 °C, 100 µL of NaOH (0.15 M), 0.2 mL of phenylisothiocyanate (PITC) was added, and incubated for 1 hour.

Number of samples analyzed: All experiments include six analyzed samples.

Number of repeated analyses: All experiments include three repeated analyses.

Number of experiment replication: All experiments include 2 replications of the experiment.

Statistical Analysis

Statistical processing of the experimental data was carried out using the ANOVA package in STATGRAPHICS Centurion XV (version 15.1.02, StatPoint Technologies) and MS Excel 2007. Statistical analysis included univariate analysis of variance (method of comparing averages using variance analysis, *t*-test) and variance analysis (ANOVA). The significance of the difference between the means of the least significant difference (LSD) results with $p < 0.05$ was considered statistically significant. All experiments were performed in triplicate and the values were expressed as mean \pm SD. The differences between the samples were assessed using the unpaired *t*-test.

RESULTS AND DISCUSSION

The content of pigments in tea

At the beginning of the active growing season (in May), the largest amount of total chlorophylls is synthesized (1.335 $\mu\text{g}\cdot\text{g}^{-1}$), followed by a smooth drop by August (1.018 $\mu\text{g}\cdot\text{g}^{-1}$). We found another pattern when studying the content of carotenoids and flavonoids (thearubigins and theaflavins). There is an increase in the content of these groups of pigments in June, a decline in July and, by August, and a slight increase again. The increase is due to unfavorable conditions – drought during these periods. Also, it was noted that the content of photosynthetic pigments shows genotypic features (Table 1).

The main photosynthetic pigment is chlorophyll *a*; carotenoids transfer additional energy to chlorophylls, performing a light-absorbing function, and divert excess energy from them, performing a light-protecting function (Endo et al., 1985; Nafissatou et al., 2011; Li et al., 2016). We noted that a significant accumulation of chlorophyll in the leaves is typical for the control cv. Kolchida. At that time, as the studied forms contain chlorophyll *a* is significantly lower (Table 1).

The content of chlorophyll *b* indicates the level of adaptation of plants to low light. 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 the sun's rays, and many of the leaves of the side surfaces are in the shade. In this case, the increased content of chlorophyll *b* is preferable for the photosynthetic activity of the leaves of this tier. We noted the same pattern

as with chlorophyll *a*: more chlorophyll *b* is accumulated by the control variety; moreover, the differences are significant or on the border of materiality, as in the case of forms 3823 and 2264. It is important not only the content of a particular pigment but also their ratio. In all the tea plants studied, the *a/b* ratio ranges from 2.813 $\text{mg}\cdot\text{g}^{-1}$ to 3.362 $\text{mg}\cdot\text{g}^{-1}$, and the differences between the forms are insignificant (Table 1).

Photosynthetic pigments are not only components of the antioxidant protection of the plant itself, but also are part of the biochemical components of the drink since the preparation of black and green tea pigments to some extent pass into the infusion (Lu et al., 2009; Li et al., 2018). Thermal processing induced the formation of degradation product: changes occur in their content: a significant part of the chlorophyll is destroyed during steam treatment, as well as during the drying process (Castillon et al., 1949; Nafissatou et al., 2011; Ošťádalová et al., 2014; Xu et al., 2018; Dini et al., 2019). Therefore, there is less chlorophyll in green tea than in raw materials, and there are even fewer green pigments in the production of black tea than in green tea. But this is good since the presence of chlorophyll in the finished tea affects its quality – the tea acquires an herbal smell and an uncharacteristic taste. The smallest amount of this pigment in green and black tea contains form 855 (0.33 $\text{mg}\cdot\text{g}^{-1}$), which is preferred. Carotenoids provide not only the plant's resistance to exogenous stress but also its value as a source of antioxidants for humans and their content in ready-made (black and green) tea is a positive fact. However, we have not detected any changes in their content, and, as a rule, carotenoids are not taken into account in the qualitative assessment of tea.

Table 1 Content of photosynthetic pigments.

Variety/Form	Pigment content in $\mu\text{g}\cdot\text{g}^{-1}$ of raw weight				
	Chl. <i>a</i>	Chl. <i>b</i>	Chl. <i>a</i> / Chl. <i>b</i>	$\Sigma\text{Car.}$	$\Sigma\text{Chl.}/\Sigma\text{Car.}$
cv. Kolchida	0.878 \pm 0.150	0.307 \pm 0.086	3.151 \pm 0.201	0.302 \pm 0.057	3.922 \pm 0.798
f. 3823	0.732 \pm 0.197	0.279 \pm 0.076	2.813 \pm 0.227	0.285 \pm 0.057	3.549 \pm 0.536
f. 582	0.726 \pm 0.261	0.257 \pm 0.100	2.958 \pm 0.339	0.269 \pm 0.091	3.650 \pm 0.359
f. 855	0.689 \pm 0.143	0.237 \pm 0.084	3.362 \pm 0.210	0.256 \pm 0.047	3.621 \pm 0.475
f. 2264	0.732 \pm 0.113	0.258 \pm 0.063	3.088 \pm 0.135	0.273 \pm 0.041	3.630 \pm 0.342
LSD ($p \leq 0.05$)	0.11*	0.05*	0.84	0.04	0.21*

Note: * $p < 0.05$ with the controlled comparison.

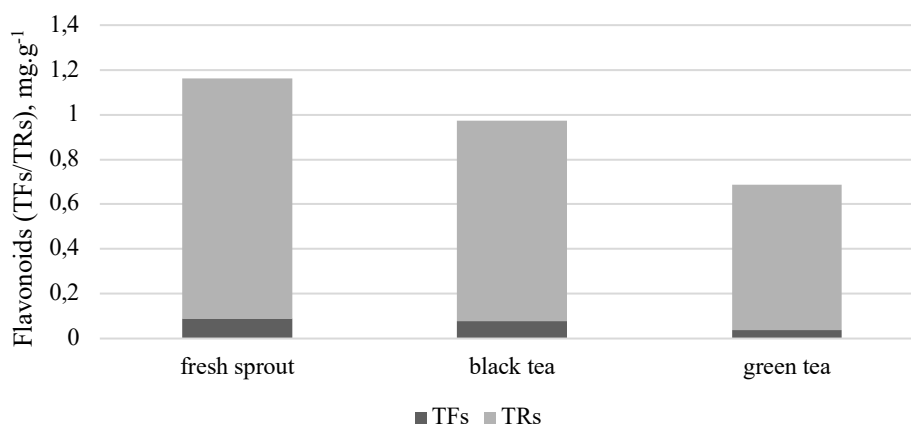


Figure 3 Content of flavonoids in Krasnodar tea, $\text{mg}\cdot\text{g}^{-1}$.

Changes occur in the content of chlorophyll: during steam tea 1:10. According to this indicator, tea made from raw

Table 2 Content of flavonoids in tea and raw materials (sprout), mg. g⁻¹

Sample	Sprout		Green tea		Black tea	
	TFs	TRs	TFs	TRs	TFs	TRs
cv. Kolchida	0.07 ±0.02	0.84 ±0.30	0.04 ±0.01	0.72 ±0.28	0.07 ±0.02	1.03 ±0.28
cv. Sochi	0.07 ±0.02	0.88 ±0.47	0.04 ±0.02	0.71 ±0.39	0.07 ±0.01	0.95 ±0.19
f. 3823	0.09 ±0.03	1.17 ±0.60	0.04 ±0.01	0.60 ±0.23	0.08 ±0.02	1.08 ±0.17
f. 582	0.11 ±0.04	1.33 ±0.65	0.04 ±0.02	0.70 ±0.32	0.09 ±0.02	1.18 ±0.32
f. 855	0.10 ±0.02	1.13 ±0.39	0.04 ±0.02	0.63 ±0.41	0.07 ±0.01	1.10 ±0.12
f. 2264	0.09 ±0.02	1.10 ±0.36	0.03 ±0.01	0.53 ±0.13	0.08 ±0.02	1.03 ±0.15

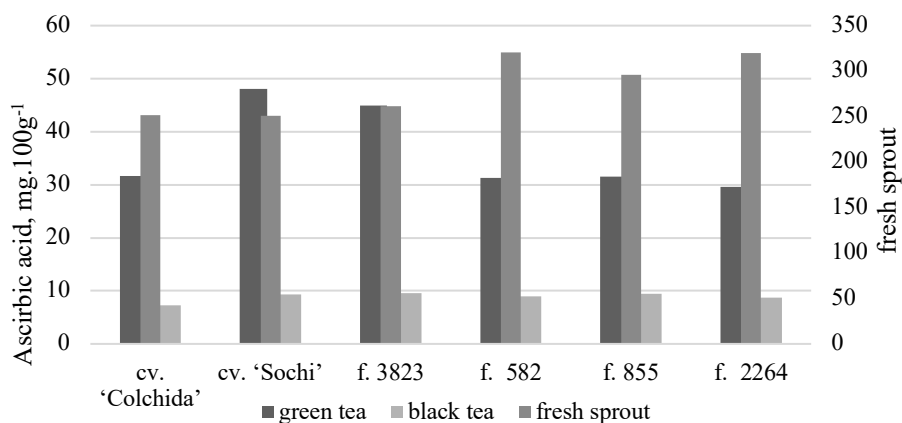


Figure 4 Changes in the content of ascorbic acid during processing, mg.100g⁻¹.

treatment, as well as during drying, a significant part of it is decayed. In the manufacture of black tea, green pigments remain even less than in green.

However, it should be noted that the presence of chlorophyll in the finished tea adversely affects the quality of the product. The smallest amount of this pigment in green and black tea contains form 855 (0.33 mg.g⁻¹), which is preferred. In respect of carotenoids, such profound changes are not observed by us, which is confirmed by the literature data, besides, carotenoids are not taken into account in the qualitative assessment of tea. However, they are an important component of the antioxidant system and they, therefore, required no less comprehensive study.

In the production of black tea, catechins are disintegrated by oxidation with the formation of flavonoids –TFs and TRs (Figure 3).

We were revealed that between the quality of raw materials and the content of flavonoids in tea there is a direct relationship (Table 2).

As we show, green tea TFs are slightly less than in sprouts and black tea. TFs are unstable compounds and during enzymatic oxidation, they easily pass into TRs, so in black tea, TRs are much more. In the varietal section, it can be noted that the raw materials of forms 855 and 582 (0.10 mg.g⁻¹ and 0.11 mg.g⁻¹ respectively), as well as black tea produced from form 582 (0.09 mg.g⁻¹), showed the highest values in the content of theaflavins, while the highest content of TRs was found in forms 582 and 3823 (1.33 and 1.17 mg.g⁻¹).

Currently, there is no single standard for the content of these pigments in the finished product. But, according to international rules, any blend of tea should have a ratio of TRs: TFs are not lower than 1:16 and in the highest quality

materials collected from experimental plants meets international requirements (Kang et al., 2017; Li et al., 2018; Palanivel et al., 2020).

Determination of ascorbic acid

The least amount of AA is contained in the raw material of forms 2264 and 582 (319 and 320 mg.100g⁻¹, respectively) (Figure 4).

In black tea, the differences between varieties are insignificant, a slightly higher content of AA is noted in black tea made from forms 855 and 3823 (13.73 and 13.07 mg.100g⁻¹, respectively).

Vitamin C is a necessary nutrient in the production of collagen. Vitamin C deficiency results in the lost formation of collagen fibers, weakening vascular walls, and causing scurvy. Ascorbic acid is also being a tool in the biosynthesis of several secondary polyphenols such as theaflavins thearubigin (Sharangi, 2009; Palanivel et al., 2020). AA also acts as an antioxidant and is believed to play an important role in the prevention of lifestyle-related diseases such as cancer (Nafissatou et al., 2011; Palanivel et al., 2020). The native level of AA is very less in tea leaves and negligible in processed tea (Palanivel et al., 2020; Zimmermann and Gleichenhagen, 2011).

Our research show, that during processing raw materials (fresh sprouts) to finished tea (Figure 1), there are significant losses (up to 90%) of AA, despite the equal conditions of collection. In the production of black tea, due to the development of oxidative reactions during fermentation, disintegrated a significant part of this vitamin on average 96 – 97% (preserved only 3 – 4%).

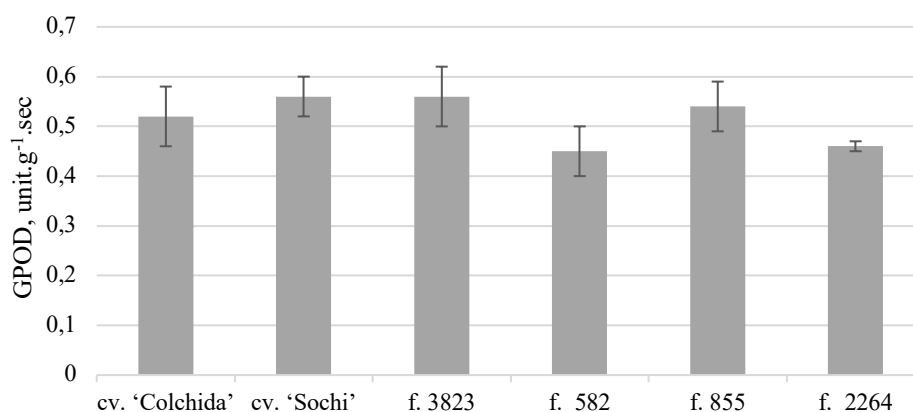


Figure 5 The activity of GPOD, g-unit in sec; LSD ($p \leq 0.05$) = 0.05.

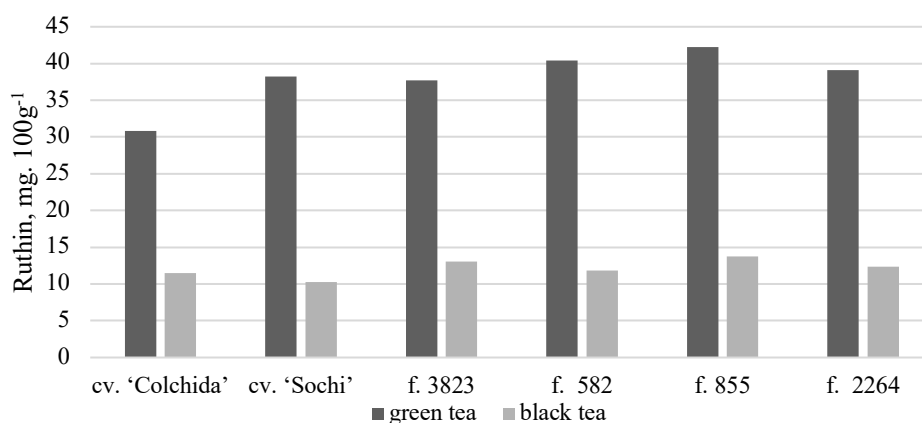


Figure 6 Content of rutin in green and black tea, mg.100g⁻¹.

In green tea, where fermentation is excluded, AA is decayed to a lesser extent. As a result, green tea is about 13% of the initial amount of AA in raw materials (sprout).

This issue requires additional research, which is planned by us in the future.

Determination of the activity of guaiacol peroxidase

At the beginning of the growing season (May), the activity of the enzyme was low – in the range of 0.363 to 0.607 g-unit in 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.

The highest activity of GPOD during the entire vegetation period is characterized by the cultivar Sochi and form 582 (about 0.56 g-unit in sec) (Figure 5). 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.

Peroxidase is considered a marker of the physiological state of plants (Marinescu et al., 1999; Pandey et al., 2017). Several scientists note that during a period of stress, the isoenzyme system is reorganized, which ensures the plant's resistance to external factors and the regulation of homeostasis (McRae and Ferguson, 1985; Castillo, 1992; Sharangi, 2009). For example, researches have demonstrated that the expression level of peroxidases and superoxide dismutase increased after the heat treatment of

creeping plants (Khan and Robinson, 1993; Huang et al., 2006).

We were shown that after May's 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 period under a stressful hydrothermal condition, 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 GPOD, as a non-specific reaction to a stress factor (Mittler, 2002; Thongsook and Barrett, 2005). However, the dynamics of enzyme activity in a 3-leaf sprout are variety-specific.

We performed a correlation analysis that showed a direct relationship between increased enzyme activity and hydrothermal factors. The most significant correlation was found between the activity of GPOD in freshly harvested 3-leaf tea sprouts and the amount of precipitation ($r = 0.86$).

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 (Kareska, 2009; Skhalyakhov et al., 2019). In the process of technological processing of raw materials to ready-made the oxidases that ensure the normal course of redox processes are important. Thus, peroxidase catalyzes the oxidation of polyphenols and certain aromatic amines using oxygen, hydrogen peroxide, or organic peroxides.

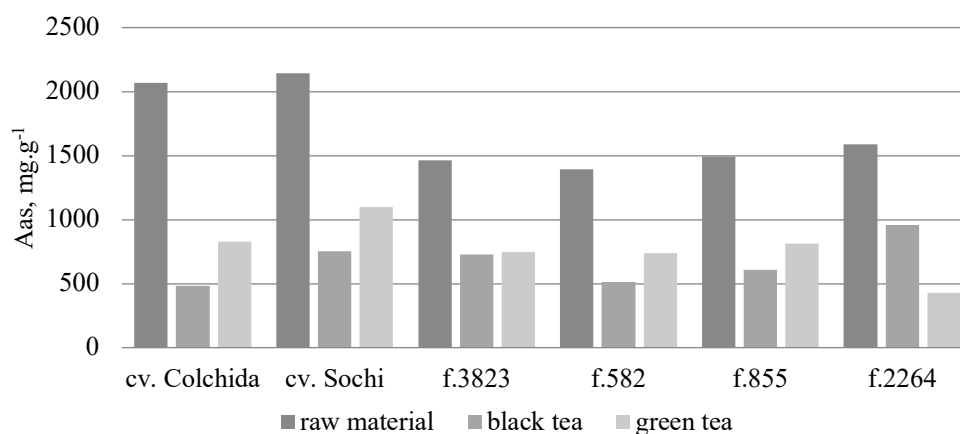


Figure 7 Content of amino acids in Krasnodar tea, mg.g⁻¹.

Given this fact, a lower level of activity of GPOD is a negative phenomenon, both for the stability of the plant itself and for the quality of the finished tea (Wright, 2005; Huang et al., 2006).

Flavonoid compounds with P-vitamin activity

Studies have shown depending on the season of tea leaf collection, the content of ruthin in green tea ranges from 36 mg.100g⁻¹ to 41 mg.100g⁻¹, and in black tea-in the range of 17 mg.100g⁻¹ – 20 mg.100g⁻¹, which is lower than in green tea. Thus, a lower content of ruthin is characterized by the cv. Sochi (on average 10 mg.100g⁻¹ in black tea and 34 mg.100g⁻¹ in green), a higher-form 582 (about 23 mg.100g⁻¹ in black tea and 46 mg.100g⁻¹ in green).

Ruthin, namely tea catechin, is very significant for human health. In the absence of it, the permeability of capillary blood vessels increases, and their strength decreases (Vinson, 2000; Sinija, Mishra, 2008; Sharangi, 2009; Ferreira et al., 2020). When acting together with vitamin C (with which it is often found in food), the latter is better absorbed and retained in the body and more successfully prevents scurvy (Skotnicka et al., 2011). Ruthin is an equally important component of the antioxidant system of tea plants, it not only takes part in the main redox reactions but also increases the absorption of ascorbic acid (Gulati and Ravindranath, 1996). The content of ruthin in black and green tea changed as the content of ascorbic acid. The ruthin on average is 3 times more in green tea than in black (on average about 38.09 and 12.12 mg.100g⁻¹ respectively). At the same time, the variability of the indicator during the season is important: a more stable content of vitamin P in tea forms 2264, 3823, 582, and 855. A variation was revealed in the content of vitamin C and P depending on genotypic characteristics (Figure 4 and Figure 6).

Content of amino acids in tea

We identified 11 AAs in Krasnodar tea, a large proportion of which is proline (30 – 70% of the total AAs content in the sample), valine (17 – 30%), and serine (about 10%) (Figure 7).

AAs are the component in tea that contributes full-bodied flavor and sweetness. Of these AAs, more than 60% is theanine, which is unique to tea. Theanine has a structure similar to that of glutamine, with its particular trait being a refined, rich flavor and sweetness (Karori et al., 2007). AAs other than theanine present in tea leaves include

proline, asparagine, arginine, serine, and others (Kumar et al., 2015).

We were shown that the largest number of AAs is synthesized in May, and in the future, the processes of their transformations and mutual conversions to other compounds are probably included in the synthesis.

It is necessary to note a strong variability in the content of AAs, and only Pro is stable enough (V, % = 4 – 26% during vegetation). When processing raw materials into ready-made tea, the number of AAs falls, which is due to oxidative deamination and their conversion into protein compounds that are involved in the formation of tea aroma.

Most of the AAs in the raw material are contained in the cv. Kolchida and Sochi. During processing, there are not only general changes – there are slightly fewer AAs in black than in green, which is understandable since it is during the fermentation of black tea that active processes of oxidative deamination take place. There are varietal differences in the metabolic reactions of converting AAs: in the black tea the AAs composition of the cv. Kolchida is more susceptible to changes, while in green tea, the composition of form 2264 changes a little bit.

CONCLUSION

We conducted a comparative biochemical analysis of the content of antioxidant substances in tea raw materials (fresh sprout) and finished black and green tea. There was a significant change in the content of flavonoids depending on the type of finished product, as well as ascorbic acid and ruthine in the processing of tea raw materials. The differences are due to the lack of fermentation process in the production of non-fermentation (green) tea, which is the most important technological method in the production of black tea. This allows green tea to retain almost all water-soluble vitamins that inhibit lipid peroxidation in cell membranes. There is a significant variation in the content of biologically active substances (flavonoids, vitamins, and amino acids), due to the genotypic characteristics of tea plants. It is important to research the rules about the accumulation and distribution of biological substances by tea plants, which will give us some scientific ideas about how to control the contents of it and increase biological active substances in the new tea cultivar.

REFERENCES

- Argunova, V. A., Bushin, P. M., Malyukova, L. M. 1994. Principles of optimal programming of tea yields and production of quality products in the development of environmentally friendly systems of mineral nutrition. *Subtropical and ornamental gardening*, vol. 38, p. 182-190. (in Russian)
- Belous, O. G. 2013. Influence of microelements on biochemical parameters of tea. *Potravinarstvo*, vol. 7, no.1, p. 149-152.
- Belous, O. G., Pritula, Z. V. 2010. Influence of trace elements on the biochemical parameters of the tea leaf (Влияние микроэлементов на биохимические показатели чайного листа). *Subtropical cultures*, vol. 1, no. 4, p. 98-101. (in Russian)
- Belous, O., Platonova, N. 2017. Influence of altitude tea plantations above sea level on the chemical composition of tea. *Sciences of Europe*, vol. 1, no. 22, p. 3-6.
- Belous, O., Platonova, N. 2018. Physiological foundations of sustainability *Camellia sinensis* (L.) O. Kuntze and *Corylus pontica* C. Koch. in the conditions of humid subtropics of Russia. *American Journal of Plant Sciences. Special Issue on Plants in Extreme Environment*, vol. 9, no. 9, p. 1771-1780. <https://doi.org/10.4236/ajps.2018.99129>
- Castillo, F. J. 1992. Peroxidases and stress. In Penel, C., Gaspar, T., Greppin, H. *Plant Peroxidases 1980-1990. Topics and Detailed Literature on Molecular, Biochemical, and Physiological Aspects*. Geneva, Switzerland : Univ of Geneva, p. 187-203.
- Castillon, L. E., Hall, C. M., O'Connor, R. T., Miller, C. B. 1949. Effect of temperature on the content of pigments of stored cottonseed. *J. Am. Oil Chem. Soc.*, vol. 26, no. 11, p. 655-659. <https://doi.org/10.1007/BF02651507>
- Dini, A., Khanamani Falahati-Pour, S., Behmaram, K., Sedaghat, N. 2019. The kinetics of color degradation, chlorophylls and xanthophylls loss in pistachio nuts during roasting process. *Food Quality and Safety*, vol. 3, no. 4, p. 251-263. <https://doi.org/10.1093/fqsafe/fyz020>
- Endo, Y., Usuki, R., Kanedaet, T. 1985. Antioxidant effects of chlorophyll and pheophytin on the autoxidation of oils in the dark. II. The mechanism of antioxidative action of chlorophyll. *Journal of the American Oil Chemists' Society*, vol. 62, no. 9, p. 1387-1390. <https://doi.org/10.1007/BF02545965>
- Ferreira, M. C. L., Lima, L. N., Cota, L. H. T., Costa, M. B., Orsi, P. M. E., Espindola, R. P., Albanez, A. V., Rosa, B. B., Carvalho, M. G. S., Garcia, J. A. D. 2020. Effect of *Camellia sinensis* teas on left ventricular hypertrophy and insulin resistance in dyslipidemic mice. *Brazilian Journal of Medical and Biological Research*, vol. 53, no. 5, p. e9303. <https://doi.org/10.1590/1414-431x20209303>
- Gai, Z., Wang, Y., Jiang, J., Xie, H., Ding, Z., Ding, S., Wang, H. 2019. The quality evaluation of tea (*Camellia sinensis*) varieties based on the metabolics. *HortScience*, vol. 54, no. 3, p. 409-415. <https://doi.org/10.21273/HORTSCI13713-18>
- Gulati, A., Ravindranath, S. D. 1996. Seasonal variations in quality of Kangra tea (*Camellia sinensis* (L.) O. Kuntze) in Himachal Pradesh. *J. Sci. Food Agric.*, vol. 71, no. 2, p. 231-236. [https://doi.org/10.1002/\(SICI\)1097-0010\(199606\)71:2<231::AID-JSFA573>3.0.CO;2-Y](https://doi.org/10.1002/(SICI)1097-0010(199606)71:2<231::AID-JSFA573>3.0.CO;2-Y)
- Gvasaliya, M. V. 2019. Perspective mutant forms of tea plants (*Camellia sinensis* (L.) Kuntze) in the collection of Russian Research Institute of Floriculture and Subtropical Crops. (Перспективные мутантные формы растений чая (*Camellia sinensis* (L.) Kuntze) в коллекции Всероссийского научно-исследовательского института цветоводства и субтропических культур). *Horticulture and viticulture*, vol. 6, p. 5-8. <https://doi.org/10.31676/0235-2591-2019-6-5-8> (in Russian)
- Kang, Y. R., Park, J., Jung, S. K., Chang, Y. H. 2017. Synthesis, characterization, and functional properties of chlorophylls, pheophytins, and Zn-pheophytins. *Food Chem.*, vol. 245, p. 943-950. <https://doi.org/10.1016/j.foodchem.2017.11.079>
- Kareska, S. 2009. Factors affecting hydrogen peroxidase activity. *ESSAI*, vol. 7, no. 27, p. 82-85. <https://dc.cod.edu/essai/vol7/iss1/27>
- Karori, S. M., Wachira, F. N., Wanyoko, J. K., Ngunge, R. M. 2007. Antioxidant capacity of different types of tea products. *Afr. J. Biotechnol.*, vol. 6, no. 19, p. 2287-2296. <https://doi.org/10.5897/AJB2007.000-2358>
- Khan, A. A., Robinson, D. S. 1993. Purification of anionic peroxidase isoenzyme from mango (*Mangifera indica* L. var. Chaunsa). *Food Chem.*, vol. 46, no. 1, p. 61-64. [https://doi.org/10.1016/0308-8146\(93\)90076-R](https://doi.org/10.1016/0308-8146(93)90076-R)
- Khan, N., Mukhtar, H. 2007. Tea polyphenols for health promotion. *Life Science*, vol. 81, no. 7, p. 519-533. <https://doi.org/10.1016/j.lfs.2007.06.011>
- Konnov, N. A., Platonova, N. B., Belous, O. G. 2019. Comparative analysis of Ruthin content in tea of different climatic zones. In *Current achievements and trends in ornamental and medicinal plants research. Jubilee scientific conference 9 may 2019 devoted to the 40th anniversary of the institute of ornamental and medic-inal plants* (Sofia, Bulgaria), p. 33.
- Kumar, R., Bisen, J. S., Choubey, M., Singh, M., Bera, B. 2015. Studies on effect of altitude and environment on physiological activities and yield of Darjeeling tea (*Camellia sinensis* L.) plantation. *J. Crop Weed*, vol. 11, p. 71-79.
- Li, X., Zhou, R., Xu, K., Xu, J., Jin, J., Fang, H., He, L. 2018. Rapid Determination of Chlorophyll and Pheophytin in Green Tea Using Fourier Transform Infrared Spectroscopy. *Molecules*, vol. 23, no. 5, p. 1010. <https://doi.org/10.3390/molecules23051010>
- Li, Y., Chen, C., Li, Y., Ding, Z., Shen, J., Wang, Y., Zhao, L., Xu, M. 2016. The identification and evaluation of two different color variations of tea. *J. Sci. Food Agric.*, vol. 96, no. 15, p. 4951-4961. <https://doi.org/10.1002/jsfa.7897>
- Wang, L., Yue, C., Cao, H., Zhou, Y., Zeng, J., Yang, Y., Wang, X. 2014. Biochemical and transcriptome analyses of a novel chlorophyll-deficient chlorina tea plant cultivar. *BMC Plant Biology*, vol. 14, p. 352. <https://doi.org/10.1186/s12870-014-0352-x>
- Lu, J. L., Pan, S., Zheng, X. Q., Dong, J., Borthakur, D., Liang, Y. R. 2009. Effects of lipophilic pigments on color of the green tea infusion. *International Journal of Food Science & Technology*, vol. 44, no. 12, p. 2505-2511. <https://doi.org/10.1111/j.1365-2621.2009.02069.x>
- Marimuthu, S., Kumar, R. R. 2001. Physiological and biochemical responses of micropropagated tea plants. *Vitro Cell. Dev. Biol.-Plant*, vol. 37, p. 618. <https://doi.org/10.1007/s11627-001-0108-9>
- Marinescu, G., Badea, E., Babeanu, C., Glodeanu, E. 1999. Peroxidase system from leaves of *Cucumis sativus* as marker of growth stimulant treatment. *Plant Peroxidase Newsletter*, vol. 15, p. 79-85.
- McRae, E. A., Ferguson, I. B. 1985. Changes in catalase activity and hydrogen peroxide concentration in plants in response to low-temperature. *Physiol. Planta*, vol. 65, no. 1, p. 51-56. <https://doi.org/10.1111/j.1399-3054.1985.tb02358.x>

- Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.*, vol. 7, no. 9, p. 405-410. [https://doi.org/10.1016/S1360-1385\(02\)02312-9](https://doi.org/10.1016/S1360-1385(02)02312-9)
- Nafissatou, D. N., Dhuique-Mayer, C., Cisse, M., Dornier, M. 2011. Identification and thermal degradation kinetics of chlorophyll pigments and ascorbic acid from ditax nectar (*Detarium senegalense* JF Gmel). *Journal of Agricultural and Food Chemistry*, vol. 59, no. 22, p. 12018-12027. <https://doi.org/10.1021/jf203582k>
- Ošťádalová, M., Tremlová, B., Straka, I., Pokorná, J., Časlavková, P. 2014. Evaluation of significant pigments in green teas of different origin. *Potravinarstvo*, vol. 8, no. 1, p. 221-227. <https://doi.org/10.5219/344>
- Palanivel, M., Venkateswaran, G., Sathish, G., Shanmugaselvan, V. 2020. Enhancing the quality of naturally oxidized tea with ascorbic acid. *International Journal of Advanced Engineering Research and Science*, vol. 7, no. 4, p. 403-409. <https://doi.org/10.22161/ijaers.74.50>
- Pandey, V. P., Awasthi, M., Singh, S., Tiwari, S., Dwivedi, U. N. 2017. A comprehensive review on function and application of plant peroxidases. *Biochem. Anal. Biochem.*, vol. 6, p. 308. <https://doi.org/10.4172/2161-1009.1000308>
- Platonova, N. B., Belous, O. G., Ostadalova, M. 2017. Comparative analysis of biochemical components of tea (Сравнительный анализ биохимических компонентов чая). *Subtropical and Ornamental Gardening*, vol. 61, p. 180-189. (in Russian)
- Platonova, N., Astanin, A., Sedykh, S., Samarina, L., Belous, O. 2019. The composition and content of phenolic compounds in tea, grown in humid subtropics of Russia. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 13, no. 1, p. 32-37. <https://doi.org/10.5219/990>
- Prokopenko, I. A., Tuov, M. T. 1994. Dependence of the yield and quality of tea production from gathering and square surface of collecting sheet (Зависимость урожайности и качества чайной продукции от способов сбора листа и площади листосборной поверхности). *Subtropical and ornamental gardening*, vol. 38, p. 161-173. (in Russian)
- Huang, R., Xia, R., Hu, L., Lu, Y., Wang, M. 2006. Antioxidant activity and oxygen-scavenging system in orange pulp during fruit ripening and maturation. *Scientia Horticulturae*, vol. 113, no. 2, p. 166-172. <https://doi.org/10.1016/j.scienta.2007.03.010>
- Sharangi, A. B. 2009. Medicinal and therapeutic potentialities of tea (*Camellia sinensis* L.): A review. *Food Res. Int.*, vol. 42, no. 5-6, p. 529-535. <https://doi.org/10.1016/j.foodres.2009.01.007>
- Shlyk, A. A. 1971. Determination of chlorophylls and carotenoids in green leaf extracts. Biochemical methods in plant physiology (Определение хлорофиллов и каротиноидов в экстрактах зеленых листьев. Биохимические методы в физиологии растений). Moscow: Nauka, p. 154-157. (in Russian)
- Sinija, V. R., Mishra, H. N. 2008. Green tea: Health benefits. *Journal of Nutritional & Environmental Medicine*, vol. 17, no. 4, p. 232-242. <https://doi.org/10.1080/13590840802518785>
- Skhalyakhov, A. A., Siyukhov, H. R., Tazova, Z. T., Lunina, L. V., Mugu, I. G. 2019. Phenolic compounds and antioxidant potential of wild-growing plant materials of the North Caucasus region. *International Journal of Engineering and Advanced Technology*, vol. 9, no. 2, p.5062-5071. <https://doi.org/10.35940/ijeat.B4046.129219>
- Skotnicka, M., Chorostowska-Wynimko, J., Jankun, J., Skrzypczak-Jankun, E. 2011. The black tea bioactivity: An overview. *Central-European Journal of Immunology*, vol. 36, no. 4, p. 284-292.
- Speckman, D. H., Stein, W. H., Moore, S. 1958. Automatic recording apparatus for use in the chromatography of amino acids. *Anal. Chem.*, vol. 30, no. 7, p. 1190-1206. <https://doi.org/10.1021/ac60139a006>
- Thongsook, T., Barrett, D. M. 2005. Heat inactivation and reactivation of broccoli peroxidase. *J. Agric. Food Chem.*, vol. 53, no. 8, p. 3215-3222. <https://doi.org/10.1021/jf0481610>
- Vinson, J. A. 2000. Black and green tea and heart disease: a review. *Biofactors*, vol. 3, no. 1-4, p. 127-132. <https://doi.org/10.1002/biof.5520130121>
- Vorobyov, V. N., Nevmerzhitskaya, Y. Y., Khusnutdinova, L. Z., Yakushenkova, T. P. 2013. Practicum on plant physiology: educational and methodological guide (Практикум по физиологии растений: учебно-методическое пособие). Kazan, Russia : Kazan University, 80 p. https://repository.kpfu.ru/?p_id=94591 (in Russian)
- Voskresenskaya, O. L., Alyabysheva, E. A., Polovnikova, M. G. 2006. Large workshop on Biocology (Большой практикум по биоэкологии). Yoshkar-Ola, Mari, 107 p. ISBN 5-94808-239-3. (in Russian)
- Willson, K. C. 1975. Studies on the mineral nutrition on tea. *Plant and Soil*, vol. 42, no. 3, p. 501-516. <https://doi.org/10.1007/BF00009939>
- Wright, L. P. 2005. *Biochemical analyses for identification of quality in black tea (Camellia sinensis)* Pretoria, South Africa : dissertation theses. Pretoria, South Africa : University of Pretoria. 216 p.
- Xu, X., Deng, J., Luo, D., Bao, Y., Liao, X., Gao, H., Wu, J. 2018. Comparative study of high hydrostatic pressure and high temperature short time processing on quality of clear and cloudy Se-enriched kiwifruit juices. *Innovative Food Science & Emerging Technologies*, vol. 49, p. 1-12. <https://doi.org/10.1016/j.ifset.2018.07.010>
- Zhang, Q., Li, T., Wang, Q., LeCompte, J., Harkess, R. L., Bi, G. 2020. Screening tea cultivars for novel climates: plant growth and leaf quality of *Camellia sinensis* cultivars grown in Mississippi, United States. *Frontiers in Plant Science*, vol. 11, p. 280. <https://doi.org/10.3389/fpls.2020.00280>
- Zimmermann, B. F., Gleichenhagen, M. 2011. The effect of ascorbic acid, citric acid and low pH on the extraction of green tea: How to get most out of it. *Food Chemistry*, vol. 124, no. 4, p. 1543-1548. <https://doi.org/10.1016/j.foodchem.2010.08.009>

Acknowledgments:

The work was carried out based on the Federal Research Centre the Subtropical Scientific Centre of the Russian Academy of Sciences (Sochi, Krasnodar region, Russia) within the research plan 0492-2021-0007.

Contact Address:

*Dr. Oksana Belous, Subtropical Scientific Centre of the Russian Academy of Sciences, Laboratory of Plants Biochemistry and Physiology, Yana Fabritsiusa st., 2/28, 354002, Sochi, Russia, Tel.: +7(918)1099115,

E-mail: oksana191962@mail.ru

ORCID: <https://orcid.org/0000-0001-5613-7215>

Corresponding author: *

Nataliia Platonova, Subtropical Scientific Centre of the Russian Academy of Sciences, Laboratory of Plants Biochemistry and Physiology, Yana Fabritsiusa st., 2/28, 354002, Sochi, Russia, Tel.: +7(918)3057387,

E-mail: natali1875@bk.ru

ORCID: <https://orcid.org/0000-0003-2392-8947>

Corresponding author: *