Chapter

The Creation of Resistant Berries' Agrobiocenosis

Zoya Evgenievna Ozherelieva, Pavel Sergeevich Prudnikov, Diana Aleksandrovna Krivushina, Marina Ivanovna Zubkova and Anna Androsova

Abstract

In *conditions* of increasing differences in the hydrothermal regime of the environment, all known adaptation mechanisms should be used fully, as at the level of individual varieties and at agrobiocenosis in general. The modern gardener needs high-productive, adapted to the growing conditions varieties of the *Fragária × ananássa* Durh. The resistant varieties of the *Fragária × ananássa* Durh. to negative environmental conditions is a basic characteristic, which shows an economic value and the effectiveness of their cultivation in an actual zone. The main physiological and biochemical indicators of resistance during autumn hardening and after temperature stress in winter are changes in the antioxidant system, interruptions of the protein-carbohydrate complex, accumulation products of membran's lipoperoxidation, and changes in the fractional composition of water in leaves. The *Fragária × ananássa* Durh. production process is characterized by the following physiological parameters: pigment analysis, photochemical activity of isolated chloroplasts, respiration, and net photosynthesis productivity. Studies of physiological and biochemical resources of resistance to abiotic stress factors and productivity of *Fragária × ananássa* Durh. are shown. As a result of the carried studies, perspective variety of the *Fragária × ananássa* Durh. ("Tzaritza") was identified for the creation of resistance berries' agrobiocenosis.

Keywords: agrobiocenosis, Fragária × ananássa Durh., abiotic stress factors, frost-resistant, resistance to spring frosts, production process

1. Introduction

Truly, strawberries are on the first place among berry crops in the world, due to their excellent taste, attractive appearance, and early fruit maturation [1–3].

The constant introduction of this culture from different climatic zones conductive to the expansion of the assortment, the involvement of new genotypes in the selection process [4]. But often the most productive, large-fruited industrial varieties have low winter hardiness. The realization of garden crops' stability and the intensity of their production processes are significantly determined by their adaptability and their ability to use fully the bioclimatic potential of the placement zone [5, 6]. Resistance to low temperatures and average daily temperature changes are the most important characteristics of the strawberry's variety in the central region [3, 7, 8]. The strawberries die in snowless winters when the temperature decrease from −15 to −18°C but can tolerate temperatures from −25 to −35°C when the level of snow cover at least 20–30 cm. The most dangerous periods in overwintering—snowless late autumn and early winter—November, December, when the snow has not yet fallen, and the air temperature decrease to −10, −15°C, can be possible freezing or death of the root system of strawberries [9]. At this time, the leaves and flower buds can freeze and in more intensive frosts—branch crowns and whole bushes. Especially the low winter-resistant varieties have suffer distress and plants that are prepared for winter badly. The second critical period for strawberries is the end of winter—the beginning of spring. The snow on the plantations is beginning to settle down and melt, and the bushes are opening [10, 11]. Alternation of thaws and frosts is also dangerous for plants [12], when the snow melts near the ground and the snow crust remains on top. At this time, there is a getting wet of bushes [10]. Visible damage after low temperatures is the death of a whole plant or damage the branch crowns and rhizome.

The frost-resistant state of plants is achieved under the condition of stopping growth and passing through the hardening phases [13]. The great significance of hardening for successful overwintering of strawberries is revealed [14]. The hardening is a difficult complex of physiological and biochemical changes which is associated with some cell dehydration and with the accumulation of protective compounds (sugars, low-molecular water-soluble proteins, amino acids, etc.), which, by increasing the concentration of cellular fluid and binding free water in the plant, prevent the formation of intracellular ice. At critical temperatures, the water outflow from the cells becomes worse significantly, and a lot of supercooled water appears, which then freezes inside the protoplast and can lead to cell death [15]. Thus, plants are characterized by an increase in the amount of bound water by the beginning of the winter period [16]. The ratio of free water to bound water is one of the essential signs of plant adaptation to a temperature decrease in the autumn-winter period. It is noted that the ratio of free water to bound water is lower in winter-hardy varieties than in non-winter-hardy ones. The state of the water regime in autumn and the effective accumulation of protective substances by winter are the important factors, which determine the successful overwintering of plants [16–18].

The research was made at the section of primary variety research of VNIISPK. The strawberry plants were planted in 2016, in the second half of summer (end of July-start of August) according to the scheme 90 × 20 cm in threefold replication, on 30 plants in every replication, randomized. We studied varieties of strawberries of different ecological and geographical origins ("Kokinskaya rannyaya," "Solovushka," "Rosinka," "Tsaritsa," "Urozhainaya TzGL" [Russia]; "Sara" [Sweden]; "Alba," "Marmolada" [Italy]; "Korona," "Sonata" [Holland]) to identify the features of functional conjugation of physiological and biochemical processes of resistance to the action of low-temperature environmental factors and productivity. **Table 1** shows the minimum and maximum air temperature in the autumn and early winter during the years of project realization.

T (°C)	2017				2018			
	Sept	Oct	Nov	Dec	Sept	Oct	Nov	Dec
Max t°C	28.0	15.5	9.0	9.0	29.8	21.5	10.6	1.5
Min t°C	−1.5	−4.8	−9.2	−5.5	−1.0	−2.8	−18.5	−17.0

Table 1.
Air temperature in the period September-December 2017–2018.

2. The study of the physiology-biochemical parameter resistance of varieties of strawberry from different ecological and geographical origins in autumn period

As known, the winter-hardy varieties of fruit crops have a higher bound/free water ratio, than non-winter-hardy [6]. In September, when the fractional composition of water was determining, the strawberry leaves showed a low ratio of bound water to free water. In October, this ratio increased by 1.8–7.7 times. In November, as far as the air temperature decreased (**Table 1**), the bound/free water ratio in leaves was higher (1.6–18.0 times) than in the previous autumn months. The largest measure of the water fraction ratio to the beginning of winter was noted in the varieties—"Solovushka," "Tsaritsa," "Sara," and "Korona" (**Figure 1**). Correlation analysis showed a high level of dependence between the minimum air temperature in autumn, the water content of strawberry leaves tissues (r = 0.81), and the bound/free water ratio (r = −0.97).

According to some of the researchers, proline has osmoprotective properties under stressful conditions [19–21]. In September-November period of time, as far as the air temperature decreased (**Table 1**), an increase in the amount of the amino acid proline was noted in the leaves. During cold adaptation, an increase in the amount of proline in strawberry plants was registered by other authors [22]. In our studies, the most intense peak of proline accumulation in all varieties was noted in October (3.27–11.21 times compared to September), while in November (1.19...1.91 times compared to October) (**Table 2**). At the same time, the varieties "Solovushka," "Kokinskaya rannyaya," and "Tsaritsa" were characterized by the highest level of the amino acid proline accumulation. The amount of proline increased by 10.38–16.20 times at these varieties in November compared to September.

Low-molecular carbohydrates play an important role as osmoprotectors alongside with proline [23]. Sugars increase the water-holding ability of protoplasmic colloids, protecting them from ice formation and excessive cell dehydration. In our studies, during 2 years, significant accumulation of sugars was noted in strawberry leaves in October compared to September, on the background of a decrease in air temperature (**Table 3**). So in 2017, the level of carbohydrates in leaves tissues increased by 1.92–4.93 times and in 2018 by 1.20–3.34 times. The maximum accumulation of sugars in 2017 was marked at varieties of strawberries "Sara," "Korona," "Tsaritsa," and "Urozhainaya TzGL" (the amount of carbohydrates in October increased by 2.37–4.93 times compared to September). At "Solovushka"

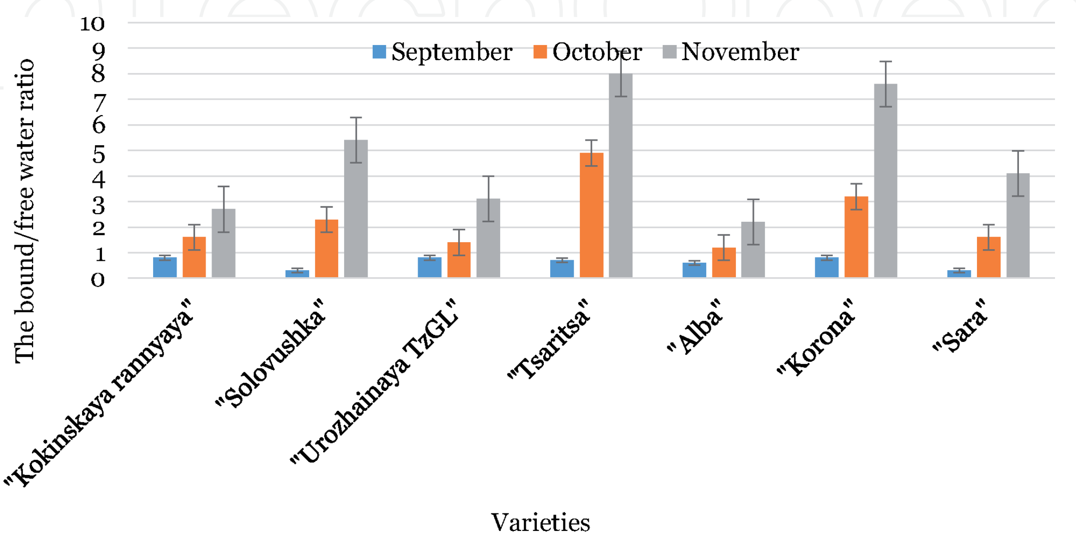

Figure 1.
The bound/free water ratio in the leaf's tissues of strawberry varieties in the autumn period.

Varieties	Proline, mg/kg		
	September	October	November
"Kokinskaya rannyaya"	2.34 ± 0.12	14.95 ± 0.79	24.28 ± 1.33
"Solovushka"	1.19 ± 0.06	13.34 ± 0.67	19.38 ± 0.90
"Urozhainaya TzGL"	1.70 ± 0.11	12.48 ± 0.68	14.93 ± 0.79
"Tsaritsa"	2.65 ± 0.11	11.32 ± 0.40	28.73 ± 1.15
"Alba"	2.03 ± 0.11	11.80 ± 0.59	14.90 ± 0.74
"Korona"	3.95 ± 0.15	14.34 ± 0.49	27.65 ± 1.11
"Sara"	6.07 ± 0.30	19.87 ± 098	27.55 ± 1.38

Table 2.
The content of free proline in the leaves of strawberry plants in autumn.

and "Kokinskaya rannyaya" varieties, the amount of sugars increased at a lower level in compared to September, by 1.95 and 2.10 times. However, in 2018, in October compared to September, the maximum level of sugar accumulation was remained only at the "Tsaritsa" variety—the amount of carbohydrates increased by 3.34 times. In addition, a high movement of sugar biosynthesis was marked at the "Solovushka" variety—by 2.20 times as compared to September. The low sugar accumulation was at the "Urozhainaya TzGL," "Tsaritsa," and "Alba" varieties by 1.20–1.57 times, in October 2018. In November 2017, as the temperature decreased, some varieties showed a decrease in the amount of sugars compare to the October level, which can be ascribed to their active use as an energy substrate for respiration processes, protein synthesis, amino acids, etc. At the same time, the sugar content decreased to a largest level at the varieties "Urozhainaya TzGL," "Sara," and "Alba" (by 1.31–1.57 times compared to October). However, sugar content continued to increase at the "Tsaritsa" and "Korona" varieties. The changes in the level of sugars were not significant at "Solovushka" and "Kokinskaya rannyaya" varieties (**Table 3**).

The determining of the total protein in leaves of strawberry showed the significant increase in its amount in October to November (**Figure 2**), which can explain the decrease in the amount of low-molecular carbohydrates observed in some varieties in November. At the background of high accumulation of proline, the "Solovushka," "Tsaritsa," "Korona," and "Sara" varieties were characterized not only by more active protein biosynthesis (the amount of peptide compounds increased by 2.8–3.5 times in November compared to October) but also had the highest protein content in November.

When resistance is forming, the functioning of the antioxidant protection system is significant, which prevents the development of oxidative stress and, in particular, peroxidation of membrane lipids (POL) at the background of adverse environmental factors. The intensity of damage to cell membranes was estimated by the accumulation of the final product of lipid peroxidation, malondialdehyde (MDA); a transition of lipid peroxidation, hydroperoxides; the content of hydrogen peroxide (as a representative of reactive oxygen species); and the activity of antioxidant enzymes: superoxide dismutase (SOD), catalase, and peroxidase.

The results of the study showed that as far as temperature of environment was decreasing, the intensity of MDA accumulation increased in all varieties. However, the intensity of damage to membrane lipids was significantly lower at "Solovushka," "Sara," "Korona," and "Tsaritsa" varieties, than in other genotypes. As can be seen from **Table 4**, the content of MDA increased by 17.5–25.7% at "Solovushka," "Sara,"

Varieties	Sucrose, mg/g					
	2017		2018			
	September		October		November	
"Kokinskaya rannyaya"	1.32 ± 0.07	3.92 ± 0.24	2.57 ± 0.11	5.94 ± 0.30	2.31 ± 0.11	8.54 ± 0.56
"Solovushka"	1.30 ± 0.06	4.97 ± 0.27	2.73 ± 0.15	10.91 ± 0.65	2.65 ± 0.13	9.95 ± 0.56
"Urozhainaya TzGL"	1.54 ± 0.08	3.85 ± 0.23	3.65 ± 0.15	4.63 ± 0.21	2.23 ± 0.10	7.78 ± 0.45
"Tsaritsa"	1.43 ± 0.05	4.68 ± 0.27	7.05 ± 0.28	15.62 ± 0.70	8.34 ± 0.29	16.56 ± 1.08
"Alba"	1.78 ± 0.09	4.24 ± 0.21	3.41 ± 0.17	6.60 ± 0.38	2.04 ± 0.11	7.35 ± 0.44
"Korona"	1.15 ± 0.05	3.84 ± 0.24	4.16 ± 0.15	7.05 ± 0.28	6.46 ± 0.26	8.90 ± 0.48
"Sara"	0.98 ± 0.05	3.37 ± 0.21	3.50 ± 0.14	6.26 ± 0.36	2.68 ± 0.12	8.00 ± 5.14

Table 3.
The content of sucrose in the leaves of strawberry plants in autumn.

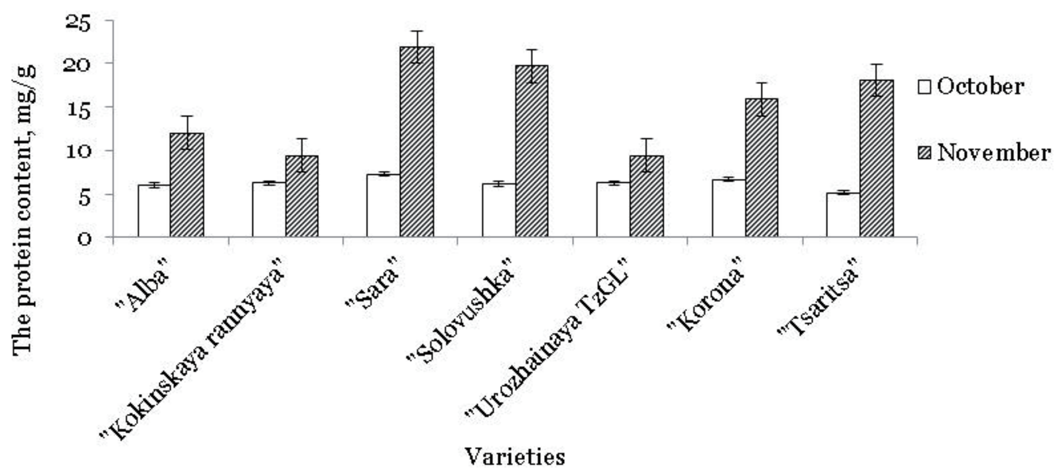

Figure 2.
The content of total protein in the leaves of strawberry plants in autumn.

"Korona," and "Tsaritsa", in November 2017 compared to October, while in other varieties it increased by 31.7–56.3%. At the same time, absolute MDA readings at "Solovushka," "Sara," "Korona," and "Tsaritsa" were lower in November than other cultivars, which indicate about a more significant damage of the structural-functional integrity of cell membranes in other varieties (**Table 4**). Different levels of MDA accumulation in the studied varieties seem to be associated with different degrees of formation of reactive oxygen species in cells and with higher activity of the antioxidant defense system that neutralizes reactive oxygen species (ROS). In this regard, it became necessary to define hydrogen peroxide as one of the representatives of ROS.

Thus, a correlation between MDA and H_2O_2 was shown, when determining hydrogen peroxide. At the same time, the dependence between these indicators changed over the years. So, the correlation coefficient between the amount of MDA and the content of hydrogen peroxide in tissues was, in October 2017, r = 0.67 and, in November, r = 0.79. In 2018, the correlation between the level of H_2O_2 and the intensity of POL in October was 0.55, but in November it increased to 0.98, which is explained by a significant decrease in the last month of autumn air temperature (**Table 1**) and the development of oxidative stress. At the varieties "Solovushka," "Korona," "Tsaritsa," and "Sara", not only the level of accumulation of hydrogen

Varieties	MDA, microMol/g			
	2017		2018	
	October	November	October	November
"Kokinskaya rannyaya"	4.1 ± 0.14	5.4 ± 0.23	10.1 ± 0.56	15.8 ± 0.84
"Solovushka"	3.8 ± 0.28	4.5 ± 0.25	9.8 ± 0.54	10.6 ± 0.48
"Urozhainaya TzGL"	3.9 ± 0.12	5.8 ± 0.17	8.1 ± 0.36	11.9 ± 0.62
"Tsaritsa"	3.7 ± 0.13	4.5 ± 0.18	7.5 ± 0.34	8.8 ± 0.37
"Alba"	4.8 ± 0.17	7.5 ± 0.15	11.4 ± 0.51	21.0 ± 1.30
"Korona"	3.5 ± 0.12	4.4 ± 0.20	8.5 ± 0.37	10.3 ± 0.46
"Sara"	4.0 ± 0.12	4.7 ± 0.20	9.5 ± 0.43	11.2 ± 0.61

Table 4.
The content of MDA in the leaves of strawberry plants in autumn.

peroxide was significantly lower, but also the absolute values of H_2O_2 were reduced in comparison with the other genotypes in October and November (**Table 5**).

Analysis of the antioxidant enzymes activity showed their significant intensity at varieties with low levels of MDA, hydroperoxides, and hydrogen peroxide, with some exceptions for superoxide dismutase (SOD). Thus, in the "Solovushka," "Tsaritsa," "Korona," and "Sara" varieties, the activity of SOD, an enzyme that recycles superoxide with the formation of hydrogen peroxide, did not significantly change in November compared to October during 2 years of research, while in the other genotypes, it increased by 14.6–31.4% (**Table 6**). On the one hand, this explains the different levels of hydrogen peroxide and hydroperoxides in the studied varieties.

In addition to the study of the activity of another antioxidant enzyme, catalase showed its significant intensification in "Solovushka," "Tsaritsa," "Korona," and "Sara" varieties. The activity of hydrogen peroxide scission by catalase at these varieties increased by 37.7–50.5% in November 2017 compared to October, while in the others by 15.7–25.5%. The correlation coefficient between the activity of the enzyme and the level of hydrogen peroxide, which is involved in lipoperoxidation of cell membranes, was r = −0.20 in October and in November r = −0.71. In 2018, the dependence between the amount of H_2O_2 and the enzymes' activity was stronger and was r = −0.84 in October and r = −0.82 in November. At the same time, the varieties "Solovushka," "Tsaritsa," "Korona," and "Sara" were characterized by a large increase in catalase activity in November 2018 (**Table 7**).

The correlation analysis showed the high level of dependence between the physiology-biochemical parameters of strawberries and the minimum air temperature in autumn (**Table 8**). In addition, the significant dependence was between bound/free water ratio, the content of proline (r = 0.98–0.99), and the sucrose (r = 0.72–0.97).

So, the multifaceted study of the physiology-biochemical parameter resistance of strawberry varieties from different ecological and geographical origins was made in autumn period. As a result of this experiment, it was found that the increase of bound water and decrease of free water in leaves were characterized in autumn period for strawberry plants on the background decrease of water content level. Changes in the composition of water fractions depended largely on the accumulation of sucrose and free proline in the leaves of strawberry during the autumn period. A high dependence between the physiology-biochemical parameters and the minimum air temperature was established during the autumn adaptation of strawberries. At the same time, "Solovushka," "Tsaritsa," "Sara," "Korona," varieties

Varieties	Hydrogen peroxide, microMol/g			
	2017		2018	
	October	November	October	November
"Kokinskaya rannyaya"	3.1 ± 0.15	15.7 ± 0.32	3.3 ± 0.18	14.8 ± 0.99
"Solovushka"	1.5 ± 0.05	3.5 ± 0.12	1.8 ± 0.10	4.2 ± 0.24
"Urozhainaya TzGL"	2.2 ± 0.17	7.4 ± 0.30	2.9 ± 0.19	9.5 ± 0.58
"Tsaritsa"	1.6 ± 0.06	2.7 ± 0.09	2.0 ± 0.13	3.6 ± 0.20
"Alba"	2.9 ± 0.15	16.9 ± 0.21	3.5 ± 0.23	21.2 ± 1.42
"Korona"	1.7 ± 0.06	2.9 ± 0.11	2.2 ± 0.14	5.3 ± 0.32
"Sara"	1.9 ± 0.10	3.9 ± 0.12	2.1 ± 0.13	4.6 ± 0.25

Table 5.
The content of hydrogen peroxide (H_2O_2) in the leaves of strawberry plants in autumn.

Varieties	SOD, c.u.			
	2017		2018	
	October	November	October	November
"Kokinskaya rannyaya"	71.0 ± 0.86	83.5 ± 3.21	87.1 ± 5.67	111.2 ± 7.23
"Solovushka"	55.5 ± 1.20	54.2 ± 1.25	65.4 ± 3.60	66.2 ± 3.97
"Urozhainaya TzGL"	69.2 ± 1.91	79.3 ± 1.29	86.7 ± 3.90	106.6 ± 5.12
"Tsaritsa"	53.4 ± 1.87	52.5 ± 1.78	65.5 ± 3.93	67.3 ± 4.03
"Alba"	53.6 ± 1.64	65.5 ± 3.04	74.3 ± 4.09	97.6 ± 5.86
"Sara"	66.9 ± 2.39	63.1 ± 2.06	70.5 ± 3.88	68.4 ± 4.10
"Korona"	52.7 ± 1.84	51.5 ± 1.55	72.3 ± 4.34	77.2 ± 4.86

Table 6.
The SOD activity in the leaves of strawberry plants in autumn.

Varieties	Catalase, ml O_2/min.			
	2017		2018	
	October	November	October	November
"Kokinskaya rannyaya"	9.8 ± 0.36	12.3 ± 0.42	7.5 ± 0.30	8.6 ± 0.34
"Solovushka"	10.5 ± 0.50	15.2 ± 0.36	9.5 ± 0.52	14.5 ± 0.87
"Urozhainaya TzGL"	10.8 ± 0.46	12.5 ± 0.40	9.1 ± 0.45	10.6 ± 0.45
"Tsaritsa"	10.7 ± 0.40	16.1 ± 0.44	12.2 ± 0.72	19.3 ± 0.87
"Alba"	11.7 ± 0.35	14.1 ± 0.47	7.2 ± 0.39	9.1 ± 0.46
"Korona"	11.4 ± 0.44	15.8 ± 0.50	10.3 ± 0.49	14.9 ± 0.77
"Sara"	12.2 ± 0.35	16.8 ± 0.50	10.2 ± 0.53	13.3 ± 0.69

Table 7.
The catalase activity in the leaves of strawberry plants in autumn.

had the highest bound/free water ratio, less damage of the structural and functional integrity of cell membranes, a low level of accumulation of hydrogen peroxide and hydroperoxides, and an increase in the activity of the antioxidant enzyme catalase,

The physiology-biochemical parameters	The correlation coefficient, r
Water content of leaves	0.81
The bound/free water ratio	−0.97
Proline	−0.98
Sucrose	−0.70
MDA	−0.92
H_2O_2	−0.82
Hydrogen peroxide	0.90
The catalase activity	−0.94
The SOD activity	−0.98

Table 8.
The correlation coefficient between the physiology-biochemical parameters of strawberry leaves and the minimum air temperature in the autumn period (min t).

which indicates a greater adaptive ability to low-temperature stresses. The analysis of another antioxidant enzyme—peroxidase—and the increase of cyanidin level were less informative for strawberry plants in forming resistance to temperature drop in the autumn period.

3. Winter hardiness of strawberries in the field

Over the years of research, we analyze the extent of winter damage to varieties of strawberries of different ecological and geographical origins, in the field. The characteristic of the variety for winter hardiness is determined on the basis of spring accounting of the degree of freezing (in points): degree of freezing by plant regrowth (April); in severe winters – by rhizome freezing [24].

The winter 2017–2018 was mild and snowy. Sharp fluctuations in temperature without snow cover were not observed, which had a favorable effect on overwintering. In January, the minimum air temperature decreased to −15.0°C and on the snow surface −12.5°C. The height of the snow cover at the end of January was 13 cm. In February, we observed a decrease in the minimum air temperature to −26.0 and − 18.5°C on the snow surface. The height of the snow cover reached −15 cm.

The winter 2018–2019 was frosty but snowy. Sharp fluctuations in temperature without snow cover are not observed too, which had a favorable impact on the overwintering of strawberry plants. In January, the minimum air temperature decreased to −24.5°C and on the snow surface −21.0°C. At the end of January, the height of the snow cover was 41 cm. In February, we observed a decrease in the minimum air temperature to −11.5 and − 11.5°C on the snow surface. The height of the snow cover reached 35 cm. So, most of the strawberry varieties survived without damage in the winter conditions of 2017–2018 and 2018–2019. The degree of freezing of most samples was 0.0 points, and some varieties had minor damage ("Sonata" and "Marmolada" up to 1.0 points). Also, the estimation of common generation of plants is demonstrative, and it was conducted in late May and early June in points. This characteristic depends largely from the hardiness and shows how the plants overwintered and in what state they enter the fruiting phase. The common condition at the varieties "Kokinskaya rannyaya," "Rosinka," "Solovushka," "Urozhainaya TzGL," "Tsaritsa," and "Sara" at the beginning and at the end of the growing season was excellent (5.0 points). The common condition of plants at the end of vegetation was excellent—80% at the most varieties. So, the weather conditions of the winter period 2017–2019 were favorable for strawberry plants overwintering.

4. Frost resistance at the beginning of the winter without snow cover and reaction on thaws of strawberry plants in winter period

Artificial freezing makes it possible to screen fruit and berry plants to determine the winter hardiness biopotential [25, 26]. In low-snow and snowless winters, the branch crowns of strawberry plants are slightly damaged by negative temperatures, but the strong freezing of the rhizomes is noted on this, as a result of this, the growth and development of plants are delayed. To determine the resistance of varieties of strawberries to early winter frost, we used freezing modes at the end of November (−15°C) and in early December (−20°C).

After exposure of a temperature −15°C, the plants of the varieties "Rosinka" and "Tsaritsa" were without damages. Insignificant damages (no more than 1.0 points) were seen in strawberry varieties "Solovushka," "Korona," and "Sara" on the top of the rhizome, as a very weak browning. The plants quickly recovered and developed well, when they were growing. Average freezing of rhizomes was detected in varieties "Alba" and "Marmolada." The rhizome tissue was light brown. The branch crowns of these varieties grew more slowly, than those of others, but later they developed normally (**Table 9**).

After an exposure of a temperature of −20°C, in early December, insignificant damages were noted at "Solovushka," "Rosinka," and "Tsaritsa" varieties (the degree of damage is not more than 1.0 points, **Figure 3**). At the same time, varieties with reversible damage to the branch crown tissues ("Korona", "Sara") were identified. Average freezing of rhizomes was detected at the varieties "Kokinskaya rannyaya," "Urozhainaya TzGL," "Alba," and "Sonata." The rhizome tissues were light brown. In the plants of these varieties, uneven growth of branch crowns was noted, but later they recovered and developed normally. The rhizomes of "Marmolada" plants are

Varieties	Point of damage to strawberries in the early winter period	
	−15°C	−20°C
"Urozhainaya TzGL" (st)	1.1 ± 0.10	2.5 ± 0.33
"Kokinskaya rannyaya"	0.7 ± 0.27*	2.5 ± 0.44
"Solovushka"	0.2 ± 0.15**	0.8 ± 0.36***
"Rosinka"	0.0 ± 0.00**	0.3 ± 0.12***
"Tsaritsa"	0.0 ± 0.00**	0.8 ± 0.20***
"Alba"	2.6 ± 0.18***	3.0 ± 0.41
"Marmolada"	2.3 ± 0.20*	3.5 ± 0.31**
"Korona"	0.5 ± 0.18	2.0 ± 0.14
"Sara"	0.8 ± 0.18	2.0 ± 0.33
"Sonata"	1.0 ± 0.00	2.3 ± 0.12

*Significantly at a significance level of $p < 0.05$.
**Significantly at a significance level of $p < 0.01$.
***Significantly at a significance level of $p < 0.001$.

Table 9.
The degree of damage to plant varieties strawberry in the beginning of winter (2017–2019).

a b

Figure 3.
The damage of branch crown tissues by a temperature of −20°C in early December, at varieties "Rosinka" (a) up to 0.5 point and "Solovushka" (b) up to 1.0 point.

very frozen. The tissues of the rhizome variety were dark brown. During regrowth, there was an inhibition in the growth of plants that died in the end (**Table 9**).

The high degree of dependence came between the bound/free water ratio and the degree of damage to strawberry plants at the beginning of winter (r = −0.76). At the same time, a moderate association was determined between the degree to plant strawberry damage, the content of free proline (r = −0.34) and sucrose (r = −0.39) in leaves tissues.

To identify the influence of positive temperature on the frost resistance of strawberry varieties, we simulated a 3-day thaw of +5°C followed by freezing at temperatures of −10°C and − 15°C in December, January, and February.

When the temperature decreased to −10°C, after a 3-day thaw +5°C in December, we noted minor damages to the apical buds of brunch crowns no more, than 1.0 point at some varieties ("Solovushka," "Urozhainaya TzGL," "Korona," "Marmolada," "Sara," "Sonata"). The "Alba" variety showed average freezing of rhizome, and its plants grew more slowly. A high regeneration of the apical buds of branch crowns was noted at most varieties, and the plants developed well. In January, after this freezing model, the damage of degree to the apical buds was no more than 1.6 points at the "Solovushka," "Tsaritsa," "Korona," "Sara," and "Sonata" varieties. The plants of their varieties recovered well after freezing. At the same time, the frost resistance was decreased at "Kokinskaya rannyaya," "Rosinka," and "Urozhainaya TzGL" varieties, which have noted average freezing of the rhizome. The "Marmolada" variety had significant freezing, and the rhizomes were brown. Plants of this variety were weakened and developed poorly. In February, when the temperature decreased to −10°C after a 3-day thaw of +5°C, the varieties "Solovushka," "Tsaritsa," "Korona," and "Sara" had reversible damages to the rhizome (the degree of damage was not more than 2.0 points). The average level of frost resistance during this period was kept by the varieties "Solovushka," "Alba," and "Sonata". Strong freezing was detected at the varieties "Kokinskaya rannyaya," "Urozhainaya TzGL," and "Marmolada," in which the branch crown did not grow and the plants died (**Table 10**).

The decrease of temperature to −15°C, after a 3-day thaw +5°C in December, increased freezing at strawberry varieties. The damage of rhizomes and annual branch crowns was not more than 2.0 points at the "Rosinka," "Solovushka," "Tsaritsa," "Korona," and "Sara" varieties. In December, the "Alba" and "Marmolada" varieties froze strongly. In January and February, under the same regime, plants of the "Solovushka," "Tsaritsa," and "Korona" varieties had reversible damage (no more than 2.0 points). Plants have recovered well after freezing. Average frost resistance was noted in the "Sara" variety. Plants of this variety grew slowly. In January and February, the varieties "Kokinskaya rannyaya," "Rosinka," "Urozhainaya TzGL," "Marmolada," and "Sonata" had strong freezing of plants. The plants of this varieties were dead after the further regrowth. In January and February, plants of the "Alba" variety froze strongly and died, after the thaw +5°C and a sharp decrease of temperature to −15°C. As a result, it was shown that the reaction of strawberry varieties to negative temperatures after the thaw increased by the end of the winter period. The most stable frost resistance during the winter thaw was shown by the varieties of "Solovushka," "Tsaritsa," and "Korona" (**Table 11**).

So, the high dependence was noted between the degree of strawberry plants freezing and the bound/free water ratio at the beginning of winter. In the early winter period, varieties of strawberries "Solovushka," "Rosinka," "Tsaritsa," "Korona," and "Sara" were characterized by frost resistance. Also, it was shown that the reaction of strawberry varieties to negative temperatures after the thaw increased towards the end of the winter period. The ability to consistently keep frost resistance during the winter thaw, showed the "Solovushka", "Tsaritsa", "Korona" varieties. As a result of artificial freezing, frost-resistant varieties of strawberries—"Solovushka," "Tsaritsa," and "Korona"—were identified. These results contributed to the development of methodological recommendation for determining the frost resistance of strawberries under controlled conditions.

Varieties	Point of damage to strawberries during the winter thaw		
	December	January	February
	+5, −10°C	+5, −10°C	+5, −10°C
"Urozhainaya TzGL" (st)	1.3 ± 0.25	2.5 ± 0.96	3.8 ± 0.46
"Kokinskaya rannyaya"	1.8 ± 0.18**	2.5 ± 0.96	3.2 ± 0.38
"Solovushka"	0.6 ± 0.24	1.4 ± 0.55*	1.9 ± 0.23***
"Rosinka"	1.8 ± 0.12**	2.4 ± 1.03	2.5 ± 0.20**
"Tsaritsa"	1.3 ± 0.12	1.6 ± 0.63	1.9 ± 0.13***
"Alba"	2.3 ± 0.27***	2.7 ± 0.62	3.0 ± 0.15
"Marmolada"	0.9 ± 0.13	3.3 ± 0.75	3.5 ± 0.29
"Korona"	1.0 ± 0.00	1.3 ± 0.48*	1.9 ± 0.29***
"Sara"	0.8 ± 0.12	0.8 ± 0.48***	2.0 ± 0.35***
"Sonata"	0.8 ± 0.12	1.6 ± 0.63	2.8 ± 0.32*

**Significantly at a significance level of $p < 0.05$.*
***Significantly at a significance level of $p < 0.01$.*
****Significantly at a significance level of $p < 0.001$.*

Table 10.
The degree of damage to strawberry plants during the thaw +5 and following temperature decreases to −10°C (2017–2019).

Varieties	Point of damage to strawberries during the winter thaw		
	December	January	February
	+5, −15°C	+5, −15°C	+5, −15°C
"Urozhainaya TzGL" (st)	3.0 ± 0.21	4.0 ± 0.58	3.5 ± 0.29
"Kokinskaya rannyaya"	2.7 ± 0.12	3.8 ± 0.43	3.3 ± 0.17
"Solovushka"	1.6 ± 0.15**	1.9 ± 0.08***	2.0 ± 0.58***
"Rosinka"	2.0 ± 0.00*	3.5 ± 0.61	3.0 ± 0.50
"Tsaritsa"	1.7 ± 0.12*	2.0 ± 0.21***	2.0 ± 0.29***
"Alba"	3.1 ± 0.24	4.2 ± 0.27	4.3 ± 0.33*
"Marmolada"	3.4 ± 0.22	3.9 ± 0.08	4.0 ± 0.29
"Korona"	1.3 ± 0.12**	2.2 ± 0.20***	1.9 ± 0.17***
"Sara"	2.0 ± 0.10*	2.4 ± 0.08***	2.5 ± 0.17*
"Sonata"	3.0 ± 0.00	3.8 ± 0.25	3.8 ± 0.18

*Significantly at a significance level of $p < 0.05$.
**Significantly at a significance level of $p < 0.01$.
***Significantly at a significance level of $p < 0.001$.

Table 11.
The degree of damage to strawberry plants during the thaw +5 and following temperature decreases to −15°C (2017–2019).

5. Study of the resistance of generative organs of strawberry to spring frosts

During the years of the project, we did not note the decrease in air temperature and on the soil surface (spring frosts) during the flowering period of strawberry varieties of different ecological and geographical origins. So, the assessment of the degree of damage to generative organs was not made in the field. To accelerate evaluation of resistance of generative organs at horticultural crops, the method of artificial freezing was used [12, 27].

Research works about resistance of generative organs of strawberry to spring frosts by artificial freezing are almost nonexistent currently [28]. During the years of research, experiments on artificial freezing allowed us to conclude that the studied varieties of strawberry are highly resistant to temperatures of −1.0 and − 2.0°C, because visible damages to generative organs were not noted (**Figure 4**).

Decrease of temperature to −2.5°C showed different degrees of damage to the generative organs. In 2018, the percent of dead flowers varied from 0.0 to 70.0% at strawberry varieties. In 2019, the presence of dead flowers was between from 0.0 and 54.6%. On average, for 2 years, dispersion analysis showed significant differences between the studied samples on the percent of flowers damage at 5% significance level. In some varieties ("Kokinskaya rannyaya," "Rosinka," "Tsaritsa"), the flowers could withstand freezing at a temperature of −2.5°C without damage. The Holland variety, "Korona", showed a small amount (5.6%) with damaged pistils. At the control variety, "Urozhainaya TzGL", the flowers were damaged to 16.4%. We noted varieties, on which the flowers were damaged from 33.8 to 46.6% - "Solovushka", "Marmolada", "Sara", "Sonata". At a temperature of −2.5°C, the highest number of damaged flowers was shown—56.3% at the Italian variety "Alba" (**Table 12**, **Figure 5**).

During the years of the research, the buds at varieties of strawberry were damaged less than flowers after an exposure to a temperature of −2.5°C. On average, for 2 years, the buds at some varieties ("Kokinskaya rannyaya," "Rosinka," "Tsaritsa") withstood

"Rosinka" "Tsaritsa"

Figure 4.
Alive flowers and buds of varieties of strawberry after exposure to the temperature of −2.0°C.

Varieties	2018	2019	Average value	Angle-arc sine √percent
	% dead flowers			
"Urozhainaya TzGL" (st)	0.0	32.8	16.4	23.9
"Kokinskaya rannyaya"	0.0	0.0	0.0	0.0
"Solovushka"	0.0	0.0	0.0	0.0
"Rosinka"	38.6	54.6	46.6	43.1*
"Tsaritsa"	0.0	0.0	0.0	0.0
"Alba"	70.0	42.6	56.3	48.7*
"Marmolada"	0.0	11.2	5.6	13.7
"Korona"	33.4	34.2	33.8	35.6*
"Sara"	37.1	30.5	33.8	35.6
"Sonata"	34.8	45.3	40.1	39.3*
$LSD_{0.05}$				32.4

*Significantly at a significance level of $p < 0.05$.

Table 12.
The percent of dead flowers of strawberry varieties after exposure to the temperature − 2.5°C, %.

freezing at a temperature of −2.5°C without damage. "Korona," "Sara," and "Sonata" varieties showed a small amount of buds (no more than 10.0%) with damaged pistils. The varieties with damaged buds from 10.5 to 20.1% were noted—"Solovushka," "Urozhainaya TzGL," and "Marmolada". At a temperature of −2.5°C, the highest number of damaged buds was shown—40.8% at the Italian variety "Alba" (**Table 13**).

Exposure to a temperature of −3.0°C increased the damage of flowers in the studied varieties of strawberry. At the same time, the significant intervarietal differences were found in sign of the degree of flower damage at the 5% significance level. A smaller percent of flower damage (6.8%) was noted at "Tsaritsa" variety. It should be noted that the flowers damaged slightly at a temperature of −3.0°C in varieties of "Kokinskaya rannyaya" and "Rosinka"—no more than 18.0%. From the data provided, it can be seen that the flowers were damaged strongly from 53.0 to 73.7% at the varieties "Solovushka," "Urozhainaya TzGL," "Alba," "Korona," "Marmolada," and "Sara." The highest percent of dead flowers after exposure to a temperature of −3.0°C was noted at the Holland variety "Sonata"—84.8% (**Table 14**, **Figure 6**).

a b

Figure 5.
The damaged flowers at "Solovushka" variety (a) and alive flowers at the "Korona" (b) variety after exposure to a temperature of −2.5°C.

Varieties	2018	2019	Average value	Angle-arc sine √percent
	% no dead buds			
"Urozhainaya TzGL" (st)	0.0	21.0	10.5	18.9
"Kokinskaya rannyaya"	0.0	0.0	0.0	0.0*
"Solovushka"	0.0	0.0	0.0	0.0*
"Rosinka"	13.8	22.8	18.3	25.3
"Tsaritsa"	0.0	0.0	0.0	0.0*
"Alba"	61.3	20.3	40.8	39.7
"Marmolada"	0.0	5.8	2.9	9.8
"Korona"	18.0	22.2	20.1	26.6
"Sara"	4.7	8,.5	6.6	14.9
"Sonata"	0.0	14.6	7.3	15.7
$LSD_{0.05}$				20.3

*Significantly at a significance level of $p < 0.05$.

Table 13.
The percent of dead buds of strawberry varieties after exposure to the temperature − 2.5°C, %.

After exposure to a temperature of −3.0°C, the buds were not damaged at "Kokinskaya rannyaya," "Rosinka," and "Tsaritsa" varieties. As we can see from **Table 15**, the buds were damaged in the range from 27.7 to 40.1% at "Solovushka," "Urozhainaya TzGL," "Korona," "Marmolada," and "Sara" varieties. The highest percent of dead buds after exposure to a temperature of −3.0°C was noted at the Italian variety "Alba" (50.4%) and the Holland variety "Sonata"—60.6%. Statistical processing of the results of artificial freezing allowed us to determine significant intervarietal differences in the degree of bud's damage at the 5% significance level (**Table 15**).

The temperature regime of −4.0°C was critical for the flowers of the studied varieties of strawberry, which were damaged from 72.3 to 100.0%. Damage of buds was noted from 43.8 to 69.1%.

Varieties	2018	2019	Average value	Angle-arc sine √percent
	% dead flowers			
"Urozhainaya TzGL" (st)	57.6	59.2	58.4	49.8
"Kokinskaya rannyaya"	0.0	25.0	12.5	20.7*
"Solovushka"	6.3	28.8	17.6	24.8*
"Rosinka"	80.5	66.8	73.7	59.2
"Tsaritsa"	0.0	13.6	6.8	15.1*
"Alba"	79.8	26.8	53.3	46.9
"Marmolada"	63.3	79.7	71.5	57.7
"Korona"	47.0	59.0	53.0	46.9
"Sara"	73.8	40.4	57.1	49.1
"Sonata"	100.0	69.5	84.8	67.0
$LSD_{0.05}$				28.2

*Significantly at a significance level of $p < 0.05$.

Table 14.
The percent of dead flowers of strawberry varieties after exposure to the temperature − 3.0°C, % (2018–2019).

a b

Figure 6.
Alive generative organs at the "Tsaritsa" variety (a) and dead at the "sonata" (b) variety after exposure to the temperature − 3.0°C.

Correlation analysis determined a high dependence of the damage degree of flowers (r = 0.97) and buds of strawberries (r = 0.98) on the intensity of the temperature of spring frosts.

Based on the research results, we offer to share the varieties of strawberries by their resistance to spring frosts into five groups:

1. Highly resistant varieties—the number of damaged flowers and buds after freezing at −3.0°C does not exceed to 25.0% and at −2.5°, C10.0%.

2. Resistant varieties—the number of damaged flowers and buds after freezing at −3.0°C is from 25.0 to 50.0% and at −2.5°C is 25.0%.

3. Medium-resistant varieties—the number of damaged flowers and buds at −3.0°C is from 50.0 to 75.0% and at −2.5°C is from 25.0 to 50.0%.

Varieties	2018	2019	Average value	Angle-arc sine √percent
	% dead buds			
"Urozhainaya TzGL" (st)	34.4	41.4	37.9	38.0
"Kokinskaya rannyaya"	0.0	0.0	0.0	0.0*
"Solovushka"	0.0	0.0	0.0	0.0*
"Rosinka"	37.8	42.4	40.1	39.3
"Tsaritsa"	0.0	0.0	0.0	0.0*
"Alba"	66.7	34.1	50.4	45.2
"Marmolada"	14.6	55.8	35.2	36.4
"Korona"	17.4	55.0	36.2	37.0
"Sara"	34.1	21.3	27.7	31.8
"Sonata"	90.1	33.1	61.6	51.7
$LSD_{0.05}$				32.1

Significantly at a significance level of $p < 0.05$.

Table 15.
The percent of dead buds of strawberry varieties after exposure to the temperature − 3.0°C, % (2018–2019).

4. Weakly resistant varieties—damaged flowers and buds after freezing at −3.0°C are more than 75.0% and at −2.5°C from 50.0 to 70.0%.

5. Unstable varieties—the number of damaged flowers and buds at −3.0°C is 100.0% and at −2.5°C more than 75.0%.

So, according to the results of ranking varieties by groups of resistance to spring frosts, we recommend two regimes. The first temperature regime is −2.5°C, which will allow to make mass rejection of unstable forms. The second regime is −3.0°C; it will make possible to select forms that are sources of high resistance to spring frosts for selection for a given sign. The high potential of resistance to spring frosts was shown by varieties of strawberries—"Kokinskaya rannyaya," "Rosinka," and "Tsaritsa."

6. Net productivity of photosynthesis, respiration intensity, potential productivity, growth rate, and yield strawberry varieties

The main elements of the production process of plants are photosynthesis and respiration. In this regard, a pigment analysis, analysis of the photochemical activity of isolated chloroplasts, the intensity of respiration, and the net productivity of photosynthesis were performed.

The efficiency of photosynthetic activity of plants depends from the development of the pigment system. Studies, which were made in leaves of strawberry plants, showed that the "Alba," "Marmolada," "Korona," and "Kokinskaya rannyaya" varieties did not differ significantly from each other in the content of chlorophyll in the leaves and the amount of green pigment was significantly lower in them than in the varieties of "Sara," "Sonata," "Solovushka," and "Tsaritsa" (**Table 16**). A similar regularity in the content of chlorophyll remained in 2019 (**Table 17**). Two other varieties were added; from them the variety "Rosinka" was referred in the group with a low content of green pigment and "Urozhainaya TzGL" in the group with high content. Higher level of chlorophyll at the "Sara," "Sonata," "Solovushka," "Tsaritsa," and "Urozhainaya TzGL" varieties is associated probably with an increased content of other carotenoid pigments in the photosynthetic apparatus. Thus, the correlation

Varieties	Pigments, mg/g		PhCA, microMol $K_3[Fe(CN)_6]$/(mg chl· h)	NPP, g/(m^2 day)	respiration intensity, ml CO_2/g·h
	Chlorophyll	Carotenoids			
"Kokinskaya rannyaya"	2.10 ± 015	0.18 ± 0.006	16.16 ± 0.45	1.38 ± 0.06	3.00 ± 0.39
"Solovushka"	2.33 ± 0.02	0.19 ± 0.010	11.19 ± 0.42	10.25 ± 0.57	3.22 ± 0.60
"Tsaritsa"	2.36 ± 0.03	0.20 ± 0.010	12.71 ± 0.70	8.53 ± 0.35	3.06 ± 0.67
"Alba"	1.98 ± 0.02	0.16 ± 0.006	6.30 ± 0.58	9.37 ± 0.44	2.85 ± 0.20
"Korona"	2.10 ± 0.10	0.18 ± 0.006	14.27 ± 0.66	0.77 ± 0.09	2.87 ± 0.57
"Marmolada"	2.02 ± 0.04	0.16 ± 0.006	7.77 ± 0.26	2.03 ± 0.24	3.61 ± 0.33
"Sara"	2.52 ± 0.02	0.20 ± 0.006	2.48 ± 0.26	3.65 ± 0.32	3.52 ± 0.44
"Sonata"	2.32 ± 0.01	0.20 ± 0.006	12.60 ± 0.66	7.97 ± 0.35	3.13 ± 0.70
$LSD_{0.05}$	0.19	0.02	1.63	0.90	0.65

Table 16.
Indicators of photosynthetic activity and respiration rate of strawberry plants in 2018.

Varieties	Pigments, mg/g		PhCA, microMol $K_3[Fe(CN)_6]$/(mg chl· h)	NPP, g/(m^2 day)	respiration intensity, ml CO_2/g·h
	Chlorophyll	Carotenoids			
"Urozhainaya TzGL"	2.15 ± 0.03	0.17 ± 0.003	8.65 ± 0.26	3.12 ± 0.36	5.03 ± 0.39
"Kokinskaya rannyaya"	2.12 ± 0.02	0.18 ± 0.002	12.88 ± 0.52	3.34 ± 0.37	5.41 ± 0.24
"Solovushka"	2.35 ± 0.03	0.20 ± 0.003	10.80 ± 0.40	8.90 ± 0.64	6.65 ± 0.26
"Rosinka"	2.25 ± 0.03	0.18 ± 0.003	11.50 ± 0.46	2.47 ± 0.27	4.30 ± 0.29
"Tsaritsa"	2.50 ± 0.02	0.19 ± 0.002	8.50 ± 0.29	9.50 ± 0.40	3.21 ± 0.28
"Alba"	2.20 ± 0.05	0.16 ± 0.003	4.49 ± 0.31	5.78 ± 0.39	6.87 ± 0.50
"Marmolada"	2.20 ± 0.03	0.17 ± 0.003	10.06 ± 1.00	2.88 ± 0.18	7.57 ± 0.33
"Korona"	2.21 ± 0.04	0.15 ± 0.003	3.53 ± 0.33	3.40 ± 0.29	4.60 ± 0.29
"Sara"	2.57 ± 0.06	0.19 ± 0.003	4.28 ± 0.53	2.66 ± 0.14	2.30 ± 0.29
"Sonata"	2.37 ± 0.02	0.19 ± 0.004	11.93 ± 0.54	7.33 ± 0.19	3.83 ± 0.33
$LSD_{0.05}$	0.09	0.01	1.44	0.74	1.02

Table 17.
Indicators of photosynthetic activity and respiration rate of strawberry plants in 2019.

coefficient between chlorophyll and carotenoids was 0.92 in 2018 and in 2019, 0.64. As a rule, carotenoids do not only have a light-absorbing function but also protect chlorophyll from photo-oxidation on the background of high solar insolation [29].

Therefore, the observed decrease of the correlation coefficient between chlorophyll and carotenoids in 2019 can be associated with a lower part of solar insolation, which is indirectly evidenced by cooler daytime conditions of the growing season (**Table 17**). As a result, the level of chlorophyll was less dependent from the antioxidant properties of carotenoids. However, the increased content of carotenoids was noted consistently in the group of varieties with high level of chlorophyll during 2 years of research.

To determine the potential abilities of the photosynthetic apparatus, we used the characteristic of the functional activity of chloroplasts on the level of light reactions [30]. As a result, it was shown that the highest speed of Hill reaction the varieties

"Korona", "Sonata", "Solovushka", "Tsaritsa", "Kokinskaya rannyaya", "Rosinka", and "Urozhainaya TzGL" had. At the same time, it should be noted that the correlation between the amount of green pigment and the speed of energy transfer in photosystems was quite low and had a negative value, during the 2 years of the study (in 2018, r = −0.23, in 2019, r = −0.22). So, at the "Sara" variety, despite high indicators of chlorophyll in the light-collecting complex of the leave, the speed of energy transfer in photosystems was low. At the same time, at the varieties "Urozhainaya TzGL", "Rosinka," and "Kokinskaya rannyaya" was a high speed of light reactions on the background of a low level of green pigment. The lack of connection between the amount of green pigment and the PhCA of isolated chloroplasts can be associated with the degree of strength of the chlorophyll-lipoprotein bond [31, 32]. According to research by Zakarian N.E. et al. [33], it was shown that the photochemical activity of isolated chloroplasts *Solanum tuberosum*, *Chrysanthemum indicum*, Trifolium pretense, Pelargonium, and Hibiscusrosa cninensis sharply decreased by 5.4–13.0 times in the lack of a labile form of chlorophyll compared to control plants where both forms of green pigment were present.

When determining the net productivity of photosynthesis (NPP) (as an integral indicator of photosynthetic activity of plants), it was shown that only the "Alba," "Sonata," "Solovushka," and "Tsaritsa" varieties were characterized by an effective accumulation of plastic equivalents for 2 years of research. At the same time, the "Korona," "Kokinskaya rannyaya," and "Urozhainaya TzGL" varieties had a low level of NPP on the background of a low content of chlorophyll and an increased rate of electron transfer to the light phase of photosynthesis. On the contrary, the Italian variety "Alba," which had a low speed of Hill reaction, was characterized by intensive assimilation of carbon dioxide and was at the level of indicators of the varieties "Sonata," "Solovushka," and "Tsaritsa" in 2018, which had an increased content of chlorophyll and had a high efficiency of NPP of chloroplasts.

In addition of research the features of photosynthetic activity, it was important to establish the effectiveness of the distribution of assimilates, namely, to consider the donor-acceptor relationship between maturing fruits and the leave's apparatus. Indirectly, we can discuss about the outflow of assimilates by studying the ratio of dry mass of forming berries to leaves in dynamics [34]. The analysis of this ratio showed that at the varieties "Alba," "Sonata," "Solovushka," and "Tsaritsa" in 2018, this indicator, on contrast to other varieties, increased most intensively in the direction of forming berries, which could indicate about the more active outflow of plastic substances into the maturing berries (**Figure 7a**). However, in 2019, at the varieties of "Solovushka" and "Sonata," the intensity of the outflow of assimilates into maturing fruits was significantly decreased compared to the first year of the study, and in "Sara," on the contrary, it was increased. Really, at "Solovushka" and "Sonata" varieties, there was a great accumulation of dry substance of leaves. So, the "Solovushka" and "Sonata" varieties increased the dry substance at 1.78–2.69 times, compared to the first decade of June to the second decade of July, and the "Sara" variety did not increase it significantly. In other varieties, the increase in dry leave's biomass in the second decade of July compared to the first decade of June was at 1.15–1.40 times. At the same time, the "Alba," "Korona," and "Tsaritsa" varieties retained an active outflow of plastic equivalents to the ripening berries in 2019.

In addition, the analysis of growth activity, which was evaluated by the speed of accumulation of raw plant substance, showed that the varieties "Alba," "Sonata," "Solovushka," and "Tsaritsa" had differences in this indicator significantly in 2018. The activity of accumulation of raw plant substance varied from 8.68 to 9.76% in these varieties (against the others from 2.41 to 5.43%) (**Figure 7b**). In 2019, as in 2018, the relative stability and high speed of accumulation of raw substance of the whole plant, showed the "Sonata" and "Tsaritsa" varieties. In 2019, the "Alba," "Sara," and "Solovushka" varieties decreased the intensity of accumulation of raw biomass of the whole plant significantly at 2.20–2.65 times. On the contrary, "Kokinskaya rannyaya," "Marmolada," and "Korona" increased it in 2019 compared to 2018.

Determining the respiratory intensity, as one of the main components of the production process in 2018 did not reveal significant differences between the studied varieties. However, in 2019, it was higher temperature conditions of the growing season at night, and strawberry varieties showed significant differences of the respiratory intensity. As a result, in 2019, there was a probability of excessive waste of plastic substances in strawberry plants that were formed as a result of photosynthesis and participate in the production process. So, the "Alba," "Solovushka," "Korona," "Kokinskaya rannyaya," and "Rosinka" varieties were characterized by a more intense breathing. At these varieties, the release of carbon dioxide varied from 5.0 to 7.57 ml of CO_2/g·h, while in other varieties from 2.30 to 4.60 ml of CO_2/g·h.

In the future, it was interesting to determine how the identified photosynthetic features of the studied varieties could affect the production process. Sometimes there could be either no direct dependent between the intensity of photosynthesis and productivity, or there could be a negative correlation between these indicators [35].

The result of the production process is evaluated by the yield or the share of useful product in the total mass of the plant. It is shown that the "Alba," "Sonata," "Solovushka," and "Tsaritsa" varieties had the highest indicators of berry yield on the background of more efficient photosynthetic activity and increased outflow of assimilates in 2018 (**Figure 8**). The varieties of domestic selection—"Tsaritsa" and "Solovushka"—were shown especially to have high yield. However, in 2019, only the "Korona" and "Sonata" produced consistently high yields. In 2019, the varieties "Alba," "Solovushka," and "Korona" decreased significantly the yield of berries at

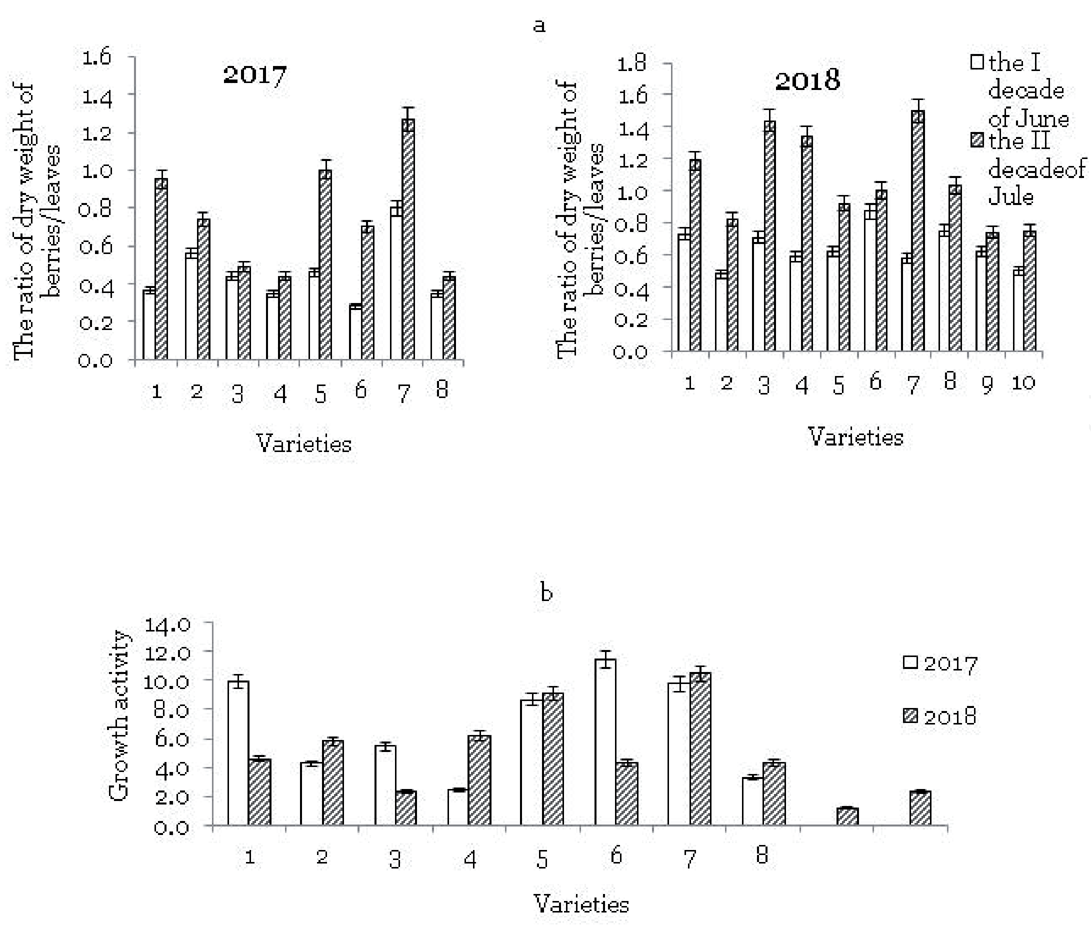

Figure 7.
The ratio of dry weight of berries/leaves (a) and the activity of accumulation of the plant raw biomass (b). Varieties: (1) "Alba," (2) "Marmolada," (3) "Sara," (4) "Korona," (5) "sonata," (6) "Solovushka," (7) "Tsaritsa," (8) "Kokinskaya rannyaya," (9) "Urozhainaya TzGL," and (10) "Rosinka".

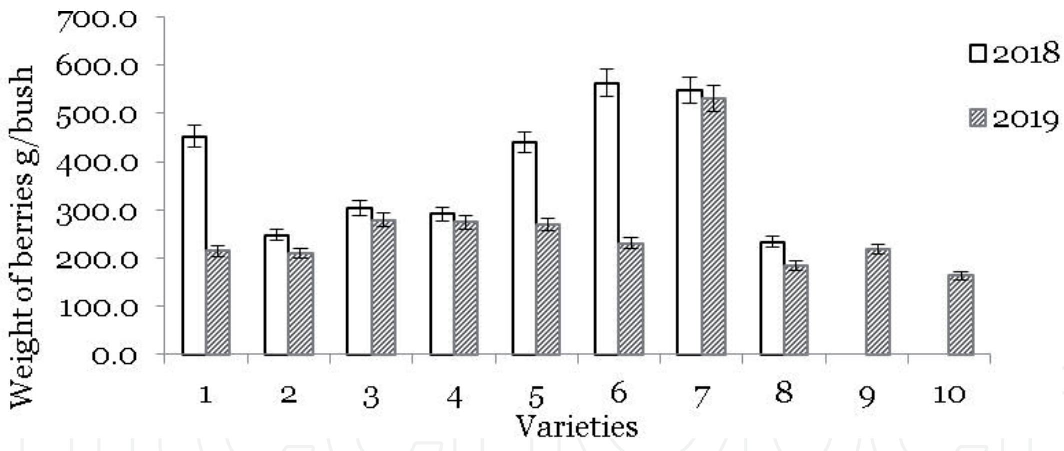

Figure 8.
The yield of the varieties of strawberry. Varieties: (1) "Alba," (2) "Marmolada," (3) "Sara," (4) "Korona," (5) "sonata," (6) "Solovushka," (7) "Tsaritsa," (8) "Kokinskaya rannyaya," (9) "Urozhainaya TzGL," (10) "Rosinka."

1.64–2.40 times as a result of both increased respiratory intensity and violations of donor-acceptor relations. The other varieties—"Marmolada," "Sara," "Kokinskaya rannyaya," "Urozhainaya TzGL," and "Rosinka"—had a low yield index to compare with "Tsaritsa" [18].

So, the research conducted during 2 years showed that only the one variety of strawberry—"Tsaritsa"—in the conditions of the middle zone of Russia had an effective work on the photosynthetic apparatus and increased outflow and accumulation of assimilates in the maturing fruit, which, as a result, affected its high yield. Other varieties showed or not stable yield over the years, such as "Solovushka," "Alba," and "Sonata" or low yield as in "Marmolada," "Sara," "Korona," "Kokinskaya rannyaya," "Urozhainaya TzGL," and "Rosinka." In these cultivars, the reasons of low yield were increased respiratory intensity or insufficient efficiency of the photosynthetic apparatus and the violations of donor-acceptor relations.

At the same time, we found a very high degree of dependence between the actual yield of strawberry varieties and the net productivity of photosynthesis ($r = 0.88$). At the same time, a moderate dependence was found between the degree of freezing of strawberry plants in winter and the actual yield on the one hand ($r = -0.47$) and between the net productivity of photosynthesis ($r = -0.36$) on the other. The calculation of the triple correlation coefficient between the actual yield, the net productivity of photosynthesis, and the degree of freezing of plants in winter showed a very high dependence ($r = 0.89$) between the studied indicators.

7. Conclusion

We had studies of physiological and biochemical processes before and after autumn hardening of strawberry varieties. We studied varieties of strawberries, based on physiological and biochemical changes, that characterized the state of plants after exposure to low positive and negative temperatures at the beginning of the winter period. We evaluated the damage of the rhizome tissues and the branch crowns of strawberry varieties of different ecological and geographical origin in the early winter period and during thaws at the winter after artificial freezing and in the field. We evaluated the damages of generative organs by method of artificial freezing during the flowering period of strawberry, and we offered a ranking for groups of resistance to spring frosts. We conducted comparative physiological studies of the photosynthetic apparatus of different varieties of strawberries of different ecological and geographical origin by productivity.

As a result of the research, it was found that in the autumn and early winter, the increase in bound water and the decrease in free water in the leaves were characteristics for strawberry plants on the background of a decrease at the level of hydration. The change in the composition of water fractions was dependent more on the accumulation of sucrose and free proline in the leaves of plants during the autumn period. At the same time, "Solovushka," "Tsaritsa," "Sara," and "Korona" had the highest bound water/free ratio by the end of autumn and beginning of winter, less damage in the structural and functional integrity of cell membranes, a low level of accumulation of hydrogen peroxide, and an intensification of the activity of the antioxidant enzyme catalase. All this indicates that these varieties are characterized by high adaptive ability in the climatic conditions of Central Russia.

As a result of artificial freezing, it was noted that the temperature decrease to −15°C at the end of November did not cause of irreversible damage at strawberry varieties. The decrease of temperature in early December to −20°C increased the damage at the studied varieties of strawberries. During the winter, it was noted that the reaction of strawberries was increasing to the thaw by the end of the winter period, which was associated with the resumption of growth processes after the influence of positive temperatures. As a result of the research, frost-resistant varieties were identified; these were "Solovushka," "Tsaritsa" (Russia), and "Korona" (Holland).

As a result of the damaging factors of the spring period, high ability of resistance to spring frost, showed the varieties of strawberry—"Kokinskaya rannyaya," "Rosinka," and "Tsaritsa," According to the results of varieties ranking by groups of resistance to spring frosts, we can recommend two regimes. The first temperature regime is −2.5°C, which will allow mass rejection of unstable forms. The second regime −3.0°C makes it possible to select forms that are sources of high resistance to spring frosts for selection for a given sign.

Researches on determine the net productivity of photosynthesis, respiration intensity, potential productivity, growth rates and yield of strawberry varieties of different ecological and geographical origin showed that the "Tsaritsa" variety in the Central region of Russia had an effective work of the photosynthetic apparatus and the outflow of assimilates into the maturing fruit, which, as a result, affected its high yield. On the results of identifying the characteristics of functional connections between physiological and biochemical processes of resistance to the action of a low-temperature environment factors and productivity of strawberry varieties of different ecological-geographical origin, we identified a variety of domestic breeding "Tsaritsa" for creation of resistance berries' agrobiocenosis.

Author details

Zoya Evgenievna Ozherelieva*, Pavel Sergeevich Prudnikov, Diana Aleksandrovna Krivushina, Marina Ivanovna Zubkova and Anna Androsova
Russian Research Institute of Fruit Crop Breeding, Orel, Russia

*Address all correspondence to: ozherelieva@vniispk.ru

References

[1] Aaby K, Ekeberg D, Skrede G. Characterization of phenolic compounds in strawberry fruits by different HPLC detectors and contribution of individual compounds to total antioxidant capacity. Journal of Agricultural and Food Chemistry. 2007;**55**:4395-4406

[2] Aitzhanova SD. Selection of strawberries in the South-Western part of the non-chernozem zone of Russia [abstract PhD dissertation in agricultural sciences]. Bryansk; 2002

[3] Eremin GV, Efimova IL. Rootstocks of Seed and Stone Crops for Modern Intensive Industrial Technologies. Developments, that Form the Modern Image of Gardening. Krasnodar: GNU SKZNIISiV; 2011. pp. 118-139

[4] Mukhanin IV, Zhbanova O. Modern assortment of strawberry for industrial cultivation. Russian school of horticulture. Strawberry. 2015;**1**:19-24

[5] Efimova IL, Shaforostova NK, Kuznetsova AP. Adaptive and productive potential of rootstocks of fruit crops in the conditions of southern gardening. Fruit and Berry Growing in Russia. 2008;**XVIII**:135-141

[6] Krasova NG, Ozhereleva ZE, Golyshkina LV, Makarkina MA, Galasheva AM. Winter Hardiness of Apple Varieties. Orel: VNIISPK; 2014. p. 184

[7] Abyzov VV. Biological features and economic value of strawberry varieties in the conditions of Central Russia [dissertation]. Michurinsk; 2008

[8] Govorov VN. Assessment of the Stability of New Varieties and Hybrids of Strawberries to the Main Fungal Diseases and Pests in the Central Zone of the Krasnodar Territory. Krasnodar; 2011. p. 22

[9] Govorova GF, Govorov DN. Strawberry: Past, Present, Future. Moscow; 2004. p. 347

[10] Shokayeva DB. Biological Bases and Regularities of Strawberry Fruiting. Orel: Kartush; 2006. p. 183

[11] Stolnikova NP. Culture of Strawberry in Western Siberia. GNU NIISS. Barnaul: IP Kolmogorov I.A; 2014. p. 182

[12] Ozhereleva ZE, Zubkova MI, Krivushina DA. Accelerated evaluation of resistance to spring frosts of strawberry varieties. Fruit and Berry Growing in Russia. 2018;**54**:123-126. DOI: 10.31676/2073-4948-2018-54-123-126

[13] Makarova NV, Stryukova AD, Antipenko MI. Comparative analysis of the chemical composition and antioxidant activity of repair and non-repair varieties of strawberry. Storage and Processing of Agricultural Raw Materials. 2014;**9**:45-48

[14] Tyurina MM, Gogoleva GA, Goloulina LK, Morozova NG, Echedi YY, Volkov FA, et al. Determination of Stability of Fruit and Berry Crops to Cold Season Stressors in Field and Controlled Conditions. Moscow; 2002. p. 120

[15] Yakushkina NI, Bakhtenko EY. Physiology of Plants. Moscow: Vlados; 2004. p. 464

[16] Matala V. The Cultivation of Strawberries. Saint Petersburg; 2003. p. 202

[17] Ozherelieva ZE, Prudnikov PS, Bogomolova NI. Frost hardiness of introduced *Hippophae rhamnoides* L. genotypes in conditions of Central Russia. Proceedings of the Latvian Academy of Sciences, Section B: Natural, Exact and Applied Sciences. 2016;**70.2**(701):88-95. DOI: 10.1515/prolas-2016-0014

[18] Prudnikov PS, Ozhereleva ZE, Krivushina DA, Zubkova MI. The formation of resistance to hyperthermia of FRAGARIA x ANANASSA DUCH varieties of different ecological and geographical origins in the autumn period. Vegetables of Russia. 2019;**6**:80-83

[19] Dzhavadian N, Karimzade G, Mafuzi S, Ganati F. Changes in the activity of enzymes and the content of proline, carbohydrates and chlorophyll in wheat caused by cold. Physiology of Plants. 2010;**57**(4):580-588

[20] Prudnikov SP, Gulyaeva AA. Features of hyperthermia effect on the hormonal system and antioxidant status of *Prunus armeniaca* L. Breeding and Selection Horticultural Crops. 2015;**3**:151-154

[21] Prudnikov PS, Krivushina DA, Gulyaeva AA. Components of the antioxidant system and intensity of POL in *Prunus cerasus* L. under the action of hyperthermia and drought. Breeding and Selection Horticultural Crops. 2016;**3**:116-119

[22] Luo Y, Tang H, Zhang Y. Production of reactive oxygen species and antioxidant metabolism about strawberry leaves to low temperatures. The Journal of Agricultural Science. 2011;**3**:89-96

[23] Chudinova LA, Orlova NV. Physiology of Plant Resistance: A Textbook for a Special Course. Perm: Perm University; 2006. p. 124

[24] Shokayeva D.B., Zubov A.A. Strawberry/ program and method of variety study of fruit, berry and nut crops. by E.N. Sedova, T.P. Ogoltsova. Orel: VNIISPK; 1999. pp. 417-443

[25] Lindén L, Palonen P, Hytönen T. Evalution of three methods to assess winter-hardiness of strawberry genotypes. Acta Horticulturae. 2002;**567**:325-328. DOI: 10.17660/ActaHortic.2002.567.69

[26] Ozhereleva ZE, Prudnikov PS, Zubkova MI, Krivushina DA, Knyazev SD. Determination of Frost Resistance of Strawberries under Controlled Conditions (Methodological Recommendations). Orel: VNIISPK; 2019. p. 25

[27] Ozhereleva ZE, Zubkova MI, Krivushina DA. Assessment of the resistance of strawberries to spring frosts. Problems of Agricultural Development in the Region. 2019;**4**(40):113-118

[28] Leonchenko VG, Evseeva RP, Zhbanova EV, Cherenkova TA. Previous Selection of Prospective Genotypes of Fruit Plants for Ecological Stability and Biochemical Value of Fruits (Methodological Recommendations). Michurinsk; 2007. p. 72

[29] Ladygin VG, Shirshikova G. Modern concepts of the functional role of carotenoids in eukaryotic chloroplasts. Journal of General Biology. 2006;**3**:163-190

[30] Gavrilenko VF, Zhigalova T. Big Workshop on Photosynthesis. Moscow: Akademiya; 2003. p. 256

[31] Sudina EG. State of Chlorophyll and its Photochemical Activity. I Union Biochemical Congress. Publishing House of the USSR Academy of Sciences; 1963. p. II

[32] Sudina EG, Lazovaya GI. The significance of the molecular organization of pigment-containing complexes for functional activity. In: Materials of the II Union Symposium on the Application of Electron Microscopy in Botanical Research. Kiev; 1967. pp. 11-114

[33] Zakarian NE, Tsovyan Zh V, Virabyan AR. About the significance

of various forms of chlorophyll in the process of photosynthesis. Scientific notes of Yerevan state university. Natural science. Biology. 1987;**2**:137-142

[34] Bobodzhanova MD. Photosynthesis, donor-scceptor relations and productivity of medium-fiber cotton (*Gossypium hirsutum* L.) [PhD dissertation in agricultural sciences]. Dushanbe; 2007

[35] Abdulaev Kh A, Giiasidinov BB, Solieva BA, Mirakilov Kh M. Paradox: photosynthesis of cotton, as a conservative feature, has not changed significantly for a hundred years of selection of new varieties. In: Materials of the Russian Scientific Conference with International Participation Plants in the Conditions of Global and Local Natural-Climatic and Anthropogenic Impacts. Petrozavodsk; 2015. p. 19