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Diagnostics of tolerance to low positive temperatures of the common millet collection during the seed germination

Aiman Rysbekova[1]*, Elmira Dyussibayeva[1], Abilbashar Seitkhozhayev[1], Irina Zhirnova[1], Nursaule Zhanbyrshina[1], Gulden Kipshakbayeva[1], Yeldos Kulzhabayev[1] and Karina Makhmudova[2]

[1]S. Seifullin Kazakh Agro-Technical University, Agronomic Faculty, Department of Agriculture and Plant Growing, Nur-Sultan; 010011, Republic of Kazakhstan
[2] Kazakh National Women s Teacher Training University, 99 Aiteke bi Street, Almaty, Kazakhstan

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ABSTRACT

Objectives of the study were the germination response of the initial breeding materials of millet (*Panicum miliaceum* L.) originating from five countries, their tolerance to the low positive temperatures during germination *in vitro* and the identification of cold-tolerant samples for further use in the breeding programs. The main problem of proso millet for early spring planting is low-temperature stress at the stage of seed germination. As a result of the laboratory screening, an unequal reaction of the studied millet genotypes by the trait of cold tolerance to low positive temperatures was revealed. The most cold-tolerant samples were K-5786, PI-531413, Zolotistoye kormovoye, Pamiati Bersiyeva, Shortandinskoye-7 and Yarkoye-6, and these genotypes demonstrated the rootlets length comparable with the standard. The mathematical analysis of the interrelation of the average data of seed viability: germination, germination energy, and coleoptiles length of the millet plantlets demonstrated that all of these traits in certain degree correlate depending on the temperature.

Key words: *Proso millet, Cold tolerance, Positive low temperature, Selection, Germination*

Introduction

Common millet or millet (*Panicum miliaceum* L.) belongs to the cultures of extreme antiquity and is one of the most important cereals in the world (Colosi and Schaal, 1997; Zhao, 2005; Wang *et al*., 2016). Millet is widespread in all continents of the globe. The five leading producers of millet are the Russian Federation, India, China, the USA, and Ukraine (Zotikov *et al.,* 2012; Wang *et al.,* 2016). Due to its precocity, drought tolerance, efficiency, and other valuable biological and economic traits, this culture can be widely used in production (Zhidkin, 1982). Proso millet, as a typical xerophyte, has a growing season of 60–100 days, and for conditions of dry steppe zone of Kazakhstan, it is one of the most adapted cultures, that defines an area of its use for food and fodder purposes (Tsygankov *et al.,* 2006). However, millet is very exacting to warm during its initial stages of vegetation that interferes with early terms of its sowing in drought areas. Millet weakly endures low temperatures at all stages of ontogenesis. Therefore, by tolerance to low positive temperatures, millet belongs to a weak tolerance group (Fedulov *et al.,* 2015).

Cold endurance is the ability of plants to endure low (0–10 °C) positive temperatures, that is peculiar to plants of a moderate climate. The cold endurance of the plants depends on the period of their ontogenesis and organs of plants (flowers> fruits and leaves> roots> stems). The most cold-tolerant plants are those of early sowing time. The cold endurance of a plant is characterized by the sum of the biological temperatures which are necessary for its development, i.e., the less sum of positive temperatures, the higher cold endurance. Very early ripening plants possess 1200 °C sum of low positive temperatures; early ripening plants possess 1200-1600 °C sum of low positive temperatures, mid-early ripening plants – 1600-2200 °C, mid-ripening plants – 2200-2800 °C, mid-late ripening plants – 2800-3400 °C and late-ripening plants – 3400-4000 °C (Yadav, 2010).

Among the various abiotic stresses, cold stress is an essential factor reducing the productivity of the cultivated plants. The limited information available suggests that the base temperature at which development stops is different for millet varieties: the rate and the duration of specific developmental processes also differ (Ong and Monteinth, 1984). Low temperature influences the growth and development of plants (Pearce, 2011). Survival of plants at low temperatures depends on their ability to acclimatization in cold conditions (Mckhann *et al.*, 2010). Obtaining of steadily high yields of millet in specific climatic zones is limited by its insufficient cold tolerance. In more northern areas this culture in some years does not manage to ripen, or frosts damage it during maturing that leads to a significant shortage of harvest and decline in quality of the final products. In specific years the death of shoots from spring frosts or the slowed-down growth of plants in a phase of shoots was observed. Being affected by low positive temperatures, millet cultivars sharply slow down their growth and do not manage to ripen during the frost-free period (Udovenko, 1998). To avoid such a situation, the creation of the new cold-tolerant cultivars and hybrids of millet, providing development in the conditions of the low temperatures and higher productivity, in comparison with the cultivars extended nowadays is necessary. Elimination of noted shortcomings at millet cultivars goes very slowly. The insufficient efficiency of breeding work with millet is connected first of all with the limitation of initial material and the applied selection methods. In the literature, there are limited works which are devoted to studying the cold tolerance of millet. In this regard, the purpose of the present research consists in a screening of the initial breeding material of millet originating of five countries on their tolerance to the lowered positive temperatures during germination *in vitro* and the identification of cold-tolerant samples for further use in the breeding programs.

Materials and Methods

Plant material

Studies were carried out at the Research Platform of Agricultural Biotechnology (RPAB) at S. Seifullin Kazakh Agrotechnical University. Thirty genotypes of the millet of various ecology-geographical origin were compared by assessment of cold tolerance (Table 1). The domestic zoned cultivar Saratovskoye-6 was taken as standard.

Determination of millet cold tolerance. Assessment of cultivars and samples of millet for their cold tolerance was carried out with use of original seeds during the phase of their germination according to a technique, when using the following indicators: germination viability of seeds, the length of the coleoptile and the intensity of sprouts' growth in conditions of the positive low temperatures at 10 and 5°C (Udovenko, 1998). During the germination, the temperature and moisture of the filter paper were under control; if it was necessary, the filter paper was additionally wetted. The measurements were carried out daily as far as day 14.

The millet well sprouts at the temperature from 10 to 45 °C, but not at 5 °C or 50 °C. The highest speed of germination occurs between 35 to 40 °C (Theisen *et al.*, 1978). The germination energy (GE), at the action of different temperatures, indicates the activity of initial seed development. In the present experiments, for determining the GE, counting the seeds was carried out on the second day. On day 14, the energy of germination of millet seeds was determined, and 30 plantlets were selected for measuring the coleoptile length of each plantlet. The average coleoptile length and standard deviations were calculated for each genotype using Microsoft Excel 6.0.

Results and Discussion

As a result of laboratory screening unequal reaction of the studied millet genotypes by the trait of cold

Table 1. The cultivars and samples of millet used for cold tolerance assessment

Cultivars and samples	Origin	Vegetation period, days
Abakanskoye kormovoye	Russia	95-98
Aktyubinskoye kormovoye	Kazakhstan	95-98
Barnaulskoye kormovoye	Russia	95-98
Zolotistiye kormovoye	Russia	95-98
Kokchetavskoye-66 standard	Kazakhstan	95-98
Kormovoye 89	Kazakhstan	94-97
Kormovoye proso	Kazakhstan	95-98
Omskoye-11	Russia	95-99
Pavlodarskoye	Kazakhstan	95-99
Pamiati Bersiyeva	Kazakhstan	95-99
Saratovskoye-3	Russia	92-96
Saratovskoye-6	Russia	92-96
Shortandinskoye-7	Kazakhstan	94-98
Yarkoye-5	Kazakhstan	95-99
Yarkoye-6	Kazakhstan	94-98
Yarkoye-7	Kazakhstan	94-98
K-10112	Russia	95-98
K-148	Kazakhstan	105-108
K-2377	Kazakhstan	103-106
K-3742	Russia	92-95
K-5786	Kazakhstan	95-98
K-8873	Kazakhstan	92-95
K-9681	Kazakhstan	105-108
K-9800	USA	94-98
K-9837	Russia	108-110
K-9989	Russia	95-99
PI-507933	Hungary	100-103
PI-531404	Russia	100-102
PI-531413	Turkey	100-102
PI-649383	USA	100-102

tolerance to low positive temperatures was revealed. In control variant (25 °C) on the day 3 about 50% of the studied samples already showed the high level of germination seed viability (> 95%) whereas in the experimental variants at 10 and 5 °C the germination seed viability was noticed only on the day 7 and 8, respectively (Table 2).

The obtained data showed that germination viability of seeds at the temperature of 25 °C of 14 samples was 100%, i.e. the germination viability of these genotypes was at the standard level. The germination viability of the other samples varied from 4 to 80%. The genotypes PI-507933, Barnaulskoye kormovoye, K-9837, K-10112, K-9989, PI-531404, K-148, and Omskoye-11 were characterized with very low germination viability. At the temperature of 10°C, only four samples (Zolotistoye kormovoye, Yarkoye-7, Yarkoye-5, and Yarkoye-6) demonstrated 100% germination viability that testified to the relative cold tolerance of these genotypes. At 5°C the following genotypes were characterized with the highest rates of germination seed viability: Zolotistoye kormovoye and Yarkoye-6 (40%), Yarkoye-7 (38%); Saratovskoye-6, Yarkoye-5 and K-9681 (32%). The analysis of data showed that among the studied collection samples, the steadiest against low positive temperatures (5 °C) were the following genotypes: PI-649383, K-9837, and the Abakanskoye Kormovoye. Indicators of GE play a direct role and are the critical factor in determining plant number per hectare, which is one of the main components of yield. The samples K-10112, Omskoye-11, PI-649383, K-3742, PI-507933, K-148, andPI-531404, possessed the weakest cold tolerance: the germination seed viability and GE of these genotypes at 5°C temperature were equal to zero.

The analysis of data for the coleoptile length, in the conditions of low positive temperatures, allowed to reveal the general trend of growth inhibition. It was shown that low temperature resulted in the reduction of the experimental coleoptile length in comparison with the control. In the control variant, the coleoptile length varied from 0.1 to 7.2 cm, while at the temperature of 10 °C – from 0.17 to 0.32 cm and at the temperature of 5 °C – from 0.14 to 0.20 cm (Table 3).

The average coleoptile length in control variant (25 °C) was 5.04 cm, at 10 °C – 0.24 cm andat 5°– 0.13 cm. The coleoptile length decreased by 20 timesat 10°C and almost 40 times at 5 °C, in comparison with the control. It should be noted that the highest rates in this trait were demonstrated by the following zoned millet samples: Kormovoye proso, Yarkoye-7, Shortandinskoye-7, Zolotistoye kormovoye, Pamiati Bersiyeva, Yarkoye-7. These genotypes were steadier against cold both at the temperature 5 °C and 10 °C; they surpassed the standard Saratovskoye-6 in germination energy. In conditions of low positive temperatures, the coleoptile emergence was not noticed in the samples of Omskoye-11, K-10112, K-3742, and PI-531404. The results for the millet plantlets root length on day 14 demonstrate dranging from 0.1 up to 6.77 cm in the control variant, while at the temperature of 10 °C the rootlets' length was 0-1.06 cm, and at the temperature of 5 °C, it was limed to 0 - 0.30 cm (Table 4).

According to the research data, it canbe noticed that at the temperature of 10 °C the genotypes Omskoye-11 and K-10112 did not form rootlets. The same was observed in the samples Omskoye-11, K-

Table 2. Effect of low positive temperatures on germination viability and germination energy of the seed millet from the collection

Genotypes	Control (25°C)		Experiment (10°C)		Experiment (5°C)	
	[1]G(%)	[2]GE	[1]G(%)	[2]GE	[1]G (%)	[2]GE
Abakanskoye kormovoye	64	5.9	40	7.4	14	8.5
Aktyubinskoye kormovoye	80	4.1	68	7.5	14	9.2
Barnaulskoye kormovoye	14	7.0	12	7.6	6	8.6
Zolotistiye kormovoye	100	4.1	100	6.7	40	9.0
Kokchetavskoye-66	54	4.6	24	7.3	8	8.7
Kormovoye-89	100	4.4	80	7.8	16	9.7
Kormovoye proso	100	4.2	66	8.4	12	9.0
Omskoye-11	4	8.0	-	-	-	-
Pavlodarskoye	100	3.9	64	7.6	28	8.7
Pamiati Bersiyeva	100	3.6	96	7.5	26	9.0
Saratovskoye-3	100	4.2	68	7.7	12	8.6
Saratovskoye-6	100	4.0	72	7.9	32	8.7
Shortandinskoye-7	100	4.0	84	7.7	24	9.0
Yarkoye-5	100	3.3	100	7.9	32	8.7
Yarkoye-6	100	3.1	100	6.8	40	9.0
Yarkoye-7	100	3.3	100	7.5	38	8.9
K-10112	10	10.6	-	-	-	-
K-148	6	5.0	6	9.3	-	-
K-2377	80	5.4	44	7.1	12	8.6
K-3742	30	4.5	6	9.0	-	-
K-5786	100	4.0	30	7.3	10	8.8
K-8873	64	5.4	40	7.6	10	8.6
K-9681	100	5.1	90	6.8	32	8.7
K-9800	44	5.6	30	7.8	10	8.6
K-9837	14	5.7	10	7.4	4	8.5
K-9989	8	5.5	10	8.0	4	9.0
PI-507933	16	7.3	2	9.0	-	-
PI-531404	8	6.2	2	10.0	-	-
PI-531413	78	4.4	80	7.9	26	9.0
PI-649383	100	4.0	52	8.5	10	8.4
Mean	66	5.0	52	7.8	19	8.8
$LSD_{0.05}$	35	1.1	30	0.5	10	0.2

[1]G – Germination, [2]GE – Germination Energy

Table 3. Effect of low positive temperatures on the coleoptile growth of millet plantlets

Genotypes	Control (25 °C)		Experiment (10 °C)		Experiment (5 °C)	
	Mean CL[1](cm)	SD[2]	Mean CL[1], (cm)	SD[2]	Mean CL[1], (cm)	SD[2]
Abakanskoye kormovoye	5.31	0.69	0.26	0.12	0.17	0.08
Aktyubinskoye kormovoye	4.9	0.42	0.26	0.11	0.17	0.05
Barnaulskoye kormovoye	2,62	3.17	0.3	0	0.17	0.06
Zolotistiye kormovoye	5.32	0.94	0.28	0.11	0.18	0.07
Kokchetavskoye-66	6.08	1.00	0.21	0.07	0.18	0.05
Kormovoye-89	3.92	1.25	0.24	0.08	0.19	0.08
Kormovoye proso	5.62	0.62	0.27	0,06	0,20	0.09
Omskoye-11	0.1	0	-	-	-	-
Pavlodarskoye	5.06	1.02	0.26	0.15	0.15	0.05
Pamiati Bersiyeva	5.82	0.80	0.30	0.10	0.20	0.09
Saratovskoye-3	5.56	0.56	0.22	0.08	0.17	0.08
Saratovskoye-6	6.5	0.98	0.26	0.13	0.16	0.07
Shortandinskoye-7	4.14	1.12	0.27	0.11	0.19	0.09
Yarkoye-5	5.35	0.89	0.21	0.10	0.16	0.05
Yarkoye-6	5.41	0.69	0.32	0.08	0.19	0.07
Yarkoye-7	5.59	0.73	0.27	0.09	0.18	0.07
K-10112	0.16	0.08	-	-	-	-
K-148	7.2	0,56	0.23	0.11	-	-
K-2377	5.3	0.91	0.29	0.12	0,15	0.05
K-3742	6.27	0.52	0.3	-	-	-
K-5786	4.46	0.46	0.23	0.08	0.20	0.01
K-8873	4.56	0.94	0.25	0.06	0.14	0.05
K-9681	4.69	0.67	0.26	0.07	0.19	0.09
K-9800	7.1	1.54	0.30	0.15	0.14	0.05
K-9837	4.84	3.34	0.26	0.05	0.15	0.07
K-9989	6.86	0.49	0.3	0.14	0.15	0.07
PI-507933	5.25	1.24	0.3	-	-	-
PI-531404	6.26	0.98	0.3	-	-	-
PI-531413	6.5	0.91	0.24	0.08	0.19	0.09
PI-649383	4.5	1.25	0.17	0.05	0.18	0.08

[1]CL – Coleoptile Length, [2]SD – Standard Deviation

10112, K-148, K-3742, PI-507933, and PI-531404 at the temperature of 5°C. The most cold-tolerant samples were K-5786, PI-531413, Zolotistoye kormovoye, Pamiati Bersiyeva, Shortandinskoye-7,and Yarkoye-6, and in these genotypes, the rootlets length was comparable with the standard.

The present study demonstrated that the lowed temperature has significant effects on millet seed germination. Selection of the samples which are tolerant to low positive temperatures leads to the creation of the genotypes capable of growing in such conditions. Tolerance of the plants to the low temperatures correlates with the minimum temperature of seed germination. Therefore, the speed of germination and the number of germinated seeds at low temperatures could be used as a criterion for evaluation of cold tolerance (Kuznetsov and Dmitrieva, 2005).

Correlation communications between germination energy (Figure 1), germination seed viability (Figure 2), coleoptile length (Figure 3) and rootlets' length (Fig. 4) depending on the temperature (25 °C, 10 °C and 5 °C) were investigated.

Data obtained confirmed that germination is a complex and dynamic stage of plant ontogeny, with several interactive metabolic processes changing from a storage phase to a mobilization phase (Bewley and Black, 1994). The mathematical analysis of the interrelation of the average data of seed germination viability, germination energy, and coleoptiles length of the millet plantlets demonstrated that all of the traits, in a certain degree, correlate depending on the temperature. In the study, a direct correlation dependence of seed germination viabil-

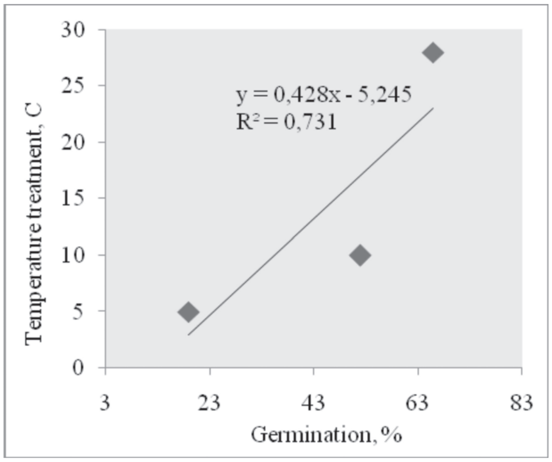

Fig. 1. Correlation dependence of germination of seeds from the temperature at the initial phase of ontogenesis

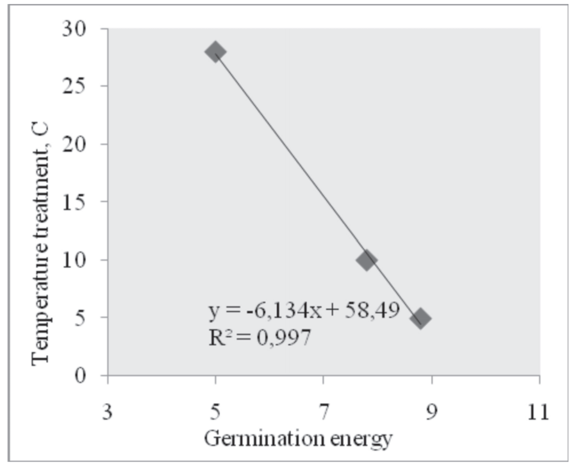

Fig. 2. Correlation dependence of germination energy of seeds from the temperature at the initial phase of ontogenesis

Table 4. Effect of low positive temperatures onto growth and development of rootlets in millet plantlets

Genotypes	Control (25° C)		Experiment (10 °C)		Experiment (5° C)	
	Mean RL[1](cm)	SD[2]	Mean RL[1](cm)	SD[2]	Mean RL[1], (cm)	SD[2]
Abakanskoye kormovoye	3.91	1.74	0.66	0.35	0.29	0.09
Aktyubinskoye kormovoye	4.24	0.07	0.65	0.27	0.27	0.05
Barnaulskoye kormovoye	5.05	3.01	0.82	0.17	0.27	0.06
Zolotistiye kormovoye	4.88	1.18	0.58	0.29	0.31	0.08
Kokchetavskoye-66	6.77	1.32	0.4	0.34	0.30	0.08
Kormovoye-89	5.27	1.19	0.55	0.41	0.28	0.10
Kormovoye proso	3.97	0.44	0.31	0.19	0.30	0.09
Omskoye-11	0.1	0	-	-	-	-
Pavlodarskoye	4.52	1.09	0.73	0.44	0.27	0.06
Pamiati Bersiyeva	4.24	0.79	0.75	0.60	0.31	0.09
Saratovskoye-3	6.33	1.6	0.46	0.28	0.27	0.08
Saratovskoye-6	5.06	1.32	0.89	0.60	0.30	0.08
Shortandinskoye-7	4.41	1.66	0.77	0.61	0.31	0.08
Yarkoye-5	4.54	0.86	0.21	0.14	0.28	0.10
Yarkoye-6	5.08	0.94	1.06	0.77	0.31	0.09
Yarkoye-7	4.08	0.70	0.64	0.34	0.31	0.07
K-10112	0.13	0.08	-	-	-	-
K-148	5.7	0	0.55	0.07	-	-
K-2377	4.25	1.09	0.74	0.34	0.27	0.08
K-3742	4.47	0.84	0.4	0.28	-	-
K-5786	3.95	0.96	0.73	0.40	0.30	0.10
K-8873	3.43	1.32	0.78	0.39	0.26	0.09
K-9681	3.29	0.71	0.59	0.38	0.29	0.09
K-9800	6.73	1.65	0.60	0.40	0.26	0.05
K-9837	6.72	1.48	0.86	0.56	0.25	0.07
K-9989	5.86	0.83	0.53	0.25	0.25	0.07
PI-507933	3.67	1.071	0.2	0	-	-
PI-531404	3.23	2.07	0.2	0	-	-
PI-531413	6.03	1.01	0.55	0.41	0.30	0.08
PI-649383	5.26	0.95	0.25	0.16	0.28	0.08

[1]RL – Root Length, [2]SD – Standard Deviation

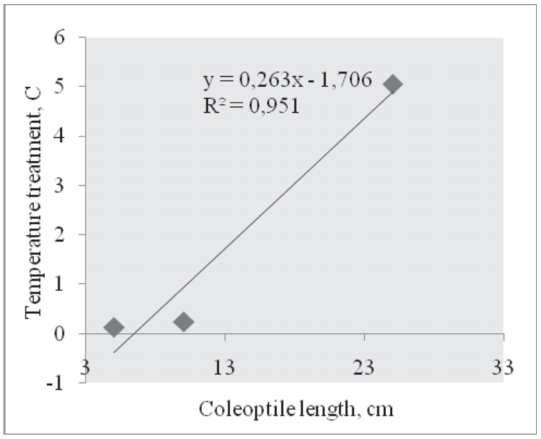

Fig. 3. Correlation communication of the coleoptile length depending on the temperature

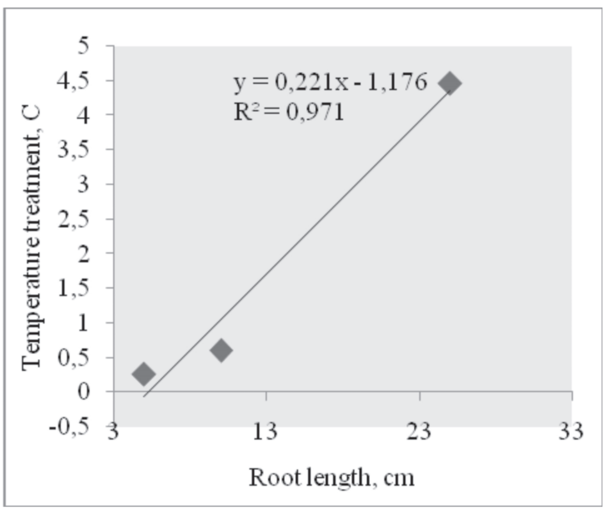

Fig. 4. Correlation communication of the rootlets length of the plantlets depending on temperature

ity on the temperature at the initial phase of on togenesis was revealed. The correlation coefficient of this trait (r) was 0.73. Similar nature of correlation communication was also shown between the germination energy and the coleoptile length depending on temperature. The correlation coefficient of germination energy and the coleoptile length depending on temperatures (r) were 1 and 0.95, respectively. It was noticed that as lower is the temperature, as higher is the coefficient of correlation.

Conclusion

Based on the experimental data analysis, it is possible to conclude that the seed germination viability of the studied millet collection considerably decreased at low positive temperatures. At temperatures of 25, 10, and 5 °C, the average seed germination declined from 66% to 52%, and 19%, respectively. Moreover, a tendency for an increased period of germination was remarked. In comparison with the control variant, at the temperature of 10 °C, the germination period was increased with 2.8 days; whereas at 5°C, it was with 3.8 days. The coleoptile length decreased at a temperature of 10 °C by 95% and at 5°C by 97%. A sharpinhibition in the development and growth of the experimental rootlets, in comparison with the control, was also detected. In the conditions of cold stress, this indicator decreased in the range from 87 to 95%.

Thus, according to the results of laboratory screening, the unequal reaction of genotypes to temperature stress (10 and 5°C) was revealed. Samples K-10112, Omskoye-11, PI-649383, K-3742, PI-507933, K-148, and PI-53140 were noticed as weakly cold tolerant. The best cold-tolerant genotypes K-5786, PI-531413, Zolotistoye kormovoye, Pamiati Bersiyeva, Shortandinskoye-7, Saratovskoye-6, Yarkoye-6, PI-649383, K-9837, and Abakanskoye kormovoye were selected. The carefully chosen valuable genotypes will be recommended for inclusion into the breeding programs for the creation of cold-tolerant millet cultivars.

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