# LARGE-GRAINED WHEATGRASS VARIETY SOVA (Thinopyrum intermedium) AS AN ALTERNATIVE TO PERENNIAL WHEAT 

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#### Abstract

For the last decades, due to climate warming, environmental threats, increasing of energy intensity of the grain production, wider usage of perennial cultures as an alternative to annual agricultural cultures, more resistant to negative biotic and abiotic environmental factors has been proposed. The large-grained wheatgrass variety Sova was created at Omsk State Agrarian University via mass selection of wintered biotypes from the population of Thinopyrum intermedium (Host) Barkworth \& D.R. Dewey obtained from The Land Institute (Kansas, USA), with following targeted cross-pollination and creation of new winter-hardy population. In 2020, the variety was registered in the State register of breeding achievements approved for use for all regions of the Russian Federation. For the first time, the biological and economical significance of the large-grained wheatgrass variety Sova as an alternative to perennial wheat is presented in this work. The variety Sova of Thinopyrum intermedium is recommended for cultivation as grain and fodder crop during four-six years, the variety forms grain with high protein content and good quality hay. The goal of this research is evaluation of economically valuable traits of new large-grain wheatgrass variety Sova under conditions of southern forest-steppe of Western Siberia, as well as to determine the correlation of spike components with plant height for increasing of the selection efficiency and thousand kernel weight. The research was carried out in the experimental field of Omsk State Agrarian University under conditions of the southern forest-steppe of Western Siberia in 2015-2019. A new wheatgrass variety Sova (Thinopyrum intermedium), winter bread wheat (Triticum aestivum L.) cv. Omskaya 4, and spring bread wheat (T. aestivum L.) cvs. Element 22 and Pamyati Azieva were compared. The spike productivity traits of 100 spikes of wheatgrass, i.e., spike weight and length, number of spikelets and grains per spike, grain weight per spike, etc., were evaluated. The thousand kernel weight and spike harvest index were calculated. The grain yield and biomass were determined. The correlations of productivity components with plant height were analyzed. The grain morphometric parameters of variety Sova and spring bread wheat Pamyati Aziev (area, perimeter, length, width, and circularity) were compared. The grain and hay quality was evaluated. For wheatgrass (Thinopyrum intermedium), winter wheat variety Omskaya 4, and spring variety Element 22, the length, width, average diameter, volume, main area, number of root tips, and total root length were determined. The biological activity of the rhizosphere of Thinopyrum intermedium compared to winter wheat variety Omskaya 4, and spring bread wheat variety Element 22 was evaluated. The soil samples for accounting of microorganisms were taken in the shoots stage, after winter survival, and in the heading stage. It was found that grain yield, biomass, and hay of the variety Sova for three years of reproduction increased every year and averaged $9.2,210.3$, and 71.0 centner per hectare, respectively. Grain quality indicators were high, the $19.4 \%$ protein and $36.3 \%$ gluten content. The variety Sova has many grains per spike, on average more than 50 , thousand kernel weight is 9.7 g , and


spike harvest index is $51 \%$. The length of all roots of Thinopyrum intermedium was 6.9-9.8 times longer compared to that of winter and spring varieties. The total number of agronomically important groups of microorganisms was 2.2 times higher, the intensity of mineralization (abundance of microorganism on starch ammonia agar to meat peptone agar SAA/MPA) was $58 \%$ higher than in winter wheat variety Omskaya 4. The rate of cellulose decomposition was 13.7 and $21.4 \%$ higher than in winter and spring wheat varieties. According to the studying the correlations between thousand kernel weight, plant height, and productivity traits suggest that the selection of biotypes with shorter stem, fewer spikelets and grains per spike is appropriate for grain weight increasing.

Keywords: Thinopyrum intermedium, spring wheat, winter wheat, breeding, perennial cultures, Sova variety, spike, valuable traits, correlations, yield, grain, hay, grain quality, roots, rhizosphere microorganisms

In recent decades, climate warming increased environmental threats and energy costs of grain production, wherefore perennial crops are proposed as an alternative to annual crops. The perennial crops are more resistant to adverse biotic and abiotic environmental factors, are able to preserve the soil cover, reduce the loss of soil moisture and nutrients, and absorb more carbon, reducing the greenhouse gases [1]. In general, in the world, greenhouse gas emissions from agriculture and land use account for more than $20 \%$ of their total amount in the atmosphere. In Russia, the Paris Climate Agreement was adopted by the Decree of the Government of the Russian Federation (September 21, 2019 No. 1228-PP), but has not yet been ratified. It is advisable to consider international practices for assessing and reducing the carbon footprint when developing the Russian system of crop production. The United States plans to radically reduce and completely eliminate pollution and greenhouse gas emissions in the agricultural sector by 2030.

In the world, annual grain crops provide about $70 \%$ of the energy consumed by humans with food, and occupy about $70 \%$ of the cultivated area [2]. An alternative strategy for use perennial crops involves domestication of wild species from the tribe Triticeae Dum. through multiple selections of plants with the desired agronomic traits.

In 2003, the Land Institute (Kansas, USA) launched a project to study perennial wild species. Among them, a gray wheatgrass [Thinopyrum intermedium (Host) Barkworth \& D.R. Dewey] population was used to obtain a perennial cereal cultivar Kernza (six-cycle breeding for improved ear productivity, grain size and threshing). As a result, the grain yield increased by $77 \%$, and the grain weight by $23 \%$ [3]. Programs for breeding perennial wheat through hybridization of cultivated wheat with perennial wild relatives were launched back in the 1930s in the USSR and the USA, but they still have not yielded the desired effect [4]. The results of these breeding programs contributed to the production of wheat forms with introgression of genes responsible for a long-term lifestyle, due to intergenomic or chromosomal translocations [5-7].

Progress has been achieved due to octaploid wheat-wheatgrass hybrids. A review on the state, achievements and prospects in using perennial wheat-wheatgrass hybrids $(2 n=56)$ from the collection of Tsytsin Main Moscow Botanical Garden RAS) [8] showed the superiority of new forms in biological and economically useful traits compared to hybrids obtained in the USSR in 1927-1969. Stable selected genotypes have good performance in terms of overwintering over 2-3 years of life, grain yield, high grain protein, baking qualities, yield of green mass for three cuttings and resistance to diseases and pests. The cultivation of perennial crops is more eco-friendly and will reduce the cost for food and feed production [8].

Hybridization of wheat species with different wheatgrass species remains an effective method for introducing alien genetic variability into the common wheat genome [9-12]. Perennial wheats have been assessed in 20 countries [13]. Perennial life cycle in plants is under polygenic control, therefor, not a single
alien introgression but a combination of seven or more pairs of chromosomes transferred from a perennial donor into the cultivated wheat genome is necessary to provide the trait expression [14]. However, along with the perennial type of development, hybrid offspring possesses many undesirable traits characteristic of wheatgrass, such as spike fragility, difficult grain threshing, asynchronous ripening, and small grain size [15-17].

According to research data from the United States, identification of the cv. Kernza genes responsible for the domestication traits, primarily improved productivity, will increase the efficiency of breeding for wheatgrass domestication [3, 18]. Kernza has good grain quality, e.g., the grain protein level, lipids, fiber, and carotenoids are significantly higher than that of ordinary wheat [1921]. It was found that for most of the intermediate wheatgrass (IWG) populations, grain antioxidant activity is higher compared to wheat [21]. Kernza ${ }^{\circledR}$ grain is used in bakery and confectionery production. Commercial products are currently marketed under the Kernza brand, owned by The Land Institute [1]. Kernza is widely used as a forage crop. In Canada, to increase its potential, crops of cv. Kernza mixed with legumes have been proposed as a long-term source of nitrogen instead of inorganic fertilizers [22].

In 2020, a unique large-grain wheatgrass cv. Sova of Omsk State Agrarian University was included in the State Register of Breeding Achievements Allowed for Use (State Register) for cultivation on all regions of Russia. The cv. Sova was created by mass selection of overwintered biotypes from the Thinopyrum intermedium population obtained from The Land Institute, followed by directed pollination and the creation of a new winter-hardy population

This work is the first to evaluate biological and economic characteristics of the large-grain wheatgrass cv. Sova from the State Register as an alternative to perennial wheat. The cv. Sova is recommended for cultivation for four to six years for grain and fodder. It forms high-protein grain and good quality hay.

Our aim was to evaluate the economically useful traits of a new large-grain wheatgrass cv. Sova in the conditions of the southern forest-steppe of Western Siberia and to reveal the correlation between the spike components and plant height to increase the efficiency of selection for improved thousand kernel weight.

Material and methods. During the field trials ( $55^{\circ} 02^{\prime} \mathrm{N}, 73^{\circ} 31^{\prime} \mathrm{E}$, the Omsk State Agrarian University, the southern forest-steppe of Western Siberia, 20152019), the weather conditions varied and were typical for this geographic location; in general, moisture supply was insufficient, the hydrothermal coefficient (HTC) varied from 0.86 to 1.07 . A new large-grain blue-gray wheatgrass cv. Sova, winter bread wheat (Triticum aestivum L.) cv. Omskaya 4, and spring bread wheat ( $T$. aestivum L.) cv. Element 22 and cv. Pamyati Azieva were involved.

In experiment 1, the traits of spike and grain were studied in the cv. Sova (a breeding nursery, manual sowing on May 15, 2015; $5 \mathrm{~m}^{2}$ plots, 40 cm row spacing, 10 cm plant spacing, sowing depth 2 cm , pure fallow predecessor, no replicates. In 2016-2017, the selection was carried out for the main spike at the stage of waxy ripeness. The ears were cut off to assess the yield components in 100 ears (i.e., ear weight and length, the number of spikelets and grains per ear, the number of grains per spikelet, grain weight per ear). The thousand kernel weight and the photosynthesis economic efficiency coefficient (Kecon.) were calculated. The hay quality was determined in 2015-2017 at the Omsk Agrochemical Center as per GOST R 55452-2013 (Moscow, 2014) in terms of the content of crude and digestible protein, fiber, feed units, metabolizable energy, calcium, phosphorus, carotene and sugars.

In experiment 2 (2017-2019), the grain yield and vegetative mass of cv . Sova were assessed in a nursery (manual sowing on August 15, 2016, $5 \mathrm{~m}^{2}$ plots,

40 cm row spacing, 10 cm plant spacing, pure fallow predecessor, 3-fold replicates; manual harvesting). Sheaves were weighed. When the moisture content reached an air-dry state, sheaves were threshed (an MPSU-1 sheaf thresher, Omsk Experimental Plant, Russia) and the grain was weighed. Grain yields were adjusted to $14 \%$ moisture content. Also, the morphometric parameters of grain (area, perimeter, length, width, and circularity) were compared in the cv. Sova and spring bread wheat cv. Pamyati Aziev (an EPSON XL 110000 scanner, Seiko Epson Corporation, Japan; Smart grain v. 1.2 software (http://www.kazusa.or.jp/phenotyping/smartgrain/installation.html). The grain quality of the cv. Sova was determined using an Infralum FT 10 M device (Lumex, Russia).

In experiment 3, the correlation between the yield components and plant height was assessed in cv. Sova (a breeding nursery, sowing in May 2017, a SSFK-7 seeder, Omsk Experimental Plant, Russia; 0.2 ha sowing area, 40 cm row spacing, sowing depth 2 cm , the fallow predecessor, no replicates). In 20182019, 50 tall plants and 50 low-growing plants were collected to determine the main spike and the plant components.

In 2018-2019, cv. Sova plants were dug out to a shovel bayonet depth at flowering stage; the roots of winter wheat Omskaya 4 and spring wheat Element 22 were also dug out at flowering stage ( 5 plants of each cultivar, no repetitions). The roots were washed from the soil and their length, width, average diameter, volume, main area, the number of root tips, and total length were estimated using an EPSON XL110000 scanner (Epson America, Inc., USA) and WinRHIZO 2016 Pro software (Regent Instruments, Inc., Canada).

The winter and spring wheat cultivars were sown in the field where experiment 3 was located (the pure fallow predecessor, 500 seeds per $1 \mathrm{~m}^{2}, 3$ repetitions). The winter wheat was sown on August 20 in 2017 and 2018, the spring wheat on May 20 in 2018 and 2019.

In experiment 4, the biological activity of the cv. Sova rhizosphere was compared to that of winter wheat Omskaya 4 and spring bread wheat Element 22 (the experimental field of the Omsk State Agrarian University, 2019). At tillering stage, after overwintering (May 22), and at earing stage (June 19), soil samples were taken to count rhizosphere microorganisms, the meat peptone agar (MPA) for saprophytic bacteria, including ammonifiers, starch-ammonia agar (SAA) for microorganisms that consume mineral nitrogen ( $\mathrm{NH}_{3}$ ), including actinomycetes, Czapek's medium for mushrooms, Mishustin's medium for oligonitrophils, Muromtsev-Gerretsen medium for phosphate mobilizing bacteria, Hutchinson's medium for cellulose-degrading microorganisms, leached agar with the addition of ammonium-magnesium salt of phosphoric acid for nitrifiers. The amount of nitrate nitrogen was determined by the disulfophenol method according to Grandval-Lyazh. To assess the soil biological activity, cellulose films were placed at a $0-20 \mathrm{~cm}$ depth in 4 replicates for each culture. The biological activity of the rhizosphere and soil was determined according to generally accepted methods [2325] in a 3-fold repetition.

Statistical analysis included calculation of mean values $(M)$, standard errors of means ( $\pm$ SEM) , and correlation analysis. The differences were assessed by the least significant difference at a $5 \%$ significance level (LSD05) according to the generally accepted methodology [26] using the package of applied statistical programs Microsoft Excel. Two-way analysis of variance was performed using the STATISTICA v. 6.0 (StatSoft, Inc., USA).

Results. Ear productivity is one of the main elements of the crop structure. In the cv. Sova, the thousand kernel weight averaged 9.7 g . It should be noted that cv. Sova had higher grain number per spike and Kecon. of ears (Table 1).

1. Yield components in Thinopyrum intermedium (Host) Barkworth \& D.R. Dewey cv. Sova ( $M \pm$ SEM; experiment 1, the field of the Omsk State Agrarian University)

| Trait | 2016 | 2017 | Average over two years |
| :--- | :---: | :---: | :---: |
| Ear weight, g | $0.79 \pm 0.19$ | $0.97 \pm 0.23$ | $0.88 \pm 0.21$ |
| Ear length, cm | $18.2 \pm 2.3$ | $19.8 \pm 2.5$ | $19.0 \pm 2.4$ |
| The number of spikelets per ear | $18.3 \pm 2.7$ | $21.0 \pm 3.1$ | $19.6 \pm 2.9$ |
| The number of grains per ear | $41.3 \pm 13.3$ | $60.0 \pm 19.2$ | $50.6 \pm 16.5$ |
| The number of grains per spikelet | $2.3 \pm 0.7$ | $2.9 \pm 0.9$ | $2.6 \pm 0.8$ |
| Ear grain weight, $g$ | $0.4 \pm 0.1$ | $0.5 \pm 0.1$ | $0.4 \pm 0.1$ |
| Thousand kernel weight, g | $10.4 \pm 1.5$ | $9.0 \pm 1.3$ | $9.7 \pm 1.4$ |
| Ear Kecon., $\%$ | $50.6 \pm 7.0$ | $51.5 \pm 8.0$ | $51.0 \pm 7.5$ |

N ot e. Kecon. - photosynthesis economic efficiency coefficient. For a description of the experiment, see the $M a$ terial and methods section.

The breeding improvement of Th. intermedium largely depends on the efficiency of selection based on the contingency of plant traits. We found a medium relationship of the thousand kernel weight with the stem height and the ear length ( $r=0.3 ; \mathrm{p}=0.05$ ) (Fig. 1). A negative correlation occurred between the thousand kernel weigh and the spikelet number $(r=-0.5, \mathrm{p}=0.01)$ and also the grains number per ear $(\mathrm{r}=-0.5 ; \mathrm{p}=0.01)$. Probably, the selection of shorter-stemmed clones, especially those with fewer spikelets in the main spike and, accordingly, fewer grains, is advisable to further increase the thousand kernel weigh.


Fig. 1. Correlation ( $r$ ) between traits of Thinopyrum intermedium (Host) Barkworth \& D.R. Dewey cv. Sova: 1 - plant height, 2 - ear length, 3 - plant weight, 4 - ear weight, 5 - stem weight, 6 - ear width, 7 - spikelet number per ear, 8 - grain weigh per ear, $9-$ grain number per ear, 10 - ear density, 11 - thousand kernel weight. The critical $r$ values at $\mathrm{p}=0.05$ and $\mathrm{p}=0.01$ are 0.27 and 0.39 , respectively (experiment 3, the field of the Omsk State Agrarian University, 2018-2019). For a description of the experiment, see the Material and methods section.
2. Grain traits in Thinopyrum intermedium (Host) Barkworth \& D.R. Dewey cv. Sova and spring bread wheat Triticum aestivum L. cv. Pamyati Azieva ( $M \pm$ SEM; experiment 2, the field of the Omsk State Agrarian University)

| Parameter | cv. Sova |  |  |  | cv. Pamyati Azieva |  |  | ${ }^{2}$ |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 2017 | 2018 | 2019 | 2017 | 2018 | 2019 | LSD05 |  |
| Area, $\mathrm{mm}^{2}$ | $9.28 \pm 1.45$ | $9.25 \pm 1.45$ | $9.03 \pm 1.41$ | $15.98 \pm 2.50$ | $15.11 \pm 2.36$ | $14.48 \pm 2.26$ | 1.1 |  |
| Perimeter, mm | $16.34 \pm 1.67$ | $16.87 \pm 1.72$ | $15.91 \pm 1.63$ | $16.82 \pm 1.72$ | $15.83 \pm 1.62$ | $14.23 \pm 1.46$ | 1.3 |  |
| Length, mm | $7.02 \pm 0.67$ | $7.35 \pm 0.71$ | $6.83 \pm 0.66$ | $6.47 \pm 0.62$ | $6.21 \pm 0.59$ | $6.45 \pm 0.62$ | 0.55 |  |
| Circularity | $0.44 \pm 0.05$ | $0.46 \pm 0.05$ | $0.46 \pm 0.05$ | $0.72 \pm 0.08$ | $0.72 \pm 0.08$ | $0.70 \pm 0.07$ | 0.25 |  |
| Width, mm | $1.68 \pm 0.25$ | $1.72 \pm 0.25$ | $1.67 \pm 0.24$ | $3.29 \pm 0.48$ | $3.30 \pm 0.48$ | $3.00 \pm 0.44$ | 0.81 |  |
| N ot e. For a description of the experiment, see the Material and methods section. |  |  |  |  |  |  |  |  |

The wheatgrass cv. Sova has a long, but small, incomplete grain, as evidenced by the reduced area and circularity compared to the spring bread wheat cv. Pamyati Azieva (Table 2, Fig. 2).


Fig. 2. Grain of the spring bread wheat Triticum aestivum L. cv. Pamyati Azieva (A) and wheatgrass Thinopyrum intermedium (Host) Barkworth \& D.R. Dewey cv. Sova (B) (the field of the Omsk State Agrarian University).

The cv. Sova is a dual-purpose crop for grain and also for green fodder and hay production. Its grain, green mass and hay yields increased over 3 years (Table 3 ). The grain protein and gluten levels in the new variety turned out to be very high. An interesting fact is that in the third year of reproduction, the grain protein was $2 \%$ higher. The wet gluten content was the highest in 2018 (see Table 3).
3. Yields and quality traits in wheatgrass Thinopyrum intermedium (Host) Barkworth \& D.R. Dewey cv. Sova ( $M \pm$ SEM; experiment 2, the field of the Omsk State Agrarian University)

| Year | Yield, c/ha |  |  | Protein, $\%$ | Gluten, $\%$ |
| :--- | :---: | :---: | :---: | :---: | :---: |
|  | grain | green mass | hay |  |  |
| 2017 | $8.3 \pm 2.1$ | $187.1 \pm 48.8$ | $63.0 \pm 15.3$ | $18.5 \pm 2.0$ | $35.6 \pm 4.1$ |
| 2018 | $9.0 \pm 2.3$ | $219.0 \pm 53.2$ | $73.0 \pm 18.6$ | $19.1 \pm 2.4$ | $37.2 \pm 4.3$ |
| 2019 | $10.4 \pm 2.7$ | $224.8 \pm 54.3$ | $76.4 \pm 18.8$ | $20.5 \pm 2.2$ | $36.0 \pm 4.4$ |
| Average | $9.2 \pm 2.4$ | $210.3 \pm 51.2$ | $71.0 \pm 17.8$ | $19.4 \pm 2.3$ | $36.3 \pm 4.2$ |
| $\quad$ LSD 05 | 1.2 | 13.8 | 5.0 | 1.3 | 1.1 |
| N o t e For a description of the experiment, see the Material and methods section. |  |  |  |  |  |

The hay quality was quite suitable for cattle feeding. The quality parameters were the best in 2015 in experiment 1 when the plants were harvested only for hay, i.e., prior to earing. In 2016-2017, the hay quality was assessed in experiment 1 after the grain was threshed (Table 4).

The perennial life cycle and well-developed root system of the wheatgrass plants significantly improve their ability to utilize soil moisture reserves. In the upper soil layer, the root system of the cv. Sova was more developed compared to the wheat plants (Table 5). In 2018, given maximum possible width and length of the roots $25 \times 25 \mathrm{~cm}$ limited by the size of soil cube with roots, the root size was $23.4 \times 24.7 \mathrm{~cm}$ in the wheatgrass vs. $8.0 \times 11.6 \mathrm{~cm}$ in winter wheat cv . Omskaya 4 and $7.2 \times 10.7 \mathrm{~cm}$ in spring wheat cv . Element 22. The average area of the main roots in the wheatgrass was larger than that of winter ( 7.9 -fold, $\mathrm{p} \leq 0.05$ ) and spring ( 8.4 -fold, $\mathrm{p} \leq 0.05$ ) wheats, and the area of all roots was 8.0 times larger ( $\mathrm{p} \leq 0.05$ ) and 8.4 times larger ( $\mathrm{p} \leq 0.05$ ), respectively, the roots were 0.3 and 0.1 mm larger in diameter, the root volume was 7.8 times and 6.2 times larger ( $\mathrm{p} \leq 0.05$ ), the length of all roots was 6.9 and 9.8 times ( $p \leq 0.05$ ) greater, the average number of root tips was 3.7 and 4.6 times greater (see Table 5). In 2019, similar differences between the cultures persisted. Consequently, the wheatgrass cv. Sova is significantly superior to both tested spring and winter wheat cultivars in all traits of the root system.
4. Hay quality parameters in wheatgrass Thinopyrum intermedium (Host) Barkworth \& D.R. Dewey cv. Sova (M $\pm$ SEM; experiment 1 , the field of the Omsk State Agrarian University)

| Year | Chemical composition, \% |  | Feed units, dry matter | Metabolizable energy, MJ/kg | Digestible protein, $\mathrm{g} / \mathrm{kg}$ | $\mathrm{Ca}, \mathrm{g} / \mathrm{kg}$ | P, g/kg | Carotene, mg/kg | Sugars, g/kg |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | protein | fibers |  |  |  |  |  |  |  |
| 2015 | $14.30 \pm 2.11$ | $21.50 \pm 1.96$ | $0.55 \pm 0.04$ | $8.55 \pm 0.51$ | $95.7 \pm 20.3$ | $3.08 \pm 0.43$ | $2.20 \pm 0.31$ | $53.00 \pm 6.11$ | $215.40 \pm 3.41$ |
| 2016 | $5.93 \pm 0.72$ | $31.50 \pm 3.21$ | $0.49 \pm 0.03$ | $7.62 \pm 0.47$ | $39.7 \pm 9.3$ | $3.83 \pm 0.52$ | $1.64 \pm 0.26$ | $18.00 \pm 3.24$ | $38.30 \pm 3.93$ |
| 2017 | $9.39 \pm 1.56$ | $28.10 \pm 3.02$ | $0.52 \pm 0.03$ | $8.44 \pm 0.52$ | $62.9 \pm 14.2$ | $4.32 \pm 0.63$ | $1.78 \pm 0.27$ | $28.00 \pm 4.49$ | $70.40 \pm 6.78$ |
| LSD05 | 6.42 | 8.57 | 0.08 | 0.96 | 33.3 | 1.05 | 0.53 | 24.0 | 131.1 |
| N ot e. For a description of the experiment, see the Material and methods section. |  |  |  |  |  |  |  |  |  |

5. Root traits in wheatgrass Thinopyrum intermedium (Host) Barkworth \& D.R. Dewey cv. Sova, winter bread wheat Triticum aestivum L. cv. Omskaya 4 and spring bread wheat $T$. aestivum L. cv. Element 22 ( $M \pm$ SEM; experiment 3, the field of the Omsk State Agrarian University)

| Trait | cv. Sova |  | cv. Omskaya 4 |  | cv. Element 22 |  | LSD05 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |  |
| Root width, cm | $23.4 \pm 2.3$ | $25.1 \pm 0.7$ | $8.0 \pm 1.0$ | $8.6 \pm 1.2$ | $7.2 \pm 0.7$ | $8.6 \pm 0.7$ | 8.79 |
| Root length, cm | $24.7 \pm 2.2$ | $26.2 \pm 0.6$ | $11.6 \pm 1.5$ | $12.2 \pm 1.5$ | $10.7 \pm 0.9$ | $11.6 \pm 0.8$ | 7.58 |
| Main root area, $\mathrm{cm}^{2}$ | $58.3 \pm 9.0$ | $57.9 \pm 7.2$ | $7.4 \pm 2.3$ | $7.6 \pm 1.9$ | $6.9 \pm 2.1$ | $6.1 \pm 1.5$ | 27.70 |
| Total area, $\mathrm{cm}^{2}$ | $183.2 \pm 28.4$ | $181.9 \pm 22.7$ | $23.1 \pm 7.1$ | $22.8 \pm 6.8$ | $21.7 \pm 4.7$ | $19.2 \pm 4.7$ | 87.18 |
| Average diamter, mm | $0.90 \pm 0.10$ | $0.70 \pm 0.05$ | $0.06 \pm 0.09$ | $0.58 \pm 0.08$ | $0.80 \pm 0.06$ | $0.60 \pm 0.07$ | 0.31 |
| Root volume, $\mathrm{cm}^{3}$ | $3.10 \pm 0.70$ | $3.21 \pm 0.70$ | $0.40 \pm 0.09$ | $0.41 \pm 0.09$ | $0.50 \pm 0.09$ | $0.30 \pm 0.10$ | 1.49 |
| Total root length, cm | $854.2 \pm 42.5$ | $826.3 \pm 56.9$ | $124.3 \pm 25.5$ | $138.5 \pm 30.3$ | $87.4 \pm 20.7$ | $96.5 \pm 19.0$ | 395.4 |
| Root tip number | $1643.0 \pm 460.0$ | $2099.0 \pm 261.0$ | $439.9 \pm 129.9$ | $450.3 \pm 131.2$ | $355.0 \pm 79.2$ | $360.9 \pm 76.3$ | 811.6 |
| Note. For a description of the experiment, see the Material and methods section. |  |  |  |  |  |  |  |

6. Rhizosphere microbial community composition (CFU/g) in wheatgrass Thinopyrum intermedium (Host) Barkworth \& D.R. Dewey cv. Sova and winter bread wheat Triticum aestivum L. cv. Omskaya 4 ( $M \pm$ SEM; experiment 4, the field of the Omsk State Agrarian University, 2019)

| Microodganisms | cv. Omskaya 4 |  |  | cv. Element 22 |  |  | Average value for cv. Sova vs. cv. Omskaya 4, \% | LSD05 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | dated |  | average | dated |  | average |  |  |
|  | 05/22 | 06/19 |  | 05/22 | 06/19 |  |  |  |
| Bacteria on MPA, $\times 10^{6}$ | 25.0 | 19.4 | $22.2 \pm 1.8$ | 31.0 | 26.1 | $28.6 \pm 1.5$ | +28.8 | 6.3 |
| Microorganisms on SAA, $\times 10^{6}$ | 15.1 | 14.3 | $14.7 \pm 0.3$ | 42.0 | 22.5 | $32.3 \pm 6.2$ | +119.7 | 6.6 |
| Oligonitrophylic bacteria, $\times 10^{6}$ | 77.0 | 69.0 | $73.0 \pm 2.5$ | 328.2 | 46.3 | $187.3 \pm 89.1$ | +156.5 | 96.0 |
| Phosphate mobilizing bacteria, $\times 10^{6}$ | 84.0 | 51.1 | $67.6 \pm 10.4$ | 209.8 | 51.6 | $130.7 \pm 50.0$ | +93.3 | 44.1 |
| Fungi, $\times 10^{3}$ | 38.0 | 28.5 | $33.3 \pm 3.0$ | 52.8 | 50.0 | $51.4 \pm 0.9$ | +54.4 | 42.5 |
| Cellulosolytics, $\times 10^{3}$ | 70.6 | 27.0 | $48.8 \pm 13.7$ | 87.9 | 31.7 | $59.8 \pm 17.7$ | +22.5 | 42.6 |
| Nitrifying bacteria, cells | 163.0 | 220.3 | $191.7 \pm 18.0$ | 131.0 | 288.6 | $210.0 \pm 49.8$ | +9.5 | 161.0 |
| Total, $\times 10^{6}$ | 201.4 | 154.1 | $177.8 \pm 14.0$ | 611.3 | 146.9 | $379.2 \pm 146.8$ | +281.4 | 147.0 |
| N ot e. MPA - meat peptone agar, SAA - starch ammoniac agar. For a description of the experiment, see the Material and methods section. |  |  |  |  |  |  |  |  |

The total number of groups of agronomically useful microorganisms in the rhizosphere of the wheatgrass cv. Sova exceeded that in winter wheat Omskaya 4 by $201.4 \times 10^{6} \mathrm{CFU} / \mathrm{g}$, or 2.1 times ( $\mathrm{p} \leq 0.05$ ). However, an increase in microbial abundance varied for each of the identified groups, being the greatest in oligonitrophylic and phosphates mobilizing bacteria (by $114.3 \times 10^{6}$ and $63.1 \times 10^{6} \mathrm{CFU} / \mathrm{g}$, respectively). The number of these microbes particularly significantly increased (up to $328 \times 10^{6}$ and $210 \times 10^{6} \mathrm{CFU} / \mathrm{g}$ ) during tillering of the wheatgrass in May when the soil moisture was still enough (more than $20 \%$ ). Oligonitrophylic bacteria can survive under a lack of soil nitrogen and fix atmospheric nitrogen [27]. Phos-phate-mobilizing microorganisms isolated on the Muromtsev-Gerretsen medium transform mineral phosphorus compounds that are difficult to access for plants in the soil, which improves phosphorus nutrition of plants. It can be assumed that in the wheatgrass of the third year of reproduction the root excrete composition is more favorable for the growth and vital activity of these microorganisms than in winter wheat (Table 6).

According to Sindireva et al. [28], the numerical ratio of different types of microorganisms serves as a better indicator of conditions than the number of one species, since the whole better reflects the total impact than the part. The ratio of groups of microorganisms on SAA to MPA assesses the intensity of mineralization of soil organic nitrogen-containing compounds while that on MPA to SAA indicates the intensity of nitrogen immobilization [29]. On average, according to preliminary data, mineralization in the rhizosphere of wheatgrass was more intense (SAA/MPA $=1.1$ ), while immobilization of nitrogen and its accumulation by organic soil compounds prevailed in the rhizosphere of winter wheat plants, as SAA/MPA $=0.66$ evidenced. The abundance of soil fungi and cellulose-decomposing microorganisms in the rhizosphere of wheatgrass was also higher than under winter wheat, by $11 \times 10^{3}$ and $18 \times 10^{3} \mathrm{CFU} / \mathrm{g}$, or by 22.5 and $54.4 \%$.

Stimulation of soil microflora in the rhizosphere of wheatgrass is apparently associated with the peculiarities of the root system of this perennial cereal crop. Thus, the total root area in wheatgrass exceeded that in winter wheat almost 8.0 -fold, and the root width 2.9 -fold (see Table 5). Cellulose test, according to the author of the method Tikhomirova [30], characterizes in general the effective fertility under the crop. We observed the highest activity of soil microflora under wheatgrass compared to winter and spring wheats, as evidenced by the intensity of cellulose decomposition in the $0-20 \mathrm{~cm}$ layer, $57.0,43.3$, and $35.6 \%$ (Table 7).
7. Cellulose decomposition (\%) in soil under wheatgrass Thinopyrum intermedium (Host) Barkworth \& D.R. Dewey cv. Sova, winter bread wheat Triticum aestivum L. cv. Omskaya 4 and spring bread wheat T. aestivum L. cv. Element 22 ( $M \pm$ SEM; experiment 4, the field of the Omsk State Agrarian University, 2019)

| Crop (A) |  | Soil layer (B) |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: |
|  | $0-10 \mathrm{~cm}$ | $10-20 \mathrm{~cm}$ | $0-20 \mathrm{~cm}$ | min-max |  |
| Wheat cv. Omskaya 4 | $49.8 \pm 4.9$ | $36.7 \pm 5.2$ | $43.3 \pm 4.1$ | $33.8-59.1$ |  |
| Wheatgrass cv. Sova | $60.9 \pm 2.9$ | $53.2 \pm 6.6$ | $57.0 \pm 2.4$ | $47.2-66.2$ |  |
| Wheat cv. Element 22 | $34.0 \pm 1.2$ | $37.2 \pm 4.8$ | $35.6 \pm 1.0$ | $31.5-41.4$ |  |

$\overline{\mathrm{Note} \text {. LSD05: } \mathrm{A}=11.0 \text {, for } \mathrm{B}=6.9, \mathrm{AB}}=15.5$. For a description of the experiment, see the Material and methods section.

In addition to traditional breeding methods, the cultivation of wild wheat relatives, such as Thinopyrum intermedium, is undoubtedly promising for agriculture which should be regenerative and resistant to climate change. Back in the 1980s, Jackson [31] suggested that the maximum sustainable crop yields can be obtained through the use of perennial crops. Based on the findings of The Land Institute, and additional breeding for winter hardiness, followed by targeted pollination of the isolated clones, we have created the Sova cultivar adapted for

Russia conditions. For Russian agriculture, the cv. Sova is the first large-grain wheatgrass crop with a thousand kernel weight of $9.0-10.4 \mathrm{~g}$ (see Table 1). Despite the fact that the cv. Sova is significantly inferior to the spring and winter wheats in thousand kernel weight, its grain can be used in bakery and confectionery products, as evidenced by the example of a similar wheatgrass cv. Kernza in the USA [1]. A further increase in the thousand kernel weight is among the main challenges of the wheatgrass breeding. Given the correlations between the spike traits (see Fig. 1), the selection of forms with a smaller number of spikelets and a smaller number of grains per main spike seems advisable to further increase the thousand kernel weight. The medium negative correlation between the plant height and the thousand kernel weight ( $r=-0.3 ; \mathrm{p}=0.05$ ) is positive in breeding forms for grain use, since taller populations are more prone to lodging.

In the works of The Land Institute, in the fourth breeding cycle, the kernel weigh had a high heritability in the wheatgrass population $\left(\mathrm{h}^{2}=0.68\right)$, but this trait was studied in plants with the largest grain weight per ear [32]. The length of the grain in the cv. Sova cultivar corresponded to that of common wheat, however, the circularity, indicating the filling of the grain, was significantly less than in the wheat cv. Pamyati Aziev (see Table 2). Breeding for an increased circularity which is closely related to the grain weight per volume is of certain value in wheatgrass breeding for grain use. The grain yield of perennial cereals is lower than that of annual crops, because part of the energy is spent on the development of the root system and branching after overwintering. However, since the cost of grain production is lower, you can get a net profit, despite the low yield of wheatgrass compared to annual cereals, as evidenced by the experience of American researchers [33]. It is possible to cultivate wheatgrass without loss for four to six years, while the appropriate agricultural technology and mineral fertilizing in the spring will increase the crop yield.

The grain protein content in the cv. Sova cultivar was high (see Table 3). The amount of protein in the wheatgrass grain is significant, but the content of high molecular weight glutenin subunits which determine the high baking quality is less than that of bread wheat. A higher total content of dietary fiber in wheatgrass flour ( $16.4 \%$ ) than in whole wheat flour ( $11.0 \%$ ) is also indicated [34]. Dietary fibers in a person's diet are helpful in controlling blood glucose after a meal. Coarse wheatgrass flour contains a significant amount of dietary fiber and antioxidants [19, 34]. It was found that for most of the wheatgrass populations, a higher antioxidant activity in grain is characteristic as compared to wheat [21].

Increasing erosion is the most common soil degradation process worldwide. Soil erosion leads to a decrease in soil fertility, pollution of receiving water bodies downstream and an increase in greenhouse gas emissions [35, 36]. The roots of the cv. Sova is significantly superior to that of spring and winter wheats, which favorably affects the biological role of this variety in increasing fertility and reducing negative erosion processes in the soil (see Table 5)

Microorganisms play an important role in the main soil processes that determine the level of root nutrition in plants. The intensity and direction of microbiological processes contribute to the better utilization of mineral and organic compounds [37]. Species and varieties have their specific root excretes which contain organic physiologically active compounds - enzymes, vitamins, growth substances, etc. [38]. Therefore, in the root zone, abundant saprophytic microflora multiplies in an abundance more significant than in the soil mass. Our experiments showed that the conditions in the rhizosphere of wheatgrass cv. Sova are more favorable for the development of agronomically important microorganisms a compared to the winter wheat cv. Omskaya 4. Their total number exceeded 2.2 times
the corresponding values in the winter crop. The mineralization (SAA/MPA) was $58 \%$ higher, the cellulose decomposition 13.7 and $21.4 \%$ higher, respectively, than in the varieties of winter and spring wheat (see Tables 6, 7). These results are consistent with reports from other research groups [39-41].

To summarize, it should be noted that the wheatgrass breeding for economically useful traits is promising to realize the genetic potential of the crop. The expansion of the cultivation areas of perennial crops will contribute to the rational use of natural resources, improve the phyto climate, balance the diet of the population and, in general, will contribute to the ecological improvement, the accumulation of carbon dioxide in the soil and the reduction of negative greenhouse effects. At present, the main breeding technique for wheatgrass is selection for economically useful traits. However, in the short term, the efficiency of marker-assisted selection (MAS) may become significantly higher. We expect that breeding progress will be accelerated through MAS programs. Their use together with new breeding tools will improve the wheatgrass yield and agronomic traits.

Thus, the grain, green mass and hay yields of the perennial wheatgrass cv. Sova for three years of reproduction increased every year and averaged 9.2, 210.3, and $71.0 \mathrm{c} / \mathrm{ha}$, respectively. The grain quality parameters of the new variety were high ( $19.4 \%$ protein and $36.3 \%$ gluten). The grain number per ear was on average more than 50 pieces, the 1000 grain weight was 9.7 g , Kecon. of ears $51 \%$. The roots of the cv. Sova variety is 6.9-9.8 times longer, and the total surface area of roots is 8.0 times larger than that of winter and spring bread wheats. The total abundance agronomically useful groups of microorganisms in the rhizosphere of the cv. Sova turned out to be 2.2 times higher, with $58 \%$ higher intensity of mineralization (by the ratio of microorganisms grown on SAA to MPA) as compared to the winter wheat cv. Omskaya 4. Cellulose decomposition was 13.7 and $21.4 \%$ higher than that of winter and spring wheat varieties. The study of correlations between the thousand kernel weight, plant height, and productivity traits revealed the expediency of selecting shorter-stemmed biotypes with a smaller spikelet number and grain number per main spike in order to further increase the grain size. The cv. Sova variety is included in the State Register of Breeding Achievements Permitted for Use and is recommended for cultivation in the regions of Russia for four to six years for fodder and grain purposes.

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