

Tolerance of gooseberry varieties to maximally low temperature in the middle of winter

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Abstract

Ozherelieva, Z. E., Prudnikov, P. S., Kurashev, O. V., & Krivushina, D. A. (2018). Tolerance of gooseberry varieties to maximally low temperature in the middle of winter. *Bulgarian Journal of Agricultural Science, 24(6)*, 1053–1058

The results of study of gooseberry varieties adaptation to negative temperatures are given in this paper. The ability of gooseberry varieties to pass the acclimatization quickly in autumn has been revealed. Owing to this ability the plants entered the dormancy period in proper time. The analysis of the fractional water composition in different periods of hardening is given. The increase of bound water and reduce of available water was noted in the tissues of one-year shoots in autumn-winter. During the successful passing the phases of hardening we noted the increase of protective sugars in the phloem of annual shoots. The potential of the resistance to low temperatures was evaluated that allowed revealing reliable varietal differences in the frost hardiness of buds and phloem of annual shoots. On the basis of the study the new gooseberry varieties have been referred to a group of frost hardy varieties.

Keywords: gooseberry; varieties; artificial freezing; adaptation; maximal frost hardiness

Introduction

Gooseberry is a valuable berry crop that is characterized by precocity, productivity and good berry transportability. The advantage of gooseberry is that its fruits can be used in a different degree of ripening. Gooseberry fruits contain sugars – 10-12%, organic acids – 1-2%, vitamin C – about 30 mg%, carotene – 0.2 mg%, vitamin E – 0.6 mg%, vitamin B9 – 5 mg%, potassium – 260 mg%, iron – 1.6 mg%, cellulose – about 2%, pectin – up to 1%. The chemical composition of gooseberry berries makes them useful not only as a dietary product of food but also for medicinal aims (Sergeyeva, 1989).

One of the limiting factors determining the tolerance of gooseberry is the ability to stand maximally low temperatures in the middle of winter. Frost hardiness is an important trait since the varieties have a different speed of preparing to the effect of negative temperatures and resistance. Gooseberry is characterized as a relatively frost hardy crop. Hardening is rather important for the formation of frost har-

diness features in gooseberry plants. Hardening is taking two stages (Tumanov, 1979). By a deep dormancy period in gooseberry plants a large number of protective high molecular compounds are formed (sugars, unsaturated fatty acids, tanning and lipid agents) and the content of bound water increases that is evident of deep reconstructions of metabolism (Kirtbaya and Sheglov, 2002). The second stage of hardening goes under small negative temperatures $-4\pm 1^{\circ}\text{C}$. During this period the frost hardiness typical for the genotypes is gained. The significant increase of frost hardiness is noted in the middle of the winter as far as cold becomes stronger.

The aim of these investigations was to study new gooseberry varieties to estimate the potential of the maximal frost hardiness.

Materials and Methods

The study was conducted in 2014-2015 in the laboratory of fruit plant resistance physiology at the Russian Research

Table 1. Maximal and minimal temperatures of the autumn-winter periods of studied years

Temp (°C)	2013			2014						2015					
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Oct.	Nov.	Dec.
Max	16.5	13.5	2.5	7.8	4.0	19.0	20.8	11.5	5.2	4.0	4.5	14.5	23.7	12.0	10.5
Min	-8.0	-7.5	-19.3	-31.0	-31.0	-10.6	-15.2	-19.8	-23.3	-24.5	-20.4	-11.2	-10.0	-16.0	-12.0

Institute of Fruit Crop Breeding (VNIISPК), Orel, Russia. The climate of the Orel region is temperately continental and characterized by the irregular distribution of temperatures during the seasons. The absolute minimum of air temperature is $-39\pm 1^\circ\text{C}$. Maximal and minimal temperatures of the autumn-winter periods of studied years are given in Table 1.

The fractional composition of water in annual gooseberry shoots was studied by Okuntzeva-Marinchik method (Baslavskaya and Trubetzkova, 1991). This method is based on the changes of the concentration of the saccharose solution when the tissues are dipped into it. The tissues of the annual shoots (0.4 g weight) were dipped into the 30% saccharose solution. A part of the water passed from the tissue to the solution reducing its concentration. As a matter of the initial volume of the solution, its first and final concentration, the quantity of the water taken from the tissue by the solution was determined. The content of the bound water was calculated taking into account the difference between the content of the total water and the water passed to the solution. Saccharose concentration in the solution was determined on the digital refractometer PAL-1 "Atago" (Japan).

At the same time the contents of sugars were determined in the bark of the annual shoots by Turkina and Sokolova method (1972). The weighted material 0.5 g was grinded in 10 ml of the ethyl alcohol heated to 80°C and put into test-tubes with the following heating on the boiling water bath during 10 minutes. The contents of the tubes were centrifuged during 10 minutes at 7000 revolutions per minute. 50 μl of 5N NaOH was added to 0.5 ml of supernatant and heated during 10 minutes on the water bath. 0.5 ml of resorcinol reagent (100 mg of resorcinol + 250 mg of thiourea in 100 ml of acetic acid) and 3.5 ml of 30% HCl were added after cooling. The tubes were heated on the water bath during 10 minutes. After cooling the optic density was determined on the spectrophotometer at 520 nm. The content value was calculated by means of the calibrated curve built for pure saccharose.

New gooseberry varieties from the VNIISPК breeding program were studied. The plant age was 8 years. Three typical bushes from each variety were taken as samples. Annual shoots of 20 cm length were used. The shoots were cut from different sides of the bushes (5 shoots per bush). The material was taken late in November when the average daily tem-

perature of the air was below 0°C . The shoots were stored in plastic bags in the freeze chamber under $-4\pm 1^\circ\text{C}$. The artificial freezing was performed in the climatic chamber "Espec" PSL-2KPH (Japan) by methodology of Turina et al. (2002).

To determine the resistance to maximally low temperature the shoots of gooseberry varieties were kept exposed to -38°C frost (during 8 hours). The freezing of Component II was done on the 15th of January. The speed of temperature lowering was gradual -5°C per hour. The damages were estimated by a method of shoot growing. One-year shoots were cut by 2-3 cm after freezing and put into vessels with water at $+21\pm 1^\circ\text{C}$. The water in the vessels was changed each 2 days. The shoots were grown during 10 days.

The degree of bud damage was determined according to the following scale: 0 – no damages; 1 – insignificant damages: parenchyma under buds was damaged; 2 – reversible damages: a part of leaf sprouts was damaged; 3 – an average point of damages: vascular system and a larger part of leaf sprouts were damaged; 4 – severe damages: apical meristems and a majority of leaf sprouts were lost; 5 – all bud tissues were lost.

The degree of bark and wood damages was determined taking into account the tissue cell growing brown on slits and cross-sections according to the following scale: 0 – no damages: tissues remained light-colored; 1 – insignificant damages: 10-20% of tissues grew brown; 2 – reversible damages: 20-40% of tissues grew brown; 3 – an average point of damages: 40-60% of tissues grew brown; 4 – severe damages: 60-80% of tissues grew brown; 5 – more than 80% of tissues was lost. The estimation of bud and tissue damages was performed by means of the binocular microscope MBC-2. The regionalized variety 'Smena' was used as a control. The experiment data were processed by means of mathematical statistics (Dospikhov, 1985) with using MS Excel. In this paper the results of studying maximal frost hardiness in the middle of winter are under consideration.

Results

The frost hardiness of gooseberry varieties was reached during the process of hardening in autumn. In October the water content of one-year shoots varied from 51.3-60.0%. The analysis of the fractional composition of water in gooseberry

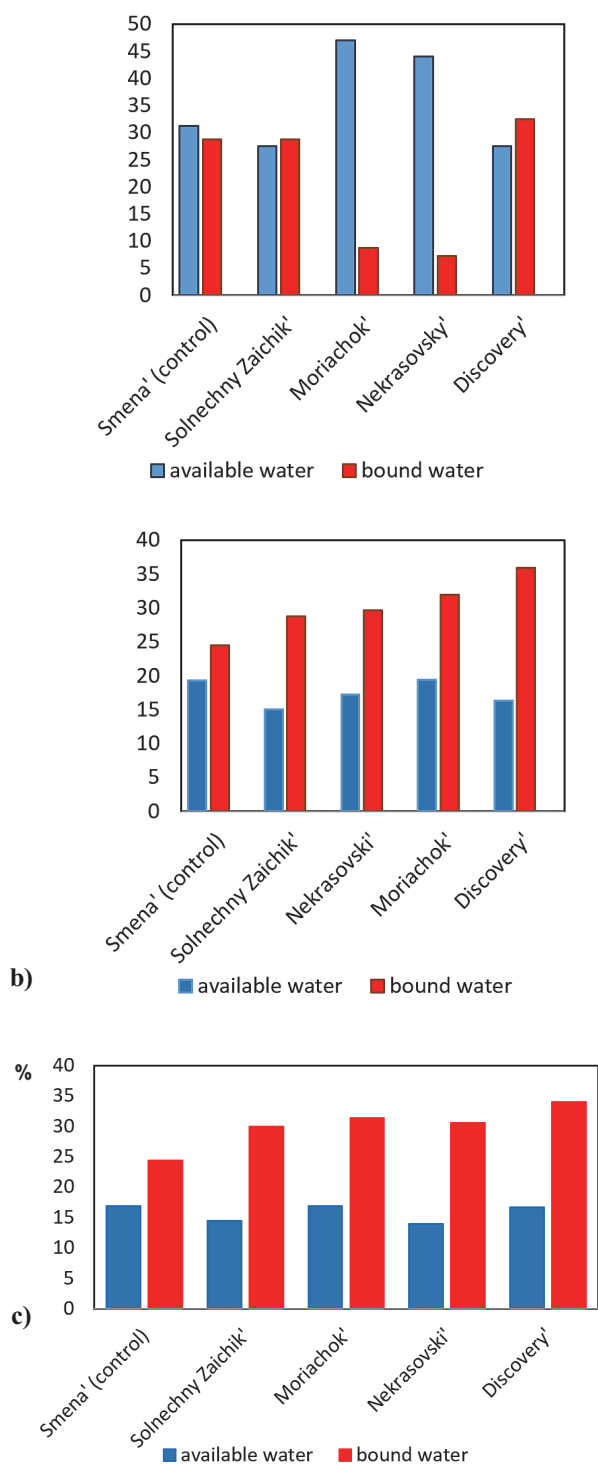


Fig. 1. Fractional water composition of gooseberry shoots in October (a), November (b) and December (c), %

shoots showed that during that period the available water predominated from 21.3-37.0% (Fig. 1a). At low temperatures in late November the level of water content in shoots declined in all gooseberry varieties from 43.8-52.2%. At the same time the quantity of bound water increased in one-year shoots to 24.5-35.9% while the content of available water declined to 15.0-19.4% (Fig. 1b). In December the further declining of the water content level was observed in one-year gooseberry shoots from 41.3-50.7%. At the same time the quantity of bound water varied within the former limits from 24.4-34.0% (Fig. 1c). The two-factor dispersion analysis revealed the reliable intervarietal difference ($F_{\text{fact}} = 5.1 > F_{\text{theor}0.01} = 4.9$) and reliable difference in the quantity of bound water in one-year gooseberry shoots during the autumn-winter period ($F_{\text{fact}} = 10.3 > F_{\text{theor}0.01} = 6.5$) as well as reliable interaction of those two factors ($F_{\text{fact}} = 4.5 > F_{\text{theor}0.01} = 4.0$).

In October the increase of the sum of sugars was noted from 46.7 to 117.7 mg/g; in November – from 197.2 to 185.6 mg/g and in December – from 165.7 to 264.0 mg/g (Fig. 2). The correlation analysis of studied traits determined the dependence between the content of bound water and protective sugars in tissues of one-year shoots ($r = 0.64$).

The two-factor dispersion analysis reliably revealed the intervarietal difference ($F_{\text{fact}} = 218.1 > F_{\text{theor}0.01} = 3.8$) and differences in sugar contents in the phloem of one-year gooseberry shoots during the autumn-winter period ($p < 0.01$), as well as the reliable interaction of these two factors ($F_{\text{fact}} = 83.8 > F_{\text{theor}0.01} = 4.1$).

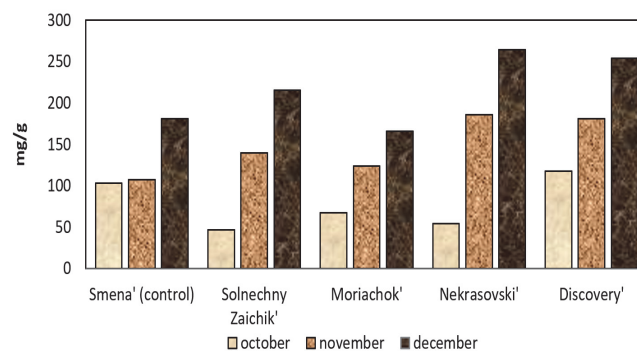


Fig. 2. Sugar sum content in the phloem of one-year gooseberry shoots, mg/g

In 2014/2015 the studied gooseberry varieties were characterized by winter hardiness in the field conditions. The one-factor dispersion analysis showed the reliable intervarietal differences in damages of one-year shoots in the field at $p < 0.05$ (Table 2).

Table 2. Winter hardiness estimation of gooseberry varieties in the field, 2014-2015

Varieties	Years of studies		Average value
	2014	2015	
Smena (control)	1.0	1.0	1.0 ± 0.4
Solnechny Zaichik	2.0	1.0	1.5 ± 0.3
Moriachok	0.0	0.0	0.0 ± 0.0
Discovery	0.0	0.0	0.0 ± 0.0
Nekrasovski	1.0	0.0	0.5 ± 0.2
LSD ₀₅			0.5

During winter (2014/2015) the air temperature was not lower than critical $-39 \pm 1^\circ\text{C}$. In connection with that the artificial freezing of one-year gooseberry shoots was performed to estimate their maximal frost hardiness. The significant variation in bud damages was noted – $V = 48.3\%$. Owing to the successful passing of hardening phases the temperature

Table 3. Damage degree of gooseberry varieties in early January at -38°C , 2014-2015

Varieties	Average point of damage		
	phloem	xylem	buds
Smena (control)	0.2 ± 0.1	0.0 ± 0.0	0.6 ± 0.2
Solnechny Zaichik	0.3 ± 0.1	0.4 ± 0.1	0.8 ± 0.2
Moriachok	0.8 ± 0.1	0.6 ± 0.2	2.0 ± 0.2
Discovery	0.6 ± 0.1	0.4 ± 0.1	1.5 ± 0.2
Nekrasovski	0.8 ± 0.4	0.5 ± 0.2	1.7 ± 0.4
Average	0.5 ± 0.2	0.4 ± 0.2	1.3 ± 0.2
V, %	13.1	16.7	48.3
LSD ₀₅	0.5	$F_{\text{fact}} < F_{\text{theor}}$	0.7

**Fig. 3. Gooseberry variety 'Moriachok'****Fig. 4. Gooseberry variety 'Nekrasovski'****Fig. 5. Gooseberry variety 'Discovery'**

lowering up to -38°C in the laboratory conditions in early January did not cause the significant damage of vegetative buds (up to 1.0 point) in 'Solnechny Zaichik' and 'Smena' (control). The vegetative buds of 'Moriachok' (Fig. 3), 'Discovery' (Fig. 5) and 'Nekrasovski' (Fig. 4) were damaged up to 2.0 point (Table 3).

Gooseberry phloem and xylem in hardened conditions demonstrated more stable frost hardiness when the variation coefficients were 13.1 and 16.7%, respectively. In all varieties the damages of phloem from 0.2 to 0.8 and xylem from 0.0 to 0.6 points were noted (Table 3). The one-factor dispersion analysis determined the reliable intervarietal differences in bud and phloem damages at $p < 0.05$. In xylem damages the intervarietal differences were insignificant.

Discussions

One of the limiting factors determining the resistance of gooseberry plants is their ability to withstand maximal low temperatures in the middle of winter. Winter hardy gooseberry cultivars suitable for the cultivation in different climates of Russia have been allocated as a result of studies in Russian scientific institutions (Yeriomina, 2003; Il'in, 2007; Petrusha, 2007; Pupkova, 2007). The foreign reports on studies of gooseberry frost resistance and winter hardiness are infrequent at present. Principally, these reports are devoted to the study of the biochemical composition, fruit quality and resistance to pests and diseases (Pluta et al., 2010). Along with this, Latvian breeders have analyzed the above-mentioned traits as well as winter hardiness of domestic gooseberry genotypes (Kampuss et al., 2010). Gooseberry is a relatively frost-resistant crop. Hardening is an important condition for the formation of the properties of its frost resistance (Tumanov, 1979). By the period of the deep dormancy, a great number of high-molecular compounds having protective effect (sugar, unsaturated fatty acids, tannins and lipoids) are formed in gooseberry plants and the content of bound water increases (Ozherelieva and Kurashev, 2014; Ozherelieva et al., 2016) and these indicate a profound restructuring of metabolism (Kirtbaya and Sheglov, 2002). By early winter, the gooseberry plants acquire frost resistance to early frosts up to -25°C (winter hardiness component I), which is determined by the hereditary potential and possibilities of its implementation in specific weather conditions, the nature and speed of the preparatory processes (autumn hardening) to overwintering (Turina, 1993). In this case, frost resistance increases significantly in the middle of winter (winter hardiness component II) as the cold increases. The potential of the maximal winter hardiness is determined by temperatures -38 – -40°C (Turina, 1993). In early winter the increase of bound water quantity in the cells of one-year shoots is connected with the change of the conformation of hydrophilous substances that provide the increase of water that is difficult of access (Kushnirenko, 1975). The frost hardiness of gooseberry varieties was reached during the process of hardening in autumn. The analysis of the fractional composition of water in gooseberry shoots showed that during low temperatures in late November the level of water content in shoots declined in all gooseberry varieties. At the same time the quantity of bound water increased in one-year shoots while the content of available water declined. In December the further declining of the water content level was observed in one-year gooseberry shoots. At the same time the quantity of bound water varied within the former limits. It was connected with the partial dehydration of tissue cells at the cost

of which the protoplasm assumed more resistant condition (Koshkin, 2010). At the same time the declination of metabolism processes, change of protoplasm penetrability for water and increase of sum of sugars happen. The sugar accumulation in plants happens during the first stage of hardening from the moment of growth stoppage. We have determined that during the phases of hardening sugars are accumulated in the phloem of one-year shoots of gooseberry. Sugars serve as an energetic material and basic substance that protects protoplasm against destroying by frost. Sugars increase the water retaining ability of protoplasm colloids protecting icing and excessive dehydration (Koshkin, 2010).

Gooseberry varieties were characterized by winter hardiness in the field conditions as during winter the air temperature was not lower than critical $-39\pm 1^{\circ}\text{C}$. In connection with that the artificial freezing of one-year gooseberry shoots was performed to estimate their maximal frost hardiness. The significant variation in bud damages was noted ($V = 48.3\%$). It is explained by the fact that the frost hardiness of buds is a variable quantity that first of all depends on the weather conditions both during hardening phases and winter periods. Gooseberry phloem and xylem in hardened conditions demonstrated more stable frost hardiness.

Conclusions

As a result of the contributed during the autumn period there was an increase of bound water and decrease of available water in tissues of one-year gooseberry shoots. A rise in protective sugars was noted in the phloem of one-year shoots. At the same time the direct relationship was determined between the increase of bound water and protective sugars. The dynamics of the water content of one-year shoots in autumn and early winter was shown. The favorable conditions of passing hardening phases contributed to the acquisition of maximal frost hardiness by gooseberry varieties which they displayed in the middle of winter.

Acknowledgments

The study was supported by Federal Agency Scientific Organizations program for support the bio resource collections.

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Received: February 14, 2018; Accepted: April 12, 2018; Published: December 31, 2018