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PHYSIOLOGICAL FEATURES OF RED CURRANT VARIETIES AND SELECTED SEEDLING ADAPTATION TO DROUGHT AND HIGH TEMPERATURE

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Abstract

Adaptation to weather conditions is an important feature of cultivars. To assess plant adaptability, we applied a physiological test of *Ribesia* (Berl.) Jancz. leaf resistance to abiotic factors during vegetation. The effect of high temperatures and drought on red currant plant photosynthetic apparatus and water regime was studied in 2011-2013 in the Central-Chernozem region (Orel region). Five varieties and three selected seedling genotypes of different eco-geographic and genetic origin were used including the derivatives of *Ribes petraeum* Wulf., *R. vulgare* Lam. and *R. multiflorum* Kit. (of these, six genotypes were originated in VNIISPK). Leaves of red currant plants are known to have a mesomorphic structure characteristic of *Ribesia* (Berl.) Jancz. subgenus. In this paper, it was shown that the morphological characteristics of leaves (i.e., shape, venation) are determined by the biological features of varieties while growing conditions alter the anatomical structure. We found a positive correlation between the leaf area and hydrothermal coefficient (r = +0.99) and negative correlation between the leaf area and environment temperature (r = –0.97). An increase in leaf thickness, the expansion of spongy parenchyma cells, a decrease in chlorophylls (both Chla and Chla + Chlb), as well as elevated carotenoid level are the response to stress factors during vegetation. The ratio coefficient of chlorophyll sum to carotenoids is considered as one of the adaptation indices. High coefficients were revealed in Hollandische Rote variety (5.14) and 1426-21-80 (5.51). Correlations between the chlorophyll sum and water loss (r = –1.00) as well as chlorophyll sum and fraction of available water (r = +0.98) were ascertained. The pigment content, fractional composition of water and water holding capacity of leaves are interconnected with the water shortage. The positive correlation of air temperature and water deficit (r = +0.84) has been noted. The total water content in red currant leaves depends on the shoot growth, leaf age, variety, meteorological conditions and is not the main indicator of resistance to high temperatures and drought. Ratios of bound and free water and water holding capacity of leaves vary depending on meteorological conditions and water availability. In 2012 the weather conditions were unfavorable, and in all red currant genotypes the increase of the coefficient of bound water to available water as well as drop in water loss were observed. According to the parameters of water regime, Hollandische Rote variety and 1426-21-80 displayed high adaptability. The laboratory diagnostic methods were confirmed by the field data of genotype resistance under high temperature and moisture deficiency. Thus, the physiological express tests are suitable for estimation of red currant plant adaptability to environmental factors in the course of breeding. A comprehensive assessment showed diverse ecological plasticity in the plants with different *Ribesia* (Berl.) Jancz. subgenus species in the pedigree. The derivatives of *Ribes petraeum* Wulf. (Hollandische Rote) and *R. multiflorum* Kit. (1426-21-80) showed the highest tolerance to drought conditions during the vegetation season in the Central Chernozem region. The varieties and seedlings derived from *R. vulgare* Lam. had low drought resistance.

Keywords: red currant, drought resistance, leaf, mesostrurture, pigments, fractional structure of water, water holding ability

Red currant is one of valuable berry crops having high yield, early maturity, and good food qualities of berries. A total of 50 to 80 % of its yield losses are accounted for the negative effect of natural climatic factors. The most important of these is an increase in the instability and stress of weather conditions [1, 2]. In the Central Black Earth Region, such stressors include drought caused by high temperatures. An increase in temperature causes morpho-anatomical, physiological and biochemical alterations that affect the growth and development of plants and can lead to a reduction in commercial yield [3-5]. Studying the morphological features, the structure of the photosynthetic apparatus and water exchange of plants with regard to the growth area are necessary for solving fundamental and applied problems of the biology of red currant. Currently, physiological and biochemical express methods are appreciated in estimation of plant resistance to adverse weather and climatic factors that makes it possible to significantly optimize breeding, to minimize yield losses and to create genotypes resistant to the destructive effect of climatic anomalies [6].

Plants have various mechanisms of adaptation to stressors [8-10]. The xenomorphic leaf structure, alterations in the pigment complex and water balance are important diagnostic features of drought resistance [11-13]. Thus, at increasing drought, palisade mesophyll cells of black currant *Ribes americanum* Mill. increase in size, and the amout of retained water in cells also increases. In some red currant varieties derived from *R. vulgare* Lam. species (Gollandskaya rozovaya, Natali krasnaya), the content of the total a and b chlorophyll and photosynthesis decrese [14-16]. A similar regularity is characteristic of strawberry, grapes, chestnut, tea plants (*Camellia sinensis*) and olive trees (*Olea evropaea* L.) under increasing temperature [17-20]. In fruit, vegetable and leguminous crops, a decrease in water retaining during the light day, an increase in transpiration and alterations in the ratio of water forms in various plant parts were observed [21-23]. Adaptation of berry crops including red currant to stressors during vegetation period is poorly studied and requires a more detailed investigation.

This paper is the first reporting application of physiological methods to detect resistance of red currant leaf photosynthetic apparatus to adverse factors during vegetation.

The aim of our study was to investigate water regime, photosynthesis and productivity in various species of *Ribesia* (Berl.) Jancz. subgenus, and also to identify varieties and breeding forms resistant to drought and elevated temperature.

Techniques. The observations were carried out in 2011-2013 at the primary nursery for red currant (All-Russian Research Institute for Breeding Fruit Crops, ARRIBFC). Aridness during the vegetation periods was assessed by a hydrothermal coefficient (HTC). Eight red currant genotypes were studied, including 6 cultivars of ARRIBFC (Dana, Niva, Roza, 1518-37-14, 1426-21-80, 1432-29-98) and 2 foreign ones (Jonkheer Van Tets, Hollandische Rote). The samples had different genetic, ecological and geographical origins and were derivatives of the *Ribes petraeum* Wulf. (Hollandische Rote, 1518-37-14), *R. vulgare* Lam. (Jonkheer Van Tets, Niva, Roza), and *R. multiflorum* Kit. (Dana, 1426-21-80, 1432-29-98) species.

Morpho-anatomical structure of leaves was assessed by studying their mesostructure [24] using a microscope Eclipse 50i (Nikon, Japan, ×400). The pigment content was determined in 80 % acetone extract [25] using a Smart Spec^{TM}Plus (Bio-Rad, USA) spectrophotometer. The water regime parameters were determined according to the developed techniques [11, 26, 27].

Dispersion, correlation and regression analyses were carried out at 95 % significance level [28] using the Microsoft Excel 2010 software package.

Results. The weather conditions varied strongly over the years of studies. From May to June of 2011, there was a combination of high water supply and elevated temperatures (HTC = 0.93; 1.12; 2.10; +30.0...+31.5 °C). 2012 was the most arid year (HTC = 0.20; 0.82; 0.40; +31.2...+32.2 °C). The vegetation period of 2013 was intermediate according to hydrothermal conditions (HTC = 0.72; 0.89; 0.68; +28.6...+31.5 °C).

Morphological variability and ecological plasticity are characteristic of *Ribesia* (Berl.) Jancz. subspecies. Red currant belongs to mesophytes. All its varieties and forms are derived from wild species (*Ribes petraeum* Wulf., *R. rubrum* L., *R. vulgare* Lam., *R. multiflorum* Kit.), growing in the intermediate zone of the Northern hemisphere in the regions with high soil humidity and average air temperature during vegetation. Europe is believed to be the origin of the *R. vulgare* Lam., while *R. petraeum* Wulf. is distributed in mountaneous regions of Europe and North Africa. The origin of *R. multiflorum* Kit. is Southern Europe [29, 30]. Study of the assimilation apparatus in varieties and selection forms of various red current species in the Russian Central Region is of special interest because specific climatic conditions significantly affect the formation and functions of leaf apparatus.

Studies have shown that some morphological features (shape, veining) of red currant leaves depended on the biological features of the variety, and the anatomical structure was affected by growth conditions. In the periods with insignificant water supply, the leaf area reduced in all studied samples compared to the optimal conditions: it was 17.64 cm^2 on the average in 2011, 13.95 cm^2 in 2012, and 14.37 cm^2 in 2013. Thereby, maximum leaf area was noted in 2011 to 2013 in 1426-21-80 sample, the derivative of *R. multiflorum* Kit. A positive relationship was between the leaf area and HTC ($r = +0.99$) and a negative one was between the leaf area and the temperature ($r = –0.97$).

The leaves of red currant plants had a mesomorphous structure characteristic of *Ribesia* (Berl.) Jancz. subspecies. The spongy parenchyma was predominant over the palisade one and the epidermal cells appeared to be large enough, being larger on the upper side of the leaf than on the lower one. High temperature and drought differently affected the sizes of the adaxial epidermal cells and the mesostructure of a leaf. In 2012 and 2013 from May to July, the main cells of the adaxial epidermis in the Hollandische Rote variety (*R. petraeum* Wulf.) and the selection form 1426-21-80 (*R. multiflorum* Kit.) affected by high temperatures (+31.2...+28.6 °C in May, and +32.2...+31.5 °C in July) somewhat elongated, which was associated with reduced cell turgor. We have noted cell shrinkage in the tangential direction (parallel to the palisade surface) in the remaining samples (Fig. 1).

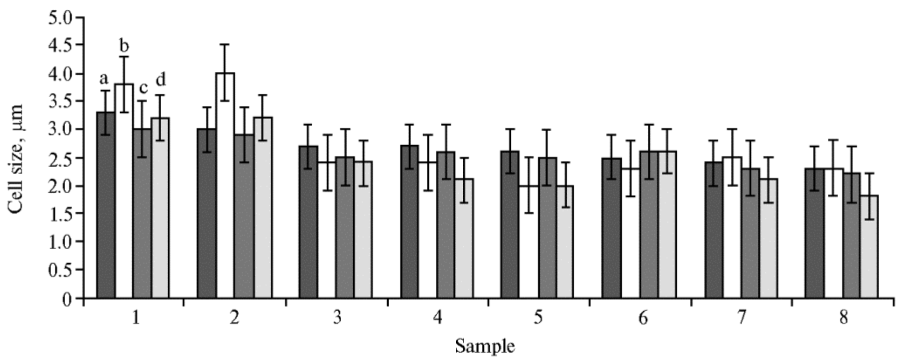

Fig. 1. Cell size in the leaf adaxial epidermis of red currant varieties and forms in May (a) **and in July** (b) **2012 and also in May** (c) **and July** (d) **2013:** 1 — Hollandische Rote, 2 — 1426-21-80, 3 — Dana, 4 — 1432-29-98, 5 — Niva, 6 — 1518-37-14, 7 — Jonkheer Van Tets, 8 — Roza (All-Russian Research Institute for Breeding Fruit Crops, Orel Province, Zhilina village).

The elevated temperature and drought in 2012 and 2013 has led to the growth of parenchymal cells and an increase in leaf thickness (Table 1). The growth of mesophyll cells occurred mainly due to the increase in the air-contained elements of spongy parenchyma which facilitated gas exchange between a leaf and environment. Cell growth in spongy parenchyma and an increase in leaf thickness compared to 2011 were found almost in all of the samples, except Niva variety, especially in Hollandische Rote plants and 1426-21-80. These changes should be viewed as a manifestation of a high adaptability of the anatomical leaf structure to stressors during the vegetation period.

1. Leaf mesostructure in the red currant varieties and forms as depended on year conditions (All-Russian Research Institute for Breeding Fruit Crops, Orel Province, Zhilina village)

Sample (B)	Year (A)								
	2011			2012			2013		
	PP	SP	TLT	PP	SP	TLT	PP	SP	TLT
Hollandische Rote	10.29	13.62	26.94	12.00	20.00	37.30	9.75	15.80	–
Niva	9.56	15.24	27.70	11.25	18.45	35.35	9.55	16.95	31.40
Dana	9.28	12.65	24.63	10.15	15.35	29.60	10.35	15.30	29.10
Jonkheer Van Tets	8.10	12.00	22.70	10.05	16.30	29.92	9.10	14.85	27.70
Roza	7.90	12.35	23.25	11.65	17.55	33.65	10.15	15.10	29.10
Hollandische Rote	7.70	14.68	25.38	9.15	18.15	30.80	8.55	16.90	29.15
Niva	7.30	10.10	20.33	10.50	15.90	30.05	9.00	12.30	24.80
Dana	6.80	11.23	20.53	7.95	13.85	25.60	8.10	13.80	25.10

N o t e. PP — palisade parenchyma, μm; SP — spongy parenchyma, μm; TLT — total leaf thickness, μm. LSD_{05} for palisade parenchyma: A = 1.57; B = 2.56; AB = 4.42. LSD_{05} for spongy parenchyma: A = 1.92; B = 3.14; AB = 5.43. LSD_{05} for total leaf thickness: A = 1.39; B = 2.27; AB = 3.93. Dash means no data.

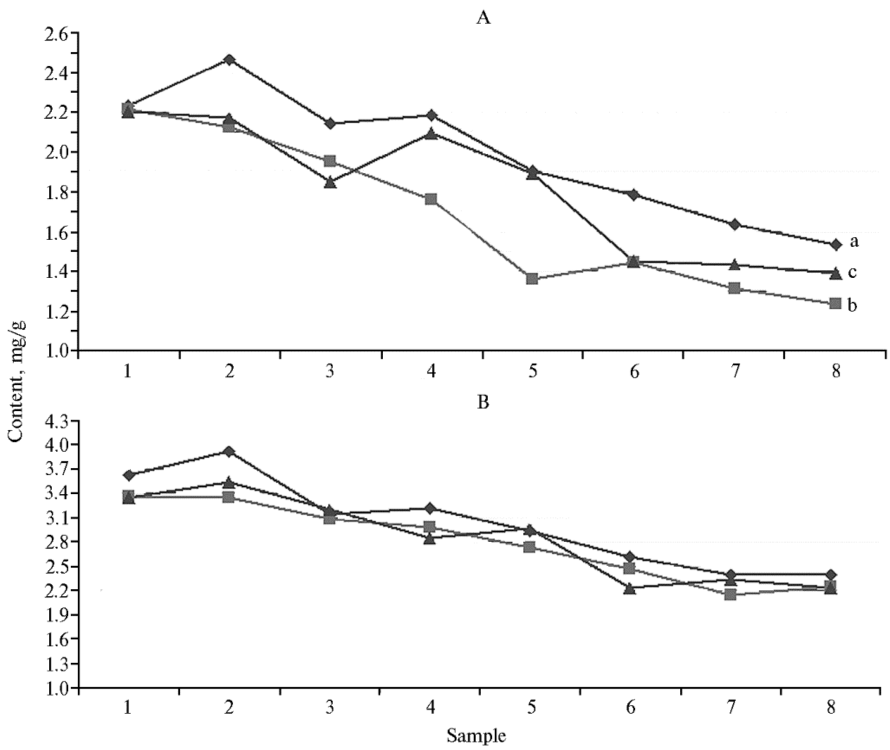

Fig. 2. Chlorophyll a (A) **and chlorophylls a+b** (B) **content during vegetation in 2011** (a), **2012** (b) **and 2013** (c) **in the leaves of red currant varieties and forms:** 1 — Hollandische Rote, 2 — 1426-21-80, 3 — Dana, 4 — 1432-29-98, 5 — Niva, 6 — 1518-37-14, 7 — Jonkheer Van Tets, 8 — Roza (All-Russian Research Institute for Breeding Fruit Crops, Orel Province, Zhilina village). A: a — LSD_{05} = 0.31; b — LSD_{05} = 0.26; c — LSD_{05} = 0.29. B: a — LSD_{05} = 0.61; b — LSD_{05} = 0.53; c — LSD_{05} = 0.58.

The weather conditions during vegetation have significantly affected assimilation apparatus. In the arid 2012, a reduction in chlorophyll a and a + b chlorophylls (1.23-2.21 and 2.15-3.36 mg/g, respectively) was noted in the leaves of all red currant cultivars compared to 2011 (1.53-2.46 and 2.40-3.92 mg/g)

(Fig. 2). It should be noted that a more drastic decrease in the pigment amount in 2012 occurred in all derivatives of the *R. vulgare* Lam. Species; the minimal one was in *R. petraeum* Wulf. and *R. multiflorum* Kit. plants. Insignificant drought relief in 2013 facilitated a small increase in the pigment content.

Drought together with high temperatures stimulated the formation of carotenoids in all of the samples of *Ribesia* (Berl.) Jancz. subgenus studied, which is consistent with findings about the protective function of this pigment group at stress [21, 31, 32]. We have found a positive correlation between the carotenoid content and temperature (r = +0.77). Some scholars in order to assess stressor resistance suggest using the coefficient of the total chlorophyll to carotenoid ratio [31, 33, 34]. Under stress conditions of 2012, the Hollandische Rote variety and 1426-21-80 plants with the coefficient of 5.14 and 5.51, respectively, were distinct in this parameter, which may indicate their resistance to drought and high summer temperatures. The hydrothermal regime affects productivity by affecting the functional state of the plants which was confirmed by high correlations between the chlorophyll a level and yield (r = +0.85), and total chlorophylls and yield (r = +0.78)

The pair correlation coefficients between total chlorophylls and water loss (r = −1.00), and total chlorophylls and free water amount (r = +0.98) confirmed the dependence of the pigment content on water regime parameters. These parameters (pigment content, water fractions, water-retaining leaf capacity) were associated with the development of water deficiency which depended on air temperature (r = +0.84). The relationship between the water regime parameters and the meteorological features of the vegetation period was described using multiple regression coefficients (Table 2).

2. Correlation between physiological parameters of the red currant samples and the meteorological factors (All-Russian Research Institute for Breeding Fruit Crops, Orel Province, Zhilina village)

Parameter	Regression coefficient
Water content	0.90·F; r = 0.22
Free water fraction	0.84·F; r = 0.33
Water loss	–0.88·F; r = 0.30

N o t e. F — hydrothermal coefficient, r — standard experiment error.

It should be noted that the total water content in red currant leaves is not the main parameter of plant resistance to elevated temperatures and drought, because it depends on a number of factors (sprout growth, leaf age, berry formation, weather conditions and variety) [35] (Fig. 3).

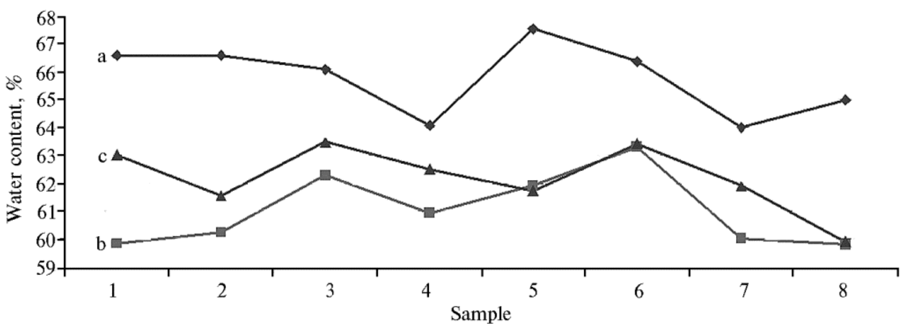

Fig. 3. Water content in leaves during vegetation in 2011 (a), **2012** (b) **and 2013** (c) **in the red currant varieties and forms**: 1 — Hollandische Rote, 2 — 1426-21-80, 3 — Dana, 4 — 1432-29-98, 5 — Niva, 6 — 1518-37-14, 7 — Jonkheer Van Tets, 8 — Roza (All-Russian Research Institute for Breeding Fruit Crops, Orel Province, Zhilina village). LSD_{05}: a — 4,38, b — 3,74, c — 3,98

The most labile parameters, depending on meteorological conditions and water supply, are the ratios of the bound and free water and the water retaining capacity of leaves [11, 36, 37]. We assessed the water retaining capacity based on water loss. Among the studied traits in the stress period, significant differences were associated with variety and specific features. In 2012 with unfavorable HTC compared to 2011, an increase in the ratio of bound and free water and a decrease in leaf water loss were observed in all the samples. In 2013, this ratio was higher than that in 2011, but lower compared to the parameter of 2012 (Table 3). The highest values of the coefficient and minimal water loss were noted in the Hollandische Rote plants and the 1426-21-80 form; this gives grounds to believe that genotypes of these *R. petraeum* Wulf. and *R. multiflorum* Kit. samples manifest a higher adaptability to high temperatures under soil moisture deficiency.

3. Main parameters of water regime in the red currant varieties and forms aas depended on year conditions (All-Russian Research Institute for Breeding Fruit Crops, Orel Province, Zhilina village)

Sample (B)	Year (A)							
	2011		2012		2013		average	
	BW/FW	WL, %	BW/FW	WL, %	BW/FW	WL, %	BW/FW	WL, %
Hollandische Rote	1.33	35.82	2.09	20.36	2.03	21.11	1.82	25.76
1426-21-80	1.31	36.16	2.41	20.62	2.09	19.37	1.94	25.38
1432-29-98	1.12	30.17	1.42	25.01	1.20	26.98	1.25	27.39
Jonkheer Van Tets	1.01	38.10	1.31	21.85	1.04	27.40	1.12	29.12
1518-37-14	0.96	40.91	1.06	27.62	1.08	26.66	1.03	31.73
Niva	0.82	42.56	1.71	24.84	1.50	25.58	1.34	30.99
Dana	0.70	40.97	1.76	25.51	1.66	25.44	1.37	30.64
Roza	0.63	39.75	0.98	26.82	0.87	27.85	0.83	31.47
LSD_{05}	0.08	2.06	0.11	3.03	0.14	1.86		

Note. BW/FW — coefficient of the bound to free water ratio, WL — water loss over 24 h, %. LSD_{05} for BW/FW: A — 2.18; B — 4.59; AB — 7.95. LSD_{05} for WL: A — 0.13; B — 0.21; AB — 0.37.

Therefore, prospects of physiological methods in assessment of resistance to unfavorable weather factors have been shown in the members of *Ribesia* (Berl.) Jancz. subgenus. The obtained results are fully consistent with data about field resistance of red currant varieties and forms under elevated temperature and moisture deficiency. This allows using the said methods for express diagnostics that significantly intensify breeding for adaptability. The complex physiological assessment has shown that the derivatives of various species of *Ribesia* (Berl.) Jancz. subgenus have different ecological plasticity which is explained by differences in their genetic and ecological and geographic origins. The highest resistance to drought during vegetation in the Central Black Soil Region was manifested in the derivatives of *Ribes petraeum* Wulf. (Hollandische Rote) and *R. multiflorum* Kit. (1426-21-80) species. Low drought resistance was noted in the varieties and forms of *R. vulgare* Lam.

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