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Biochemical Characteristics of Drought-Resistance of the Scab-Immune and Non Immune Apple-Tree Varieties

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ABSTRACT

This work outlines the findings of physiological-biochemical and anatomic research of the apple-tree varieties differing in ecological-geographical origin with varying scab-immunity under the drought conditions. The most significant parameters, responsible for drought adaptability of the apple-trees. The study was given to peculiarities of moisture status, pigment complex during the drought season of vegetation. The data on the mechanisms of the chlorophylls (a+b), carotinoids protective action to improve the drought resistance of apple-trees are presented. The enhanced immunity of variety was established to have positive impact on its drought resistance, improves its ecological plasticity. The highly drought-resistant apple-tree varieties were singled out for growing in the North-Caucasian region of the Russian Federation.

Keywords: *Apple-Tree, Scab, Drought Resistance, Water Content, Chlorophylls Totality, Carotinoids, Xeromorphic Characters.*

INTRODUCTION

In the North-Caucasian region of the Russian Federation the apple-tree (*Malus domestica Borkh.*) is one of the most important food crops, it tops the list of the horticulture products, both for its importance and the volume of production. The apple tree occupies in the range from 60% to 95% of the cropland acres in the different horticulture zones. In recent years the need for the higher drought resistance of the cultivated apple-tree varieties grew considerably, which is due, first of all, to the global warming and aridization of climate processes (NENKO et al. 2016; KISILEVA et al. 2016; GONCHAROVA 2011).

Also one of the reasons, limiting the high yield of apple-tree, is significant damage, caused by diseases, particularly by scab, the agent of which is *Venturiainae qualis (Cooke) G. Winter* fungus. The drop in the apple-tree fruits yield caused by this disease comes to 40%, and in the whole scale distribution years – about 80% of the yield is lost. Between 2001 and 2015 13 epiphytoties of scab pathogen were noticed in all horticulture zones of the North-Caucasian region of the Russian Federation. Under these circumstances the high-priority task of the modern horticulture is the creation of the drought-resistant, scab-immune and highly resistant to it apple-tree varieties.

According to the information available, the contribution of selection to the yield enhancement of the critical agricultural crops for the last decades makes 30-70%, and with regard for the probable climatic changes the role of this factor will grow constantly (ZHUCHENKO 2013). The physiological biochemical studies are utterly necessary to assess impartially the adaptability of the fruit crop varieties to the growth environment.

At the present time the sufficient research is available in this direction for the different horticultural crops (pear, peach, apple-tree, Arctic kiwi, hydrangea, freesia) in the different edaphoclimatic zones (KISILEVA 2009; MENGetal. 2004; BESEDINA et al. 2017; NENKO et al. 2014; RYNDIN et al. 2014; BELOUS et al. 2017). The discovery of the physiological-biochemical parameters, responsible for the drought and scab resistance, singling out

on their basis the varieties mostly adapted to conditions of the Russian south, featuring the enhanced protective mechanisms, will finally favour the reduction in losses and higher yield of apple-tree.

The present work aims at finding out the criteria of the physiological-biochemical adaptation of the scab-immune and non-immune apple-tree varieties to the summer drought, at singling out the most drought-resistant varieties to grow in conditions of the North-Caucasian region of Russia.

MATERIAL AND METHODS

The physiological-biochemical research was conducted in 2014-2016 on the basis of Pilot production farm "Centralnoye", Common Use Center "Instrument and Analytical" and physiology and biochemistry laboratory of the Federal State Budgetary Scientific Institution North Caucasian Federal Scientific Centre for Horticulture, Enology and Viticulture. The objects of research were scab-immune and non-immune apple-tree varieties differing in ecological-geographical origin:

- Rassvet /dawn/ – scab-immune, early-summer maturation variety of NCRRIH&V selection. The early maturity is high, it enters fruiting on M9 stock the 2nd year after planting. Fruiting is regular. The fruits may amount to 135-160 g.
- Fortuna – scab-immune, summer maturation variety of NCRRIH&V selection, drought- and frost-resistant. The 6-7-year trees crop capacity on small-stature form of stock amounts to 37 t/ha. The fruits may be as much as 180-235 g.
- Soyuz - scab-immune, summer maturation variety of NCRRIH&V selection. Early - producing, fruiting on M9 stock the 2nd-3rd year after planting. The crop capacity amounts to 28-32 t/ha. The fruits may be as much as 355g.
- Dayton – autumn maturation variety of American selection. Winter-hardy, scab-immune, very early-producing and productive. The tree is medium-grown. The fruits amount to 200 g.
- Ligol – winter maturation variety of Polish selection. It is notable for high crop capacity, early entrance to fruiting, sufficient winter-hardiness, scab immunity; it is prone to periodicity of fruiting.
- Prikubanskoye - winter maturation variety of NCRRIH&V selection. Drought resistance is high; the frost tolerance is above the average, relatively scab-immune. It enters fruiting the 3rd-4th year. The fruits amount to 210-250 g.
- Rodnichok - summer maturation variety of NCRRIH&V selection. The high field scab immunity, high drought- and frost-resistance. Early-producing, it enters fruiting on M9 stock the 2nd year after planting. The fruits amount to 210 g.
- Erli Mac – early-summer maturation variety of American selection. Highly winter-hardy, scab non immune. It enters fruiting the 2nd-3rd year after planting. The fruits amount to 110-120 g.

The scab-immune variety is known to be fully resistant to scab pathogen, it may never be down with scab, since it has Vf immunity gene. The scab-immune variety has no Vf scab immunity gene and in certain conditions it may be scab-sensitive. The peculiarities of moisture status, chlorophylls (a+б) and carotinoids content were determined in accordance with the conventional methods (GONCHAROVA 2005; GAVRILENKO et al. 1975; Nenko et al. 2012). The anatomic research of leaf blade was conducted by the methods using a light microscope "Olympus" BX 41 (PAUSHEVA 1980). The measurement data were processed by the conventional methods of variation statistics (DOSPEKHOV 1979).

THE RESULTS AND DISCUSSION

The condition of the apple-tree plants in summer period was estimated by the physiological-biochemical parameters of leaf apparatus, since a leaf is a very plastic vegetative organ, very responsive to the change in ecological factors. The drought affects adversely the water status of vegetative organs, photosynthetic parameters and water transport over the shoots of plants that have an effect on the figures of apple-tree yield (KUZNETSOV, DMITRIEVA 2006; HENFREY et al. 2015).

The summer period in Pilot production farm "Centralnoye" in Krasnodar city in 2014-2016 was hot. In July and August of 2014 the air temperature reached 35-37°C, in 2015 39-38°C, in 2016 37°C. The precipitation depth in August as compared with June and July, lowered, and in August of 2014-2015 the drought was registered (0 mm of precipitation at the air temperature of 36 and 38°C, respectively). The essential figures, which are necessary to be taken into account

when estimating the degree of the varieties' drought resistance, are those of the moisture status. In August, at the time of the largest impact stress factors the highest water content values leaf apparatus tissues were noticed in Fortuna, Soyuz, Prikubanskoye, Rodnichok apple-tree varieties and made 67.54-70.19 % of the substance wet weight (Table 1).

Table1. *The moisture status parameters of the scab-immune and non-immune apple-tree varieties leaf apparatus in August of 2014-2016 (mean values).*

Variety	Water content, %	Free water, %	Bound water, %	Bound / Free
Immune:				
Rassvet	61.21	30.95	69.35	2.24
Fortuna	67.54	19.83	80.17	4.04
Soyuz	68.61	17.67	82.33	4.66
Dayton	60.69	31.72	68.28	2.15
Non-immune:				
Ligol	58.19	32.28	67.72	2.09
Prikubanskoye	69.19	18.76	81.24	4.33
Rodnichok	70.19	19.54	80.46	4.11
Erli Mac	51.10	60.68	39.32	0.64
$HCP_{0.05}$	0.45	0.26	0.28	0.42

The lowest values of the leaf apparatus water content were registered for Erli Mac variety – 51.10% of the substance wet weight.

The quantitative measures of bound water, preconditioning the drought resistance of Fortuna, Soyuz, Prikubanskoye, Rodnichok plants, are higher than those of other varieties under study and made 80.46-82.33 % of wet weight.

The change in the bound water to free ratio is among the adaptive reactions to the drought conditions of environment. The high figure of bound to free water ratio is an evidence of the high drought resistance of variety. The figure of bound to free water ratio was mostly high for Fortuna, Soyuz, Prikubanskoye, Rodnichok varieties and made 4.04-4.66.

The productivity of variety is a function to great extent of the leaf apparatus condition, determining the net productivity of the leaves photosynthesis. The drought and higher-temperatures of summer period inhibit the process of photosynthesis, which belongs to the temperature-dependent processes. The suppression of the photosynthetic processes starts at the relatively low temperatures, but a repair process is also fast to come about. That's why a conclusion may be made about the functional resistance of assimilation apparatus in the absence of leaf qualitative changes, visible by the changes in photosynthesis activity under the impact of high temperature (CHIRKOVA 2002).

The study of chlorophylls accumulation dynamics showed the stability of content of the chlorophylls ($a+b$) totality within summer vegetation period, noticed in Fortuna, Soyuz, Prikubanskoye, Rodnichok varieties, that implies the better adaptation of plants to conditions of cultivation.

The quantitative figures of chlorophylls content within summer remained invariably high in these varieties and were in August in the range from 2.66± 0.24 to 3.87± 0.27mg/g of dry weight (Table 2).

Table2. *The parameters of the leaf apparatus color combination of the scab immune and non immune apple-tree varieties in August of 2014-2016*

Variety	C_a, mg/g	C_b,mg/g	$C_{(a+b)}$,mg/g	$C_{a/b}$	Carotinoids, mg/g	$C_{(a+b)}$ / carotinoids
Immune						
Rassvet	3.10 ± 0.03	1.01± 0.11	4.11 ± 0.23	3.06 ± 0.25	1.90 ± 0.32	2.16 ± 0.11
Fortuna	2.75± 0.15	0.99 ± 0.14	3.73 ± 0.03	2.79± 0.31	1.74 ± 0.24	2.14 ± 0.15
Soyuz	2.49 ± 0.06	0.85 ± 0.16	3.35 ± 0.13	2.91 ± 0.04	1.47 ± 0.13	2.28 ± 0.08
Dayton	2.87 ± 0.24	1.19± 0.35	4.06 ± 0.40	2.42 ± 0.21	1.86 ± 0.12	2.18± 0.27
Non immune						
Ligol	3.62 ± 0.04	1.30 ± 0.08	4.92 ± 0.30	2.77 ± 0.18	2.20 ± 0.31	2.23 ± 0.12
Prikubanskoye	2.91 ± 0.27	0.96 ± 0.29	3.87± 0.27	3.02 ± 0.10	1.82± 0.24	2.13 ± 0.15
Rodnichok	2.54 ± 0.19	0.93 ± 0.17	3.47 ± 0.38	2.74 ± 0.37	1.51 ± 0.26	2.29 ± 0.37
Erli Mac	2.49 ± 0.13	0.96± 0.24	3.45 ± 0.29	2.61 ± 0.14	1.38 ± 0.12	2.50± 0.14
$HCP_{0.05}$	0.52	0.15	0.45	0.61	0.27	0.63

As for the rest of varieties under study, the dynamics of change in the synthesis of green pigments showing the decreasing tendency in August is in evidence. The slight drop in chlorophylls (*a*+*b*) totality in the scab-immune Rassvet and Dayton varieties apple-tree down to 4.11 ± 0.23 and 4.06 ± 0.40 mg/g of the dry weight respectively is sooner a protective reaction of plants, aimed at the elimination of the high temperature and water stress impacts.

The dramatic drop in the quantitative figures of chlorophylls in Ligol and Erli Mac varieties down to 4.92 ± 0.30 and 3.45 ± 0.29 mg/g of dry weight respectively, results from the lower drought resistance of the scab-damaged leaf apparatus, since these varieties are not immune to this disease.

The evidences of resistance to drought and fungal diseases are known to have effect on each other through the metabolic processes. The scab infection, damaging a leaf, provokes a premature leaf fall, the plant fails to prepare adequately for winter and it freezes worse. The immunodefences of the frozen plants go down, they are more susceptible to scab and their drought resistance lowers.

An important parameter in physiological research is chlorophyll *a* to chlorophyll *b* ratio (*a*/*b*), characterizing the degree of pigmented system tolerance to illumination. Chlorophyll *b* regulates the degree of plants adaptation to the high intensity of light, its content grows in the case of adaptation to the lack of illumination (GREEN et al. 1991).

The low value of chlorophyll *a*/*b* ration was noticed in Dayton, Erli Mac varieties (2.42± 0.21 and 2.61± 0.14mg/g of dry weight respectively), that occurs probably due to the thickness of crown in these varieties. The rest of varieties under study shows the value of *a*/*b* chlorophyll ratio be in the range from 2.74± 0.37 to 3.06± 0.25 mg/g of dry weight.

The carotinoids are another important element of pigmented complex. They protect the chlorophylls from excess light and oxidation by oxygen, generating in the photosynthesis process. The increased accumulation of carotinoids in unfavorable conditions of summer vegetation period is necessary to stimulate the adaptive reactions and to lower general stress (KARNAUKHOV 1988). The highest quantitative figures of carotinoids were noticed for Ligol, Rassvet, Dayton varieties and ranged from 1.86 ± 0.12 to 2.20 ± 0.31mg/g of dry weight.

The quantitative ratio of chlorophylls totality to carotinoids – is an important physiological character, reflecting the degree of the plants adaptation to unfavorable factors of environment. The lesser is chlorophylls totality to carotinoids ratio, the more resistant is variety. The least values of chlorophylls totality to carotinoids ratio were noticed for Fortuna (2.14 ± 0.15) and Prikubanskoye (2.13 ± 0.15) varieties. These apple-tree varieties adapt through the synthesis of the larger quantity of chlorophyll *b* and carotinoids, protecting chlorophyll *a* from the excess solar insolation, typical of August.

Soyuz and Rodnichok varieties show the mean values of chlorophylls totality to carotinoids ratio (from 2.28 ± 0.08 to 2.29 ± 0.37), nevertheless, they proved to be highly drought-resistant in the field conditions, for they are triploid by origin, making them more adaptive to unfavorable conditions (NENKO et al. 2013).

Table3. *The leaf blade parameters of the scab-immune and non immune apple-tree varieties in August of 2014-2016.*

Group of varieties	Overall thickness, мк	Upper epidermis,	Palisade parenchyma, мк	Spongy parenchyma, мк	Lower epidermis, мк	Palisade index	Stomata quantity, шт	Stomata width, мк	Stomata length, мк
Highly drought-resistant	192.7 ± 0.22	11.0± 0.34	98.1 ± 0.14	76.6 ± 0.29	7.1 ± 0.14	1.28 ± 0.05	269.5± 0.54	53.0 ± 0.07	30.0 ± 0.13
Drought-resistant	184.8± 0.12	10.0 ± 0.43	84.3 ± 0.11	83.5 ± 0.29	7.0 ± 0.21	1.01 ± 0.14	245.1± 0.73	54.1± 0.28	31.1 ± 0.23
Non resistant to drought	177.6 ± 0.52	10.0 ± 0.16	80.3 ± 0.17	80.3 ± 0.20	7.0 ± 0.45	1.00± 0.04	215.2± 0.34	56.2 ± 0.24	34.2 ± 0.17
$HCP_{0.05}$	13.73	0.75	3.45	3.15	0.73	0.01	17.94	1.45	1.51

The results of research of the moisture status and color combination for the leaves of the scab-immune and non-immune apple-tree varieties agree with the data of the leaf blade anatomic research, conducted in parallel within the same dates of summer vegetation period. It was made clear that the largest quantitative parameters of a leaf blade (overall thickness of

leaf blade, cuticle, upper and lower epidermis, palisade parenchyma, palisade index, stomata quantity per unit of leaf surface) and the least quantitative parameters of stomata linear dimensions, preconditioning the plants drought-resistance, were noticed in Fortuna and Prikubanskoye varieties. These varieties were singled out into a highly drought-resistant group (table 3).

The signs of the leaf blade xeromorphic structure are expressed less clearly for Rassvet, Dayton, Ligol apple-tree varieties; they were singled out as the drought-resistant ones.

Erli Mac, a non-immune to scab apple-tree variety, featuring the least quantitative parameters of the leaf blade (overall thickness of leaf blade, cuticle, upper and lower epidermis, palisade parenchyma, palisade index, stomata quantity per unit of leaf surface) and the largest quantitative parameters of stomata linear dimensions, was singled out as a non drought-resistant on the basis of the leaf blade anatomic parameters.

Erli Mac variety behaves according to genotype, showing the low adaptive capacity for extreme factors of a summer period.

It is worth noting that the leaf blade quantitative parameters of Soyuz and Rodnichok varieties were not considered in comparative characteristics of varieties, for they are triploid by origin and the large size of their mesophilic cells is a genetically caused character.

The field studies in the no irrigated gardens revealed, that the improved scab-immunity of variety has a positive effect on its drought resistance.

The lack of necrotizing damages and leaf fall in a drought period was shown by the scab-immune Fortuna and Soyuz apple-tree varieties and by the non-immune to scab Prikubanskoye and Rodnichok varieties (Table 4).

Table4. *The results of field studies for the scab-immune and non-immune apple-tree varieties in August of 2014-2016*

Variety	Damage of leaves, point	Leaf fall, point	Productivity, t/ha
Immune			
Rassvet	0.5	0.5	22.5 ± 0.57
Fortuna	0	0	22.7 ± 0.21
Soyuz	0	0	23.1± 0.17
Dayton	0.5	0.5	14.7 ± 0.19
Non immune			
Ligol	0.5	0.5	23.0 ± 0.15
Prikubanskoye	0	0	22.4 ± 0.27
Rodnichok	0	0	18.0 ± 0.81
Erli Mac	1.0	1.0	16.5 ± 0.34
$HCP_{0.05}$	-	-	1.31

The 0.5 point damage and leaf fall was noticed for Rassvet, Dayton and Ligol varieties. 1.0 point – for Erli Mac variety.

All the studied apple-tree varieties were notable in 2014-2016 for productivity ranging from 22.4 ± 0.27 to 23.1 ± 0.17 t/ha, except Dayton, Rodnichok, Erli Mac varieties, the productivity value of which averaged from 14.7 ± 0.19 to 18.0 ± 0.81 t/ha.

A good taste and external appearance of the fruits are the major elements of the apple-tree fruits commercial characteristics. The high tasters judgment was given to the fruits of the scab immune Soyuz (4.9 points), Rassvet and Fortuna (4.8 points) apple-tree varieties.

Whereas Rassvet, Fortuna, Soyuz and Erli Mac varieties enjoyed the highest estimate of external appearance (4.7-4.8 points).

Dayton and Rodnichok varieties proved themselves sufficiently drought-resistant by the physiological-biochemical parameters, but in the field conditions provided second high yield, nevertheless, they preserve their importance as a genetic source of the high drought resistance for further selection.

CONCLUSIONS

The physiological-biochemical adaptation of the scab-immune and non-immune apple-tree varieties to the summer drought is achieved at the cost of increased contents of bound water, carotinoids, lower chlorophylls (*a*+*b*) totality to carotinoids ratio.

The optimum quantitative ratio of chlorophylls totality to carotinoids, reflecting the degree of the plants adaptation to unfavorable environmental factors, was noticed in Fortuna and Prikubanskoye

varieties. These varieties, singled out as the highly drought-resistant ones, showed the characters of the leaf blade xeromorphic structure, preconditioning the drought resistance of plants. It has been established that the tendency to the higher drought-resistant appears along with improved scab-immunity of variety.

As it becomes evident from the physiological-biochemical and anatomic figures of laboratory and field studies (damage and leaf fall), Fortuna, Soyuz, Prikubanskoye and Rodnichok apple-tree varieties proved themselves as the highly drought-resistant ones in the North-Caucasian region of the Russian Federation.

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