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Physiological Foundations of Sustainability *Camellia sinensis* (L.) O. Kuntze and *Corylus pontica* C. Koch. in the Conditions of Humid Subtropics of Russia

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Abstract

The study of the water status and activity of catalase in Chinese tea plants (*Camellia sinensis* (L.) O. Kuntze) and hazelnut (*Corylus pontica* C. Koch.). The indicators, which not only describe the physiological state of plants in the stressful period, but also allow it to identify more adaptive varieties within a given area was determined. Analysis of catalase activity data in Chinese tea and hazelnut revealed the presence of similar patterns: there is a change in enzymatic activity in response to hydrothermal factors. It is established that the stable varieties *Camellia sinensis* (L.) O. Kuntze and *Corylus pontica* C. Koch. characterized by a more active catalase, which is of particular importance during droughts. The water regime parameters variance scale for *Camellia sinensis* was developed, which allows differentiating varieties into groups of varying degrees of stability. It is shown that all methods that are used to estimate stability of plant species to extreme environmental conditions are based on several basic principles connected with peculiarities of adaptation mechanisms. The result was a selection of diagnostic indicators proposed for assessing adaptability: the water content of the leaf tissue, water-holding capacity of leaves, and concentration of cellular juice sprouts and activity of catalase.

Keywords

Chinese Tea (*Camellia sinensis* (L.) O. Kuntze), Hazelnut (*Corylus pontica* C. Koch.), Stable, Water Regime, Enzymatic Activity

1. Introduction

Among the wide ranges of modern problems developed by plant physiologists and biochemists, the problem of stress and adaptation is relevant [1] [2] [3]. Its solution requires a comprehensive approach to the study of the adaptation mechanism, which is possible only on the basis of improving the known and developing new methods of functional diagnosis of plants and criteria for assessing their adaptive potential, based on the use of stable features associated with the main physiological functions, such as water status, the power of the pigment system, enzyme activity, hormonal status, etc. [4]-[9].

Issues related to plant adaptability are particularly acute in the subtropical zone of Russia, which is specific to soil and climatic conditions, where mass pest and disease damage are often observed, long dry periods, which lead to oppression, decrease in productivity and even premature death of plants [10] [11]. Subtropical crops are very demanding to the conditions of growth, maintaining and increasing their adaptive capacity is an important task. The search for anatomical and morphological and physiological and biochemical indicators that determine the level of stability contributes to a better understanding of the nature of the phenomenon and the successful development of diagnostic methods. However, historically developed adaptation of plants to the conditions of existence affects the whole complex of functional processes. The study of the relationship between physiological and biochemical processes in plants, and changing external factors, allows to identify the differences between stable and unstable plants.

2. Objects, Material and Methods

The work was carried out on the basis of Federal state budgetary scientific institution "All-Russian research Institute of floriculture and subtropical crops" 1989-2015.

The objects of the researches' were plants *Camellia sinensis* (L.) O. Kuntze (local population, varieties of Colchida, Sochi, Karatum) and *Corylus pontica* C. Koch. (varieties Cherkesskiy-2, President, Futkurami, Lombard Red).

Determination of physiological and biochemical characteristics, was carried out by classical methods:

- The concentration of cell sap (CCS)—using a field Refractometer, with simultaneous recording of temperature and air humidity [12];
- Water deficiency—comparison of water content in the selected leaves, with its quantity in the same leaves which are in a condition of full saturation with water [13];
- Determination of the bound water—to change the concentration of the standard sucrose solution after its interaction with the leaf cutting [14];
- Coefficient of heat drought resistance—change in thickness of the lamina before and after exposure to stress using field tularemia [15];
- Activity of the enzyme catalase—gasometrical method by volume of released

oxygen [16].

Processing of experimental material was carried out by ANOVA method using mathematical software STATGRAPHICS Centurion XV.

Physiological parameters are very labile, so the indicator organs were previously installed, taking into account the age of the plant, the physiological maturity of the diagnosed organ, its location, etc. For the diagnostic purpose of *Camellia sinensis*, indicator organs are physiologically mature 1st - 2nd sheet, located after the so-called "fishy" on the shoot of the growing season this year ("fishy » the leaf differs from the normally developed leaves and is a good reference point in the selection of samples) [10]. When diagnosing stability of *Corylus pontica*, used physiologically mature leaves located on the escape of the middle tier [17].

3. Results and Discussion

The zone of humid subtropics in Russia is unique in its climatic conditions (including the selection of stress factors), and plants grown in this region have specific biology, therefore, the task is to identify the General mechanisms of resistance of crops to specific environmental factors, as well as to outline the essence of the basic principles and methods of diagnosing their resistance.

In the subtropical zone of the Krasnodar region precipitation is distributed very unevenly, with the required amount of 500 - 600 mm during the growing season in some years, they fall no more than 150 - 200 mm, while most often they have a storm character. Summer period in subtropics of Russia is characterized not only by uneven precipitation, but also by high temperatures. This creates conditions on the coast for annual recurring dry periods, in which there is a suspension of plant growth, withering and even drying. The influence of stressors leads to significant losses in the yield of subtropical fruit crops (the fall of the ovary) and tea (due to the cessation of the formation of sprouts).

When diagnosing crop resistance to drought and high temperatures, an assessment of the complex parameters of the water regime we were used [1] [18]. These parameters are characterized by greater liability and high responsiveness to the action of many abiotic factors, so it was necessary to observe special care in the selection of plant material. As a result, the water status was studied *Camellia sinensis* (L.) O. Kuntze и *Corylus pontica* C. Koch., defined indicators that not only fully and clearly describe the physiological state of plants in the stressful period, but also allow you to identify more adaptive varieties for the local area. Scientifically valuable is that the laboratory-field methods used to assess stability are based on field observations of the state of plants with the study of changes in water exchange, especially during drought. Methods closely related to the water status of crops are: determination of water deficiency of leaves, determination of fractional composition of water and identification of parameters of concentration of cellular sap (CCS).

Based on the results of a long-term research of a culture *Camellia sinensis*, a scale of changes in the parameters of the water regime was developed, which allows differentiating varieties into groups of different degrees of stability [10]. When assessing the condition of plants in the optimal period, it is necessary to pay attention to the fact that the leaves of resistant varieties contain water 50% and above, the water deficit in them does not exceed 10%, the water-retaining ability of tissues from 20% to 25% (**Table 1**). CCS of tea sprouts in the optimal period is always kept at the level of 8%, regardless of the degree of stability of the variety. In less stable varieties, the hydration of leaf tissues is 40% - 50%, the water deficit is 10% - 15%, and the loss of water by tissues is not more than 25% - 40%. In unstable plants, these parameters change to a strong degree even under normal conditions. The water content of the tissue sheet is less than 40%, the water deficit is above 15%, water loss is high—more than 40%. Moreover, the tea culture requires a period of time in determining the water retention capacity of at least 6 hours.

In the period of unfavorable environmental factors, the most drought-resistant varieties the water content drops to 50% - 60%, *i.e.* in the process of wilting the leaves lose no more than 10% water, and water deficit increasing by 10%. The plants average resistance water content is reduced to 20% - 50%, the water deficit increases by 15% - 25%, and CCS increased to 15% - 18%. The greatest changes occur in unstable plants. Water content is less than 20%, water deficiency of leaves is more than 40%, water loss is 50% from the initial, and CCS is more than 18%.

When establishing water status of *Corylus pontica* C. Koch. it is revealed that in the optimal period the hydration of leaf tissues averaged 62.5%; the ability to retain moisture cells of hazelnut leaves in the whole culture is quite high (from 38% to 56%), due to the xerophytes nature of leaf tissues. The content of free and bound water varies slightly, but in unfavorable hydrothermal period there is a wide variation due to varietal differences. Thus, in comparison with the control (Cherkesskiy-2) experimental varieties differ somewhat reduced water content of

Table 1. The scale parameter changes of the water regime of the leaves for the comparative evaluation of drought resistance of plants *Camellia sinensis* (L.) O. Kuntze.

The conditions of evaluation	Characteristic	Degree of stability		
		high	avarage	low
Optimal condition	The water content of leaves	50 - 70	40 - 50	<40
	Water holding capacity (after 6 hours)	20 - 25	25 - 40	>40
	Water deficit	7 - 10	10 - 15	>15
	CCS sprouts	≤8	≤8	≤8
In the period droughts	The water content of leaves	50 - 60	20 - 50	<20
	Water holding capacity (after 6 hours)	10 - 15	15 - 40	>40
	Water deficit	<10	10 - 40	>40
	CCS sprouts	≤15	15 - 18	>18

leaf tissues, which is associated with lower water-holding capacity of the cells (**Table 2**); the coefficients of variation for the entire period of research do not exceed an average of 12.4% - 14.5%, which indicates the stability of this indicator. There is a higher content of water bound in one fraction in the variety President, which is important in adapting plants to the effects of elevated temperatures against the background of moisture deficiency (**Table 2**). Data analysis carried out in the most stressful conditions by hydrothermal periods, allowed us to draw the following conclusions: if in may the studied plants, the magnitude of the water deficit ranged from 14.9% (Lombard red) to 17.6% (President), then when the dry season minimum water deficit is observed in the cultivars President and Cherkesskiy-2, and the maximum value in the variety Futkurami. At the same time, the variety Futkurami at the onset of even a short dry period (July) marked a significant decrease in water retention capacity, which implies the absence of a significant mechanism of stability. Taking into account the full characteristics of the water status in varieties *Corylus pontica* C. Koch., both in times of stress and in optimal weather conditions, we conclude that the varieties such as Cherkesskiy-2 and the President are found more stable.

Along with the considered methods of assessment of plant resistance in the diagnosis of complex adaptability to extreme environmental factors, other methods based on the account of a variety of physiological parameters are used [4] [9] [18] [19]. For example, for a plant the mechanisms functioning at the level of enzyme systems and providing adaptation of plants to adverse environmental conditions are significant. These mechanisms are manifested in the form of changes in the concentration of enzymes and/or the activity of their multiple molecular forms. It is the enzymatic processes that characterize the features of metabolism in plants of different types [20]. Various physiological features, including biological stability, are connected with their action. When working with enzyme systems must take into account their reaction to changes in temperature, as enzymes, there has been a specific response to this factor. One of the main redox enzymes of plants, including cultures studied by us, is catalase. Change of its activity testifies to change of adaptability of plants in response to adverse influence of environment therefore; we studied dynamics of enzymatic activity of cultures, both during a vegetative period, and in a varietal section [19]. The study of enzyme activity in the leaves *Camellia sinensis* in dynamics revealed that the least active leaf catalase in July, characterized by high temperatures and maximum

Table 2. Water status of varieties *Corylus pontica* C. Koch. in the stressful period (in%), average for three years.

Varieties	Water content, %		Water deficit, %		Water-holding capacity, %		Free water, %		Bound water, %	
	average	V, %	average	V, %	average	V, %	average	V, %	average	V, %
Cherkesskiy-2	67.21 ± 5.00	12.2	16.07 ± 5.84	24.9	52.55 ± 7.65	13.8	46.6 ± 3.6	21.5	53.4 ± 1.5	14.7
Lombard red	62.78 ± 3.11	12.6	16.88 ± 5.38	24.3	47.91 ± 9.53	14.4	48.2 ± 2.4	22.8	51.8 ± 6.1	14.4
President	65.77 ± 2.96	12.4	16.71 ± 3.30	24.5	48.38 ± 7.00	14.4	39.3 ± 1.5	20.4	60.7 ± 2.4	15.9
Futkurami	64.59 ± 3.03	12.5	17.82 ± 3.04	23.7	43.25 ± 7.30	15.2	49.7 ± 3.2	22.9	50.3 ± 8.2	14.2

illumination. At the same time, in the tea sprouts, in contrast to the leaves, in July there was a maximum of enzymatic activity (from 52 to 74 ml O^2/g), and in August there was its decline to 39 - 33 ml O^2/g. Moreover, during this period, the activity of catalase in the flash on average 2.1 - 3.0 times lower than in mature leaves. In September, the process of flash formation is completed, so in the future the determination of enzymatic activity was carried out only in the leaves. With the weakening of heat (in September) enzyme activity is maximal, in the future there is another decline in activity-in October, but less significant than in July. This is the period of physiological aging of the leaf, at the same time, the period of active reproductive activity, accompanied by no less active formation of the root system, which involves the outflow of substances to these organs and the strengthening of physiological processes in the appropriate cellular structures [10] [21].

If we trace the influence of leaf age on enzyme activity, the following picture is obtained (**Table 3**): the greatest activity of the enzyme is observed in the 1 - 4 sheets after the so-called "fishy» on the escape. This is a completely physiologically matured leaves, characterized by active metabolic processes. It is no coincidence that these leaves were chosen as indicator objects for physiological studies on tea. Then, with aging of the leaf there comes a loss of catalase activity, which confirms the point of view on these processes Tsagareli M. L. and Pruidze G.N., linking the downturn with the slowing of metabolic processes in older leaves and decreased intensity of breath.

Determination of catalase activity in the leaves of *Corylus pontica* it showed that in General, in culture it ranges from 355.6 ml O^2/g (in July) to 462.0 ml O^2/g (in August), but the differences are insignificant (LSD ($P \le 0.05$) = 43.5). In this process varietal characteristics was traced (**Figure 1**).

During the three-year observations in the unfavorable growing season (July), the lowest enzyme activity was observed in the variety Futkurami, Lombard red varieties and President—1.2 times higher than Futkurami. Moreover, the variety Futkurami throughout the research period was characterized by a reduced

Table 3. The catalase activity of the leaves *Camellia sinensis* (L.) O. Kuntze depending on the age of the sheet, the average for eleven years.

Sequential number of the leaf on the sprout	$\bar{x} \pm S\bar{x}$	σ^2	V, %
Sprout	48.0 ± 5.2	24.2	34
1 - 2	93.8 ± 3.4	16.8	12
3 - 4	107.1 ± 4.5	16.9	6
5 - 6	76.9 ± 7.3	28.7	8
7 - 8	76.1 ± 4.5	62.5	7
9 - 10	48.7 ± 0.7	22.9	12
11 - 12	38.8 ± 0.6	16.8	10
13 - 14	38.9 ± 2.5	26.2	30

enzymatic activity compared to other varieties, which is likely to be its varietal feature. At the same time, the increase in enzymatic activity during drought in the Lombard red variety indicates the inclusion of its protective mechanisms.

To confirm the reliability of the studies, statistical processing of experimental data was carried out with the calculation of the pair correlation coefficients between abiotic factors and physiological parameters, according to the methodological guidelines for conducting field experiments taking into account the specifics of perennial crops. The result was the selection of diagnostic indicators proposed for the assessment of adaptability, and included in the basis of the General methodological recommendations developed in 2017 for the diagnosis of the functional state of subtropical, peanut, flower and ornamental crops and tea plants [1].

Of all assessed stressors, a reliable dependence for *Camellia sinensis* (L.) O. Kuntze exists between enzyme activity—temperature and enzyme activity—solar insolation (Table 4). So, if the temperature rise to an average of 26˚C - 28˚C causes an increase in catalase activity, then with a further increase in temperature—there is a sharp decline in it. The temperature factor that leads to overheating of the leaf surface and, strengthening of water loss causes a significant increase in CCS and metabolic disorders, accompanied by inactivation of enzymatic activity. The calculation of the correlation confirms our conclusion: the correlation between temperature and enzymatic activity is negative and, on average, ranges from −0.7 to −0.8. But the more important factor for changing the activity of catalase is the illumination, which is caused by the manifestation of

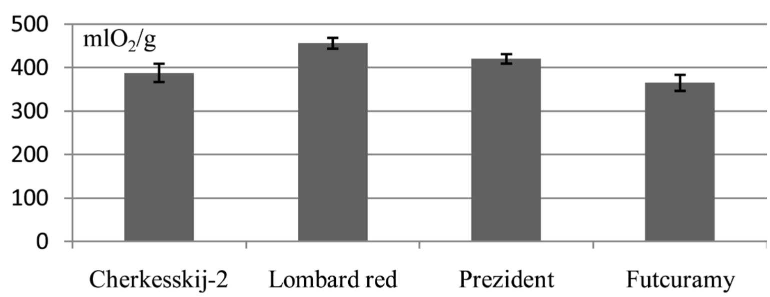

Figure 1. The catalase activity in the leaves of *Corylus pontica* C. Koch., average for three years.

Table 4. Correlation matrix of connection between physiological and biochemical parameters and stress factors (for diagnosis of resistance *Camellia sinensis* (L.) O. Kuntze).

Characteristic	Temperature, ˚C	Illumination, LX	Physiological activity radiation, μv/m^2	Relative humidity, %
Water deficit, %	0.89…0.90	0.68…0.71	0.66…0.73	−0.67…−0.75
CCS of sprouts, %	0.92…0.99	0.54…0.63	0.64…0.68	−0.37…−0.40
Water content, %	−0.77…−0.79	−0.55…−0.69	−0.57…−0.68	0.72…0.83
The coefficient of wilting, units.	0.85…0.88	0.65…0.79	0.62…0.77	−0.87…−0.90
The catalase activity, ml O^2/g	−0.71…−0.80	−0.77…−0.82	−0.77…−0.85	0.34…0.44

photostress. With increasing illumination the activity of the enzyme decreased (**Table 4**).

Within the studies on the adaptability of *Corylus pontica* C. Koch., it was shown that all physiological parameters characterizing the stability of culture are closely connected not only with each other, but also with hydrothermal factors, which allowed building correlation and regression models (**Table 5** and **Table 6**).

Studies have shown that hydrothermal factors closely correlate such physiological and biochemical parameters like water content, water-holding capacity of leaf tissue and the activity of catalase, which is reflected in the development of guidelines for the diagnosis of culture to stress factors [17].

The experimental data showed that with all the variety of specific methods for assessing the resistance of different plant species to extreme environmental conditions, all methods are based on several basic principles associated with the peculiarities of adaptation mechanisms. The main characteristics of the approaches and methods of diagnosis of resistance *Corylus pontica* and *Camellia sinensis* to ecological stresses are given in the **Table 7**.

Table 5. Correlation matrix of connection between physiological and biochemical parameters and stress factors (for diagnosis of resistance *Corylus pontica* C. Koch.)

Characteristic	Air temperature, ˚C	The amount of precipitation, mm
Water content, %	−0.81…−0.89	0.66…0.79
Water-holding capacity, %	−0.83…−0.90	−0.62…−0.72
Associated fraction of water, %	0.72…0.75	−0.77…−0.79
The catalase activity, ml O^2/g	−0.80…−0.86	0.54…0.60

Table 6. Regression analysis of the sustainability relationship of *Corylus pontica* C. Koch. to stress factors.

Characteristic	The equation of linear regression
Water content, %	$Y = 10.69 + 2.07 * F1 + 0.081 * F2, R^2 = 0.46$
Water-holding capacity, %	$Y = 2.67 + 1.41 * F1 + 0.15 * F2, R^2 = 0.58$
Free fraction of water, %	$Y = 38.36 - 0.66 * F1 + 0.88 * F2, R^2 = 0.10$
Associated fraction of water, %	$Y= 0.88 + 2.86 * F1 + 0.84 * F2, R^2 = 0.23$
The catalase activity, ml O^2/g	$Y = 60.06 + 12.03 * F1 + 25.07 * F2, R^2 = 0.60$
Where, F1 is the temperature in ˚C; F2—rainfall in mm	

Table 7. Basic physiological features for the diagnosis of stability *Corylus pontica* C. Koch. and *Camellia sinensis* (L.) O. Kuntze to the stressors of moist subtropics.

Culture	Diagnostic indicator	Diagnosable symptom
Corylus pontica C. Koch.	The water content of the leaf tissue, water-holding capacity of the leaves	drought tolerance
	The activity of catalase	Heat tolerance, acoplasticos
Camellia sinensis (L.) O. Kuntze	The concentration of cellular juice flushes, the activity of catalase	Drought tolerance, heat tolerance

4. Conclusions

Thus, the use of physiological methods during periods of low soil moisture, air and high temperatures allows to determine the influence of abiotic stressors of the nature on the condition of the plants and to identify features of the formation of their resilience.

The water status of such crops as *Camellia sinensis* and *Corylus pontica* has been studied. The indicators that not only describe the physiological state of plants in the stressful period, but also allow identifying more adaptive varieties for the area was defined. Analysis of catalase activity data in cultures revealed the presence of similar patterns: there is a change in enzymatic activity in response to changes in climatic conditions, and primarily hydrothermal factors. Moreover, *Camellia sinensis* (maximum activity of the enzyme is in favourable and on the temperature and water regime period (may), by July the level of activity diminishes according to the stressful effects, and of to the end of the vegetation period (August) there has been some post-stress recovery activities.. At the same time, catalase in the leaves of *Corylus pontica* in the post-stress period shows a large recovery activity, as a result, the activity in August is fully leveled to the optimal level.

We have revealed that the stable varieties of the studied cultures are characterized by a more active catalase, which is of particular importance in the drought period. Given the fact that catalase is an element of the mechanism of plant stability in response to the stress factor, reducing its activity can be used to diagnose the functional state of crops.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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