Resistance of winter soft wheat varieties to low negative temperatures

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Abstract. The problem of resistance of winter wheat to low negative temperatures is one of the most important in the agriculture economy, the solution of which will contribute to the increase of yield and gross grain harvesting in the Russian Federation. Therefore, the creation of winter wheat varieties capable of resisting the effects of frost during winter is very relevant. The aim of the study is to identify high frost-resistant winter wheat varieties for use in the breeding process by direct and indirect methods of assessment, and to define the possibility of using free proline content to detect genotypes resistant to low negative temperatures. The paper presents the results of complex studies on frost resistance of winter soft wheat varieties by three scientific centers: Agricultural Scientific Center Donskoi, National Grain Center named after P.P. Lukyanenko and Federal Rostov Agrarian Scientific Center. Therein is shown the possibility of using an indirect method - defining free proline content as a method of assessing frost resistance of samples. After freezing plants grown in sowing boxes at four different temperatures, varieties were identified that significantly exceeded the Tarasovskaya 29 classifier in plant safety: Polina, Volnitsa, Volny Don, Ascetic, Don Bezostaya, and Don 107. Samples that were distinguished by the safety of plants in direct evaluation methods also showed a high content of free proline in laboratory and field experiments. Correlation analysis between the values of various methods for assessing frost resistance showed their close relationship. Varieties of winter soft wheat characterized by high frost resistance, on average, accumulate more of this amino acid in the leaves in laboratory and field experiments.

1. Introduction
One of the important conditions for increasing grain production in the country is a deep knowledge of the biological characteristics of varieties, the study of their resistance to negative environmental factors [1,2].

In conditions unfavorable for the development of plants, they die on large areas, which causes enormous damage to agricultural production and, of course, affects the economy. The losses are especially great in countries of temperate and northern latitudes, in which valuable crops are exposed to be frozen during the winter period [3,4].

To further increase the production of grain, especially winter wheat, it is necessary to create high-yielding varieties that are resistant to adverse environmental factors in certain soil and climatic conditions [5,6,7].

This problem is the most urgent in the field of biological and breeding science, since an in-depth study of the processes and factors that determine high stability will serve as the main material for a more
effective fight against environmental factors that adversely affect the plant organism and inhibit the creation of more valuable varieties [8, 9, 10].

2. Materials and methods

Studies were conducted in 2014 to 2016.

As an object of research, 15 varieties of winter soft wheat of three scientific centers were used: Donskaya Bezostaya, Don 107, Ascetic, Raisin, Lydia, Captain, Lilith, Kaprizulya, Krasa Dona, Volnitsa, Volny Don, Polina (Donskoy Research Center), Tarasovskaya 29 (FRANCE / ANC "Donskoy"), Don Lira (FRANCE), Tanya (NCP named after P.P. Lukyanenko). As a classifier variety for frost resistance, the Tarasovskaya 29 variety was used.

Frost resistance was defined in three ways: by freezing seed boxes in low temperature chambers; Don method; shelving method.

Defining of free proline concentration in seedlings and leaves of winter common wheat varieties in laboratory and field experiments was made according to the method described by Bates L.S.

3. Results

Studying the frost resistance of winter wheat for more than two centuries has led to the development of a number of assessment methods - direct and indirect [11]. One method for directly assessing the frost resistance of winter wheat varieties is to freeze plants in sowing boxes in low-temperature chambers.

The safety of plants at a temperature of minus 19 °C was in the range from 58.5% (Kaprizulya) to 91.5% (Don 107). The meaning of the classifier variety Tarasovskaya 29 amounted to 76.6% (Table 1).

| Variety                   | Safety of plants, % |
|---------------------------|---------------------|
|                           | Minus 19°C | Minus 20°C | Minus 21°C | Minus 22°C |
| Tarasovskaya 29 *         | 76.6       | 55.3       | 28.6       | 13.1       |
| Don 107                   | 91.5       | **83.8**   | **49.0**   | **40.1**   |
| Captain                   | 75.8       | 61.7       | 32.9       | 20.3       |
| Raisin                    | 76.6       | 63.0       | 32.4       | 12.0       |
| Krasa Dona                | 75.3       | 66.7       | 31.0       | 20.1       |
| Ascetic                   | 82.3       | **78.1**   | **39.9**   | **35.3**   |
| Donskaya Bezostaya        | 88.3       | **78.1**   | 47.0       | **39.3**   |
| Lydia                     | 75.2       | 62.1       | 29.4       | 16.0       |
| Volnitsa                  | 81.3       | **76.0**   | **39.9**   | **33.7**   |
| Volny Don                 | 83.5       | **78.3**   | **41.5**   | **36.4**   |
| Polina                    | 80.0       | **72.3**   | 37.7       | **32.0**   |
| Kaprizulya                | 58.5       | 57.0       | 23.1       | 7.0        |
| Lilith                    | 64.4       | 58.4       | 25.2       | 4.2        |
| Tanya                     | 64.2       | 58.0       | 23.4       | 6.0        |
| Don Lira                  | 72.4       | 67.3       | 31.0       | 21.3       |
| HCP05                     | 13.1       | 14.1       | 10.8       | 13.9       |

* - classifier variety.

At a temperature of minus 20 °C, the safety of plants varied from 55.3 to 83.8%. Significantly high frost resistance (HCP05 = 14.1%) was obtained in the following varieties: Polina (72.3%), Volnitsa (76.0%), Ascetic (78.1%), Donskaya bezostaya (78.1%), Volny Don (78.3%) and Don 107 (83.8%). High safety of plants after freezing at a temperature of minus 21 °C was shown by the samples: Volnitsa...
(39.9%), Ascetic (39.9%), Volnyi Don (41.5%), Don bezostaya (47.0%) and Don 107 (49.0%), frost resistance of the Tarasovskaya 29 classifier was 28.6%. A further decrease in temperature at the tillering node to minus 22 °C contributed to a decrease in the safety of plants in the experiment. The percentage of preserved plants ranged from 4.2 to 40.1%. At a given temperature, on average, for 3 years, the following varieties were distinguished: Polina (32.0%), Volnitsa (33.7%), Ascetic (35.3%), Volny Don (36.4%), Don Bezostaya (39.3 %) and Don 107 (40.1%), significantly exceeding the frost classifier variety. The smallest number of living plants had the samples of Lilith, Kaprizulya and Tanya (4.2 ... 7.0%).

The next method used to assess frost resistance is the Don method. Its essence lies in the direct effect of low negative temperatures on the tillering nodes of plants. At minus 19 °C, the safety of the studied varieties ranged from 17.5 to 55.4%. Tarasovskaya 29 on average retained 32.0% of plants. Varieties Polina (44.5%), Volnitsa (47.3%), Ascetic (49.6%), Donskaya Bezostaya (50.8%), and Volny Don (50.8%) significantly exceeded the classifier (HCP = 7%) and Don 107 (55.4%). Low safety was noted in the samples of Kaprizulya, Lilith and Tanya (17.5 to 23.9%) (Figure 1).

![Figure 1. Plants safety of winter soft wheat varieties whereas using Don method for defining frost resistance, 2014 to 2016.](image)

At a freezing temperature of minus 20 °C, safety values decreased from 13.1% (Kaprizulya) to 43.8% (Don 107). High frost resistance was shown by the varieties Polina, Volnitsa, Ascetic, Donskaya Bezostaya and Volny Don (28.3 to 43.8%).

One form of application of a provocative background in assessing the frost resistance of plants is the use of the shelving method. This method allows to obtain reliable results on the degree of survival of winter wheat plants only in years with sufficient intensity of the temperature factor. In our studies, only the winter of 2014-2015 allowed the differentiation of varieties by frost resistance. Plants safety varied from 9 to 64.1% (Figure 2).
Less than 10% of living plants are found in samples of Tanya and Lilith. The maximum values were in the varieties Donskaya Bezostaya (64.0%) and Don 107 (64.1%).

In addition to the above methods, we conducted experiments in the laboratory to determine the content of free proline in seedlings and leaves of winter common wheat. This amino acid allows plants to resist and withstand adverse environmental conditions [10]. In a laboratory experiment, before cooling of the seedlings of the studied varieties, no significant differences in the content of proline were revealed. After exposure to low positive temperatures, an increase in the amount of free amino acid in the leaves occurred. The content of free proline ranged from 334.9 μg/g (Kaprizulya) to 365.9 μg/g (Don Bezostaya) (Figure 3).

![Figure 2](image-url)  
**Figure 2.** Safety of plants of winter soft wheat varieties whereas using shelving method, 2014 to 2015.

![Figure 3](image-url)  
**Figure 3.** Content and increase in content of free proline after cooling, 2014 to 2016.
The increase in free proline after cooling ranged from 56.9 to 82.9 μg / g. The maximum values of the content of this amino acid and its increase relative to the initial level were observed in the highly frost-resistant varieties Volny Don, Don 107 and Don Bezostaya.

In the winter period of 2014-2015, maximum proline concentrations were detected at the beginning and end of winter, when low negative air temperature was observed (Figure 4).

![Figure 4. Average content of free proline in fall-winter-spring period, 2014 to 2015.](image)

With increasing air temperature in early December and January, there was a decrease in the amount of free proline in the leaves of winter common wheat plants. The minimum concentration of 297.6 μg/g was detected in warm weather after the resumption of spring vegetation.

According to the studied varieties, differentiation was observed according to the content of free proline in the leaves of winter common wheat (table 2).

| Variety      | Content of free proline in leaves, µg/g |
|--------------|----------------------------------------|
|              | December 1 | January 13 | February 4 | February 19 | April 10 |
| Tarasovskaya 29 * | 707.4       | 374.3      | 315.9      | 728.5        | 298      |
| Don 107      | 826.8       | 446.4      | 341.5      | 921.1        | 296.8    |
| Captain      | 695.3       | 430.9      | 316.5      | 705.5        | 291.3    |
| Raisin       | 684.2       | 353.5      | 301.6      | 677.1        | 299.2    |
| Krasa Dona   | 801.6       | 417.9      | 340.2      | 781.5        | 298.3    |
| Ascetic      | 866.4       | 446        | 358.8      | 916.3        | 300.1    |
| Donskaya Bezostaya | 784.2     | 438.3      | 340.2      | 919.4        | 305.2    |
| Lydia        | 644.2       | 412.5      | 310.3      | 687          | 291.7    |
| Volnitsa     | 848.5       | 440.4      | 343.2      | 899.5        | 299.2    |
| Volny Don    | 870.5       | 443.7      | 353.4      | 918.5        | 302.5    |
| Polina       | 830.4       | 435.1      | 336.5      | 804.6        | 301.1    |
| Kaprizulya   | 663.9       | 385.8      | 304.3      | 665.1        | 291      |
| Lilith       | 690.6       | 379.5      | 297        | 663.8        | 297.7    |
| Tanya        | 723.4       | 387.6      | 321.8      | 693.6        | 299.1    |
| Don Lira     | 761.1       | 422        | 334.4      | 730          | 293.4    |
| HCPo5        | 112.5       | 65.6       | 48.2       | 53.8         | 16.9     |

* - classifier variety
At the beginning of winter, there were favorable conditions for hardening winter soft wheat plants and accumulating the content of free proline. The Tarasovskaya 29 classifier had an amino acid amount of 707.4 μg/g. Significantly (HCP05 = 112.5 μg/g) exceeded this variety, such samples as: Don 107 (826.8 μg/g), Polina (830.4 μg/g), Volnitsa (848.5 μg/g), Ascetic (866.4 μg/g), and Volny Don (870.5 μg/g). On February 19, when the air temperature dropped to minus 15 °C, the maximum value of free proline in winter wheat leaves was obtained. It ranged from 663.8 to 921.1 μg/g. The highest concentration was observed in the varieties Polina, Volnitsa, Volny Don, Ascetic, Donskaya Bezostaya and Don 107 (804.6 - 921.1 μg/g).

In the winter period of 2015 to 2016, the content of free proline increased until the middle of winter, when the maximum values were obtained (716.2 μg/g). Then, by spring, with a decrease in frost resistance of winter soft wheat plants, a decrease in the amount of this amino acid in the leaves occurred (Figure 5).

The lowest level of free proline was also observed in spring in steadily warm weather (296.7 μg/g). The maximum values of this amino acid by variety were recorded in samples taken on January 27. They ranged from 538.2 μg/g to 845.4 μg/g (table 3).

**Table 3.** Content of free proline in fall-winter-spring periods, 2015 to 2016.

| Variety             | November 20 | December 27 | January 27 | February 27 | March 24 |
|---------------------|-------------|-------------|------------|-------------|----------|
| Tarasovskaya 29 *   | 359.7       | 518.4       | 720.8      | 374.3       | 298      |
| Don 107             | 372.4       | 615.2       | 765.2      | 446.4       | 296.8    |
| Captain             | 352.6       | 467.9       | 717.9      | 430.9       | 291.3    |
| Raisin              | 349.7       | 496.4       | 646.4      | 353.5       | 299.2    |
| Krasa Don           | 355.3       | 537.2       | 687.2      | 417.9       | 298.3    |
| Ascetic             | 362.1       | 600.7       | 790.7      | 446.0       | 300.1    |
| Donskaya Bezostaya  | 373.2       | 595.4       | 845.4      | 438.3       | 305.2    |
| Lydia               | 353.7       | 522.3       | 685.2      | 412.5       | 291.7    |
| Volnitsa            | 363.6       | 561.8       | 788.5      | 420.8       | 293.3    |
| Volny Don           | 363.5       | 594.2       | 801.1      | 441.5       | 299.8    |
High values \( (\text{HCP}_{05} = 77.0 \, \mu g/g) \) were obtained in the varieties Polina, Volnitsa, Volny Don, Ascetic, Don 107 and Donskaya Bezostaya \((760.5 \text{ to } 845.4 \, \mu g/g)\). A low concentration was noted in the varieties of Kaprizulya \((538.2 \, \mu g/g)\), Tanya \((620.1 \, \mu g/g)\) and Lilith \((625.0 \, \mu g / g)\).

In spring, the content of free proline ranged from 291.0 to 305.2 \( \mu g/g \) and no significant differences between the studied varieties were revealed.

According to the results of the correlation analysis between the values of direct and indirect methods for assessing frost resistance, the following results were obtained. Between the frost resistance, determined in the seed boxes, and other methods for assessing the safety of plants, strong positive relations are observed \((r = 0.83 - 0.97 \pm 0.14–0.03)\) (table 4).

**Table 4.** Correlation coefficients between values of direct and indirect methods for assessing frost resistance, 2014 to 2016.

| Correlated attributes                  | Frost resistance (boxes) | Frost resistance (Don method) | Frost resistance (racks) | Proline (before cooling) | Proline (after cooling) | Proline (increase) | Proline (field experiment) |
|---------------------------------------|--------------------------|-------------------------------|--------------------------|--------------------------|------------------------|-------------------|---------------------------|
| Frost resistance (boxes)              | 1.00                     | 0.97*                         | 0.83*                    | 0.49                     | 0.94*                  | 0.93*             | 0.95*                     |
| Frost resistance (Don method)         | 1.00                     | 0.76*                         | 0.50                     | 0.90*                    | 0.90*                  | 0.95*             | 0.90*                     |
| Frost resistance (racks)              | 1.00                     | 0.24                          | 0.74*                    | 0.76*                    | 0.71*                  | 0.71*             | 0.95*                     |
| Proline (before cooling)              | 1.00                     | 0.55                          | 0.41                     | 0.48                     |                        |                   |                           |
| Proline (after cooling)               | 1.00                     | 0.99*                         | 0.94*                    |                          |                        |                   |                           |
| Proline (increase)                    | 1.00                     | 0.94*                         |                          |                          |                        |                   |                           |
| Proline (field experiment)            |                          |                               |                          |                          |                        |                   | 1.00                      |

*- authentically at 5% significance level.

Close positive relationships were found between the content of free proline in leaves of winter soft wheat (in field and laboratory experiments) and all direct methods for assessing frost resistance \((r = 0.74 - 0.95 \pm 0.17–0.05)\).

### 4. Discussion

The use of direct and indirect methods for assessing the frost resistance of winter soft wheat varieties allows to distinguish genotypes with high resistance to negative low temperatures. However, for breeding work to create highly frost-resistant winter wheat varieties, methods are needed that allow quick and high-quality selection of samples with high resistance to low temperatures. One of such methods may be the determination of free proline in winter wheat plants, but for greater efficiency and ease of use of this method, further study is necessary.
5. Findings
As a result of studies using various methods of assessing frost resistance, samples with high resistance to low negative temperatures were identified: Polina, Volnitsa, Volny Don, Ascetic, Donskaya Bezostaya and Don 107; these varieties are recommended to be used in the breeding process as sources of high frost resistance. The correlation coefficients of the values of direct and indirect methods for assessing frost resistance suggest the possibility of using the determination of the content of free proline as a way of identifying resistant genotypes to low negative temperatures. Varieties of winter soft wheat, showing a high safety of plants, on average accumulate more this amino acid.

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